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CHECKLIST OF THE FRESHWATER MOLLUSCA OF TURKEY (MOLLUSCA: GASTROPODA, BIVALVIA)

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ABSTRACT

Considering the studies related to the freshwater molluscs in Turkey, it can be seen that the first checklist was done in freshwater and brackish water in 1999 and 72 taxa were determined. One of the checklists published in 2006 included just the freshwater pulmonate species and in this checklist 28 taxa were reported. Another checklist on prosobranchia was also released in the same year and 80 taxa were identified. Any studies on this subject have not been done since 2006. In this paper all studies related to freshwater were recorded, and the existence of 204 taxa (164 species belonging to Gastropoda and 40 species belonging to Bivalvia) were given. A checklist about the Bivalvia in Turkey was also presented for the first time in this paper.

KEYWORDS: Freshwater, mollusca, gastropoda, bivalvia, Turkey, checklist

INTRODUCTION

Freshwater molluscs key component role in aquatic ecosystems, providing food for many fish species [1, 2] and vertebrates [3]. According to Wosu, 2003 they prove immensely beneficial both economically and medicinally [4]. They have been important to humans throughout history as a source of food, jewellery, tools and even pets. Freshwater molluscs play significant role in public and veterinary health [5]. Some freshwater molluscs are vectors of diseases of humans and livestock, serve as the intermediate hosts for a number of infections such as helminth diseases caused by trematodes [6]. Among the most important diseases of humans and animals are fascioliasis (caused by Fasciola hepatica and F. gigantea) and opisthorchiasis (caused by Opisthorchis felineus). Freshwater molluscs fall into two main groups, the Bivalvia and the Gastropoda, with the latter dividing into two informal groups, the prosobranchs and the pulmonates [7].

The aims of this study are to summarize the available information about the mollusca diversity in the freshwater of Turkey and to determine the distribution of species richness.

MATERIALS AND METHODS

To preparing of the Turkish freshwater malacofauna, related publications on freshwater bivalvia and gastropoda from Turkey were analyzed. 143 papers (between 1841 and 2019) were used in the checklist. The first taxonomic studies in Turkey about freshwater mollusks were performed by Reclu in 1841 [8].

While preparing the checklist, all the studies that have been performed were taken into account. Studies were conducted so as to provide geographical distribution in Turkey and accordingly the country was divided into eight separate regions and therefore the regions in which most and least studies were performed were determined. ArcGIS 9.3 software was used while conducting these operations.

RESULTS

As a result of the studies conducted on gastropoda and bivalvia in Turkey, the presence of 204 taxa have been reported (Table 1).

DISCUSSION

In Turkey, when we look at the studies performed so far, it can be observed that the studies are focused on the Mediterranean, Aegean, Marmara and Central Anatolia regions. According to the number of species found and the scientific study average, the least studied regions are Eastern Anatolia and Eastern Black Sea regions (Figure 1).
### TABLE 1
Checklist of Freshwater Mollusca of Turkey (Gastropoda and Bivalvia)

<table>
<thead>
<tr>
<th>Species no.</th>
<th>Classification</th>
<th>Area</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Class: Gastropoda Subclass: Neritimorpha Order: Cycloneritimorpha Superfamily: Neritoidea Family: Neritidae Genus: Theodoxus</td>
<td>Sarıkum Lake (Sinop), Balık Lake, Uzun Lake, Göc Lake, Çernek Lake, Lake Sapanca, Terkos Lake, Istranca Stream, Büyük Menderes River, Yuvarlakçay (Köyceğiz), Poyrazlar Lake, Taşkıngı Lake, Büyük Akgöl Lake</td>
<td>[9, 10, 11, 12, 13, 14, 15, 16]</td>
</tr>
<tr>
<td>2.</td>
<td>Species</td>
<td>Theodoxus fluviatilis Linnaeus, 1789</td>
<td>Lake Eğirdir, Kovaďa Channel, Lake Kovaďa</td>
</tr>
<tr>
<td>3.</td>
<td>Species</td>
<td>Theodoxus heldreichi heldreichi Martens, 1879</td>
<td>Kiremihanenler, Diündenbaş Source (Antalya), Aksu (Kahramanmaraş), Sakarya River, Manavgat (Antalya), Yarımburgaz (İstanbul), Bıyıças, İzmir, Yenişehir (Antalya), Beçapınar Source (Manisa), Akpınar Source (Manisa), Nif River, Çifteer (Eskişehir), Çakal Lake (Soluç), Gökpinar (Denizli), İşikli (Dinar), İnilice (Fethiye), Maden Stream, Beşgöl, Başgöz, KümbețiPINar Sazak, Hacapınar, Gülbahçe Village Stream, Muradiey (Manisa), Malatya, Antakya, Denizli, Mersin Limonlu (Lamos), Gölbaşı Lake, Azaplı Lake, İnêkli Lake, Ceyhan River (Adana), Gökşu (Silifke), Soğuksu (Anamur), Antalya, Eskişehir, Şulat (Afyonkarahisar), Sandıklı (Afyonkarahisar)</td>
</tr>
<tr>
<td>4.</td>
<td>Species</td>
<td>Theodoxus syriacus Bourguignat, 1852</td>
<td>Adana, Gökşu (Silifke), Çennet Çehennem (Silifke), Erdenli (Mersin), Bücek (Kozan), Elmalıheçe (Mardin), Çırlı (Diyarbakır), Tigris River, Tülüük (Diyarbakır), Silvan (Diyarbakır), Derik (Mardin), Aynizelihya source (Diyarbakır), Tila Lake village source (Diyarbakır), Örnök village source (Diyarbakır), Bağveren village source (Diyarbakır), Nasirli village source (Diyarbakır), Devegeneval water (Diyarbakır), Sıhur (Mardin), Fabrika stream (Diyarbakır), Nusaybin (Mardin), Suruç (Urfa)</td>
</tr>
<tr>
<td>5.</td>
<td>Species</td>
<td>Valvata cristata Müller, 1774</td>
<td>Döşeme Alti Lake (Antalya), Kırkgöz (Antalya)</td>
</tr>
<tr>
<td>6.</td>
<td>Species</td>
<td>Theodoxus altai Schütz, 1965</td>
<td>Lake Sapanca</td>
</tr>
<tr>
<td>7.</td>
<td>Species</td>
<td>Theodoxus danubialis Pfeiffer, 1828</td>
<td>Lake Gölbasi (Hatay)</td>
</tr>
<tr>
<td>8.</td>
<td>Species</td>
<td>Theodoxus jordani Sowerby, 1832</td>
<td>Ceylanpinar, Habur River</td>
</tr>
<tr>
<td>9.</td>
<td>Species</td>
<td>Theodoxus cinctellus Martens, 1874</td>
<td>Yamsaz Lake (Antalya)</td>
</tr>
<tr>
<td>10.</td>
<td>Species</td>
<td>Theodoxus pallasi Lindholm, 1924</td>
<td>Ildre Springs, Gülbahçe Village Stream, Balikhova Spring, Urla Mineral Springs, Yuvarlakçay (Köyceğiz)</td>
</tr>
<tr>
<td>11.</td>
<td>Species</td>
<td>Theodoxus gloerii Odabaşi and Arslan, 2015</td>
<td>Sakarya River, Balıkdamlı wetland (Eskişehir)</td>
</tr>
<tr>
<td>13.</td>
<td>Species</td>
<td>Valvata cristata Müller, 1774</td>
<td>Lake Gölbasi (Hatay)</td>
</tr>
<tr>
<td>14.</td>
<td>Species</td>
<td>Valvata saucyli Bourguignat, 1853</td>
<td>Işıklı Lake, Lake Gölbasi (Hatay)</td>
</tr>
<tr>
<td>15.</td>
<td>Species</td>
<td>Valvata macrostoma Mörch, 1864</td>
<td>İzmir, Alaşehir, Kazağan Lake (Coraklar Spring), Efes Lake (Selçuk), Narlıkuyu (Bornova), Manavgat (Bornova), Kocasu (Bornova), Lake Gölbasi (Hatay), Gökşu Springs, Kani Fountain, Üçparı Village, Oğlanagaş Pond, Akhisar reservoir, Akpınar Stream, Akyağa Kadın River branch, Poyrazlar Lake, Taşkıngı Lake, Büyük Akgöl, Acilar Lake, Abant Lake, Yenicağa Lake, Azaplı Lake, İnêkli Lake, Ceyhan River basin</td>
</tr>
<tr>
<td>16.</td>
<td>Species</td>
<td>Valvata piscinalis Müller, 1774</td>
<td>Tuzla Stream, Dümrek Brook, Karamenderes Stream (Canakkale), Cire Brook, Lake Eğirdir (İsparta)</td>
</tr>
<tr>
<td>No.</td>
<td>Genus:</td>
<td>Species</td>
<td>Location</td>
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<tr>
<td>18.</td>
<td>Borysthenia</td>
<td>Borysthenia naticina</td>
<td>Lake Sapanca, Karatay Lake, Istranca Stream, Lake Egirdir, Kova da Channel, Lake Kovada, Yuvarlaçtay (Köyceğiz)</td>
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<tr>
<td>20.</td>
<td>Viviparus</td>
<td>Viviparus contectus</td>
<td>Karaoz Pond (Isparta), Beysehir Lake (Konya), Lakes Region, Egirdir Lake (Isparta)</td>
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<td>21.</td>
<td>Viviparus</td>
<td>Viviparus mamillatus</td>
<td>Gerc Lake, Cermek Lake, Terkos Lake</td>
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<tr>
<td>22.</td>
<td>Viviparus</td>
<td>Viviparus acerosus</td>
<td>Küçük Akgöl, Taşkışlı Lake, Büyük Akgöl, Yeniçağa Lake, Karamurat Lake, Lake Sapanca</td>
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<td>24.</td>
<td>Viviparus</td>
<td>Viviparus syriacus</td>
<td>Melanoides tuberculata Müller, 1774</td>
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<td>25.</td>
<td>Viviparus</td>
<td>Viviparus praemorsa</td>
<td>Melanopsis praemorsa</td>
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<tr>
<td>26.</td>
<td>Viviparus</td>
<td>Viviparus acerosus</td>
<td>Melanopsis acicularis stussineri</td>
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<tr>
<td>27.</td>
<td>Viviparus</td>
<td>Viviparus praemorsa</td>
<td>Melanopsis costata</td>
</tr>
<tr>
<td>28.</td>
<td>Viviparus</td>
<td>Viviparus praemorsa</td>
<td>Melanopsis costata costata</td>
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<tr>
<td>29.</td>
<td>Viviparus</td>
<td>Viviparus acicularis stussineri</td>
<td>Lake Sapanca</td>
</tr>
<tr>
<td>30.</td>
<td>Viviparus</td>
<td>Viviparus syriacus</td>
<td>Lake Sapanca</td>
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<tr>
<td>31.</td>
<td>Viviparus</td>
<td>Viviparus acicularis stussineri</td>
<td>Lake Sapanca</td>
</tr>
<tr>
<td>32.</td>
<td>Viviparus</td>
<td>Viviparus syriacus</td>
<td>Lake Sapanca</td>
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<tr>
<td>33.</td>
<td>Viviparus</td>
<td>Viviparus syriacus</td>
<td>Lake Sapanca</td>
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<td>34.</td>
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<td>Viviparus syriacus</td>
<td>Lake Sapanca</td>
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<td>35.</td>
<td>Viviparus</td>
<td>Viviparus syriacus</td>
<td>Lake Sapanca</td>
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<td>36.</td>
<td>Viviparus</td>
<td>Viviparus syriacus</td>
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<td>37.</td>
<td>Viviparus</td>
<td>Viviparus syriacus</td>
<td>Lake Sapanca</td>
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<tr>
<td>38.</td>
<td>Viviparus</td>
<td>Viviparus syriacus</td>
<td>Lake Sapanca</td>
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</tbody>
</table>
39. *Bithynia pseudemmericia* Schütt, 1964
   Kirkçoğ (Antalya), Panarbaşı (Antalya), Yeniköy spring, spring Astyada, Eber Lake, Afyon, Isparta, Lake Eğirdir, Kovada Channel, Lake Kovada, Avlan Lake (Konya), Elmalı (Antalya), Lake Hazar (Elazığ), Istranca Stream (İstanbul), Malatya, Tunceli [16, 17, 24, 28, 31, 33, 45, 54, 55, 56]

40. *Bithynia phialensis* Conrad, 1852
   Lake Gölbasi (Hatay) [35]

41. *Bithynia badiella* Kuster, 1852
   Gelbula village, Tarsus [22, 57]

42. *Bithynia pesicii* Gloer and Yıldırım, 2006
   [16, 17, 24, 28, 31, 33, 45, 54, 55, 56]

43. *Bithynia yildirimi* Gloer and Georgiev, 2012
   Karsanli village [58]

44. *Bithynia timmii* Ogden and Arslan, 2015
   Lake Uluabat [59]

45. *Bithynia kayrae* Ogden and Ogden, 2017
   Çanakkale [60]

**Genus:** *Pseudobithynia*

46. *Pseudobithynia pentheri* Sturany, 1905
   Karpuz Sekisi, Ambar Park Brook, Kayseri [56, 61]

47. *Pseudobithynia yildirimi* Odacan, Kebapci and Akin, 2013
   Tuzla Stream, Biga Peninsula [42]

48. *Pseudobithynia adiyamanensis* Gürel, 2017
   [62]

49. *Pseudobithynia adiyamanensis* Gürel, 2017
   Karpuz Sekisi, Ambar Park Brook, Kayseri [56, 61]

50. *Pseudobithynia yildirimi* Odacan, Kebapci and Akbulut, 2013
   Tuzla Stream, Biga Peninsula [42]

51. *Pseudobithynia adiyamanensis* Gürel, 2017
   [62]

52. *Pseudobithynia gulgocuica* Gürel, 2018
   Mersin-Aydıncık Yeniköş Soğukus spring [63]

53. *Pseudobithynia cocossica* Gürel, 2019
   Kocaeli - Törbüzek stream [150]

**Family:** *Bythinellidae*

54. *Bythinella opaca* Frauenfeld, 1857
   Belgrad Forest (İstanbul) [22]

55. *Bythinella occasiuncula* Boeters and Falkner, 2001
   Kırk Oluç spring (Odenişi), İzmir [64]

56. *Bythinella kazdaghenis* Odabası and Georgiev, 2014
   Canakkale- Ayaçma Deresi, South of Evciler village at the Mount Ida (Kaz Dağı) [65]

57. *Bythinella anatolica* Yıldırım, Kebapçi and Bahadır Koca, 2015
   Manisa Spil mountain Çırıcalıdede hill [66]

58. *Bythinella istanbulensis* Yıldırım, Kebapçi and Yuço, 2015
   İstanbul Belgrad forest [66]

59. *Bythinella turbochica* Yıldırım, Kebapçi and Bahadır Koca, 2015
   Aydin-Incirliova, Karagözler Village, Karapınar Spring [66]

60. *Bythinella wilkei* Yıldırım, Kebapçi and Bahadır Koca, 2015
   Kocaeli- Maşukiye, Kartepe [66]

61. *Bythinella veltii* Gürel, 2017
   Balikesir-Dursunbey Osmanye village [62]

**Genus:** *Potamopyrgus*

62. *Potamopyrgus antipodarum* Gray, 1843
   Deveönü, Çakal Lake (Selçuk), Finike (Antalya), Akyaka (Muğla), Kirkçoğ (Antalya), Gümüldür (İzmir), Sarıçay creek, Ildır Springs, Mene- men, Çeşme (İzmir), Çay (Afyon), Çıvırl (Denizli), Yuvvalıkçay (Köyceğiz), Karkamış Dam Lake (Gaziantep), Sea of Marmara [10, 25, 28, 29, 33, 39, 40, 43, 67, 68, 69, 70]

**Family:** *Hydrobiidae*

63. *Anadoludamnicola glori* Koşal Şahin, Koca & Yıldırım, 2012
   Akçadağ (Malatya), İnek Spring, Malatya, Tunceli [31, 54, 71]

64. *Anadoludamnicola glori brevis* Koşal Şahin, Koca & Yıldırım, 2012
   Malatya, Takas Village [31, 71]

**Genus:** *Hemite*

65. *Hemite ceyhanensis* Gürel, 2019
   Osmanye Hemite bridge [150]

**Subfamily:** *Belgrandiellinae*

66. *Belgrandiella* Şanlıurfa, Siverek [27]
65. Belgrandiella edessana Schütt, 1993
   Belgrandiella cavernica Boettger, 1957
   Belgrandiella adsharica Lindholm, 1913

66. *Hydrobia ventrosa* Montagu, 1803
   *Belgrandiella cavernica* Boettger, 1957
   *Belgrandiella adsharica* Lindholm, 1913

67. *Hydrobia ventrosa* Montagu, 1803
   *Belgrandiella cavernica* Boettger, 1957
   *Belgrandiella adsharica* Lindholm, 1913

68. *Hydrobia soosi* Wagner, 1928
   *Hydrobia soosi* Wagner, 1928
   *Hydrobia soosi* Wagner, 1928

69. *Hydrobia anatolica* Schütt, 1965
   *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805

70. *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805

71. *Subfamily: Hydrobiinae*  
   *Genus: Hydrobia*

72. *Hydrobia ventrosa* Montagu, 1803
   *Hydrobia soosi* Wagner, 1928
   *Hydrobia anatolica* Schütt, 1965

73. *Hydrobia soosi* Wagner, 1928
   *Hydrobia soosi* Wagner, 1928
   *Hydrobia soosi* Wagner, 1928

74. *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805

75. *Hydrobia anatolica* Schütt, 1965
   *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805

76. *Hydrobia soosi* Wagner, 1928
   *Hydrobia soosi* Wagner, 1928
   *Hydrobia soosi* Wagner, 1928

77. *Hydrobia soosi* Wagner, 1928
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87. *Hydrobia soosi* Wagner, 1928
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76. *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805
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83. *Hydrobia acuta* Draparnaud, 1805
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   *Hydrobia acuta* Draparnaud, 1805
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87. *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805
   *Hydrobia acuta* Draparnaud, 1805

88. *Genus: Pseudorientalia*
   *Pseudorientalia natolica* Radoman, 1973

89. *Pseudorientalia ceriti* Gürlek, 2017
   *Pseudorientalia ceriti* Gürlek, 2017
   *Pseudorientalia ceriti* Gürlek, 2017

90. *Pyrgorientalia zilchi* Schütt, 1964
   *Pyrgorientalia zilchi* Schütt, 1964
   *Pyrgorientalia zilchi* Schütt, 1964

91. *Genus: Kirelia*
   *Kirelia carinata* Radoman, 1973
   *Kirelia carinata* Radoman, 1973
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<th>No.</th>
<th>Genus/M. Common Name</th>
<th>Location/Model</th>
<th>Reference(s)</th>
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</thead>
<tbody>
<tr>
<td>92.</td>
<td><em>Kirelia murtici</em></td>
<td>Martići spring (Antalya), endemic</td>
<td>[84]</td>
</tr>
</tbody>
</table>
| 93. | Genus: *Falsipyrgula*  
*Falsipyrgula pfeiferi* | Lake Eğirdir, Kovada Channel, Lake Kovada | [17] |
| 94. | | Lake Beysėhir (Konya) | [22] |
| 95. | | Lake Beysėhir, Beysėhir Lake (Konya) | [32, 76] |
| 96. | Genus: *Falsibelgrandiella*  
*Falsibelgrandiella bunarica* | Pinarbaşı spring (Gedelek), Gemlik (Bursa) | [84, 85] |
| 97. | Genus: *Graecoanatolica*  
*Graecoanatolica lacustristurca* | Lake Eğirdir, Kovada Channel, Lake Kovada, Lake Köşkpinar spring | [17, 18, 86, 87, 88, 89] |
| 98. | | Gemiş spring (Denizli), Gökgöl Lake, Işikli Lake (Denizli) | [73] |
| 99. | | Kocapinar spring (Isparta) | [73] |
| 100. | | Aci Lake | [73, 84, 90] |
| 101. | | Lakes Region | [73] |
| 102. | | Pınar Gözü spring (Burdur) | [73] |
| 103. | | Muğla, Antalya, Kırkgöz springs, Korkuteli Yanıkpinar, Gömbe Upland | [22, 73, 91] |
| 104. | | İnçirlipnar spring, Ulupınar spring (Afyonkarahisar) | [73] |
| 105. | | Sinop Sankum Lake | [92] |
| 106. | | Mağla Akyaka-Gökoğa karst spring | [92] |
| 107. | Genus: *Heleobia*  
*Heleobia longiscata* | Lake Gölbaşı | [35] |
| 108. | Subfamily: Horatiinae  
Genus: *Horatia*  
*Horatia parvula* | Tokat, Isparta Keçiborlu stream | [22] |
| 109. | Genus: *Sadleriana*  
*Sadleriana affinis* Frauenfeld, 1863 | Karpuzatan (Kayseri), | [22] |
| 110. | | Bursa, Sakarya, Bilecik | [22, 90] |
| 111. | | Yarıncın Spring, Gömbe Spring (Antalya), Kümbetpinar Spring, Yarışlı Village Spring, Yeşilova (Burdur), Burdur Lake Basin, Kümbetpinar, Kocapinar Village, Yarışlı Lake | [18, 91, 93] |
| 112. | | Lake Sapanca | [34] |
| 113. | | Eregli (Konya) | [94] |
| 114. | Subfamily: *Islamianae*  
Genus: *Islamia*  
*Islamia bunarbasia* Schütz, 1964 | Antalya-Kırkgöz karst springs | [84] |
| 115. | | Antalya-Kırkgöz karst springs | [84] |
| 116. | | Antalya-Kırkgöz karst springs | [84] |
| 117. | | Burdur-Aziziye Çibiş spring | [95] |
| 118. | Genus: *Tefennia*  
*Tefennia tefennica* Schütz and Yıldırım, 2003 | Başpinar Spring (Tefenni, Burdur) | [18, 76, 96] |
| 119. | Genus: *Orientalina*  
*Orientalina capitulacas* Schütz and Şeşen, 1993 | Golbaşı Lake (Adıyaman), Azaplı Lake (Adıyaman) | [27] |
### Genus: Turkorientalia

120. *Turkorientalia anatolica*
   Radoman, 1973
   - Yerçey Spring (Yarılış Spring), Yeşiüova (Burdur)
   - [32, 84, 97]

### Genus: Sheitanok

121. *Sheitanok amidicus*
   Schütt & Sechen, 1991
   - Adiyaman, Diyarbakır, Mardin, Siirt, Şırnak,
   - [98]

### Genus: Torosia

122. *Torosia proscwitzi*
   Gloer & Georgiev, 2012
   - Toros, Gölhisar, Burdur
   - [58]

### Genus: Sivasi

123. *Sivasi bodoni Koşal Şahin, Koca & Yıldırım, 2012*
   - Sivas-Gürün Gökpnar
   - [71]

### Family: Lithoglyphidae

124. *Lithoglyphus naticoides*
   Pfeiffer, 1828
   - 7DU9LOODJH.ÕUNSÕQDU0D
   - [13, 14, 48]

### Pulmonate

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<th>Species no.</th>
<th>Classification</th>
<th>Area</th>
<th>Source</th>
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<td>Class: Gastropoda</td>
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<td>Family: Acroloxidae</td>
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<td><strong>Genus: Acroloxus</strong></td>
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<td></td>
<td><em>Acroloxus lacustris</em> Linnaeus, 1758</td>
<td>Gökpınar pond (Denizli), Gökgöl Village source (Dinar-Çivri arası), Yayla Lake (Buldan), Kirkçeş (Antalya), Körfez (Antalya), Lake Eğirdir (Isparta), Lake Sapanca (Sakarya), İzmir, Tefenni Başpınar Village Kirkpinar, Matla, Tunceli, Büyük Menderes River</td>
<td>[11, 18, 23, 28, 31, 33, 34, 45, 54, 67, 72, 89, 99, 100]</td>
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<td>2.</td>
<td>Family: Bulinidae</td>
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<td><strong>Genus: Bulinus</strong></td>
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<td><em>Bulinus truncatus</em> Audouin, 1827</td>
<td>Lake Eğirdir (Isparta)</td>
<td>[101]</td>
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<td>3.</td>
<td>Family: Lymanaeidae</td>
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<td><strong>Genus: Galba</strong></td>
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<td></td>
<td><em>Galba truncata</em> Müller, 1774</td>
<td>Ceylanpınar (Urfa)</td>
<td>[36]</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>Lake Sapanca, Lake Beyşehir (Konya), Antalya, Konne Spring (Eğirdir, Isparta), Lake Eğirdir (Isparta), İzmir, Isparta, Balıkgaya, Erenli-Zonguldak, Toros Dağları, Bucak Yazılı Village Sulama Kanalı, Pınarbaşı Kaynağı Kestel Village Bucak, Kuşbaba Village (Başgöz), Ağlasun, Soğanlı Village, Örencik Village, Kurna Village, Terzıpınar (Gölehisar), Közpınar (Gölehisar), Çataloluk Spring, Sarı Seki, İncirpinar (Gölbüş Village), Ağlasun Dereköy, Terkos Lake, Erciyes Mountain (Kayseri), Istranca Stream, Sarçay creek, Matla, Tunceli, Mersin Limonlu (Lamos) Stream, Down Sakarya River (Karasu), Eleşkirt, Dolutaş village (Ağrı), Nuh’un Gemisi (Ağrı), Gölbüş Village, Azaplı Lake, İnekli Lake, Ceyhan River basin</td>
<td>[14, 16, 18, 20, 23, 27, 30, 31, 39, 45, 46, 51, 53, 72, 88, 89, 91, 99, 100, 102, 103, 104, 105, 106]</td>
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<td>5.</td>
<td>Family: Lymanaeidae</td>
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<td>Subfamily: Lymanaeinae</td>
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<td><strong>Genus: Lymanea</strong></td>
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<td><em>Lymanea stagnalis</em> Linnaeus, 1758</td>
<td>Lake Sapanca, Beyşehir (Konya), Lake Eğirdir (Isparta), Terkos Lake, Isparta, Eğirdir (Isparta), Erciyes Mountain (Kayseri), Down Sakarya River (Karasu), Gökpınar Stream, Saz Lake (Doğubeyazıt-Ağrı), Poyrazlar Lake, Abant Lake, Yeniçağa Lake, İskil Lake (Denizli)</td>
<td>[9, 13, 14, 45, 46, 47, 48, 89, 100, 102, 103, 104, 105, 106]</td>
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<tr>
<td>Genus: Radix</td>
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<td>6. <strong>Radix auricularia</strong> Linnaeus, 1758</td>
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<td>Genus: Stagnicola</td>
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<td>10. <strong>Stagnicola palaustri</strong> Müller, 1774</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>Bohu, Izmir, Denizli, Aydin, Antalya, Burdur, Kutahya, Afyon, Niğde, Konya Ceylanpınar (Urf) Mersin, Adana, Antakya, Diyarbakir, Mardin, Urla, Gölbasi, Inokli, Azapli, Adiyaman, Akgoğ (Gebe kirse), Ağlasun (Burdur), Karataş Lake (Burdur), Akyaka (Muğla), Kızılıcahamam, Beşgöl Spring, Cernke Lake, Lake Beyseri (Konya), Lake Eğirdir (Isparta), Isparta, Ceylanpınar (Urf), Karm Lake, İlivat Lake, Erciyes Mountain (Kayseri), Sarışçay creek, Lake Eğirdir, Kövada Channel, Lake Kovada, Nuh'un Gemisi (Ağrı), Sarsu (Ağrı), Patnos Dam Lake (Ağrı), Abant Lake, Çubuk Lake, Gölbaşı Lake, Azapli Lake, Inokli Lake, Ceyhan River basin</td>
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**Genus: Stagnicola** |

<table>
<thead>
<tr>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>Patlak Stream (İzmir Lake), Abant Lake (Bolu), Gümüşöl Pond (Eskişehir), Çavuşçu Lake, Deveoğun Pond (Afyon), Akarçay Stream (Afyon), Geminş Village Pond (Çardak), Fidanlık Ditch (Eğirdir), Akkçeçil Village Lake (Eğirdir), Yilanlı Sazi Pond (Beyser), Kürtüş Ditch</td>
</tr>
</tbody>
</table>

**Genus: Radix** |

<table>
<thead>
<tr>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isparta, Yağrı Lake, Karneali Lake, Erciyes Mountain (Kayseri), Malatya, Poyrazlar Lake, Süülük Lake, İskılı Lake (Denizli)</td>
</tr>
</tbody>
</table>

**Radix balthica** Linnaeus, 1758 |

<table>
<thead>
<tr>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hacilar Village, Lake Gölbaşı (Hatay), Lake Sapanca, Karacaoğlan (Karşı) Dam Lake, Ağlasun Backöy, Burdur Lake, Örençik Village, Koca Pınar Village, Eşenler Village, Hacilar Village, Panurbaş Village, Karataş Lake, Çatalölök Spring, İncirliçan (Gölbaşı Village), Terkos Lake, Akyaka Kadın River branch, Down Sakarya River (Karasu), Değirmendere Stream (Tunceli), Lake Eğirdir, Lake Kovada Abant Lake (Bołu), Emir Lake (Antakya), Akşehir Lake (Konya), Apolyont Lake, Sapanca Lake, Abant Lake, Marma Lake, Köprüören Stream (Tavşanlı), Hamidiye Village Stream (Eskişehir-Konya yol), Çavuşçu Lake, Eber Lake, Develi, Köşkpinar Spring (İsparta), Eğirdir Lake, Hendek (Beysere), Yılanlı Sazı Pond (Beysere), Bakaran Village Stream (Beysere), Akgöl (Gebekirse), Ağlasun (Burdur), Karataş Lake (Burdur), Akyaka (Muğla), Kızılıcahamam, Beşgöl Spring, Balıklı Lake, Uzun Lake, Grev Lake, Cernke Lake, Derbent Dam Lake, Lake Sapanca (Sakarya), Antalya, Konne Spring (Eğirdir, Isparta), Lake Eğirdir (İsparta), Isparta, Karm Lake, Karneali Göl, Susam Lake, İlivat Lake, Süülük Lake (Bozkır), Erciyes Mountain (Kayseri), Eğirdir (İsparta), Güzélhisar Reservoir, Alaqua (İzmir), Ozbek Village, Pınarlı Stream, Urla (İzmir), Karagöl Lake, Seferihisar (İzmir), Gökös Springs, Muradiye (Manisa), Oğlançaga Pond, Menderes (İzmir), Sarıçay creek, İstranca Stream, Mersin Limonlu (Lamos) Stream, Yuvarlakçay (Köyçeği), Eleşkirt, Uludal Village (Ağrı), Değirmendere (Ağrı), Sarsu (Ağrı), Doğubeyazıt Balık Stream (Ağrı), Saz Lake (Ağrı), Karaca Village (Ağrı), Taşlıçay (Ağrı), Admov Stream (Ağrı), Hamur suyu (Ağrı), Poyrazlar Lake, Taşkışl şeh Lake, Abant Lake, Ye niçağa Lake, İskılı Lake (Denizli), Ceyhan River basin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>[12, 13, 17, 18, 24, 28, 36, 39, 50, 53, 88, 89, 100, 102, 104, 105, 107, 108, 150]</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Location</th>
</tr>
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<tbody>
<tr>
<td>[13, 31, 45, 47, 53, 100, 102, 109, 110]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>[10, 12, 13, 14, 16, 17, 18, 19, 23, 24, 28, 30, 34, 35, 39, 45, 46, 47, 53, 54, 67, 72, 89, 91, 99, 100, 102, 105, 111, 150]</td>
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<table>
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<tr>
<th>Location</th>
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<tbody>
<tr>
<td>[70]</td>
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</tbody>
</table>

<table>
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<th>Location</th>
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<tbody>
<tr>
<td>[9, 12, 13, 18, 19, 23, 27, 28, 33, 34, 39, 53, 57, 67, 72, 88, 89, 91, 99, 100, 102, 103, 104, 112]</td>
</tr>
</tbody>
</table>
## Stagnicola tekecus Glöer and Yildirim, 2006

Konne Spring (Isparta)  

### Family: Physidae  
### Subfamily: Physinae

<table>
<thead>
<tr>
<th>Genus: Physa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physa fontinalis Linnaeus, 1758</td>
</tr>
<tr>
<td>Abant Lake (Bolu), Eber Lake, Çay Township, Gemiş Village Pond, Çardak Township, Çötel Village Pond, Çivril, Çaplı Village Pond, Pınarbaşı, Dinar, Eğirdir Lake, Apolyont Lake, Kırkgöz (Antalya), Kurttepe Stream (Kayseri), Çernek Lake, Derbent Dam Lake, Lake Eğirdir (Isparta), Karnı Lake, Kızılçay Lake, İvat Lake, Lake Konya (Seydişehir), Dipsız Lake (Seydişehir), Süülüklü Lake (Seydişehir), Eğirdir Lake (Seydişehir), Erciyes Mountain (Kayseri), Gölbasi Lake, Azaplı Lake, İneki Lake, Tercos Lake, Akçaçınar Stream, Akyaka Kadin River branch, Gelbula village, Dokuş Pınar Water, Han Yeri Village, Kayaonu (Kara Taş), Kayseri, Sarıçay creek</td>
</tr>
</tbody>
</table>

## Stagnicola kayseris Glöer and Yildirim, 2006

Kayseri Karpuz Sekisi basin gelbula village, Kayseri Pastirmaclar Park Garden |

### Genus: Physella |

| Physella acuta Draparnaud, 1805 |
| Kürüşt Village, Serik, İ lacepınar, Karadayi Village, Serik, Çarşamba Stream, Antalya, Beyşehir (Konya), Konne Spring (Eğirdir, Isparta), Lake Eğirdir (Isparta), Ceylanpınar (Urla), Çernek Lake, Derbent Dam Lake, Malatya, Gülbahçe Village, Muradiye (Manisa), Güzelihsar Reservoir, Aliaga (Izmir), Uşun Village Trough, Muradiye (Manisa), Göksu Springs, Muradiye (Manisa), Kars Fountain, Uşun Village, Muradiye- Manisa, Bornova Stream, Bornova (Izmir), Seferihisar Reservoir, Seferihisar (Izmir), Lake Eğirdir, Kovada Channel, Lake Kovada, Sarıçay Creek, Istranca Stream, Down Salesa River, Tunceli, Değirmendere Stream, Ağlasun Stream, Isparta Stream, Mersin Limonlu (Lamos) Stream, Terkos Lake, Karasu, Yuvarlakçay (Köyceğiz), Taşkısağı Lake, Ceyhan River basin |

## Ancylus fluviatilis Müller, 1774

Ayazma Stream (Çanakkale), Gürne Stream (Edremit), Daloglu Garden Ditch (Turgutlu), Koçak Stream (Umuru), Kestane Water Ditch (Buldan), Bakaran Village Stream (Beyşehir), Taşlıca Village (Beyşehir), Aykırcacahi Village Stream (Elmalı), Karacay Stream (Tınike), Çarşamba Stream, Antalya, Lake Eğirdir (Isparta), İzmir, Isparta, Ceylanpınar (Urla), Tefenni Başpınar Village, Ağlasun Dereköy, Erciyes Mountain (Kayseri), Eğirdir (Isparta), Değirmendere Stream, İzmir, Özbek Village, Pınarlı Stream, Urla (İzmir), Yiğıtler Stream,
Genus: *Ferrissia*

   Isparta, İzmir [23, 100]

Subfamily: Planorbinae
Genus: *Planorbarius*

17. *Planorbarius corneus* Linnaeus, 1758
   Isparta, Malatya, Yuvarlakçay (Denizli), Maltepe Village, Menemen (İzmir), Oğlançaylı Stream, Urla (İzmir), Gülbahçe Village, Muradiye (Manisa), Göksu Springs, Muradiye (Manisa), Kama Fountain, Uçınar Village, Muradiye (Manisa), Gökpınar Stream [9, 10, 13, 18, 23, 31, 45, 47, 53, 88, 89, 100, 102, 103, 104]

Genus: *Antisus*

18. *Antisus spirorbis* Linnaeus, 1758
   Toprakkale (Erzurum) [20]

19. *Antisus leucostoma* Millet, 1813
   Lake Gölbahşi (Hataş) [35]

20. *Antisus vorticulus* Troschel, 1834
   Afyon [112]

Genus: *Bathyomphalus*

21. *Bathyomphalus contortus* Linnaeus, 1758
   Yâyla Lake (Buldan), Omerçukuru Pond (Sarpöl), Kocapınar (Gravgaz-Burdur), Akyaka (Mağla), Kızılcabaham, Beşgöl Spring, Konne Spring (Eğirdir, Isparta), Kırkçöz (Antalya), Lake Eğdir (İsparta), Antalya, İzmir, Armut Village Kendir Pond, Akçaköy Kara Ahmet Spring, Yarışlı Village, Karaçay Lake, Buldan Dam Lake (Denizli), Güzelhisar Reservoir, Aliağa (İzmir), Gediz Stream, Maltepe Village, Menemen (İzmir), Oğlançaylı Pond, Menderes (İzmir), Değirmendere Stream, Lake Eğdir, Kovada Channel, Lake Kovada, Gökpınar Stream, Yuvarakçay (Köyçeğiz), Eleşkırt, Dolutas village ( Ağrı), Poyrazlar Lake, Taşkışla Lake, Büyük Akgöl, Abant Lake, Yen'içığı Lake, Çubuk Lake, Ceyhan River basin [10, 13, 17, 18, 23, 28, 29, 33, 67, 89, 91, 99, 100, 104, 105, 106, 111, 116, 117, 150]

Genus: *Gyraulus*

22. *Gyraulus albus* Müller, 1774
   Konne Spring (Eğirdir, Isparta), Lake Eğdir (İsparta), Gavur Lake (Seydişehir) [53, 89, 99, 100, 115]

23. *Gyraulus ehrenbergi* Beck, 1837
   Yenipınar (Gaziantep), Toprakkale (Erzurum) [20]

24. *Gyraulus piscinarum* Bourguignat, 1852
   Lake Gölbahşi (Hataş), Lake Beyshehir (Konya), Yazi Lake, Duraca Göl, Mersin Limonlu (Lamos) Stream, Ceyhan River basin [30, 35, 48, 53, 88, 150]

25. *Gyraulus piscinarum acutissimus* Schütt & Şesen 1993
   Vilayet Adiyaman, Azaplı Lake (Adiyaman) [27]

26. *Gyraulus ephraticus* Mousson, 1874
   Ceylanpunar (Urfa) [36]

27. *Gyraulus laevis* Alder, 1839
   İzmir, Akçaköy Stream, Alanköy Pond, Taşınar Village, Özbek Village, Pınarlı Stream, Ural (İzmir), Gülbağ kö Village Stream, Muradiye (Manisa), Güzelhisar Reservoir, Alağa (İzmir), Üçparmak Village, Muradiye (Manisa), Göksoya Springs, Muradiye (Manisa), Kam Fountain, Uçınar Village, Muradiye (Manisa), Gökpınar Stream [18, 23, 29, 106]

28. *Gyraulus parvus* Say, 1817
   Derbent Dam Lake [12]

29. *Gyraulus crista* Linnaeus, 1758
   Afyon, Sarçay creek [18, 39, 112]
30. Gyraulus nedyalkovi Glöer & Georgiev 2012
Kazanli village [58]

Rize, Fındıklı District, Çalgıayan River [118]

32. Gyraulus pamphylicus Glöer & Rähle, 2009
Antalya [119]

33. Gyraulus argaeicus Sturany, 1904
Lake of Soysalı village [58, 120]

34. Genus: Planorbis

Planorbis planorbis Linnaeus, 1758
Gelbula village, Han Yeri Village, Akşehir Lake (Konya), Niğde, İznil Lake (Çakarca), Kırkgöz (Antalya), Srıkkaraağaçlar Stream, Sarkan Lake, Lake Sapanca (Sakarya), Sarkan Lake (Şirin), Beştehir (Konya), Komte Spring (Eğirdir, Isparta), Lake Beştehir (Konya), Lake Eğirdir (Isparta), Izmir, Lake Abant, Isparta, Kırızlı Alabalık tesisleri, Koca Pınar village, Burdur Kayazalı Village, Yamanlı Lake, İncirliören (Gölbaşı Village), Terkos Lake, Karın Lake, Karneali Göl, Kızılot Lake, Durova Göl, İliv Lake, Dipsız Lake (Bozkır), Dipsız Lake (Seydişehir), Gavur Lake (Seydişehir), Söllükli Lake (Seydişehir), Sarıçay Creek, Akhisar reservoir, Istranca Stream, Malatya, Akçaşınar Stream, Akyaka Kadin River branch, Down Sakarya River (Karasu), Lake Eğirdir, Kovada Channel, Yuvarlaçay (Köyçeğiç), Nuh'un Gemisi (Ağrı), Doğuübayız Bardaklı Village Saç Lake (Doğuübayız Saçlığı) (Ağrı), Işıkli Lake (Denizli), Yeniçağa Lake, Ceyhan River basin

35. Planorbis carinatus Müller, 1774
Antalya, Beştehir (Konya), Lake Eğirdir (Isparta), Isparta, Karataş Lake, Tefenni Bağcılar Stream Village, Karın Lake, Kızılot Lake, Kovalı Lake (Seydişehir), Dipsız Lake (Seydişehir), Erciyes Mountain (Kayseri), Malatya, Güzeltihas Reservoir, Aliaga (İzmir), Oğlanagaşı Pond, Menderes (İzmir), Akhisar reservoir, Down Sakarya River (Karasu), Taşköşüş Lake, Gölbasi Lake, Azaplı Lake, İnekli Lake, Işıkli Lake (Denizli)

36. Planorbis cilicicus Sturany, 1904
Mersin-Arslanköy (Efrenk) [102, 120]

37. Planorbis intermixtus Mousson 1874
Ağrı Doğuübayız marshes, Lake Sapanca [48, 70]

38. Genus: Hippeutis

Hippeutis complanatus Linnaeus, 1758
Ceylanpinar (Urfa), Afyon, Lake Eğirdir [17, 36, 112]

39. Genus: Segmentina

Segmentina nitida Müller, 1774
Melen River [13]

40. Order: Stylommatophora
Superfamily: Succineoidea
Family: Succineidae
Subfamily: Oxyllomatinae
Genus: Oxyloma

Oxyloma elegans Risso, 1826
Istranca Stream, Akyaka Kadin River branch, Akçaşınar Stream, Mersin Limonu (Lamos) Stream, Yuvarlaçay (Köyçeği), Gölbasi Lake, Azaplı Lake, İnekli Lake, Ceyhan River basin [10, 16, 19, 27, 30, 150]

BIVALVIA

<table>
<thead>
<tr>
<th>Species no.</th>
<th>Classification</th>
<th>Area</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Class: Bivalvia</td>
<td>Derbent Dam Lake, Lake Sapanca, Terkos Lake, Sarıçay creek, Akhisar reservoir, Down Sakarya River (Karasu), Lake Eğirdir, Kovada Lake, Beştehir Lake, Burdur Lake, Sapanca Lake, Taşköşüş Lake</td>
<td>[9, 12, 13, 14, 39, 45, 46, 122, 123, 150]</td>
</tr>
<tr>
<td>No.</td>
<td>Genus / Species</td>
<td>Localities</td>
<td>Reference(s)</td>
</tr>
<tr>
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<td>-----------------</td>
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<td>--------------</td>
</tr>
<tr>
<td>1</td>
<td><em>Dreissena</em></td>
<td>Büyükk Akgöl, Abant Lake, Yenice aş Lake, Ceyhan River basin</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Dreissena polymorpha gal-landi</em> Locard, 1893</td>
<td>Sapanca Lake, Uluaabat Lake</td>
<td>[108]</td>
</tr>
<tr>
<td>3</td>
<td><em>Dreissena polymorpha anato-lica</em> Locard, 1893</td>
<td>Eğirdir Lake (Isparta), Beyşehir Lake (Konya)</td>
<td>[108]</td>
</tr>
<tr>
<td>4</td>
<td><em>Dreissena polymorpha sioaffi</em> Locard, 1893</td>
<td>Frat River</td>
<td>[108]</td>
</tr>
<tr>
<td>5</td>
<td><em>Dreissena polymorpha ar-nouldiformis</em> Schütt, 1993</td>
<td>Taşköşli Ilica Mudurnu (Bolu)</td>
<td>[108]</td>
</tr>
<tr>
<td>6</td>
<td><em>Dreissena bondourensis</em> Fischer, 1866</td>
<td>Burdur Lake</td>
<td>[108]</td>
</tr>
<tr>
<td>7</td>
<td><em>Dreissena iconica</em> Schütt, 1991</td>
<td>Konya Ereğli</td>
<td>[108, 124]</td>
</tr>
<tr>
<td>8</td>
<td><em>Dreissena capulatus</em> Schütt, 1993</td>
<td>Gölbâsi Lake, Adıyaman, Ceyhan River basin</td>
<td>[108, 150]</td>
</tr>
<tr>
<td>9</td>
<td><em>Dreissena dilavii</em> Abich, 1859</td>
<td>Erzurum Horasan</td>
<td>[108]</td>
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<tr>
<td>10</td>
<td><em>Dreissena rostiformis</em> Ushayes, 1838</td>
<td>Iznik Lake</td>
<td>[108]</td>
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<td>11</td>
<td><strong>Superfamily: Dreissenoidae</strong></td>
<td>Family: Dreissenidae</td>
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</tr>
<tr>
<td></td>
<td>Genus: <em>Corbicula</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Corbicula fluminalis</em> Müller, 1774</td>
<td>Gavur Lake (Kahramanmaraş), Tigris River</td>
<td>[125, 126, 150]</td>
</tr>
<tr>
<td>12</td>
<td><strong>Superfamily: Sphaerioida</strong></td>
<td>Family: Sphaeriidae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genus: <em>Pisidium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pisidium casertanum</em> Poli, 1791</td>
<td>Kırkgöz (Antalya), Çarşamba Stream, Beğöl Spring, Karaçay (Finike), Sarıkum Lake (Sinop), Kamlarlı Lake, Susam Lake, Özbek Village, Panarlı Stream, Urla (İzmir), Gülübahçe Village Stream, Muradiye (Manisa), Göksu Springs, Muradiye (Manisa), Kadıovacık Fountain, Çeşme (İzmir), Oğlanagaşı Pond, Menderes-İzmir, Sarıçay creek, Akhisar reservoir, Istranca Stream, Tunceli, Lake Eğirdir, Kovada Channel, Gölbâsi Lake, Yuvarlakçay (Köyceğiz), Acaar Lake, Gölbâsi Lake, Azaplı Lake, İneklı Lake</td>
<td>[10, 12, 13, 15, 16, 17, 27, 28, 33, 39, 53, 54, 127, 150]</td>
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<tr>
<td>13</td>
<td><em>Pisidium obtusale</em> Lamarck, 1818</td>
<td>Kovalı Lake (Seydişehir)</td>
<td>[53]</td>
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<tr>
<td>14</td>
<td><em>Pisidium personatum</em> Malms, 1855</td>
<td>Sarıkum Lake</td>
<td>[12]</td>
</tr>
<tr>
<td>15</td>
<td><em>Pisidium subtruncatum</em> Malms, 1855</td>
<td>Kızılol Lake</td>
<td>[53]</td>
</tr>
<tr>
<td>16</td>
<td><em>Pisidium annandalei</em> Prashad, 1925</td>
<td>Susam Lake</td>
<td>[53]</td>
</tr>
<tr>
<td>17</td>
<td><em>Pisidium lilljeborgii</em> Clessin, 1886</td>
<td>Tunceli</td>
<td>[54]</td>
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<tr>
<td>18</td>
<td><strong>Genus: Sphaerium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Sphaerium rivicola</em> Lamarck, 1818</td>
<td>Gavur Lake (Seydişehir)</td>
<td>[53]</td>
</tr>
<tr>
<td>19</td>
<td><em>Sphaerium corneum</em> Linnaeus, 1758</td>
<td>Gölbâsi Lake, Azaplı Lake, İneklı Lake</td>
<td>[27, 150]</td>
</tr>
<tr>
<td>20</td>
<td><em>S. corneum f. manilanum</em> Westerlund, 1871</td>
<td>Kovalı Lake (Seydişehir)</td>
<td>[53]</td>
</tr>
<tr>
<td>21</td>
<td><strong>Genus: Unio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Unio mancus</em> Lamarck, 1819</td>
<td>Karaoğul (Elmalı), Taşköşlü Lake, Gediz Delta (Burdur), Kırkgöz (Antalya), Kızılcabamam, Kovalı Lake (Seydişehir), Lake Sapanca, Güzelihasır Reservoir, Aliağa (İzmir), Göksu Springs, Muradiye (Manisa), Oğlanagaşı Pond, Menderes (İzmir)</td>
<td>[13, 14, 25, 28, 29, 53, 68, 116]</td>
</tr>
<tr>
<td>22</td>
<td><strong>Order: Unionoida</strong></td>
<td>Family: Unionidae</td>
<td></td>
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<tr>
<td></td>
<td>Genus: <em>Unio</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Unio mancus</em> Lamarck, 1819</td>
<td>Tigris River, Çağçık River (Mardin), Fabrika Stream (Diyarbakır), Tigris (Diyarba- kir), Kırkçeşme (Mardin) Bektaşğa Pond</td>
<td>[12, 128, 129]</td>
</tr>
</tbody>
</table>
In the Mediterranean, Aegean, Marmara and Central Anatolia regions, the most common prosobranch species are *Theodoxus* and *Melanopsis*. Apart from these, *Islamia*, *Graecoanatolica*, *Bithynia*, *Pseudobithynia* and *Pseudamnicola* species are distributed extensively. Again endemism is very high in these genus. Planorbid and Lymnaeid which belong to Pulmonata snails are observed frequently in internal waters of Turkey. Although it appears that the number of studies conducted in the Mediterranean Region is directly proportional to the number of species found, the zoogeographical location and formation of this region has increased this richness. The Mediterranean was formed geologically in a close period. In the Late Miocene times, it used to stretch to most of Eastern Europe and part of Asia 11.6 to 5.3 million years ago. In the time of the Mio-Pliocene, this large but shallow sea was divided into brackish and fresh water basins. Today the Mediterranean region has many freshwater stream systems and many endemic species. Hydrobiidae and

| 23. | Unio mancus eucirrus Bourguignat, 1860 | Gölbashi Lake, Azaplı Lake, İnekli Lake, Bektuşa Pond | [12, 27] |
| 24. | Unio pictorum Linnaeus, 1758 | Akşehir Lake, Taşköprü, Eber Lake, Küçük Akgöl, Büyük Akgöl, Beşgöl Spring, Balki Lake, Uzun Lake, Lake Sapanca, Terkos Lake, Gavur Lake (Kahramanmaraş), Lake Sapanca, Down Sakarya River (Karasu), Küçük Akgöl, Büyük Akgöl | [9, 12, 13, 14, 16, 25, 28, 33, 45, 46, 130, 131] |
| 25. | Unio stevenianus Krynicki, 1837 | River Karasu basin, Lake van | [132] |
| 26. | Unio terminalis Bourguignat, 1852 | Adıyaman Azaplı Lake | [133] |
| 27. | Unio terminalis delicatus Lea, 1863 | Lake Gölbashi, Kirikhan, Hatay | [134, 135] |
| 28. | Unio delicatus Lea, 1863 | Ceyhan River basin (Gavur lake, Hemite, Sir dam lake) | [150] |
| 29. | Unio crassus Philipsson, 1788 | Sarma Village, Sarma Stream, Muradiye (Manisa), Lake Sapanca, Gölbashi Lake, Azaplı Lake, İnekli Lake | [27, 29, 131] |
| 30. | Unio tigrinus Bourguignat, 1853 | Lake Gölbashi | [136] |
| 31. | Unio tigrinus Bourguignat, 1852 | Lake Gölbashi | [150] |
| 32. | Anodonta piscinalis Nilsson, 1823 | Tigris River | [126] |
| 33. | Anodonta pseudodopsis Locard, 1883 | Lake Gölbashi, Balki Lake | [35, 139, 140] |
| 34. | Anodonta anatina Linnaeus, 1758 | Lake Sapanca | [131] |
| 35. | Anodonta woodiana Rea, 1834 | Lake Sapanca | [131] |
| 36. | Potamida seminatata Lamarck, 1819 | Lake Gölbashi, Azaplı Lake, İnekli Lake, Ceyhan and Seyhan rivers | [27, 141, 142, 150] |
| 37. | Leguminia wheatleyi Lea, 1862 | Mardin, Lake Gölbashi | [143, 144, 145] |
| 38. | Family: Margaritiferidae | Orontes River | [146] |
FIGURE 1
Distribution and density of malacological studies in Turkey according to the eight regions. (The color is darkening from the least studied region to the most studied region).

FIGURE 2
Zoogeographical distribution of the Prosobranch species.

Bithyniidae are the most frequent groups in these waters with many Gastropod species [147]. In studies conducted to date in Turkey, 113 species from subclassis of Prosobranchia and 11 subspecies (totally 124 species) were identified. 63% of these species are Endemic and this subclass demonstrates very high level of endemism. 21% of the detected species are Palearctic and 13% are of...
Levant origin. The other 3% are from the Mediterranean, Afro-tropical and Australasia (Figure 2).

In previous studies conducted on Prosobranches, Yıldırım (1999) stated that there were 60 species and 12 subspecies and the endemism was close to 50% [32]. In another study performed by Yıldırım et al. in 2006, it was stated that the number of species reached up to 80 [76]. The acceptance of a zone as a zoogeographic region suggests that the endemism must reach 50% [32; 116]. According to this theory; the fact that 63% of the Prosobranches living in Anatolia are endemic species, makes the region a zoogeographic one.

At the end of the study, a total of 40 species were identified from the subclassis of Pulmonata. Only 20% of these species are endemic. 46% of the remaining species are Palearctic, 17% are Levant, 12% are Holarctic, 3% are Indotropical, and 2% are Afro-tropical region members (Figure 3). In the study conducted by Yıldırım et al. (2006) it was mentioned about the existence of 28 species in Turkey while he did not incorporate *Radix balthica* species as there are no sufficient data [61]. This checklist included 11 species and *Radix balthica* (= *Lymnaea balthica* f. *ovata*), which was recorded by Sturany (1905), Forcart (1953) and Göting (1961) [102; 109; 110].

Studies involving freshwater Bivalves made up to now have detected 33 species and 7 subspecies. 45% of these species are Palearctic, 23% are Holarctic, 22% are Endemic and 10% are Middle East.
and 5 species belong to the genus *Dreissena*.

In the study conducted by Modell (1951) he mentioned about their migration route of bivalves living in Turkey, the migration route he revealed complies with zoogeographic origins identified in our study [148]. In the aforementioned study, Central European forms, Nile-Mediterranean forms, South Asian forms and species from the Caucasus were mentioned. Many of the species given in the checklist confirm this theory in terms of origin.

9 species and 1 subspecies of the *Sphaeriidae* family were given in Bivalves. However, the species are often zoogeographically from distant regions and this fact raise suspicion about the accuracy of the diagnosis. It is suggested that this family should be revised in future studies.

With this study, zoogeographical distribution of freshwater molluscs of Turkey and their current systematic situation were demonstrated. While species list was prepared all malacological studies performed in Turkey up to now were taken into consideration. In addition to systematic studies, parasitological and water quality studies were added to the records provided in the articles. However, many taxonomic errors were encountered in these studies. Especially species names and localization records in water quality studies are quite complicated and contain contradictions. Even if aforementioned studies are included in the checklist, revisions and more careful definition are required in further studies.

**REFERENCES**


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Two important rice varieties, Karacadağ Karakılçık (dark) and Karacadağ Beyaz (white) rice varieties are grown in the upper Mesopotamia region in Southeastern Turkey. Although the physical and mechanical characteristics of these rice varieties are different from each other, they are harvested using the same combine-harvester and thresher. Thus, high kernel losses and energy consumption occur during the harvesting stage. The rice stem cutting and kernel cracking properties have to be known for each variety for reducing grain losses and energy consumption. The exact knowledge of rice stem cutting energy and kernel cracking energy are the main parameters for optimizing design of cutting elements in harvesting, threshing, sorting, transporting and milling machines.

The purpose of this study was to compare the relationships between rice stem cutting properties and kernel cracking force under load position for two local rice varieties of Karacadağ Karakılçık and Karacadağ Beyaz.

The experiments in this study were conducted at stem moisture contents of 70.81 %, 41.60 % and 7.5 w.b, and kernel moisture contents of 24.10 %, 19.30 % and 8.7 % w.b. The cutting energy of stems were examined at three internode positions as lower (IN1), medium (IN2) and higher (IN3) mm down from the panicles for Karacadağ Karakılçık and Karacadağ Beyaz rice varieties. The stem cutting and kernel cracking forces were measured via Llyood Universal Testing Machine.

The results indicate that the energy requirement of Karacadağ Karakılçık variety is greater than that of the Karacadağ Beyaz variety. Differences with regard to the cracking force and energy were determined between the horizontal and vertical sides. The cracking force of rice at horizontal orientation was determined to be higher than the cracking force at vertical orientation.

**Keywords:** Rice Stem, Kernel, Cracking, Shearing, Force, Energy.

**INTRODUCTION**

Rice (Oryza sativa L.) is an important staple food and the main source of income for about half of the world’s population and is cultivated in more than 100 countries in the world and it will continue to be the mainstay of life for future generations as well [1, 2]. Therefore, rice has got a strategic importance in the human diet. Turkey is one of the leading rice producers in terms of climate, soil and environmental conditions. Southeastern Turkey is among the important rice producers in the region. Rice varieties with two different qualities are grown in this region with 95 % of the cultivation area and production located in Şanlıurfa, Diyarbakır and Mardin provinces (especially, the Karacadağ region) where manual harvesting is mostly performed with some combine harvester use as well. Manual rice harvesting results in an increase in grain loss and also paddy stems are burned in the field which is detrimental to the environment. Combine harvesters are used to prevent this issue. However, various problems occur during harvesting via combine harvesters. The main problem is the high rate of loss of broken rice kernel during harvesting, threshing and post harvesting processing stages. Losses in rice production due to the use of unsuitable machinery and techniques occur in this region during harvesting season at an estimated ratio ranging between 25 % to 30 % [3]. This value is quite high. It must be reduced to a reasonable level. Rice breakage is closely related to rice variety. As is known, harvesting and threshing operations are known as crucial and influential processes on quantity, quality and production cost of rice. However, combine harvesters have negative impacts on quantity and quality of paddy grains which seriously affect the profitability of the crop since majority of the losses are due to improper adjustment of the machines according to rice varieties and crop conditions. Therefore, it is very important to choose adequate and proper parameters during harvesting processes to minimize the level of grain damage and to increase grain quality [2-4]. Moreover, both the quality and market value of broken rice are reduced significantly [5]. The objective of good harvesting is to maximize grain yield while minimizing grain losses and quality deterioration.
The income of farmers will increase with decreasing harvest loss.

Various important parameters such as stem shearing strength, kernel hardness, kernel rupture force, the relationship between cutting properties of rice stem and rice kernel cracking properties toward resistance have to be known separately for each rice variety in order to reduce these losses. Hardness is the resistance of the individual grain to deformation under applied forces. Percentage presence of broken rice grains is an important quality criterion for the rice industry [3, 6, 7] since the economic value of rice strongly depends on the presence of unbroken grains. Stem cutting force and kernel cracking properties are important mechanical properties when designing machines for grinding, harvesting, and milling, cleaning and separating biological materials. The engineering design and efficient operation of post-harvest processing machines and equipment require adequate knowledge of engineering properties of the bio-materials [8, 9].

Two important rice varieties are grown in southeastern Turkey. These varieties have different stem and kernel characteristics. However, both varieties are harvested with the same combine-harvester during the harvesting period resulting in excessive grain loss. Therefore, it is necessary to reduce these losses. The physical and mechanical properties of rice stem and rice kernel have to be known for selecting the design and operational parameters of equipment relating to harvesting, threshing and processing. In addition, stem cutting force and kernel cracking properties as well as the relationships between them have to be known for minimum energy and kernel losses.

Several studies have been conducted to determine the mechanical properties of plants. It was observed as a result of a literature survey that no study has been carried out until now regarding the correlation between the dependence of rice stem cutting properties on internode diameter and kernel cracking force for under compression. Therefore, the objective of this study was to assess the relationship between the maximum rice stem cutting force and cutting energy along the length of rice stem and kernel cracking force and energy under compression and three orientation loading conditions.

### Materials and Methods

**Experimental materials and moisture content.** Two rice varieties, KaracaDağ Karakılçık and KaracaDağ Beyaz, were obtained during harvesting season in 2016 from a commercial farm in Diyarbakır province located in southeastern Turkey with panicles (Figure 1). The harvested rice samples were transported to the laboratory at the Department of Agricultural Machinery and Technologies Engineering, Dicle University, Agriculture Faculty, Turkey and stored at a temperature of 4 °C for 4 weeks until uniform moisture content was obtained for all samples. The average rice physical properties measured are given in Table 1.

![FIGURE 1](image)

**FIGURE 1** Rice panicles - left: Beyaz (white), right: Karakılçık (Dark).

Four samples of 25 g stem for each variety were weighed and dried in an oven of 103°C for 24 h which were then reweighed in order to determine the average moisture content of rice stem. The average moisture contents were obtained as 70.81 %, 41.60 % and 7.5 % w.b., respectively. In addition, four samples 25 g kernels of rough rice from each variety were randomly selected prior to the cracking tests which were dried in an oven of 103 °C for 24 h and reweighed [10] for m.c. measurements. The average moisture content of rice kernels varied between 8.70 % to 24.10 % for both varieties.

**Stem cutting properties.** The mechanical experiments were carried out in two stages. Stem cutting properties were measured for two varieties in the first stage. The rice stems were cut manually prior to the cutting tests at ground-level at three internodes, namely; the first, second and third in-
ternodes and the internodes were labeled as IN1, IN2, and IN3, respectively (Figure 2). The fourth and the other lower stem internodes from the ear were not considered because these internodes are usually left on the field [11, 12]. The stem internodes were separated according to their position below the ear [9, 13]. Diameters were measured for each internode using a digital caliper with an accuracy of 0.01 mm. Three diameter measurements were taken for each sample after which their average was calculated. The internode diameter of the stem (mm) was converted to cross-section area in mm².

**FIGURE 2**
Rice stem internodes: IN1, IN2, and IN3: The first, second, and third internodes, respectively

Cutting and cracking tests were conducted using an Instron Universal Materials Testing Machine, Lloyd LRX Plus, as shown in Figure 3. The applied force was recorded as a function of displacement. The rice stem specimens for the cutting tests were obtained from randomly selected whole plants. The rice stem samples were placed flat on the table during the tests. Loading was applied in vertical direction [14-16]. Cutting measurements were performed at 30 mm/min fixed cutting speed for all tests.

The rice stem cutting energy was calculated by measuring the surface area under the force-deformation curve via material testing machine [4, 8, 9, 13, 15, 17, 18]. A computer data acquisition system recorded all force-displacement curves during the cutting process.

**FIGURE 3**

Kernel cracking force. Rice kernel cracking (compression, rupture) tests were carried out during the second stage via Instron material test device at three orientation positions (x, y and z side) and three moisture content values are shown in Figure 3. A total of 50 brown rice kernels were randomly selected during the cracking experiments which were then used for each variety, position and moisture content.

A special supporting point was made for the tests in order to determine the breaking force of rice kernel at three different points (Figure 4). The loaded rate was set at 30 mm/min [5]. Cracking energy values were calculated from the area under the cracking force-deformation curve up to the point of fracture [5]. The area under the cracking force-deformation curve was obtained by using a NEXYGEN computer program.

Experimental design and data analysis. This study was planned as a completely randomized block design. The mechanical properties, cutting force and cracking force were determined with twenty-five replications in each treatment. Experimental data were analyzed using analysis of variance (ANOVA) and the means were compared at the 1 and 5 % levels of significance using the Tukey multiple range tests in JMP software, version 11.

**RESULTS AND DISCUSSION**

Cutting force and cutting energy of rice stem. The main values for the cutting force and energy of rice stems for both varieties at different moisture content are presented in Table 2. Variance analysis results indicate that the cutting force and energy values of rice stems are closely related to stem moisture content and variety. The stem cutting
force requirement increased linearly with increasing moisture content. The obtained cutting force values varied from 72.19 N to 17.74 N and 55.35 N to 15.43 N, for Karakılıç and Beyaz varieties, respectively (Table 2). However, no significant differences were determined among the varieties. The maximum values were observed for the Karakılıç variety. The results indicated that the stem resistance of Karakılıç variety was higher in comparison with that of the Beyaz variety. This is an indication that the energy requirement for the cutting of Karakılıç variety is higher in comparison with that of the Beyaz variety. Thus, these values have to be considered before starting the design of a harvester or threshing. Similar results were obtained for cutting energy subject to moisture content. The cutting energy decreased with decreasing moisture content. The maximum cutting energy was obtained at 70.81 % with a moisture content of 10.85 Ncm and 8.70 Ncm for Karakılıç and Beyaz varieties, respectively. The lowest values were obtained at a moisture content of 7.50 % for both varieties (Table 2). Similar results were found by Yore et al. [13] for rice straw.

The average values for cutting force and energy of internodes with three stem diameters are presented in Table 3. As shown in Table 3, the cutting force and energy values were observed to decrease at a statistically significant level (p<0.01) from the lower (IN3) to the upper region (IN1). Statistically significant differences were observed between the internodes and varieties. Cutting forces and energies for three internodes and both varieties were found to be significantly different. Higher cutting force energy and values were found in the third internode (IN3) in comparison with other internodes for both the Karakılıç and Beyaz varieties due to the accumulation of more mature fibers and a higher diameter of the stem [5]. However, both the cutting force and cutting energy of Karakılıç variety values were greater at a statistically significant level (p<0.01) according to Tukey’s multiple range test at the 5 % level.

**TABLE 2**

The change of mean cutting force and energy depend on moisture content for Karakılıç and Beyaz paddy varieties.

<table>
<thead>
<tr>
<th>Moisture content, % w.b.</th>
<th>Karakılıç ‡</th>
<th>Beyaz ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cutting force, N</td>
<td>Cutting energy, Ncm</td>
</tr>
<tr>
<td>70.81</td>
<td>72.19 ‡</td>
<td>10.85 ‡</td>
</tr>
<tr>
<td>41.60</td>
<td>60.65 ‡</td>
<td>8.70 ‡</td>
</tr>
<tr>
<td>7.50</td>
<td>17.74 ‡</td>
<td>3.15 ‡</td>
</tr>
<tr>
<td>Mean</td>
<td>50.19 ‡</td>
<td>7.56 ‡</td>
</tr>
</tbody>
</table>

*All data represent the average of three replications with 15 values.

**means followed by the same letter in each column are not significantly different by Tukey’s multiple range test at the 5 % level.
variety. This effect could be related to higher stem resistance of stem wall thickness of Karakılçık cross-sectional area compared with that for the Beyaz variety. It was concluded that significant energy savings can be made when the cutting height is increased towards the third internode to the first internode during rice harvesting. Similar results were obtained by Alizadeh et al. [19] for rice stem and by Li et al. [5] for two different rice varieties.

In addition, these results are in agreement with those of Yore et al. [13] for rice straw, Tavakoli et al. [11] for the shear strength of wheat and barley straw and Zareiforoush et al. [12] for rice straw, Chandro et al. [9] for wheat and rice straw and Alizadeh et al. [19] for rice stem. Tavakoli et al. [20] compared the mechanical properties for two rice straw varieties. The results indicate that the cutting force and energy requirement were different for both varieties.

**Kernel cracking force and energy.** The results for rice kernel cracking force and cracking energy values subject to moisture content are shown in Table 4. The effect of moisture content on kernel cracking force and energy was observed to be statistically significant (p<0.05). The cracking force and cracking energy values increased significantly (p<0.01) from 24.10 to 8.70 % kernel moisture content. The relation was found to be linear. However, no significant differences were determined between 19.30 and 8.70 % moisture content. While the maximum forces and energy values were obtained at 8.70 % moisture content, the lowest values were obtained at 24.10 % moisture content for both varieties. On the other hand, the mean values of the cracking force and energy for the Beyaz variety were significantly higher (p<0.05) in comparison with those for the Karakılçık variety. Whereas, the stem cutting force and cutting energy values of Karakılçık variety were found to be higher in comparison with those of the Beyaz variety (Table 3). Accordingly, it can be indicated that there is no correlation between rice stem strength and kernel cracking force. The cutting resistance of rice stems can be low, while kernel cracking force can be higher. Similar results were obtained by Bamrunwong et al. [21] for rice and by Tavakoli et al. [22] for barley grain. These results are also consistent with the findings of Zareiforoush et al. [12] for paddy grains. Altuntas and Yildiz [23] reported that moisture content increased grain rupture force. However, according to Bamrunwong et al. [21] breaking deformation slightly decreased at higher moisture content percentage. Both the maximum cracking load and tensile strength values decreased with moisture content increase which is in agreement with the results reported by Cao et al. [24] for rice kernel.

### TABLE 3
The change of mean cutting force and energy depend on stem region internodes) for stem of Karakılçık and Beyaz paddy varieties.

<table>
<thead>
<tr>
<th>Stem diameter, mm</th>
<th>Variety</th>
<th>Karakılçık*</th>
<th>Beyaz**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cutting force, N</td>
<td>Cutting energy, Ncm</td>
<td>Cutting force, N</td>
</tr>
<tr>
<td>1.5 (upper region, IN1)</td>
<td>33.92c</td>
<td>4.80c</td>
<td>22.94c</td>
</tr>
<tr>
<td>3.5 (middle region, IN2)</td>
<td>48.98b</td>
<td>6.44b</td>
<td>40.42b</td>
</tr>
<tr>
<td>4.5 (lower region, IN3)</td>
<td>67.67a</td>
<td>11.46a</td>
<td>53.72a</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>50.19a</td>
<td>7.56a</td>
<td>39.02b</td>
</tr>
</tbody>
</table>

*All data represent the average of three replications with 15 values.
**means followed by the same letter in each column are not significantly different by Tukey’s multiple range test at the 5 % level.

### TABLE 4
The change of mean kernel cracking force and energy depend on kernel Moisture content for stem of Karakılçık and Beyaz paddy varieties.

<table>
<thead>
<tr>
<th>Moisture content, % w.b.</th>
<th>Variety</th>
<th>Karakılçık*</th>
<th>Beyaz**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cracking force, N</td>
<td>Energy, Ncm</td>
<td>Cracking force, N</td>
</tr>
<tr>
<td>24.10</td>
<td>86.74a</td>
<td>3.79a</td>
<td>119.47a</td>
</tr>
<tr>
<td>19.30</td>
<td>120.10b</td>
<td>6.26b</td>
<td>148.59b</td>
</tr>
<tr>
<td>8.70</td>
<td>141.19b</td>
<td>6.43b</td>
<td>135.47b</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>116.00a</td>
<td>5.47a</td>
<td>134.50b</td>
</tr>
</tbody>
</table>

*All data represent the average of three replications with 15 values.
**means followed by the same letter in each column are not significantly different by Tukey’s multiple range test at the 5 % level.
The test results for rice kernel cracking force and energy values subject to loading position are shown in Table 5. The cracking force and energy values for both varieties were found to be different depending on loading position. On the other hand, it was obvious that the cracking force on the vertical side (x-x orientation) was always larger than that on the horizontal sides.

Higher mean values for both the cracking force and energy were obtained for the Beyaz variety. The cracking force values varied from 40.28 N to 191.74 N depending on loading position. Highest cracking force and cracking energy values were obtained for the Beyaz variety as 134.50 N and 6.54 Ncm. Maximum values were obtained for Y orientation. Minimum values for both the Karakılıç and Beyaz varieties were obtained at x loading position. However, the maximum energy values were observed at Y and Z loading positions for both varieties. It was obvious that the cracking force on the vertical side (x-x orientation) was always larger than that on the dorsal side (y-y orientation).

The test results for rice kernel cracking force and energy values subject to both loading position and moisture content are shown in Table 6. As can be seen from the table, the effect of moisture content, variety and loading position on cracking force and energy were found to be significant (p<0.05). The cracking force increased linearly with the decrease in moisture content for all loading orientations. It was also observed based on statistical analyses that the interactions between the moisture content x variety, moisture content x loading orientation, variety x loading orientation and moisture content x variety x loading orientation were found to be significant at 1 %. The results indicate that the cracking force of rice kernel decreased depending on the moisture content for both varieties at all kernel loading positions (X, Y, Z). Grain moisture content increased from 8.7 % to 24.10 %, while grain cracking force decreased for both varieties and all loading positions. However, highest cracking forces at all moisture content levels were obtained for both varieties loaded along the Y loading position at x-x orientation. The lowest cracking force and energy values were obtained at X and Z loading positions at y-y axis. This is an indication that the value of kernel cracking force and energy at the vertical position was higher than the horizontal position and according to results it can be concluded that there is a strong relationship between moisture content and cutting force and cutting energy. Similar results were obtained by Resende et al. [25] for rice and by Zhang et al. [26] for rice kernel.

Moreover, kernel detachment force (KDF) from panicle was measured from the top to the bottom using a digital force-gage (FG-20) at a resolution of ±0.01 N. Kernel detachment force (KDF) from panicle is given in Table 7. Maximum values for KDF were obtained at a moisture content of 24.10 % for both varieties. KDF decreased with decreasing moisture content. While a maximum values of 12.32 N and 10.18 N were obtained at 24.10 % the lowest values of 4.35 N and 3.25 N were obtained at a moisture content as of 8.70 % for the Karakılıç and Beyaz varieties, respectively. Differences were also determined among varieties. However, according to results given in Tables 6 and
7, there is no relation between kernel grain detachment force from panicles and grain cracking force subject to moisture content. Whereas the direction of the applied force and grain detachment force from panicles are important in determining the magnitude of the threshing force and energy during the harvesting and threshing operations [27]. The threshing force of some rice varieties were determined by Alizadeh and Allameh [27].

As a result, the harvesting-threshing machines as well as the sorting and cleaning machines used during paddy harvesting have to be adjusted according to the characteristics of the paddy variety. Otherwise, the value of broken grain ratio and energy consumption will increase due to product impact when harvest-threshing and cleaning operations are performed on the same machines for all varieties. The machine settings must be adjusted separately for each variety in order to prevent this.

**CONCLUSIONS**

In this study, the mechanical properties of two varieties of rice stem at three internode positions were compared at three moisture content values. Higher cutting force energy and values were found for the third internode (IN3) in comparison with the other internodes for both the Karakılçık and Beyaz varieties. However, results indicate that the average cutting strength and cutting energy of Karakılçık variety were significantly higher in comparison with those of the Beyaz variety. The cutting location and diameter of the stem are significant factors for cutting force and energy.

However, the highest kernel cracking force and cracking energy values were found for the Beyaz variety at all moisture content values and loading orientations. It can be put forth that the Karakılçık rice variety stem has more resistance in comparison with the Beyaz variety. In conclusion, there are strong relationships between moisture content and cracking force, energy and loading orientation. Increasing moisture content increased cracking force and energy. The cracking force values of rice kernel at the Y loading position (x-x axis) were higher in comparison with those at X and Z loading positions (y-y horizontal axis) at all moisture content values. However, no close relations were observed between the rice stem cutting force and energy values and rice kernel cracking force.

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INVESTIGATION OF THE EFFECT OF TRIBULUS TERRESTRIS ON THE OXIDANT AND ANTIOXIDANT MECHANISMS IN CARBON TETRACHLORIDE-INDUCED LIVER INJURY

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ABSTRACT

Today, the diseases of the liver is a common problem worldwide. The liver is a critical internal organ involved in many vital functions including the human skeletal metabolism and detoxification of a variety of medications. Experimental studies in animal models have been popular in the 20th century in order to determine the antioxidant and hepatoprotective effects of herbal medications against liver damage. Against the carbon tetrachloride (CCI4), known for its toxic effect to the liver, this present study aimed to investigate the effects of Tribulus terrestris on the oxidant and antioxidant mechanisms and whether it is effective as a liver protective agent. In the study, 35 Wistar-Albino male rats aged 8-12 weeks were used. The rats were assigned to five groups of seven rats each. Excluding the control group, all rats in other groups were fed with standard diet, plus they were given CCI4 at a dose of 0.8 ml/kg administered intraperitoneally for 14 days. The extract of Tribulus terrestris plant was administered to the rats in groups 3, 4, and 5 for a period of eight weeks after the administration of CCI4 for a period of 14 days. It was observed in the study that the serum levels of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), malondialdehyde (MDA); and the levels of total antioxidant capacity (TAC) and total oxidant capacity (TOC) were significantly elevated in group 2 compared to group 1 after the administration of CCI4 (p<0.01). Tribulus terrestris extract administration is followed by significantly decreased levels of AST and ALT in group 4 and 5 compared to group 2 and the levels of MDA, TAC, and TOC were found to be significantly decreased in group 4 compared to group 2 (p < 0.01). Tribulus terrestris extract has been observed to suppress the oxidative stress and enhanced the antioxidant system, especially increasing doses have been seen to be more effective.

KEYWORDS: Carbon tetrachloride, Tribulus terrestris, total antioxidant capacity, total oxidant capacity

INTRODUCTION

The liver is a critical internal organ involved in many vital functions including the human skeletal metabolism and detoxification of a variety of medications. For this reason, the liver is susceptible to the effects of a wide variety of factors including toxic, microbial, metabolic, circulatory, and neoplastic insults [1]. Today, the diseases of the liver is a common problem worldwide. Occurring due to a wide range of etiological factors, the acute and chronic diseases of the liver are still very common with remarkably high incidence rates [2, 3]. Different etiological factors (viral infections, metabolic disorders, exposure to toxic substances, etc.) are known to be associated with alterations observed in the enzymatic and non-enzymatic oxidative capacities of the hepatocytes [4]. In general, a significant number of chemicals, such as various environmental toxicants and some medications which are considered to be useful clinically, can cause serious cellular damage in different organs, especially in the liver, by activating the production of reactive oxygen species (ROS) [5]. Therefore, the inhibition of hepatic inflammation and fibrosis is critical in order to prevent the development of liver cirrhosis and hepatocellular carcinoma. A number of studies have reported that the free radicals and ROS play a critical role in several stages independently inducing and maintaining the progression of liver fibrosis [6, 7]. A variety of xenobiotics are known to cause hepatotoxicity. One of these molecules is carbon tetrachloride (CCI4) [8, 9]. CCl4 is usually used experimentally to induce liver damage, fibrosis, and carcinoma in rodents [10]. Even a single dose of a CCl4 can lead to a centrilobular necrosis and steatosis [8] while the repeating doses cause liver fibrosis, cirrhosis and hepatocellular carcinoma [9]. CCl4 induces the production of several types of ROS. Current studies have recently directed towards finding the natural antioxidants that may help prevent the oxidative damage.

In the CCl4 induced toxicity, the cellular damage is directly proportional to the increase in lipid peroxidation and it has been determined that this toxic effect is triggered by the formation of free rad-
is associated with liver damage and fibrosis [11, 12].

Antioxidants may provide benefits in the prevention and treatment of CCl₄-induced liver damage. As herbal medications are cheap, easily accessible and as they cause toxic or side effects at relatively lesser rates, experimental studies in animal models have been popularized in the 20th century to determine the antioxidant and hepatoprotective effects of them against liver damage induced by different chemical substances [13].

The species, Tribulus terrestris L., which is investigated in our study, belongs to the Tribulus genus under Zygophyllaceae family. From this family, 5 genus and 6 species grow in our country. The only species growing in our country is Tribulus terrestris under the genus Tribulus genus. The active substances contained in T. terrestris are therapeutic for several diseases. There is a limited number of studies conducted in Turkey, investigating this plant, which grows widely in our country. The usual effects of Tribulus terrestris are aphrodisiac, antimicrobial, diuretic, anthelmintic, anti-inflammatory, anti hypertensive, cardiotonic, vasodilator, anticancer, liver protective, cholesterol lowering, antihiperlipidemic, antidiabetic, and analgesic [14]. The absence of side effects during its use for the above-mentioned effects also increases the interest in the plant. Tribulus terrestris contains several distinct active substance groups including saponins (protodioscin, furostanol), glycosides, flavonoids, alkaloids, resins, tannins, sugars, sterols, and essential oils. More than 20 saponins have recently been identified in Tribulus terrestris. Tribulusalide A and B are hepatoprotective lignamides and have been isolated from the fruits of Tribulus terrestris [15]. There is limited research on the plant of Tribulus terrestris to study its effect against liver damage.

In this study, the effects of Tribulus terrestris on the oxidant and antioxidant mechanisms have been examined and it has been investigated whether it is effective as a liver protective agent against CCl₄, which is known for its hepatotoxicity.

MATERIALS AND METHODS

In the study, 35 Wistar-Albino male rats aged 8-12 weeks were used. The rats were assigned to five groups of seven rats each. The rats in the control group were fed with a standard diet for 14 days. The rats in the group 2 and 3 were fed with a standard diet and they were administered 0.8 ml/kg CCl₄ intraperitoneally for 14 days. The rats in the groups 3, 4, and 5 received the extract of Tribulus terrestris plant for a period of 8 weeks following the intraperitoneal administration of 0.8 ml/kg CCl₄ for a period of 14 days. The body weights of the rats were measured at the beginning and at the end of the study. All rats were decapitated in an appropriate environment under appropriate conditions. The rats in group 1 and 2 were decapitated 2 weeks after the baseline and the rats in group 3, 4, and 5 were decapitated 10 weeks after the baseline. After decapitation, the blood samples were collected to investigate the study parameters. After collecting the blood samples in gel tubes for biochemical tests, they were centrifuged at 4000 rpm for 5-10 minutes, eliciting the serum. The local ethics committee on animal research of Firat University (FÜHADYEK) approved the study (31.03.2017/2017/07, decision no: 81, Protocol no: 2017-39) and the study was conducted in compliance with the ethical principles of standard experimental animal research. The levels of AST, ALT, and ALP were measured with a Siemens Advia 2400 autoanalyzer spectrophotometrically.

The serum total antioxidant capacity (TAC) was measured colourimetrically with the autoanalyzer using a Fully Automated 3rd Generation rat kit. The ASSAY KIT Catalog number: RL0017 LOT: RL024 was used and the measurements were performed at 660 nm. The results were reported in units of mmol Trolox Equiv / L.

The serum total oxidant capacity (TOC) was measured with the autoanalyzer colourimetrically using Fully Automated rat kits. ASSAY KIT Catalog no: RL0024 LOT: RL026 was used and measurements were performed at 530 nm. The results were reported in units of μmol H₂O₂ Equiv/L.

MDA is one of the end-products of lipid peroxidation. The serum levels of MDA were measured by the HPLC method using a Techopak 10 C-18 (25 cm x 3.9 mm) column at a wavelength of 254 nm and a flow rate of 1.5 ml/min [16, 17].

All interventions to be applied to the animals were performed in compliance with the principles announced by the local ethics committee on animal research of Firat University (FÜHADYEK). The care of the animals took place at Firat University Experimental Animal Application and Research Center. Carbon tetrachloride, which was used in this study to induce toxicity, was provided by the company Merck.

A total of 5 groups were made, assigning 7 rats to each, using random sampling. The groups and the summary of the procedures applied in each group are listed below:

- **Group 1:** Control group
- **Group 2:** CCl₄ group
- **Group 3:** CCl₄ + 0.5 g/kg Tribulus terrestris
- **Group 4:** CCl₄ + 1 g/kg of Tribulus terrestris
- **Group 5:** CCl₄ + 2 g/kg Tribulus terrestris
**Tribulus terrestris Administration.** The extract of Tribulus terrestris was prepared by dissolving in water. The doses of the extract administered orally by group were 0.5, 1.0, and 2.0 mg/kg for group 3, 4, and 5, respectively.

**CCl4 Administration.** Dissolving 0.8 ml/kg CCl4 in 0.8 ml/kg liquid oil, all rats except the ones in the control group received an intraperitoneal administration of 0.8 ml/kg CCl4 daily for 14 days in addition to the standard feed.

**Termination of The Study.** 24 hours after the end of the period when Tribulus terrestris administrations were made, blood samples were collected from all of the animals under sodium pentobarbital anesthesia (pentobarbital sodium, 40 mg/kg, i.p.) [12] and then the blood samples were prepared for performing the laboratory analyses. All data were described as mean ± standard error (SE). A one-way analysis of variance (ANOVA) was used to evaluate the data.

**RESULTS**

The blood serum levels of AST, ALT, and ALP are presented in Table 1. The levels of MDA, TAC, and TOC are presented in Table 2.

Statistically significant differences were observed between the groups (p < 0.01). After the period of CCl4 administration, the serum levels of AST, ALT, and ALP elevated in group 2 compared to those levels in group 1 (Table 1), however, it was observed that the levels of AST (Figure 1) and ALT (Figure 2) statistically significantly decreased in group 4 and 5 where the extract of Tribulus terrestris was applied (p < 0.01).

The serum levels of MDA, TAC, and TOC statistically significantly elevated in group 2 compared to group 1 (Table 2). It was observed that the levels of MDA (Figure 4), TAC (Figure 5), and TOC (Figure 6) significantly decreased in group 4 compared to group 2. The decrease in the TOC levels in group 5 was found out to be decreased close to the control levels measured in group 1 with no statistical difference between these two groups (p<0.01).

### TABLE 1

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<tbody>
<tr>
<td>AST (U/L)</td>
<td>173.71±20.69c</td>
<td>1453±142.62a</td>
<td>1392.57±83.38a</td>
<td>925.88±83.38b</td>
<td>854.13±92.31b</td>
<td>P&lt;0.001</td>
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<tr>
<td>ALT (U/L)</td>
<td>75.05±16.45c</td>
<td>1095.54±93.04a</td>
<td>1058.68±90.21a</td>
<td>852.24±88.58b</td>
<td>795.07±94.48a</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>255.85±15.17c</td>
<td>428.39±37.33a</td>
<td>411.33±35.13a</td>
<td>395.65±22.12ab</td>
<td>363.16±22.17b</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

The data are presented as means and standard errors. a, b, c: There is a statistically significant difference between the measured levels of the parameters when they are marked with different letters on the same line.

### TABLE 2

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<tbody>
<tr>
<td>MDA(μM/L)</td>
<td>0.11±0.3c</td>
<td>0.55±0.6a</td>
<td>0.44±0.6b</td>
<td>0.36±0.5c</td>
<td>0.25±0.3bc</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>TAC (ng/L)</td>
<td>1.75±0.3c</td>
<td>1.11±0.6b</td>
<td>1.19±0.7b</td>
<td>1.55±0.8a</td>
<td>1.65±0.5ab</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>TOC(μM/L.T.E/L)</td>
<td>17.48±2.05c</td>
<td>31.19±3.45a</td>
<td>28.08±2.01ab</td>
<td>26.12±1.91b</td>
<td>19.32±2.21b</td>
<td>P&lt;0.005</td>
</tr>
</tbody>
</table>

The data are presented as means and standard errors. a, b, c: There is a statistically significant difference between the measured levels of the parameters when they are marked with different letters on the same line.
DISCUSSION

Carbon tetrachloride is metabolized by the cytochrome P450 enzyme system in the agranular endoplasmic reticulum in the hepatocytes to its toxic metabolites, which are the free radical metabolites trichloromethyl (CCl₄) and trichloromethyl peroxyl (CCl₃O₂). It is reported that these molecules, then, react with the unsaturated fatty acids in the cell membrane and induce lipid peroxidation or impair the cell membrane integrity by binding to the protein and fat molecules, leading to an eventual hepatic injury [18, 19, 20]. In the studies where CCl₄ was administered to the rats chronically to induce hepatic injury [21, 22, 23, 24], a severe form of necrosis surrounded by a fibrous tissue composed of fibrocytes, fibroblasts, and collagen bundles originating especially from the portal region was observed in the hepatic tissues. In these studies; fatty degeneration, pseudolobe formation with inflammatory cell infiltrates, micro-macrovacular steatosis, balloon-like or vacuolar degeneration, and lymphocytic cell infiltrates were also observed. The AST/ALT ratio is a good marker for alcoholic liver disease. The elevated levels of AST and ALT are definitely indicative of hepatocellular damage [25]. A study was conducted to investigate the protective effects of *Tribulus terrestris* in diabetes mellitus (DM). In the pathogenesis of diabetes, it is known that the level of reactive oxygen species (ROS) increases. For the purpose of that study, the rats were assigned to six groups and were treated with either of the following, including the saline solution, glibenclamide (Glib), or *Tribulus terrestris* extract, for 30 days. Diabetes was induced in the rats by Streptozotocin (STZ) in the first group and these rats were treated with saline solution. In the second group, diabetes-induced rats were treated with Glib at a dose of 10mg/kg of body weight. In the third group, diabetes-induced rats were treated with the extract of *Tribulus terrestris* at a dose of 2 g/kg of body weight. Diabetes was not induced in the rats in the fourth, fifth and sixth groups and the rats in either group were treated with either of the following, including the saline solution, Glib, and the extract of *Tribulus terrestris* respectively. At the end of the experiment, serum and liver samples were collected for biochemical and morphological analysis.
Serum alanine aminotransferase (ALT) and creatinine levels were determined. In addition, the levels of malondialdehyde (MDA) and the reduced glutathione (GSH) levels were tested. The Tribulus terrestris extract decreased the serum ALT and creatinine levels significantly (P < 0.05) in the diabetic groups. The treatment with the extract decreased the levels of MDA in the liver both in the diabetic (P < 0.05) and non-diabetic groups (P < 0.01). In addition, the reduced levels of GSH in the liver increased significantly in the diabetic mice treated with the extract of Tribulus terrestris (P < 0.01). Histopathologic examination showed a remarkable improvement in the liver in the rats treated with this plant. That study proposes that the protective effects of Tribulus terrestris can be utilized to block the oxidative stress in the rats with STZ-induced diabetes [26].

Lipid peroxidation causes the oxidative degradation of antiatherosclerotic fatty acids, leading to impairments in the structural integrity and membrane functions. The studies have shown that lipid peroxidase increases significantly during hyperalumuria. Tribulus terrestris lowers the levels of free radicals responsible for the lipid peroxidation, causing decreased levels of malondialdehyde. This decrease demonstrates that Tribulus terrestris has the capacity to scavenger the free radicals and to ameliorate the injury caused by these molecules. Being conducted based on the published data in the literature, the study confirmed the protective effect of Tribulus terrestris treatment against the oxidative stress due to free radicals by balancing the activity of the elevated antioxidant enzymes and gene expression. As a result, Tribulus terrestris reduced the oxidative stress, allowed for the appropriate functioning of the kidneys, and ameliorated kidney damage. In our study, it has been observed that this plant relieves the stress, allowed for the appropriate functioning of the kidneys, and ameliorated kidney damage. In our study, it has been observed that this plant relieves the

In the study conducted by Ilhan and Seckin (2005), investigating the role of Nigella sativa in preventing the liver fibrosis induced by CCl4, the mean levels of AST, ALT, and MDA were found to be significantly higher in the rats with CCl4 induced hepatotoxicity compared to the control group [28]. The study by Tanriverdi (2005) reported liver injury induced by chronic CCl4 administration and higher activity levels of AST and ALT in the CCl4 group. In another study (Ustundag et al., 2005), where intraperitoneal CCl4 had been administered to the rats 3 days a week for a duration of 5 weeks, it was found out that the plasma and liver tissue levels of MDA were elevated in the CCl4 group and the levels of AST and ALT were approximately five folds greater than those levels in the control group [29]. These studies proved that the serum levels of AST, ALT, and MDA were elevated in the CCl4 administered rats. Özenirler et al. (1996) reported an increased emergence of lipid peroxidation in the hepatocytes of rats exposed to CCl4 and occurrence of centrilobular necrosis with fatty degeneration in the liver [30]. Yang et al. (2007) induced liver injury with CCl4 in 30 rats to investigate the liver protecting effects of pycnogenol. They demonstrated that CCl4 administered in a single dose of 1.25 ml/kg resulted in hepatotoxicity and increased the serum levels of AST and ALT. AST and ALT are the most commonly used markers in the routine practice to determine liver injury [31]. AST is not only available in the liver but it is abundant in the heart, muscles, brain, pancreas, kidneys, lungs, leukocytes and erythrocytes. However, ALT is abundant only in the liver. Approximately 80% of the AST in hepatocytes are in the mitochondria. However, the predominant form of ALT is non-mitochondrial. Therefore, in mild hepatocellular injury, cytoplasmic AST and ALT are released into the serum if the cell membranes of hepatocytes are insulted and the mitochondrial membrane is intact. In more severe forms of hepatocellular injury, the mitochondrial membrane is damaged too, resulting in the release of mitochondrial AST [32].

Shallan et al. (2008) investigated the antioxidant effect of Solanum nigrum L. in hepatotoxicity by administering 1 ml of 10% CCl4 orally in corn oil to the rats and found out that the serum activities of ALT and AST increased significantly in the rats intoxicated with CCl4 compared to the control group, however, they did not observe any significant findings in the activity ALP. They have suggested that the increased activities of AST and ALT due to the hepatic injury is an indicator of the membrane integrity of the hepatocytes. They associated the recovery of serum transaminase levels with the improvements in the hepatic parenchyma and hepatic regeneration [33].

The study has demonstrated that CCl4 impairs the antioxidant defense mechanisms in the lung tissue, leading to oxidative injury. MDA is one of the products synthesized as a consequence of lipid peroxidation and it is a commonly used parameter to indicate the oxidative injury [34]. The increased levels of MDA indicated that CCl4 led to the induction of lipid peroxidation and an eventual oxidative injury in the lung tissue. Similarly, Ganie et al. [35] reported in a study on rats that the exposure to CCl4 led to the induction of lipid peroxidation [35]. The reactive radical trichloromethyl peroxyl, which is produced during the processing of CCl4 in the liver, affects the membrane lipids, primarily the unsaturated fatty acids, leading to the progression of the hepatic injury by insulting the energy metabolism and protein synthesis [36]. The results of those studies are consistent with the results of our study.

Lipid peroxidation is the most critical effect induced by the free radicals [37]. Both experimental and clinical studies have demonstrated that lipid pe-
oxidation is associated with the development of fibrosis in the liver. The study by Yuce et al. demonstrated that the MDA levels in the CCl4 administered groups were significantly higher compared to the control group and the quercetin-treated group [39]. These findings are consistent with the data in the literature, reporting that CCl4 augments lipid peroxidation [40]. In the studies investigating the acute [41, 42, 43, 44] and chronic injuries [45, 46] induced with CCl4, it has been demonstrated that the serum activities of AST, ALT, and ALP were elevated in association with the severity of the emergent injury. The levels of AST, ALT, and ALP were similar in the CCl4 administered group.

It is reported that the activities of the antioxidant enzymes will be reduced when the cellular antioxidant defense systems are overwhelmed due to the long-term increases in free radicals and occurrence of augmented lipid peroxidation. In parallel to the increase in the lipid peroxidation following the administration of CCl4, the increased activity of glutathione peroxidase, which is one of the cellular antioxidant defense mechanisms, has revealed that the cellular antioxidant system could not be overcome at the blood level [47, 48]. Therefore, it was observed in our study that the total oxidant level was increased with the administration of CCl4, and the total antioxidant levels were increased with the administration of Tribulus terrestris containing antioxidants.

Xu et al. [49] measured the levels of AST, ALT, MDA, GSH, CAT, and SOD in the group where they induced liver toxicity with the administration of CCl4. They reported that there was an increase in the levels of AST, ALT, and MDA and there was a decrease in the levels of GSH, SOD, and catalase. They argued that 5-4-Dihydroxy-3 5 -di-methoxy 7-o-β-D glucopyranose flavone, they used against the hepatotoxicity, could be used as a preventive agent.

In the current study, it has been observed that the severe liver injury occurred in the groups where CCl4 was administered, which is a finding consistent with the reports in the literature. In the group where only CCl4 was administered, the levels of AST, ALT, ALP, and MDA significantly increased and the total oxidant level was high whereas the total antioxidant level was low. In the groups which were treated with the extract of Tribulus terrestris plant at different doses following the administration of CCl4, there was a reduction in the levels of AST, ALT, ALP, and MDA; a reduction in the levels of AST, ALT, GSH, SOD, and CAT; and an increase in the total oxidant levels.

CONCLUSION

In this current study investigating the effects of the extract of Tribulus terrestris on the hepatotoxicity induced by CCl4, it was observed that the extract of

*Tribulus terrestris* suppressed the oxidative stress and enhanced the antioxidant system, being more effective especially in higher doses. *We are of the opinion that the extract of* *Tribulus terrestris* can be beneficial in human beings when used either for preventive purposes or to treat liver injuries and it can be considered as a supplement in the treatment of liver diseases, becoming one of the agents in adjunctive treatments.

REFERENCES


ASSESSMENT OF NATIONAL RURAL DEVELOPMENT STRATEGY WITH R’WOT TECHNIQUE SAMPLE OF TURKEY

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ABSTRACT

The study was focused on investigation of National Rural Development Strategy: 2014-2020 document of Turkey. The document had entered into force on 21 February 2015 with Official Gazette no:29274. The strategy was prepared under collaboration of five ministries. Agriculture and Forestry (formerly Food, Agriculture and Livestock), Development, Foreign Affairs – EU Directorate (formerly European Union (EU) Affairs, Environment and Urbanization and Internal Affairs. The prioritisation of rural areas in Turkey was analyzed DQGDVVHVVHGZLWK5¶:275DQNLQJ6:27 analysis results, it was detected that infrastructure and irrigation investments, forestation and land consolidation need to be increased.  The National Rural Development Strategy has five strategic objectives. 'Betterment of the rural environment and maintaining the sustainability of natural resources', which is the second strategic objective, should be prioritised due to the results retrieved.

KEYWORDS:
R’WOT, Rural development, National Rural Development Strategy, Turkey.

INTRODUCTION

The rural areas are not just determined regarding physical endowments, but they also stand for a complicated structure indicating economic and social differences compared with the urban districts. It is not fair to define rural areas as places which provide job and living environment for the population living there; the rural areas are multi-functional areas where ecological balance is provided as well [1].

The initiatives that are taken to sustain rural areas’ existence and to improve the living conditions of rural society, which has less economic and social opportunities compared to urban one, are generally called “Rural Development” [2]. Taşcioğlu [3] defines rural development as “all moral and material support, training initiated for the betterment of people or communities living and working in rural areas”.

Rural development policies cover all policies that include advances as a result of attempts executed by the society and public authorities as a whole to improve economic, societal and cultural opportunities for individuals living in rural areas, to increase their welfare level and to maintain the participation of rural population to the national development. The main objective of rural development strategy and policies is to maintain development of backward societies in economic and socio-cultural areas through self-help or external help method [4].

The development plans for five years had started to be prepared and put in issue in Turkey since 1963. The Rural Development concept was mentioned directly in the eighth development plan (2001-2005), upon legal preparations made before the plan. The policy documents for the first rural development were released in the 9th Development Plan (2007-2013). These documents are the National Rural Development Strategy and Rural Development Plan.

The National Rural Development Strategy Document aims to increase the awareness on development in rural and urban areas, decrease unfavourable effects of migrations on rural area, and strengthen rural policy management on central and local levels and to contribute to EU accession process [5].

There are various studies conducted on the field. Before proceeding, it is essential to overview these researches.

Oddershede et al. [6] assessed the priorities of training, infrastructure, economy and environment sub-factors for tourism, service, industry and maritime sectors with Analytical Hierarchy Method (Analytical Hierarchy Process) for Chile’s rural areas. Regarding the use of analytical hierarchy method in rural development in their study conducted at areas. Hu et al. [7] assessed population, area, industry, social and environment criteria with AHP by using Rural-Urban Development Index and Rural-Urban Change Index between 1994 -2010. Another study including the use of AHP in rural areas was
conducted in solar system houses in rural areas of Bangladesh [8].

The R’WOT technique was used in Turkey for the first time by Yılmaz [9] in assessment of project success status regarding the development of beekeeping. Sönmezler [10] applied R’WOT technique for evaluating alternatives for a festival project and selection of the most suitable alternative.

Öztürk and Tonuk [11] stated in their study conducted for Devrekani Basin that the central government and the local public should consider solid waste disposal facilities, residential and industrial wastewater treatment plants as the most important threat.

Gürbüz and Öztürk [12] found out in the R’WOT technique applied for shipping department of the textile industry. The analysis revealed that possessing sufficient technological and business facilitator equipments (forklifts, pallets, shelves, palm, etc.) was the most important priority of SWOT factor within the SWOT group. Öztürk [13] pointed out in their study conducted at Sarskam Conservation Area that “existence of other natural areas for Sinop” was the most important priority within opportunities revealed as factors of SWOT analysis. Stavroulakis and Riza [14] used R’WOT technique in their study on the health sector.

In this study, prioritization the SWOT analysis in the “National Rural Development Strategy: 2014-2020” document prepared by the Ministry of Food, Agriculture and Livestock by the RWOT technique and evaluating the strategic objectives within the scope of the determined strategies were conducted.

MATERIALS AND METHODS

The main material of the study is National Rural Development Strategy: 2014-2020 document, which was prepared by the Agriculture and Livestock Ministry and entered into force with 21 February 2015 dated and 29274 numbered Official Gazette. The SWOT analysis prepared within the scope of National Rural Development Strategy: 2014-2020 and the determined strategically purposes constitute the material of the study.

In this study, R’WOT Technique was used as a hybrid method in which SWOT Analysis, Ranking Analysis and Linear Combinations analysis are used together. SWOT analysis was prioritised by 40 experts working on rural development.

Analysis Used within the Scope of R’WOT Technique. SWOT Analysis, which is in use since 1970s with the purpose of business management, has been considered as a tool of analysis and planning for various policy fields in the following years. SWOT is an abbreviation consisting of the first letters of strengths, weaknesses, opportunities and threats. With this method, which has the analysis principle to examine the parameters of the existing structure, analysis of both quantitative and qualitative properties can be done and a strategic view can be created with the examination of SWOT matrix regarding the existing situation [15].

The opportunities are properties of the external environment preparing advantageous conditions for a place or objective out of a pre-determined objective or objective group. In addition, the threats are also external factors as environmental development creating problems against reaching the objective or objectives of the SWOT matrix. The specific opportunities as the strengths of superior properties helping to reach these determined objectives and weaknesses that prevent reaching these objectives and including limited experience in some subjects are the internal factors of the matrix [16].

In determination of policies for rural areas of a country, internal (strengths and weaknesses) and external (opportunities and threats) factors of the potential of the rural areas in the focused country can be determined with the help of the SWOT technique. The technique helps selection of an appropriate strategy set in conformity with the country's objectives to explicitly know and analyse the strengths and weaknesses of rural policies of a country. The methodology encompasses two phases as follows.

Ranking Analysis. In this analysis, the ranking is conducted to determine the relative priority values of SWOT groups and SWOT factors within each SWOT group. This ranking operation is conducted using “nine-point scale”. In this scale are accepted accordingly:

1- Weakly important
3- Low importance
5- Important at a medium level
7- Very Important
9- Extremely important

Additionally, “2, 4, 6 and 8 values” can be used as medium values [17].

Linear Combinations Analysis. In this technique, relative priority values of each SWOT factor are multiplied with the relative priority values of SWOT group connected to these factors. In this way, the relative priority values of SWOT factors are put into the same scale, and they can be compared with each other. In conclusion, the order of importance is obtained by multiplying relative priority values of SWOT factor with the relative priority values of SWOT group connected to this factor. Accordingly, ‘linear combination’ analysis was conducted mathematically [18].

The linear equation used in this technique can be indicated as follows:
TABLE 1
Stages of R’WOT Technique

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>1. Stage</td>
<td>SWOT Analysis</td>
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<tr>
<td>2. Stage</td>
<td>Comparisons for Each SWOT Group</td>
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<tr>
<td>3. Stage</td>
<td>Comparisons for SWOT factors within Each SWOT group</td>
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<tr>
<td>4. Stage</td>
<td>Determination of final priority value of each SWOT factor</td>
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SWOT analysis is conducted for Superiorities, Weaknesses, Opportunities and Threats.
4 SWOT Groups, consisting of Superiorities, Weaknesses, Opportunities and Threats, are compared with each other.
For each SWOT group, SWOT factors found within these groups are compared.
Here, the relative priority values of each SWOT group calculated based on the comparisons of 2nd Stage are multiplied separately with the relative priority values of each SWOT factors found in this group according to the 3rd stage comparisons.

Pji= Wji Xji
Here,
Pji = (j) SWOT group (i) final priority value in SWOT factor,
Wji = (i) SWOT factor included by (j) SWOT group's relative (at the same time, final) priority value,
Pji = (j) SWOT group (i) relative priority value in SWOT factor [17].

Stages of R’WOT Technique. R’WOT technique consists of four stages. The first stage is SWOT solutions; the second stage is comparisons for SWOT group, the third stage is comparisons for SWOT factors within each SWOT group, and the last stage is determining the final priority of each SWOT group (Table 1).

Subsequent to prioritisation with R’WOT technique, strategic objectives stated in National Rural Development Strategy (2014-2020) were assessed.
The strategies of National Rural Development Strategy (2014-2020) are given below;

Strategic Objective 1. Improvement of rural economy and increasing employment opportunities. Under this title, it is intended to improve the institutional capacity of producer organisations for agriculture and food sector, to process and market agriculture-food products, to maintain training and consultancy services for producers. In addition, following objectives are to modernise agriculture and food enterprises and to increase rural and national food security. It is planned to improve rural tourism to diversify the rural economy and water aquaculture additionally.

Strategic Objective 2. Developing the rural environment and maintaining sustainability of natural resources. It is planned with this strategy to popularise environment-friendly agricultural practices, to improve organic farming, to prevent environmental pollution caused by agricultural activities and to reform the pasture. Improvement of the irrigation infrastructure, promoting land consolidation, developing income generating activities for forest products, reforming forests, promoting forestry and supporting income generating activities in

and around the protected areas are the following objectives under this title.

Strategic Objective 3. Developing social and physical infrastructure of rural settlements. Under this title, it is planned to improve the transport network, to develop drinking water infrastructure, to improve waste management system, to cope with potential natural disasters, to encourage local architecture in the settlement and to develop infrastructure to protect cultural heritage.

Strategic Objective 4. Developing human capital of rural society and reducing poverty. It is planned to facilitate formal and non-formal education services to rural areas, to increase accessibility to preventative health and to strengthen the social services for disadvantaged individuals under this orientation.

Strategic Objective 5. Improving the institutional capacity for local development. The primary goals of this strategic objective are creating national and rural development cooperation, developing innovative approaches in provision of public services, improvement of service delivery capacity of town, village neighbourhood and municipalities.

RESULTS AND DISCUSSION

By reviewing the SWOT analysis conducted by Food, Agriculture and Livestock Ministry (formerly), a total of 51 SWOT factors, consisting of 15 strengths, 12 weaknesses, 12 opportunities and 12 threats as SWOT groups, were determined. The results of R’WOT technique, applied to 40 persons, were provided in Table 2. Accordingly, while the most important priority in SWOT groups is strengths (0.2744), the lowest priority is threats (0.2372). The group members were indicated consecutively below.

Strengths. Increasing forestation (S1), infrastructure investments (S2), increasing land consolidation (S3), diversity of crafts and local products (S4), existence of young population despite the
immigration (S5), diversification of non-agricultural economic activities and household income (S6), foundation of Development Agencies (DA) (S7), existence of rich natural resources, increasing protected areas and unpolluted agricultural area (S8), experience of cooperatives (S9), potential of organic farming (S10), existence of sample enterprises and practices (S11), increase of mechanization in agriculture (S12), wideness of grasslands (S13), increasing awareness about agricultural organization (S14), foundation of Agriculture and Rural Development Support Institution (ARDSI) (S15).

**Weaknesses.** Soil erosion threat due to the increased fallow land (W1), existence of scattered and small agricultural parcels (W2), insufficient statistics in rural areas (W3), scattered and sectional settlement of many villages due to topography and climate (W4), lack of coordination among institutions (W5), lack of modern agricultural knowledge and absence of professional management in producer organizations, unconscious practices in agricultural irrigation (W6), lack of social facilities and activities (W7), free family labor in agriculture, social security problem (W8), lack of investment due to non-accumulation of capital in agricultural enterprises (W9), low productivity in agricultural production (W10), Lack of storage and marketing infrastructure of products W11), lack of accessibility to elderly care services (W12).

**Opportunities.** Increase of legal regulations regarding treasury lands in rural areas for conformity with the EU legislation (O1), increasing supports for pressurized irrigation (O2), popularization of information and communication technology (O3), increasing interest on nature tourism (O4), longing for traditional village life (O5), size of internal demand and existence of close external markets (O6), strengthening women labor (O7), development of innovative approaches in service provision to rural areas (O8), diversification of grant programs towards rural areas and improving capacity of institutions and citizens to prepare and apply projects (O9), heading for branding in agricultural production due to the increased demand for qualified agricultural products (O10), maintaining financial support from international institutions and experience sharing and cooperation (O11), heading for renewable energy (O12).

**Threats.** Increasing natural factors damaging infrastructure (T1), rising regional differentiation in development levels of rural areas (T2), pressure of energy demand on natural resources (T3), declining interest of youth in agriculture (T4), drought risk caused by climate change (T5), environmental pollution caused by manufacturing industry facilities (T6), neglecting implementation of urban-focused social policies in rural areas (T7), aging of rural population due to the immigration to urban districts (T8), rising pollution in water resources (T9), high input cost (T10), lack of regulations of the local administration units (towns-headmen) (T11), increased use of productive agricultural lands for non-agricultural purposes (T12).

While the highest priority in strengths is existence of youth population despite immigration (0.0864), the lowest priority is developing infrastructure and irrigation investments (0.0467), while the highest priority in weaknesses is lack of modern agricultural knowledge and absence of professional management in producer organisations. Unconscious practices in agricultural irrigation (0.0975). The lowest priority is existence of scattered and small agricultural parcels (0.0723) (Table 2).

Longing for traditional village life (0.1012) is the top priority in opportunities. Declining interest of youth in agriculture (0.0956) is the top priority in threat factor within all SWOT sub-factors. While the most important priority in opportunities group is longing for traditional village life (0.02439). The least priority in strengths group is developing infrastructure and irrigation investments (0.0128) (Table 2).

**CONCLUSION**

The National Rural Development Strategy (2014-2020) has 5 Strategic Objectives (SO). These are “Improvement of rural economy and increasing employment opportunities (SO 1)”, “Developing the rural environment and maintaining sustainability of natural resources' (SO 2)”, “Developing social and physical infrastructure of rural settlements (SO 3)”, “Developing human capital of rural society and reducing poverty (SO 4)” and “Improving the institutional capacity for local development (SO 5)” when these strategies are examined with R’WOT technique's priorities. The steps to be taken according to the priority order were given below.

45% of the municipalities found in rural areas do not have a solid waste treatment system in Turkey. 80% of the villages do not have a sewage system. The internet is limited in rural areas [20]. Solid waste storage facilities, internet accessibility, and sewage system need to be completed in rural areas. Furthermore, completion of infrastructure in places, where there is no sufficient agricultural infrastructure, is essential. In addition, project designing for irrigation systems to promote pressurised irrigation and closed conduit systems instead of open systems for minimising the water distribution loses are essential as well (SO 2).
<table>
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<th>Total Point</th>
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The forest existence is approximately 21.7 million hectares in Turkey. 1.65 million hectares of forest area had been destroyed within 98653 forest fires between 1937, when fire statistics record-keeping had started and 2014 [19]. Fight with forest fires should be increased and rehabilitation of degraded forest areas should be focused on (SO 2).

Agricultural lands’ being scattered and agricultural production in Turkey has been declining sectionally. It is scattered and fragmented agricultural land in Turkey caused a reduction in agricultural production. Land consolidation needs to be widespread to increase the productivity of agricultural land (SO 2).

The organic farming lands and lands suitable for organic farming constitute a small share of total agricultural lands. The ratio of organic farming in total agriculture is approximately 0.5% [20] (SO 2).

Eliminating insufficiencies in product processing of farmers, taking criminal actions against those involved in unlicensed production and increasing research and development studies are among the main things to do for improving organic farming.

Turkey needs to determine objectives to decrease greenhouse gas emissions and consumption of fossil fuel, to increase energy efficiency, to protect and improve forests and to take the active role in international climate policies (SO 3) [21].

Even though the ratio of female participation in the labour force is higher in developed countries, this ratio is low in many developing countries and Turkey. Due to SO 4 empowerment of women in the rural areas should be considered and supported [22]. The empowerment refers to increasing potential of women employment via training programmers and increasing opportunities to market their products and promising payment equality with males should be considered within the strategy for young females as well as the middle and old aged women farmers.

Considering the priorities of RWOT technique conducted within the scope of the study. It is seen that the prior strategy to be implemented to develop rural development in Turkey is “Improvement of the rural environment and maintaining the sustainability of natural resources.”

REFERENCES


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EFFECTS OF NITROGEN FERTILIZATION METHODS ON MACRO AND MICRO-NUTRIENTS IN WHEAT LEAF

Fulya Tan¹, Cihangir Saglam

Namuk Kemal University, Agricultural Faculty, Department of Biosystems Engineering, Tekirdag, Turkey

ABSTRACT

The aims of this study were to compare the Nitrogen-fertilization methods; Anhydrous ammonia method (M-1), traditional method (M-2), and control method (M-3) and to study the effects on leaf macro-and micro nutrients in the winter wheat plants. The research has been conducted in the ecological conditions of Tekirdag during the 2014–2015 and 2015–2016 growing seasons. The experiment has been arranged in a randomized complete block design with three replications. Leaves collected during three different developmental stages (I-II-III periods) of wheat have been tested for macro and micro-nutrient analysis. Results showed that M-1 method has a positive effect on leaf macro and micro nutrients. The highest leaf N content (3.45%, 3.68%) has been detected in the application of anhydrous ammonia in both year.

KEYWORDS:
Leaf analysis, fertilization, anhydrous ammonia, wheat, nitrogen

INTRODUCTION

Wheat agriculture is very important compared to other agricultural crops in terms of being the main source of human nutrition. Especially in our country (Turkey), wheat production is becoming increasingly important. World wheat production is 751.5 million tons, Turkey has 3% of the total wheat production. Cereals have the largest share of 49.9% in the 23.9 million hectares of arable land in our country. Wheat, in total grain production has the largest share with 67% [1, 2]. The macro and micro nutrients of crops depends on environmental conditions and agronomic practices, including fertilization [3, 4]. The use of nitrogenous fertilizers in cereal cultivation is important. Therefore, nitrogen fertilizers are used widely in agriculture as the main sources of crop nutrients. It is applied two or three times to meet the nitrogen requirement in the wheat growing [5]. Farmers generally use leaf color as a visual indicator to determine the N need of wheat. Chlorophyll levels can be predicted with high accuracy using the vegetation indexes [6, 7].

The most commonly used nitrogenous fertilizers in our country are urea (46%) and nitrate (23-26%). Generally, farmers have been use nitrogen fertilizer in quantities much larger than the nitrogen amounts required by the soil [8, 9, 10]. The use of anhydrous ammonia is also important because it reduces the use of nitrogenous fertilizers in large quantities. But, due to the chemical properties of anhydrous ammonia, its use in agriculture is not common. It also requires special equipment for application [11, 12]. Although anhydrous ammonia is the fertilizer with the highest nitrogen content, there is no use for this purpose in our country cultivation. Anhydrous ammonia is performed once in the pre-sowing period in developed countries such as America and Canada [13].

The main purpose of this study is to compare the fertilization methods and to examine the possible effects of anhydrous ammonia on leaf macro- and micro nutrients in wheat.

MATERIALS AND METHODS

The experiment was conducted in Tekirdağ (41°8′N, 27°39′W and 41°18′N, 27°47′W) during the 2014-15 and 2015-16 growing seasons. The average temperatures of the growing periods in these years were 14.8 °C and 14.9 °C, respectively. The wheat cultivar used in the first season was Anapo and in second season Esperia. Winter wheat (Triticum aestivum L.) was sown on October 28, 2014 and November 12, 2015, with a row spacing of 18 cm and a seeding rate of 180 kg ha⁻¹.

Soil sampling and analysis. The results of some basic physical and chemical analyses of the 0–20 cm soil layer are listed in Table 1. The soil textures of the experimental site have a low calcareous structure in the first year and a clay-tanned structure in the second year.

Leaf analysis. The leaf analysis in the experiment was carried out in three periods (Table 2). The nitrogen content determined with the Kjeldahl method. Phosphorus, potassium, calcium, magnesium, copper, zinc and manganese were determined with DTPA-ICP method.
### TABLE 1

Physical and chemical properties of soil

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<th>Year II</th>
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<td>Mn</td>
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*N (Total nitrogen), P (phosphorus), K (potassium), Ca (Calcium), Mg (magnesium), Cu (copper), Zn (zinc) and Mn (manganese)*

### TABLE 2

Dates of period

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### TABLE 3

The total nitrogen levels applied in different fertilization methods

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<tr>
<td>M-I</td>
<td>18.0 kg da⁻¹</td>
<td>18.0 kg da⁻¹</td>
<td>Pre-sowing</td>
</tr>
<tr>
<td>M-II</td>
<td>18.9 kg da⁻¹</td>
<td>20.3 kg da⁻¹</td>
<td>Pre-sowing; Tillering; Stemelongation</td>
</tr>
<tr>
<td>M-III</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### TABLE 4

Physical and chemical properties of Anhydrous ammonia

<table>
<thead>
<tr>
<th>Physical Form</th>
<th>Gas (liquid under pressure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Colorless gas and liquid,</td>
</tr>
<tr>
<td>Odor</td>
<td>Strong</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>-33° C at 1 atm</td>
</tr>
<tr>
<td>Melting point</td>
<td>-78° C</td>
</tr>
<tr>
<td>Ph</td>
<td>Approximately 12.0</td>
</tr>
<tr>
<td>Density</td>
<td>0.696 g/L</td>
</tr>
</tbody>
</table>

**Experimental design.** The experiments were arranged in the field using a randomized complete block design with three replications. In this research, three different fertilization methods were used: Anhydrous ammonia method (M-1), Traditional method (M-2) and Control method (M-3). Nitrogen ratio used application was determined according to the soil analysis before sowing the crop. In the Anhydrous ammonia method, nitrogen fertilization was performed only once before sowing. In the traditional method, the farmer applied the nitrogen fertilizer determined according to soil analysis (18.0 kg da⁻¹) in three different periods. In the control method, nitrogen fertilization was not performed.

The fertilization period, N fertilization numbers and total nitrogen levels applied in different fertilization methods are given in Table 3.

**Anhydrous ammonia (NH₃) and application.** Anhydrous ammonia is rich in nitrogen (%82). Chemical and physical properties of Anhydrous ammonia are shown in Table 4 [12]. Anhydrous ammonia application was applied on 25th October, 10th November (two days before sowing) in wheat at the period before sowing 2014 and 2015, respectively. It was injected with 50 cm spacing and 20 centimeters beneath the soil surface with traditional knife type injection unit [5, 11].
The effects of methods and periods on the macro and micro-nutrients of the wheat (Year I)

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>Cu (Ppm)</th>
<th>Zn (Ppm)</th>
<th>Mn (Ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.44±0.72</td>
<td>0.43±0.05</td>
<td>2.61±0.29</td>
<td>0.55±0.08</td>
<td>0.108±0.01</td>
<td>8.34±0.84</td>
<td>19.06±9.48</td>
<td>99.0±48.87</td>
</tr>
<tr>
<td>2</td>
<td>2.50±0.62</td>
<td>0.33±0.03</td>
<td>2.29±0.08</td>
<td>0.45±0.12</td>
<td>0.123±0.02</td>
<td>7.33±1.35</td>
<td>13.16±4.36</td>
<td>95.0±44.60</td>
</tr>
<tr>
<td>3</td>
<td>2.39±0.69</td>
<td>0.18±0.02</td>
<td>1.22±0.04</td>
<td>0.70±0.13</td>
<td>0.200±0.03</td>
<td>5.17±0.35</td>
<td>5.07±0.19</td>
<td>63.5±63.45</td>
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**F Values**

<table>
<thead>
<tr>
<th>Period</th>
<th>Method</th>
<th>R²</th>
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<tbody>
<tr>
<td>39.007</td>
<td>44.890</td>
<td>79.164</td>
</tr>
<tr>
<td>6.679</td>
<td>29.181</td>
<td>41.507</td>
</tr>
<tr>
<td>8.452</td>
<td>2.922</td>
<td></td>
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</tbody>
</table>

*For each parameter, means followed by different letters are significantly different according to Tukey HSD (P < 0.05);

Micro-Nutrients; Ca (Calcium), Mg (magnesium), Cu (copper), Zn (zinc) and Mn (manganese)

The effects of methods and periods on the macro and micro-nutrients of the wheat (Year II)

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>Cu (Ppm)</th>
<th>Zn (Ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.24±0.67</td>
<td>0.32±0.02</td>
<td>2.79±0.34</td>
<td>0.53±0.06</td>
<td>0.140±0.02</td>
<td>8.74±1.14</td>
<td>17.16±7.71</td>
</tr>
<tr>
<td>2</td>
<td>2.88±0.77</td>
<td>0.16±0.05</td>
<td>2.77±0.29</td>
<td>0.43±0.11</td>
<td>0.091±0.02</td>
<td>9.10±1.51</td>
<td>13.05±2.78</td>
</tr>
<tr>
<td>3</td>
<td>2.03±0.51</td>
<td>0.10±0.01</td>
<td>2.20±0.42</td>
<td>0.69±0.18</td>
<td>0.138±0.06</td>
<td>8.74±0.58</td>
<td>8.67±1.66</td>
</tr>
</tbody>
</table>

**F Values**

<table>
<thead>
<tr>
<th>Period</th>
<th>Method</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>64.889</td>
<td>490.978</td>
<td>1.688</td>
</tr>
<tr>
<td>10.576</td>
<td>2.268</td>
<td>0.296</td>
</tr>
<tr>
<td>3.559</td>
<td>10.898</td>
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</tr>
</tbody>
</table>

*For each parameter, means followed by different letters are significantly different according to Tukey HSD (P < 0.05);

Macro-Nutrients; N (nitrogen), P (phosphorus), K (potassium),

Micro-Nutrients; Ca (Calcium), Mg (magnesium), Cu (copper), Zn (zinc) and Mn (manganese)

Statistics analysis. To evaluate statistical significance between the periods and methods in terms of macro and micro-nutrients of wheat, the data was analyzed using the ANOVA procedure, and significant differences among means were determined by Tukey HSD test and correlation test was performed between all parameters (SPSS v.18.0).

RESULTS AND DISCUSSION

The effects of methods and periods on the macro and micro nutrients of the wheat leaf in the first season are given in Table 5. The effects of methods and periods on the macro and micro nutrients of the wheat leaf in the second season are given in Table 6. The correlations between all parameters are given in Table 7.

While the effect of periods on macro nutrients (N, P, K) was statistically significant, the effect of the methods was only significant for N concentrations. The effect of periods on micro nutrients were statistically significant, except for Mn concentrations. Different nitrogenous fertilizer applications were important on N, Mg, Mn, Zn and Cu concentrations.

The highest N concentrations was measured in the M-1 method (3.456%). The N concentrations at the M-1 in 2015 and 2016 were 3.456 and 3.686%, respectively, whereas the average N concentrations at the M-2 method were 2.503 and 3.060% in the same two years, reductions of 19.96% and 16.98%.

Compared with the M-3, the total N concentration decreased by 38.95% in year I and by 34.27% in year II. In the present research N content was correlated with the method (R² = 979, P < 0.05) and period (R² = 724, P < 0.01) (Table 7). In the present study, the leaf-N concentrations in the M-1 method during the all periods ranged from 3.44 to 4.24 %, which were similar to those found by Wyckoff [12]. According to Wyckoff [12], the leaf N in anhydrous ammonia applications is higher than urea applications. Similar results were found in our study. Leaf nitrogen in the M-2 method is very low compared to the M-1 method. At the same time, the nitrogen content did not have adequate levels in some periods.
The P concentrations was affected by the periods and the highest P concentrations was measured in the M-1 method both year. The P concentrations was 0.343% in year I, which was 16.62% higher than the M-3. However, there was no significant difference among the methods year I compared to the M-3 method. There was a significant correlation between P concentrations and periods ($R^2 = -0.822$, $P < 0.01$). The P concentration increased significantly under the fertilization applications, also according to [14].

The K concentrations was not affected by the methods [14], where the average levels were 2.19 and 2.47 % in year I and in year II, respectively, although there was a significant increase to 11.21% in year I and 5.27% in year II compared with the M-3. The K concentrations was affected by the periods in year I. There was a significant correlation between K concentrations and periods ($R^2 = -0.712$, $P < 0.01$). The K concentration correlated strongly negatively with Mg concentration ($R^2 = -0.605$, $P < 0.05$). Similar results were obtained by Jaskulska et al. [15].

The Ca concentrations was affected by the periods and the highest Ca concentrations was measured in the M-1 method both year. The Ca concentrations in period I, was 26.44% lower than the period 3. The Ca concentrations in year I and year II were 0.68 and 0.64%, respectively. The average Ca concentration in M-1 method was 0.66%. The Ca concentrations in the M-1 were 33.83%, 23.25% higher than the M-3 in year I and II, respectively. Correlation coefficient ($R^2 = -0.568$, $P < 0.05$) between Ca concentrations and methods was relatively low, although it was statistically significant.

The Mg concentration was not affected by the periods and methods in the second year, although the Mg concentration was a significant both period and method in the first year. The highest Mg concentrations were measured in the M-1 method both year. The Mg concentrations in the M-1 were 12.27% and 23.31% higher than the M-2 and M-3 methods, respectively. The Mg concentration correlated strongly positively with Ca concentration ($R^2 = 0.806$, $P < 0.05$). Similar results were obtained by Jaskulska et al. [15].

The methods had significant effects on the Cu concentration during year I and II. The Cu concentrations at the M-1 in year I and II were 7.98 ppm and 10.01 ppm, respectively, whereas the average Cu concentrations at the M-3 method were 6.35 ppm and 8.41 ppm in the same two years, reductions of 20.43% and 15.99%. But, there was no significant correlation between Cu concentrations both methods and periods.

The Zn concentration was not affected by the periods and methods in the second year, although the Zn concentration was a significant both period and method in the first year. The highest Zn concentrations were measured in the M-1 method both year. The Zn concentrations in the M-1 were 42.5% higher than the M-3. There were a significant correlation between Zn concentrations and periods ($R^2 = -0.694$, $P < 0.01$), with methods ($R^2 = -0.507$, $P < 0.05$).

Methods applied in the experiments had significant effects on the Mn concentrations. The highest Mn concentrations was measured in the M-1, M-2 and M-3 both year, respectively. The Mn concentrations was 142.33 ppm in the M-1, which was 68.1% higher than the M-3 methods during year I also year II.
CONCLUSION

The results of this research demonstrate that the application of Anhydrous ammonia had significant effects on the macro and micro-nutrients in wheat leaf. Anhydrous ammonia enhanced the macro and micro-nutrients in wheat leaf compared with the control method in each year. Especially when we examine the leaf of the plant in terms of nitrogen content, in the leaf N concentration increased significantly compared with the M-3 method. It was determined that the plant has nitrogen content which is needed in the plant development periods in M-1.

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REFERENCES

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THE RELATIONSHIP BETWEEN EPILITHIC DIATOM COMMUNITIES AND WATER QUALITY VARIATIONS ACROSS TOHMA STREAM (MALATYA-TURKEY)

Vesile Yildirim*, Nilgun Baran

Firat University, Faculty of Science, Department of Biology, Elazig, Turkey

ABSTRACT

Epilithic diatom community of Tohma Stream, as well as physicochemical properties of the stream were studied through samples collected from the specified 4 stations in monthly periods for one year. 70 species from 21 genera were identified in the study. In epilithon in the stations, *Navicula* spp. (16 taxa) was the most remarkable genus in terms of both number of species and the number of individuals from species and this was followed by *Cymbella* (13 taxa) and *Nitzschia* (9 taxa). The water quality in the Tohma stream varied at different stations based on levels of pollution. The epilithic diatom communities responded to changes in the aquatic environment. Dominant species had variation among the stations. Dominant species of the station 1 were *Cocconeis placentula*, *Diatoma vulgaris*, *Cymbella affinis* and *Gomphonema olivaceum*. *Navicula cryptcephala*, *C. minutula*, *Ultaria ulna*, *Nitzschia amphibia*, and *N. thermalis* were dominant diatoms of the stations 2 and 3. Dominant diatoms of the station 4 were *Achnanthidium minutissimum*, *N. palea*, *Gomphonema parvulum*, and *Surirella angustata*, diversity of diatoms also varied in all stations in parallel to water quality. Also, station 4 in Beyler Brook in which wastes of 1<sup>st</sup> and 2<sup>nd</sup> Organized industry zones are discharged joins to Tohma stream.

We concluded that Tohma stream has a water quality gradient and this led to apparent variances in species composition of diatom across the stream. The results have confirmed that epilithic diatoms are crucial biological indicators to evaluate ecological quality of Tohma stream.

KEYWORDS:
Tohma Stream, Epilithic diatoms, Water quality, Pollution

INTRODUCTION

Rivers and streams are complex ecosystems that are influenced by numerous environmental factors in spatial or temporal scale. Among these factors, external effects such as climate, geomorphology, use of land in the basin, release of domestic and industrial wastes into water resources without treatment, unconsciously applied agricultural activities, devastation of vegetation, destruction of streambeds negatively influence aquatic environments either directly or indirectly. Diatoms are the most varied group of algae in rivers and streams. They constitute a major part of total algal biomass in several environments and are a high quality food source for those at higher trophic levels in aquatic food web. There are studies indicating that distribution of diatoms is based on environmental factors such as climate and hydrologic regimens, geomorphological characteristics, use of soil, nutrient, ion concentration, and herbivorous creatures [1].

Diatoms, the most important members and primary producers in stream ecosystems, are extremely sensitive to physical and chemical changes and valuable indicators of steam ecosystems because they are influenced from the changes in their habitat and have short lasting generations and taxonomic diversity [2]. While the number and diversity of diatoms in aquatic ecosystems also provide information about productivity of their environment, some species that are indicators of pollution become important criteria for identification of degree of pollution in this environment as well [3].

Tohma Stream, our study area, is the largest stream of the city after Euphrates River being the eastern border of Malatya and it is 52.5 km long. Tohma Stream is affected by intensive agricultural lands, the use of stream as garbage dump, drawing water from the stream for irrigation, discharge of wastes from organized industrial zone into the stream, and domestic and industrial wastes.

The aim of the present study is to determine the structure of epilithic diatom community of this stream and how epilithic diatom communities reflect environmental changes between upper and lower parts of Tohma Stream.
In the present study, 4 stations that were considered to characterize Tohma Stream were chosen. Figure 1 shows condition of the stream and locations of sampling stations.

Station I: It is the closer to the resource among all stations, has higher current velocity, its base is gravelly.

Station II: Its base is gravelly, partly fine sandy, it is an area where stockbreeding and agricultural lands are intensive. Current rate and volume of water increase and decrease periodically based on seasons.

Station III: The stream is distributed in a wide area in this station. This station with low current rate has a fine sandy base structure. It is an agriculture-intensive area.

Station IV: It is the location where Beyler brook in which wastes of 1st and 2nd Organized industry zones are discharged joins to Tohma stream. Sometimes color of water turns black due to pollution resulting from waste water. Current rate and volume of water are lower than the other stations.

The distance between the first and last stations is about 25 km.

In 4 stations chosen from Tohma Stream, pH and electrical conductivity were measured using mobile Hanna HI 9812 pH/EC/TDS device; whereas, dissolved oxygen and temperature of surface water were measured using mobile Lutron DO-5511 digital oxygen meter, \textit{in situ}. Nitrate and phosphate values were measured using Nova 60 Merck Spectro Quant device on water surface. Other analyses were performed on samples brought to the laboratory. Ca, Mg and organic matter were determined in accordance with method in APHA [4]. All of them were made to be determined of water quality and to be found out its relationship with algea.

Epilithic diatoms were collected monthly by pooling rock scraping from 5 to 8 rocks, depending on size, into plastic containers. In each case, the upper surfaces of the rocks were scraped using a knife. To prepare permanent diatoms slides, sub-samples were taken and strong acidic solution (50:50 nitric/ sulphuric acid) was added to digest organic material. These samples were boil on a hot plate for 15 min to expedite the digestion process, and subsequently left to cool. Then samples were neutralized by rinsing with distilled water, and dried on cover-slips, mounted on the slides using balsam. Individual numbers were obtained by counting at least two hundred valves on each slide and results are expressed as (\%) relative abundance. Biological diversity (H') was determined by the methodology of Shannon and Weaver [5]. Species were identified according to Krammer and Lange-Bertalot [6, 7].

**RESULTS**

Mean values of physicochemical variables of Tohma Stream showed differences between stations (Table 1). While mean temperature of water was lower in the station 1 than the other stations, mean concentration of DO was higher in the station 1 than the other stations, particularly quite higher than station 4. There were no significant differences among the stations in terms of pH and mean concentrations of Ca$^{2+}$ and Mg$^{2+}$. Mean concentrations of EC, PO$_4^-$, and NO$_3^-$ were found to be higher in the station 4 compared to the others.

A total of 70 diatom taxa were observed in the epilithic samples (Table 2). Members of Pennales were more dominant than the members of Centrales both in taxon richness and the abundance (i.e. number of individuals). In the present study, both the number of species and community structure greatly varied among the stations.
While Cymbella, Navicula, and Nitzschia were the diatom genera represented by the highest number of species, dominant species varied based on the stations. Dominant species of the station 1 were Cocconeis placenta, Diatoma vulgaris, Cymbella affinis and Gomphonema olivaceum. Navicula cryptocephala, C. minuta, Ulhnaria ulna, Nitzschia amphibia, and N. thermalis were dominant diatoms of the stations 2 and 3. Dominant diatoms of the station 4 were Achnanthidium minutissimum, N. pala, Gomphonema parvulum, and Surirella angustata. Some species were observed in very low relative density only in some of the stations. C. aspera, N. bacillum, N. dubium, and N. angustata were observed only in the station 3; C. arcus, N. salinarum, N. constrictum only in the station 2; D. ovalis, D. tenuis, D. elliptica, N. gracilis and N. reinhardtii only in station 1 (Table 2). Diversity of diatoms also varied in each station in parallel to water quality (Figure 2).

DISCUSSION

Cocconeis placenta, Cymatopleura solea, Cymbella affinis, C. helvetica, C. minuta, C. prosstrata, Gomphonema olivaceum, G. parvulum, Gyrosigma acuminatum, Navicula cryptocephala, N. lanceolata, N. praetexta, N. radiosa, N. triplunctata, Nitzschia amphibia, N. pala, N. thermalis, Surirella ovata, S. ovalis and Ulhnaria ulna from Bacillariophyta were found in all habitats during the study. Nitzschia pala, Navicula cryptocephala, and Ulhnaria ulna from these species are among the species with high tolerance according to biological classifications of water quality, can survive in very polluted environments [6], especially N. pala is accepted to be an indicator for organic pollution [8].

While Navicula was the genus represented with the highest number of species among epilithic flora in all stations, this was followed by the genera Cymbella, Nitzschia and Gomphonema. Chesman indicated in their study that the genera Cymbella, Navicula and Nitzschia were cosmopolitan and the fact that the species Navicula and Nitzschia were identified also in all stations in the present study supports the idea that species of these genera are cosmopolitan [9].

Station 1 in Tohma stream is a stream region which is closer to the resource that is not influenced by domestic waste water and industrial wastes. Diatoma vulgaris was the dominant species in the station 1, which was followed by Cymbella affinis, Gomphonema olivaceum and Cocconeis placenta. Previous studies also stated that these species were dominant in regions that were closer to resource and where the water was clean [10]. Stations 2 and 3 are the ones influenced by agricultural lands and domestic wastes. Navicula cryptocephala, C. minuta, Ulhnaria ulna, Nitzschia amphibia, and N. thermalis were important in these stations in terms of relative density. The study by Brown et al. indicated that these species were tolerant to organic pollution [11].

Kelly reported that Ulhnaria ulna was a very common taxon in fresh waters, found in waters with pH higher than 7, and had considerably high ecological tolerance [12]. This species was found in high relative density in all stations in Tohma Stream as well. Navicula trivalis was indicated to be a species which has high tolerance to alpha mesosaprobic conditions and is commonly found in waters from those with high electrolyte content to hard water [13]. Brown et al., also stated that the species Melosira varians, Cymbella minuta, Ulhnaria ulna, Navicula cryptocephala and Nitzschia amphibia were tolerant to organic pollution [11]. These species had important relative density in stations 3 and 4 where pollution was intensive in Tohma Stream. Some taxa of the genus Cymbella are indicators for waters that are rich in oxygen and poor in organic nitrogen compounds [14]. Cymbella affinis, described as the indicator for clean waters, had substantial relative density in station 1 of Tohma Stream.

Achnanthes from algae identified in Tohma is a diatom distributed all around the world. Diatoms, which prefer eutrophic water and were adapted to polluted waters, such as Cyclotella meneghiniana, Rhoicosphenia curvata and Cocconeis placenta were identified in the stream as well as Navicula minima being the indicator for mesotrophic waters [14].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
<th>Station 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>mgL^{-1}</td>
<td>13.3±4.09</td>
<td>15±5.60</td>
<td>15.58±4.23</td>
<td>17.08±4.79</td>
</tr>
<tr>
<td>EC</td>
<td>µS/cm</td>
<td>479.25±32.01</td>
<td>560.83±68.65</td>
<td>601.58±81.74</td>
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<tr>
<td>pH</td>
<td>-</td>
<td>8.32±0.42</td>
<td>8.42±0.27</td>
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<td>8.6±0.46</td>
</tr>
<tr>
<td>DO</td>
<td>mgL^{-1}</td>
<td>8.36±1.55</td>
<td>7.54±1.03</td>
<td>6.91±0.85</td>
<td>4.46±0.92</td>
</tr>
<tr>
<td>PO_{4}-P</td>
<td>mgL^{-1}</td>
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<td>0.52±0.75</td>
<td>0.37±0.26</td>
<td>1.02±1.84</td>
</tr>
<tr>
<td>NO_{3}-N</td>
<td>mgL^{-1}</td>
<td>0.006±0.007</td>
<td>1.241±0.498</td>
<td>1.200±0.424</td>
<td>1.725±0.524</td>
</tr>
<tr>
<td>Ca</td>
<td>mgL^{-1}</td>
<td>38.41±11.78</td>
<td>42.66±9.35</td>
<td>41.08±16.14</td>
<td>52.83±15.81</td>
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<tr>
<td>Mg</td>
<td>mgL^{-1}</td>
<td>17.25±7.30</td>
<td>18.41±8.85</td>
<td>19.00±6.25</td>
<td>19.91±10.12</td>
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<tr>
<td>Organic matter</td>
<td>mgL^{-1}</td>
<td>0.53±0.28</td>
<td>0.60±0.31</td>
<td>0.67±0.49</td>
<td>0.72±0.42</td>
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</table>
## Table 2

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
<th>Station 4</th>
</tr>
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<tr>
<td>Cyclotella meneghiniana Kützing</td>
<td>2.00</td>
<td>1.15</td>
<td>0.60</td>
<td>1.15</td>
</tr>
<tr>
<td>Achnanthidium minutissimum Kützing</td>
<td>1.38</td>
<td>1.27</td>
<td>1.34</td>
<td>6.89</td>
</tr>
<tr>
<td>A. lanceolata (Brebisson) Grunow</td>
<td>0.07</td>
<td>0.33</td>
<td>0.27</td>
<td>0.04</td>
</tr>
<tr>
<td>Melosira varians Agardh</td>
<td>0.94</td>
<td>0.13</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>Amphora ovalis (Kützing) Kützing</td>
<td>2.70</td>
<td>2.40</td>
<td>2.38</td>
<td>0.41</td>
</tr>
<tr>
<td>Cocconeis placentula Ehrenberg</td>
<td>5.55</td>
<td>1.60</td>
<td>0.19</td>
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<td>0.04</td>
<td>0.45</td>
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<tr>
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<td>0.59</td>
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<td>N.reinhardtii Grunow</td>
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<td>-</td>
<td>0.10</td>
<td></td>
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<tr>
<td>Nitzschia amphibia Grunow</td>
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<td>2.98</td>
<td>3.60</td>
<td>4.68</td>
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<tr>
<td>N. angustata Grunow</td>
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<td>2.25</td>
<td>3.54</td>
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<td>0.45</td>
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<td>0.91</td>
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<td>Ulmaria ubna (Nitzsch) P.compere</td>
<td>4.95</td>
<td>6.23</td>
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In the present study, water temperature, pH, electrical conductivity, phosphate and nitrate values were higher and the dissolved oxygen was lower in station 4 compared to the other stations. Station 4 is influenced by the wastes of 1st and 2nd Organized Industry Zones. There was a weak smell during the sampling and color of water was different in station IV compared to the other stations. It is known that oxygen is used during decomposition of organic material in water and recovery of oxygen consumed is quite low in surface waters. This reflects on naturally inhabiting organisms. In the present study, lower reproductivity determined in the station IV is considered to be associated with this pollution. Electrical conductivity resulting from mineral substances and dissolved salts found in water was recorded to be high especially in station IV. In this station, release of wastes from organized industry zones into water had an important effect on increased electrical conductivity of the water and water temperature of the stream. Compared to the other stations, Nitzschia palea was found more in station IV. Pollution had a negative impact on diversity of diatoms and led to increase of dominant taxa but decrease of diversity. Several researchers reported that this species was an important indicator for organic pollutions in streams [15, 16, 17]. Nitzschia palea is also described to tolerate heavy metals and to be the characteristic species of streams and lakes with heavy metal contamination [13]. Duong et al. determined in their experimental study that increased Cd concentration considerably increased development of N. palea. Achnanthes minutissima, Gomphonema parvulum, and Surirella angusta reached significant relative density in station 4 [18]. In their studies Denisinger et al., Medly and Clements stated that A. minutissima was resistant to toxic compounds [19, 20]. In their study, Nakanishi et al. reported that Achnanthes minutissima and Surirella angusta were the species tolerant to Cu pollution in rivers, consequently A. minutissima could be used as an indicator for heavy metal pollution [21]. Species diversity is frequently used in ecological status assessment for explaining spatial and temporal patterns of biotic communities. In some studies, it was found that diversity of species could either increase or decrease as a result of the population increase [22]. In the present study, the diversity index was lower in station 4 than the other stations; however, there was no significant difference among the other 3 stations. Therefore, diversity can be used to evaluate high levels of pollution and stream sites. Diversity was also successfully used as the indicator for organic pollution before [22]. When considering the physicochemical results of the study area, WT, EC, NO$_2$, NO$_3$, pH were significant factors in community structure of diatoms.

Diatom composition was substantially influenced by high concentrations of EC due to wastes of industrial zone in the station 4. Gray and Becker stated that high levels of EC, TN, K$^+$ and Cl$^-$ concentrations resulting from domestic and industrial wastes affected community structure of diatoms in lower parts of streams [23]. While diversity of species decreased, the individual numbers of species increased in the station 4. EC is a good monitor that can reflect total concentrations and contents of ions in water bodies. In the present study, mean concentration of EC was 479 µS/cm in the station 1, it was 1372 µS/cm in the station 4. Diversity value of this station was lower than the other stations. In studies conducted in rives of USA, EC was reported
to be a factor influencing species composition of diatom and benthic diatom distribution [24, 25].

There is a contradiction that Tohma Stream has the appearance of a stream with characteristic of clean water by containing individuals that are sensitive to pollution and also contains species that are tolerant and resistant to pollution. However, this may be resulted from the fact that resources affecting organisms found in a certain region for biologically monitoring streams join to the environment. Settlements close to stations of the study area where samples were taken increase the possibility for the resources that may cause organic pollution to enter the environment. External factors lead to alternations in turbidity, color, particles, pH, temperature, and minerals contained in water [26], this may cause diatom flora to change in time. Consequently, diatoms identified in Tohma Stream showed significant differences in sampling stations chosen along the stream. These differences were observed in diversity, mean relative density, and also species composition. While some species decreased throughout inputs of contamination, their relative density increased. Diversity remained distinctly limited especially in station 4 with intensive pollution for a year compared to the other stations, thus diversity values were in parallel to variation of water quality. Community structure also reflected changes in chemical composition of water well. The present study revealed that species composition of diatoms could vary based on varying ecological conditions too. There were pressure of both organic pollution and industrial pollution and accordingly differences in diatom diversity and community structure in the stations selected during the study. Cosmopolitan species and an advantage of wide distribution of these were determined.

There was large variability in physical and chemical variables and in diversity indices between upstream and downstream waterways of Tohma, but there was no significant difference in physical and chemical variables between the stream samples of Station 2 and 3. However, the composition and spatial patterns of diatom assemblages were very different among the stream stations. The gradients in concentration of EC, TN, WT, and major ions were identified as the primary determinants for the spatial patterns of diatom assemblages. Results of the present study showed that the benthic diatom composition was more sensitive and accurate than the routine investigation of water physical and chemical parameters. Therefore, it can be recommended to use diatoms, along with water physical and chemical parameters, for surface water quality assessment.

REFERENCES


DETERMINATION OF THE DRIFT POTENTIAL OF SOME NEW GENERATION NOZZLE TYPES IN WIND TUNNEL

Ali Bolat*
Eastern Mediterranean Agricultural Research Institute, Adana, Turkey

ABSTRACT

The purpose of this study was to determine drift potential for some nozzle types (AIXR110015, XR80015VS, AI110015VS, IDK90-015C and TX80015). To detect drift potential of used nozzles, spray pattern displacement and droplet sizes were measured to compare the nozzles. All spray tests were done in a wind tunnel at three different nozzle pressures (3; 4 and 5 bar) and at three wind velocity (1.5; 2.5 and 3.5 m.s⁻¹) by spraying tap water. According to the results obtained, the higher wind velocities were produced higher displacements in spray pattern according to the spray pattern without wind. AI and IDK nozzles were produced higher \(D_{\text{v}0.5}\) of droplet sizes that means less drift prone droplets comparing with the other nozzles at the same pressures.

KEYWORDS:
Spray drift, Wind velocity, Spray pattern, Droplet size

INTRODUCTION

The use of pesticides has become essential to the large-scale agricultural production; however, they pose risks to the environment due to several factors, among them, the spray drift [1]. Spray drift is the movement of a pesticide through the air, during or after application, to a site other than the intended target [2, 3]. Drift is considered to be the most challenging problem facing applicators and pesticide manufacturers. Although drift may occur as vaporized active pesticide from the application site, it is usually the physical movement of very small drops from the target area at the time of application. Spray drift started with the invention and use of pesticides. Spray drift has become a serious concern because the pesticides used today are more active and many are non-selective. Usually, only a small amount of pesticide is needed to cause injury to non-target plants and animals that are susceptible to the pesticide.

Briefly, drift of pesticide is formed in two different ways. The first one is unsuitable meteorological conditions, drop diameters and equipment effect, and the second is resulting from evaporation of the pesticide due to its chemical properties [4]. Nozzles play an extremely important part in drift management by having a major effect on determining spray droplet size. They are inexpensive but can be the most important sprayer part regarding spray drift. The spray nozzle and operating pressure determines how effective the spray deposit is and how much spray may drift [5]. While elimination of all drift is impossible, new nozzle technology is extremely effective in reducing it. Drift can also be kept within reasonable amounts by avoiding spraying at times and places when drift potential is high and by using proven drift-reducing procedures and equipment. Drop diameters are the most important factors affecting the spray drift [1], even the drop size of 400 μm stated that the effect of drift in certain ratios. Some nozzle types produce smaller drops (10-200 μm) due to their structural features. These nozzles were able to provide better surface covering and because of the its high covering rates it constitutes better biological effect. However, decreasing the droplet size from 200 to 20 μm will increase coverage by a 10 fold factor, in contrarily a 20 μm water drop will travel less distance before it completely evaporates in less than 1 second. Droplets less than 100 μm in size obtain a horizontal trajectory in a very short time and evaporate very rapidly. The pesticides in these droplets become very small aerosols, which will move up into the atmosphere and will not fall out until picked up in falling rain. Droplets over 150 μm in size resist evaporation to a much greater degree than smaller droplets due to their larger volume. From these and other research results, we can conclude that there is a rapid decrease in the drift potential of droplets as their diameter increases to about 150 μm [7]. Small droplets produced by sprayer nozzles in the nozzle market are moved towards the outside of the target affecting by meteorological conditions [8, 9]. For this purpose, in recent years manufacturers have produced some new nozzle types to reduce drift. (AI, AIXR, IDK, XR etc). Feature of the these spray nozzles can be described thanks to the its pre-orifice, as falling of the pressure inside the nozzle and the reduction of the small scale drops rates thus, the fact that applications which are performed with these nozzle types are less affected by wind is
proved. The average drift distance increases with wind velocity, but when the drop size grows this ratio decreases [10, 1]. Another different design of new spray nozzles is the air-induction/venturi nozzles. They are designed to “entrap” air into the spray drop inside the spray nozzle. Air is introduced into the nozzle through a small opening in the nozzle as a result of a venturi effect. The reduced pressure or vacuum created within the nozzle will draw air into the spray solution, forming air bubbles. The air and liquid exit the nozzle as a larger spray droplet with the potential to get more pesticide product to the target and reduce drift. Air-induction/venturi nozzle systems appear to be very promising for improved application efficiency and reduced drift, especially for use with systemic herbicides. These nozzles are relatively new, so more research and experience is needed to verify this concept.

The objective of this study was to determine efficiency of some new generation nozzles in reduce spray drift potential by measuring droplet sizes and spray pattern displacements under different wind velocities and spray pressures in a wind tunnel.

MATERIALS AND METHODS

The Spray Pattern Displacements tests were performed in wind tunnel of Spraying Laboratory, Department of Agricultural Machinery and Technologies Engineering, University of Çukurova Adana/Turkey. Spraying nozzles used in the experimentation were: (1) Air induction nozzle (AI110015), (2) Air induction flat fan nozzle (AIXR110015), (3) Air induction compact nozzle (IDK90015), (4) Standard flat fan nozzle (XR80015) and (5) Hollow cone nozzle (TX80015). The nozzles were operated at three wind velocity (1.5; 2.5 and 3.5 m.s\(^{-1}\)), at three different nozzle pressures (3, 4 and 5 bar) in the wind tunnel to determine the displacement in spray pattern of nozzles. Flow rates of the all used nozzle types in different pressure levels are given in Table 1.

As shown in Table 1, selected nozzles achieved same flow rate in the same pressure. Thus, data taken from nozzle spray pattern and droplet sizes at the same pressure could be compared directly.

**Determination of droplet characterization in different nozzles in different pressure.** In this research, droplet sizes produced by the nozzles at operating pressure were determined by Malvern particle sizers (Malvern Spraytec -Open Spray). The all nozzle types of droplet size distribution were determined in three different pressure (3, 4 and 5 bar) in a 60 second periods. \(D_{V0.5}\), \(D_{V0.1}\) and \(D_{V0.9}\) of droplet characteristics were measured, and relative span factor (R) was calculated for homogeneity coefficients of droplet size of each nozzles by using formula below (Equation 1).

\[
R = \frac{D_{V0.9} - D_{V0.1}}{D_{V0.5}} (1)
\]

Where; R: Relative span factor, \(D_{V0.5}\): 90 percent of spray volume is in droplet smaller than this value, \(D_{V0.1}\): 10 percent of spray volume is in droplets smaller than this value, \(D_{V0.5}\): Volume median diameter (%50 of the volume).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Flow rates of nozzle used in three pressure (l.min(^{-1}))</th>
</tr>
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<tbody>
<tr>
<td>Nozzles Type</td>
<td>3 bar</td>
</tr>
<tr>
<td>AI 110015</td>
<td>0.380</td>
</tr>
<tr>
<td>AIXR110015</td>
<td>0.380</td>
</tr>
<tr>
<td>IDK 90015</td>
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<tr>
<td>XR 80015</td>
<td>0.380</td>
</tr>
<tr>
<td>TX 80015</td>
<td>0.380</td>
</tr>
</tbody>
</table>

**FIGURE 1**
Schematic view of wind tunnel
Determination of Spray Pattern Displacement (SPD). The spray pattern displacement trials were performed in the wind tunnel. The wind tunnel had a working section of 1.3 m wide, 1.2 m height and 5.5 m long and was built of plywood material for low turbulence (Fig 1). To measure spray pattern displacements of nozzles at the different wind velocity and operating pressure, a special patternator grooved with 5 cm interval was used (Fig 2).

In order to carry out the tests, required wind velocity is provided by axial fans. Air is sucked from the outside in tunnel entrance shaped venture by means of axial fan. In order to provide uniform air flow to area where tests will be performed, flow regulators were located both the entrance of the wind tunnel and behind of the fan. Flow regulators were produced 3 cm diameter and 25 cm length of plastic pipe. Nozzles which are used in the tests were located middle section of the tunnel. Liquid flow to the nozzle inside the tunnel was provided by air pressure generated by the air compressor. Necessary settings were made from the spray control unit in order to automatic cut of the liquid flow (spraying). Wind velocity of 1.5; 2.5 and 3.5 m.s⁻¹ in tunnel were measured at the nozzle height with an anemometer (Thies Clima/Germany).

Spray liquid in the patternator grooves was collected into glass bottles (height 5 cm; diameter 3.5 cm) placed under each groove. After spraying, liquid collected in the bottles was weighted by using an electronic balance with three replicates for each nozzle size.

Obtained data was analyzed for average liquid flow in each bottle to calculate and evaluate spray pattern displacements using Equation 2 [11].

\[
D_c = \frac{\sum_{i=1}^{59} V_i d_i}{D \sum_{i=1}^{59} V_i}
\]  

Where; \(D_c\): Distance to centre of mass (cm), i: Number of patternator channel (i=1,…,59), \(V_i\): Liquid volume at \(i^{th}\) channel of the patternator, \(d_i\): Channel width (cm)

According to the Equation 2, higher \(D_c\) indicates greater movements of spray patterns and consequently greater drift potential.

Determination of Percentage Deviations.
The following Eq. 3 was used for the calculate the Percentage deviations for all treatments [12].

\[
PD = \frac{D_1 - D_2}{D_2} \times 100
\]  

Where; PD: Percentage deviations (%), \(D_1\): Spray pattern under the nozzles with effect of wind (cm), \(D_2\): Spray pattern under the nozzles without effect of wind (cm).

RESULTS AND DISCUSSION

Droplet sizes measurements for each nozzles at operating pressure are given in Table 2.

As it can be seen in the Table 2, the highest \(D_{v0.5}\) was obtained from IDK90015 nozzle under 3 bar pressure as 360.9 μm and the lowest \(D_{v0.5}\) was obtained from TX80015 under 5 bar pressure as 107.0 μm. As expected, increasing in the operating pressures resulted in decreases in \(D_{v0.5}\). But AI, AIXR and IDK nozzles tested in this research produced less drift prone droplets since \(D_{v0.5}\)'s of these nozzles were higher than 150 μm. That means spray applications with AI, AIXR and IDK nozzles are moresafe in terms of sprayer operators and environmental risks. Concerning uniformity (R) of droplets produced by nozzles tested, XR that is a standard flat fan nozzle and TX hollow cone nozzles tested produced partially better R value. As known, R value of closer to1 means better droplet uniformity for any of spray pattern. But, R value for air induction nozzles not so bad because of smaller than 2 of R value.
TABLE 2
Droplet sizes measurements for each nozzles at operating pressures

<table>
<thead>
<tr>
<th>Nozzle Type</th>
<th>Pressure (bar)</th>
<th>$D_{v0.1}$ (μm)</th>
<th>$D_{v0.5}$ (μm)</th>
<th>$D_{v0.9}$ (μm)</th>
<th>Relative Span (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI110015</td>
<td>3</td>
<td>138.0</td>
<td>354.9</td>
<td>699.3</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>136.7</td>
<td>324.4</td>
<td>666.7</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>122.6</td>
<td>281.2</td>
<td>611.2</td>
<td>1.73</td>
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<tr>
<td></td>
<td>3</td>
<td>96.3</td>
<td>201.4</td>
<td>396.6</td>
<td>1.54</td>
</tr>
<tr>
<td>AIXR110015</td>
<td>4</td>
<td>94.5</td>
<td>194.8</td>
<td>433.6</td>
<td>1.68</td>
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<td></td>
<td>5</td>
<td>91.4</td>
<td>191.8</td>
<td>390.4</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>142.0</td>
<td>360.9</td>
<td>700.6</td>
<td>1.54</td>
</tr>
<tr>
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<td>4</td>
<td>127.2</td>
<td>306.6</td>
<td>646.6</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>112.7</td>
<td>269.6</td>
<td>600.3</td>
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<tr>
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<td>3</td>
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<td>138.9</td>
<td>255.9</td>
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<tr>
<td>XR80015</td>
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<td>135.4</td>
<td>245.1</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>62.0</td>
<td>127.2</td>
<td>243.0</td>
<td>1.42</td>
</tr>
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<td>3</td>
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<td>132.7</td>
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<tr>
<td>TX80015</td>
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<td>124.8</td>
<td>227.0</td>
<td>1.31</td>
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<tr>
<td></td>
<td>5</td>
<td>55.1</td>
<td>107.0</td>
<td>194.3</td>
<td>1.30</td>
</tr>
</tbody>
</table>

TABLE 3
Spray pattern displacements in different wind velocities for each type of nozzles

<table>
<thead>
<tr>
<th>Nozzle Type</th>
<th>Operated Pressure (bar)</th>
<th>Spray pattern displacement (cm) Wind velocity (m.s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>AI110015</td>
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<td></td>
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<td>36.51</td>
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<td></td>
<td>3</td>
<td>25.04</td>
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<tr>
<td>AIXR110015</td>
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<td>28.69</td>
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<tr>
<td></td>
<td>5</td>
<td>31.93</td>
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<td></td>
<td>3</td>
<td>14.87</td>
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<td>16.51</td>
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<tr>
<td></td>
<td>5</td>
<td>19.79</td>
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<td></td>
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<td></td>
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<td>44.90</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>52.45</td>
</tr>
</tbody>
</table>

FIGURE 3
Percentage deviations in spray patterns at wind velocity of 1.5 m.s⁻¹
The spray pattern displacements calculated for each operating condition are given in Table 3.  
As seen in Table 3, increasing pressure and wind velocity resulted in increases in the spray pattern displacements in all types of nozzles used in the research. The maximum pattern displacements among the nozzles were obtained working with hollow cone nozzle (TX80015) as 60.44 cm. But, lower levels of pattern displacements were observed with the air induction type nozzles (AI and IDK nozzles. Especially IDK90015 nozzle type formed the lowest drift distance as 18.81 cm in - with wind velocity of 3.5 m.s⁻¹. Air induction modification (AIXR) of XR nozzles achieved lower displacement in spray pattern. This means AIXR nozzles will be less drift potential comparing with standard XR nozzles at the same operating conditions. Comparing XR and TX nozzles, even though both nozzles have same spray angel and flow rate at the same operating parameters, TX nozzles achieved higher spray pattern displacements that means higher drift potential. The results of percentage deviation (%) in nozzles’ spray patterns for all wind velocities (1.5; 2.5 and 3.5 m.s⁻¹) conditions are given in Fig 3, 4 and 5.

According to data presented Fig-3, 4 and 5, increases in wind velocities and pressures also resulted in increases in percentage deviations (%) of spray pattern. Especially, having lower Dᵥ₀.₅ nozzle (TX80015) produced higher deviations in each wind and pressure conditions. Lower spray pattern deviations were produced with air induction sets of nozzles used in the research.
CONCLUSION

The following results can be drawn from this research:

- Air induction nozzles (AI, AIXR and IDK) achieved better performance in terms of drift reduction.
- The smallest droplets were obtained with cone nozzle (TX80015) for all operating pressures (3, 4 and 5 bar) and this resulted in higher displacement in spray pattern.
- As expected, increasing in wind velocities and pressure resulted in higher deviation on nozzle spray pattern.
- All the results shown that Air induction nozzles may reduce amount of drift prone droplets, but lower wind velocity and operating pressure should be preferred in pesticide applications as possible as.

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BIOREMEDIATION AND "GREEN CHEMISTRY"

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ABSTRACT

Bioremediation is basically a process which represents the ability of microorganisms to decompose different dangerous contaminants, and it has an increasingly key role in detoxification of contaminated soil and groundwater. These processes are getting their place owing to capacity of enzyme metabolism of microorganisms to transform organic contaminants into pollutants and less dangerous compounds. But, we should not forget that this method cannot always be applied. Among the available options for purification of contaminated soils, bioremediation is the best because it is less disturbing to environment and from the economic point of view-it costs less.

KEYWORDS:
Land, pollution, bioremediation, microorganisms

INTRODUCTION

With the industrial revolution there has been intensive human impact on nature and the world around him. The level of environmental awareness was very low, and pursued as far as productivity and cost-effectiveness, which did not take into account the distortion of the natural balance and now the necessary means to eliminate the negative consequences of industrialization, if it is still possible. According to the policy of sustainable development technology development should be in the direction of eliminating the negative effects to the environment. However, not all countries are in a financial position that in due time to leave old technologies. Also, nowadays are present the consequences of the operation and need to be eliminated. The twentieth century will be remembered as the century of the oil. With the emergence of oil as a fuel has been the most intense economic growth and the entire modern civilization is based on the use of oil [1, 2, 22].

The green chemistry is the scientific field that involves chemical research and engineering, whose focus is to promote the design of products and processes that minimize the use and formation of hazardous substances. While the chemistry of the environment is dealing with the natural environment and chemical pollution in nature, the green chemistry aims to decrease and prevent the formation of contamination at its sources. In the form of chemical philosophy, the green chemistry is applicable to organic chemistry, inorganic chemistry, biochemistry, analytical chemistry, and even physical chemistry. The focus of the green chemistry is on industrial applications, regardless of the type. Click of chemistry is often cited as a style of chemical synthesis that is consistent with the goals of green chemistry. The objective is minimization of hazards and maximization of efficiency. Examples of key events in green chemistry are: the use of supercritical CO₂ as green solvents, the use of an aqueous solution of hydrogen peroxide to clean the oxidation and the use of hydrogen in asymmetric synthesis. Green chemistry is used in the supercritical water oxidation reactions in water, and reactions in the solid state.

Bio-remediation technology is a selective method that least disturbs the environment, particularly in the application of in situ and corresponds to the strategy of sustainable development in the ecosystem security. The effectiveness of bioremediation of land depends on a number of parameters: environmental factors, additive and availability of nutrients and the technical characteristics of the plants. However, under optimal conditions for decontamination, the process does not remove all the contaminants, but the effectiveness and economic viability of bio-process depends on the identification of critical factors and their optimization.

METHODS AND MATERIALS

Biological methods of decontamination. Biological methods are applied on land contaminated with radio-nuclides and can be used in combination with other methods. The most common used bioremediation processes for this pollutant are: biotransformation - where the contaminated molecules are converted into less hazardous or non-hazardous molecules; biodegradation - where the organic substance are destroy the smaller organic and inorganic
molecules; Biomineralization - where they are degraded by organic materials to inorganic, such as CO₂ and H₂O. All three processes can be applied in situ and ex situ. Strategies that are recommended for the removal of metals and radionuclides include microbial leaching, microbial surfactants, volatilization and bioaccumulation.

In any case, bioremediation processes are provided by the same as those that occur in nature. Depending on the location and contaminants, bioremediation may be safer and less expensive than alternative technologies such as incineration and landfilling. It can also be applied to phytoremediation, which involves the use of plants for extraction, sequestration and/or detoxification of pollutants that are present in the soil.

This method is considered the cheapest and simplest cleaning area. Also, the plants can be used to monitor the effects of soil remediation. This applies to plants with great potential for the accumulation of heavy metals and radionuclides, but when it comes to food for human consumption, it can be dangerous if it contains these dangerous substances [3-5].

The mechanisms of phytoremediation:
- Phytostabilization - includes the use of plants containing/immobilizing pollutants throughout: the absorption and accumulation by means of the root system, to adsorb onto the surface of the root and the precipitation in the root zone;
- Phytodegradation/phytotransformation – including decomposition of pollutants through: metabolic processes (internal) and release enzymes into the soil;
- Phytovolatization - absorption and transpiration of pollutants into the atmosphere by using of plants;
- Rhizodegradation – decomposition of pollutants in the soil due to the interaction of microbes/root/soil;
- Phytohydraulics – involves the use of plants to monitor the migration of contaminants.

**Definition and the way of functioning of the bioremediation.** Bioremediation is a process that uses microorganisms for removal or degradation of toxic substances into less toxic or non-toxic substances. In this process, the microorganisms through their enzymes degrade (metabolize) the organic contaminants from the soil or the water and transform it into non-toxic end products, above all, to carbon dioxide and water. In this process, therefore, occurs the interaction between plants and microorganisms, and the end result is a cleansing and healing of the land. Bioremediation is a natural process, which is to be carried out in soil and water and without human influence, but it would last much longer. The process is completely harmless to human health and, most importantly, there is no additional environmental pollution. Bioremediation technology, creating optimal conditions for growth of microorganisms and increase of their numbers, support the detoxification of certain quantities of contaminants.

In order to successfully carry out the process of bioremediation, it is necessary to know the characteristics of the contaminant, localities and microorganisms. From these parameters depends the running time of bioremediation, which can often reach several years. Some readily biodegradable contaminants can be degraded in less than a year, while the high molecular weight contaminants degrade significantly longer. The most important characteristics of the contaminant which is necessary to determine are the possibility of bio-degradation, solubility in water, the coefficient of absorption of the land and chemical reactivity.

Characterization and description of the site include the determination of the depth and the surface distribution of the contaminant, the concentration of the contaminant in the locality, soil type or class of water with their properties (pH, organic matter content, the content of microelements* and microelements, etc.), Presence or absence of substances that are toxic to microorganisms, the presence of other electron acceptors, and so on. When the microorganisms are concerned, it is necessary, first of all, that they are active, i.e., to have the ability for biodegradation of contaminants and their populations in the locality is large enough, in order to more efficiently decompose the contaminant.

Bioremediation researchers have come up with a lot of information about the mechanisms of bio-oxidation, the resulted products and the influence of reaction conditions before the bioremediation technology began to be applied commercially. Microorganisms which are able to degrade a variety of classes of compounds, and under aerobic and under anaerobic conditions, were quite well-tested, as well as their need for an appropriate pH, nutrients, oxygen, temperature, redox potential and moisture. Depending on the amount of the available oxygen in the soil remediation can take place under aerobic and anaerobic conditions.

**Aerobic and anaerobic degrading of land.** Aerobic degradation is performed by aerobic microorganisms, and to it, in addition to oxygen a significant effect has the presence of mineral salts, temperature and pH. Aerobic microorganisms require salts of nitrogen, phosphorus, potassium, magnesium, iron, zinc and others. The largest growth of bacteria and fungi oxidants hydrocarbons is observed in the temperature range of 25 - 40 °C. However microorganisms show great adaptability in terms of growth, as well as the temperature. The optimum pH for the biodegradation is between 7
Degradation of oil decreases with increasing sediment depth and anaerobic. The methyl group on the ends of the molecule of an alkane and aromatics (toluene, xylene) are subject to oxidation reactions, where the first is formed alcohol, then aldehyde and in the end the carboxylic acid. Microbiological degradation of toluene may result in benzaldehyde and benzoic acid. Alkyl group is subject to reactions of subterminal oxidation provide the ketone or hydroxy-derivative. Thus, from hexane is derived 2-hydroxyhexane and 2-ketohexan, etc. Alkanes are subject to the reactions of dehydrogenation: from n-heptane creating 1-heptene. Cycloalkanes are subject to oxidation reactions: may result in hydroxylation of cyclohexane to form cyclohexanol, may be created-keto derivative to form a cyclohexanone and can take place and the reaction of dehydrogenation of cycloalkanes.

Aromatic compounds are subject to hydroxylation reactions and formation of ketone. Hydroxylation is nonspecific and sometimes leads to the formation of a ketone or quinone. The most common product is dihydrodiol when the two OH groups are introduced to two adjacent C-atoms. From benzene may derive phenol and hydroquinone. Depending on the substrate and the microorganism can occur various products when opening the aromatic rings. There may be opened only one, several or all of the rings. In aerobic conditions by opening the ring in the molecule is formed two carboxylic or on carboxylic and one hydroxyl groups. Degradation of benzene bacteria begins forming cis-dihydrodiols, followed by dehydrogenation to catechol and then comes to the ring-opening.

Bacterial degradation of naphthalene is the same as for benzene – first occurs deoxygenation (cis-1,2-dihydrodiol) followed by dehydrogenation (1,2-dihydroxynaphthalene), and finally comes to the opening of the ring.

The use of micro-organisms (decontamination process) as biodegradation agents is in constant increasing due to the enormous biodiversity and unrivalled catabolic potential. Degradation abilities are conditioned by catabolic genes and enzymes. In addition, microorganisms have different mechanisms for adaptation to hydrophobic substrates such as: modification of the cell membrane, production of surface-active substances, or the use of an efflux pump to decrease the concentration of the various components of toxicity.

The system for the biodegradation in microorganisms is organized in a way that the starting compounds in a larger number of peripheral pathways to transform certain central by-products, such as catechol, homogentisate or protocatechuate which are converted to the intermediate cycle of tricarboxylic acids.
Anaerobic process takes place under the action of anaerobic microorganisms and is so slow that its importance is negligible. However, it was found that anaerobic digestion can take effect after the oil had previously been exposed to aerobic microorganisms. Anaerobic decomposition of hydrocarbon is possible in the deeper layers of oil, i.e., in the depth of oil sites without air supply. Actuators of anaerobic degradation are usually sulfate-reducing bacteria. The reaction of sulphate under the influence of microorganisms represents oxidation-reduction process. Thereby the sulfates are reduced to hydrogen sulfide and hydrocarbons are oxidised.

Under anaerobic conditions degradation processes are more specific, for example, pure cultures of bacteria can reward benzylsuccinate toluene, and from kisil a metilbenzysuccinate. The aromatics can lead to the reduction of double bonds, such as tests with microbiological cultures showed a reduction of one or more double bonds in the benzene ring under anaerobic conditions: benzene can occur cyclohexene, toluene from a 4-methylcyclohexanol.

**Microbiological communities and design processes.** In order the removal of the contamination was effectively it is necessary to provide adequate oil-oxidizing microorganisms in sufficiently large numbers, as well as the optimal conditions for their growth and development as sufficient quantities of nitrogen and phosphorus. The most commonly is applied autochthonous micro flora, which is isolated from the soil and multiplied in bioreactors. Apart from yeasts of the genera Candida (C. lipolytica, C. tropicalis), Hansenula, Torulopsis, Rhodotorula and fungi from the genera Aspergillus, Penicillium, Fusarium, Trichoderma and other primary role in the biodegradation of petroleum hydrocarbons are bacteria, including species from the genera Pseudomonas, Vibrio, Arthrobacter, Aeromonas, Acinetobacter, etc. [4].

Lack of catabolic pathway for biodegradation of certain xenobiotic today goes beyond the techniques of bioengineering, because biochemical pathways can evolve. In this regard, the exchange of genetic information Pseudomonas soya beans that is able to degrade a wide range of chlorobenzoate and chlorophenols. If it is carried out genetic engineering of microorganisms is referred to as process of Bioaugmentation [5]. Bioremediation depends not only on the type and concentration of pollution and present microbiological community, but also of hydrogeochemical soil characteristics.
When designing bioremediation process must prevent the further pollution spreads due to penetration in the ground water. Which variant of the cleaning will be applied depends on the type of contamination, the nature of the terrain, etc. It can be applied technical, spontaneous bioremediation, a combination of both, or a mixture of bioremediation with non-biological treatment. As the usual concentration of pollution in groundwater is less than in the zone of pollution sources, then can be applied different procedures for the original zone and the widespread stain. Factors affecting the design of bioremediation process are: the objectives to be achieved in the treatment of land, spread of pollution (type, concentration and location), type of biological process that efficiently transforms pollution, transport dynamics of the land.

## RESULTS AND DISCUSSION

**Types of soil remediation.** Bioremediation can be divided into two basic types:

a) Passive bioremediation, which applies when the natural conditions are suitable for taking place of bioremediation without human intervention;

b) Technical (engineered) bioremediation, which is applied when it is necessary to add substances that stimulate microorganisms.

Passive Bioremediation involves the reduction of the concentration of pollution by natural processes. Passive Bioremediation is applied when the natural biodegradation degree of pollution is faster than the degree of migration of pollution. This rela-
tive ratio depends on the type and concentration of pollution, microbial communities and hydrogeochemical characteristics of the soil. It is mainly used to clean the aquifer where the source of pollution has been removed, although it can also be used when the source is still present or if there are removed some dangerous points.

Natural removal of contamination may be due to the destructive (aerobic and anaerobic biodegradation, abiotic oxidation, hydrolysis) and non-destructive (sorption, dilution (dispersion and infiltration), volatilization) process. Advantages of natural removal are the lower cleaning costs and minimal disruption of the landscape. Disadvantages include the inability to remove high concentrations of pollution, it may cause migration of pollution, time degradation of severe fraction is long, cannot always achieve the target value for the concentration of pollution for a reasonable time, the conditions on the site may be unfavorable, you need a long-term monitoring. In order the passive bioremediation was favored the following conditions are required: a steady flow of groundwater, the presence of minerals that protect to prevent changes in pH environment, the presence of high concentrations of oxygen, nitrate, and sulfate, iron (III). Passive bioremediation relies on the natural abilities of the present microbial communities.

The ability of natural microorganisms to perform passive bioremediation must be proven by laboratory tests conducted on specific land. These tests must be carried out before the establishment of passive bioremediation that would be accepted as a legitimate technique for cleaning. In addition, the efficiency of passive bioremediation must be proven by field monitoring that includes chemical analysis of pollution, the final electron-donor and/or other reactants or products characteristic of the biodegradation process.

Passive bioremediation can be used alone or in conjunction with other remediation techniques. Similarly, this process can be applied after the "pump it-and-treat" technique or after a technical bioremediation to prevent migration of pollution from a given locality. Primarily, in the application of passive bioremediation is that the land must be fully tested. The parameters that need to pay attention are: type, quantity and distribution of pollution, contamination susceptibility to biodegradation by microorganisms in the soil, groundwater flow when pumping is not performed (including seasonal fluctuations), the period of migration stains and intimacy and sensitivity of potential receptors, which can be harmfully favored if they come into contact with the pollution. If our information on all of these parameters is available, we can use a mathematical model to determine the relationship of migration and biodegradation.

Key characteristics of the land suitable for spontaneous bioremediation are: a steady flow of groundwater (speed and direction) through the season: - seasonal variations in the thickness of water table <1m, seasonal variations in regional trajectory of the flow fulfilled the expectation. If expectations are not met and pollution is spreading, must continue to apply further corrective measures.

Other technical terms for bioremediation are "enhanced" bioremediation and "biorestoration". Technical bioremediation is faster than passive, because it’s carried out the stimulation of microbiological degradation of the pollution through the control of concentrations of oxygen, nutrients, moisture, content, pH, temperature, etc. Technical bioremediation is applied when it is important that cleaning is carried out for a short time or when the stain is rapidly expanding. Its application reduces costs due to shorter treatment and fewer land samples and analysis, and it is important for political and psychological needs because the community is highly exposed to contamination. It is subject to variations due to geological, hydrological and chemical characteristics of the soil and the necessary biochemical process, and an important aspect when establishing the technical bioremediation is whether is treated the land or water.

In situ and ex situ bioremediation. Depending on the execution site of decontamination, bioremediation technologies are divided into two subcategories: in situ and ex situ. In situ bio-technologies are carried out directly on the site of contamination, while the ex situ bio-technologies the contaminated soil is removed from the site of contamination and transported to the "processing/treatment." locality. In situ bioremediation technologies are substantially more cost-effective than ex situ technologies, because they allow the treatment of contaminated soil directly to the site of contamination, while avoiding the costs of excavation and transportation.

In situ bioremediation technologies are very effective when subsurface soil is very permeable, when includes land located in the depths of a maximum of 8 - 10 meters and when groundwater is present at depths below 10 meters. The depth of the contamination is a very important factor that determines whether the in situ bioremediation will be applied. In the case that the contaminations are penetrated to the proximity of groundwater, it is necessary the excavation of contaminated soil, or applied ex situ bioremediation technology in order to avoid contamination of groundwater. The permeability of the land also has great significance. Land with low permeability is not suitable for in situ bioremediation. In case that in situ bioremediation technologies applied without additional aeration, the effective diffusion of oxygen, which would enable a satisfactory level of bioremediation can be achieved for the majority of land in the depths of a few to thirty centimetres. Some of the most im-

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portant in situ bioremediation technologies are su-
perficial landfarming, bioventilation, biorasplasing, biostimulation and phytoremediation.

In situ bioremediation is based on the treat-
ment of contaminated soil or water at the same lo-
cation where it is detected for the presence of con-
taminants. The main goal of aerobic in situ biore-
mediation is to provide oxygen and nutrients neces-
sary for the growth of microorganisms in order to
effectively degradation of contaminants. This group
of methods, among others, involves the bioventila-
tion and insertion of hydrogen-peroxide. In the sys-
tem of bioventilation air from the atmosphere is
injected into the contaminated soil or water, and
oxygen, which in this way reaches to the system,
uses the microorganisms for own biosynthesis, as
well as the degradation of contaminants. By inject-
ing hydrogen peroxide and its circulation through
the contaminated soil, provides the oxygen stimu-
lating effect on microorganisms, which, through its
activities, contribute to the degradation of contami-
nants.

Ex situ bioremediation techniques are faster,
easier to control and allow the decomposition of a
wider spectrum of contaminants than in-situ biore-
mediation. These techniques involve the excavation
and treatment of contaminated soil before, and
sometimes, after performing of bioremediation. The
most common method of ex situ bioremediation is
mixing of contaminated soil with appropriate
amounts of water in a special 'bioreactor' with the
addition of microorganisms. Information in the
field of molecular ecology can be useful for the
development of bioremediation method and evalua-
tion of environmental impact. These information’s
have special application in the process of bioaug-
mentation or the introduction of exogenous micro-
organisms in contaminated soil or groundwater.

**The nature of soil pollution.** Some types of
microorganisms can use oil as a carbon source, a
kind of oil kills or inhibits, while some species are
not affected. Physical and chemical characteristics
of oil affect the degree and speed of its degradation.
Physical effects of importance to biodegradability
are: viscosity, photolytic activity, evaporation, me-
chanical dispersion, dissolution, bioemulsification
and sorption. Viscosity affects the spread of oil
slicks, and thus an increase in the surface suitable
for microbial attack, which tend to focus on the
interface of the oil - water. Low viscosity oil spilled
in the cooler temperatures is more resistant to bio-
degradation. Photolytic products are more polar
and therefore more susceptible to the biodegradation
of the compounds from which they arise. Liquid hy-
drocarbons are susceptible to biodegradation than
those in solid form.

As a special factor influencing the biodegrada-
tion can be considered concentrations of the indi-
vidual components in the oil. Certain compounds in
high concentrations, like phenol, m- and p-cresol,
can be degraded due to their toxicity, while at low
concentrations subject to the biodegradation. As it
regards the chemical composition of the oil, n-
alkanes are degraded faster than the other groups of
the compound. Also alkanes of medium chain
length are less toxic and decompose quickly from
n-alkane chain of large length (in excess of 30 car-on atoms) or a cycloalkane. Degradation of al-
kanes inhibits their ramifications, especially at low
temperatures.

Volatile compounds, benzene, toluene, ethyl
benzene and xylenes (BTEX), relatively easily are
degraded due to several factors: they are relatively
soluble in water, can serve as the primary electron
donor for many bacteria, they are quickly destroyed
and decomposed by bacteria which degrade BTEX
grow rapidly in the presence of oxygen. The PAHs
are slowly degraded due to the complex structure,
low solubility and strong alveolar features. The
half-life degradation of PAHs low molecular mass
(naphthalene, alkyl naphthalene) takes few days in
the atmosphere, week in the water, months in the
soil and about a year in sediment. The PAHs of
higher molecular mass, with five or six-membered
condensed rings in the molecule are more stable,
with a half-life of several weeks to several years.
The efficiency of biodegradation of individual
PAHs in sediment decreases with the number of
condensed rings in the molecule, so that the five-
member and six-member PAHs are very difficult to
to get degraded.

The factors affecting the efficiency of bio-
remediation. The success of the bioremediation
process apart from microorganism capability to
degrade contaminant as a carbon source must be
taken into account other factors such as easily
adoptable source of nitrogen and phosphorus (nutri-
ents), humidity, temperature, oxygen (aeration)
and the presence of surfactants. In addition, important
are the characteristics of the soils, such as pH, min-
eralogical composition and the content of organic
substances.

Polluting substances represents a carbon
source for microorganisms, and mainly contaminat-
ed soil is poor in nitrogen and phosphorus. The
inclusion of these components leads to increased
growth of microorganisms and accelerates the deg-
radation of contaminants. It is customary to add
nutrients to the soil in order to establish the mass
ratio of carbon: nitrogen: phosphorus (C: N: P) 120:
10: 1, which is about the relationship of these ele-
ments in the biomass. Addition of nutrients is re-
ferred to as bio stimulation, and can be used miner-
al fertilizer or organic fertilizer (manure, activated
sludge). The optimum soil moisture for bioremedia-
tion process is 12-30% or 40-80% saturation capa-
city. Insufficient humidity limits and reduces the
growth of microorganisms and reduces excessive humidity of aerating soil.

Temperature affects the microbial growth, the composition of microbial communities and the rate of degradation of pollutants. In addition, since the temperature depends on the viscosity, solubility, physical nature and chemical composition of petroleum pollutants. Biodegradation of hydrocarbons can be carried out in a wide temperature range. Isolated are the psychrophilic mesophilic and thermophiles microorganisms that use hydrocarbon oil as the sole source of electrons and atoms [4].

The degradation of petroleum pollutants is the fastest and most complete exercised under aerobic conditions. The availability of oxygen depends on the intensity of the total microbial consumption and soil types. In order to increase the concentration of oxygen in the contaminated environment using a number of methods, such as rolling over, forced aeration, by mechanical mixing, bioventilation, the introduction of air and addition of alternative sources of oxygen such as hydrogen peroxide or, preferably, magnesium peroxide.

pH soil determines the type of bacteria that is available for biodegradation. Most bacteria correspond to a neutral pH, and mushrooms slightly acid environments. Usually is the optimum pH for bioremediation in the range of 6 to 8. If the soil is acidic, lime is added, and if it is too alkaline pH is adjusted by adding ammonium sulphate.

Surfactants are compounds that reduce the surface tension of the water and increase the solubility of hydrophobic substances in water. Hydrocarbons bind to the particles of soil, surfactants and soil in the pores of aiding the process of desorption of the non-polar compounds from the soil particles, thereby increasing their bio-adaptability. May be used surfactants both from chemical and biological origin. Chemical of surfactants used in the bioremediation must be biodegradable and that do not inhibit the growth of micro-organisms. Some micro-organisms synthesize biosurfactants glycolipids such as rhamnolipids in strains of Pseudomonas aeruginosa or trehalose ripples in the genus of Rhodococcus, lipopeptides such as surfactant in in the genus of strains of Bacillus subtilis, the polymers as emulsion in bacteria Acinetobacter calcoaceticus. Although it was confirmed that the biosurfactants are formed and in the soil, is generally considered to be in situ bioremediation is not achieved in an effective concentration of surfactant. As an alternative there is the process where rhamnolipids products in a separate proceeding, and then added to the plant for bioremediation [10-15].

The texture of the soil affects the permeability, the moisture content and the total density of the soil. Finely splashed soils are less permeable than land with large particles. Soils with low permeability are usually plastered and impede the distribution and transport of moisture, nutrients and air. To such land during the bioremediation can be added agents such as straw or sawdust to achieve the desired texture. The speed and degree of degradation also affects the type of contaminated soil, clay content and organic substances, and the stake of individual fractions of sand. Land which includes sand and gravel in the overwhelming amount has good drainage capacity, i.e., very shortly retains water and is permeable to air.

CONCLUSION

The market of remediation technology is constantly increasing. Although the share biological methods on the market, about 10%, bioremediation has significant advantages as compared to other technologies in terms of cost and efficiency in the removal of the pollutants. This technology infringes at least the environment, particularly in the application of in situ, and is convenient to strategy of sustainable development. However, bioremediation is not universal and is not applicable to all pollutions. The effectiveness of bioremediation of land depends on a number of parameters: environmental factors, additive and availability of nutrients and the technical characteristics of the plant. It is important to point out that even under optimal conditions, the process does not remove all the contaminants, but the effectiveness and cost-effectiveness of the biological process depends on the identification of critical factors and their optimization.

In the countries of the Western Balkans are yet to develop remediation technologies, and in a world where bioremediation is already commercially used on an industrial scale for more than 10 years constantly improve techniques to expand the list of contaminants that treatment can be applied, and the process is accelerated with the aim of increasing efficiency. They study the metabolic pathways and the role of certain strains of the microbial communities. The ecotoxicological methods are introduced for monitoring and evaluation of current processes, studying the distribution of contaminants before and after the applied treatment. Of particular importance are strategies to increase the bioavailability of contaminants, as well as introducing biological steps in processes based on chemical or physical methods [16-22].

REFERENCES


EFFECT OF NITROGEN SOURCES ON GROWTH, PROTEIN, TOTAL LIPID AMOUNT AND PIGMENT CONTENT IN CHLORELLA VULGARIS

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ABSTRACT

The discovery of valuable metabolites and microalgae containing high lipids has made these organisms economically very important. The aim of this study is to investigate the effects of different nitrogen sources on growth, protein, lipid amount and pigment content of Chlorella vulgaris. The use of ammonium nitrate as a nitrogen source resulted in high concentrations of biomass, protein and lipid while the use of sodium nitrate as a nitrogen source resulted in high concentrations of chlorophyll and astaxanthin. Concentrations of biomass, protein and lipid increased by about 200%, 127.50% and 112.14%, respectively, on day 6 in media where ammonium nitrate was used as a nitrogen source while concentrations of chlorophyll a, chlorophyll b and astaxanthin increased by 45.71%, 192.87% and 118.94%, respectively, on day 6 in media where sodium nitrate was used as a nitrogen source. In conclusion, the amount of lipid and valuable metabolites in Chlorella vulgaris grown in media with appropriate nitrogen sources can be significantly increased.

KEYWORDS:
Chlorella vulgaris, Nitrogen sources, Growth, Pigment, Chlorophyll a, Chlorophyll b, Astaxanthin

INTRODUCTION

The search for alternative energy sources has exponentially increased in recent years in response to environmental pollution caused by fossil fuels. Non-renewable fossil fuels are not considered environmentally friendly as they are the main sources of greenhouse gases responsible for global warming. Biodiesel has received great interest in recent years as a renewable energy source due to its environmental benefits. Biodiesel is a monoalkyl ester of long-chain fatty acids derived from renewable sources. Microalgae lipids are regarded as feedstocks of the future for sustainable biodiesel production because the growth rate and photosynthesis efficiency of microalgae are much higher than those of traditional terrestrial plants [1, 2]. However, the biggest obstacle in the production of microalgal oil is high cost. Microalgae can adapt to extreme environmental conditions, fix CO2 from the atmosphere and achieve high growth rate using nutrients in wastewater. If microalgae are grown in wastewater, they do not only utilize nutrients and remove waste materials, but also decrease costs by producing oil. This is considered a useful approach to both purify wastewater and produce microalgae biodiesel [2]. Biomass productivity of and lipid accumulation in algae are some of the key parameters affecting economic feasibility in the production of biodiesel. The main objective of an ideal process is, therefore, to obtain high amounts of biomass and cells with high lipid content. However, this is not always possible because cells with high lipid content are usually produced under stress conditions, such as nutrient limitation, which inhibits cell growth and results in low lipid productivity [3].

In recent years there have been many studies on changes in growth, and protein and lipid content of microalgae grown under stress conditions [4-8]. Microalgae are known to undergo biochemical changes as a survival strategy under stress conditions. Studies show that especially nutrient stress causes an increase in lipid content of microalgae [9, 10]. Most studies investigate the importance of medium composition of microalgae in lipid yield and biomass yield, and report that one of the most interesting chemical elements in this regard is nitrogen. Numerous studies, therefore, focus on selecting the appropriate nitrogen source and determining the optimum concentration [11]. Nitrogen is the most important element affecting microalgae growth, and protein and lipid content. Nitrogen is an essential component of macromolecules such as DNA, RNA, protein and chlorophyll. Nitrogen plays an important role in the synthesis of both protein and nucleic acids so that a decrease in nitrogen in a medium limits the synthesis of protein and nucleic acids, resulting in an increase in lipid content. Many studies report major changes in the amount of protein and lipid in microalgae subjected to nitrogen stress [10, 12-16]. Many oleaginous microorganisms grown in nitrogen-limited media accumulate lipids [17, 18]. Not only the amount of nitrogen but also different nitrogen sources affect cell growth, and lipid, protein and pigment content.
of microalgae [3, 11, 19].

Microalgae are a very large group of aquatic organisms representing an enormous amount of biodiversity, with approximately 40,000 species identified so far. One of the most important members of this group is *Chlorella vulgaris*, which is a eukaryotic microalgae. *C. vulgaris* (2-10 μm in diameter) is spherical and like plants due to its structural properties. Proteins play an important role in the composition and chemistry of microalgae. In addition to their main role in cell reproduction, development and renewal, proteins also act as chemical messengers, regulators of cellular activities and defense mechanisms against invasion by foreign pathogens. The total protein content of *C. vulgaris* constitutes about 42 to 58% of the dry weight. Lipids are a heterogeneous group of compounds that are soluble in non-polar solvents and partially soluble in water. Lipids constitute about 5-40% of the dry weight of *C. vulgaris* grown under optimum conditions [20]. These compounds are synthesized by chloroplasts and also found in cell walls and membranes of organelles (chloroplasts and mitochondrial membranes). The lipid content of *C. vulgaris* can reach 58% under adverse growth conditions. The most abundant pigment in *C. vulgaris* is chlorophyll and can reach a peak of 1-2% of the dry weight [21]. *C. vulgaris* also contains a significant amount of carotenoids, which are responsible for protecting chlorophyll molecules from degradation during exposure to radiation and oxygen. These pigments are known to have multiple properties such as antioxidant activity, cholesterol regulation, prevention from chronic diseases (cardiovascular and colon cancer), and reinforcement of the immune system [22].

Based on the above-mentioned literature, the main aim of this study is to investigate the effect of different sources of nitrogen on biomass, protein, lipid, chlorophyll and astaxanthin content of *C. vulgaris*.

**MATERIALS AND METHODS**

*C. vulgaris* was isolated from Keban Dam Lake, Turkey and grown in Jaworski’s medium containing the following components; 80 mg NaNO₃, 36 mg Na₂HPO₄·12H₂O, 20 mg Ca(NO₃)₂·4H₂O, 12.4 mg K₂HPO₄, 50 mg MgSO₄·7H₂O, 2.25 mg EDTAFeNa, 2.25 mg EDTANa₂, 2480 μg H₂BO₃, 15.9 mg NaHCO₃, 1390 μg MnCl₂·4H₂O, 1000 μg (NH₄)₆Mo₇·4H₂O, 40 μg biotin, 40 μg cyanocobalamin (B₁₂) and 40 μg thiamin (B₁). The medium was sterilized for 30 min, at 121 °C and an atmospheric pressure of 1. *C. vulgaris* was inoculated into 500-mL Erlenmeyer flasks containing 100 mL of medium. The Erlenmeyer flasks were incubated in a climate cabinet at 23 ±1 °C under a 16h light/8h dark regime and 55 μmol photon m⁻²s⁻¹ light intensity. Having been taken from the media that reached a certain density, 10-mL stock cultures were grown in Jaworski’s medium while working cultures were grown in media in which the nitrogen source was replaced by NaNO₃, NH₄NO₃ and (NH₄)₂SO₄. The cultures were left to grow for 10 days at 23 ±1 °C under a 16h light/8h dark regime and 55 μmol photon m⁻²s⁻¹ light intensity. The Erlenmeyer flasks were shaken for three times a day.

**Determination of Cell Number.** *C. vulgaris* was microscopically counted using a plankton counting chamber. Simultaneously, optical density (OD) values of the samples were determined at 680 nm using a visible density spectrophotometer, and then both measurements were compared. A standard optical density was generated to calculate the number of individuals based on their optical density. These calculations were repeated three times.

**Total protein analysis.** Lowry (1951) method was used for total protein analysis. Based on this method, 0.1 mL DOC solution was added on a 1-mL sample and the sample was kept at room temperature for 10 min. Afterwards, 0.1 mL TCA was added on the sample which was, then, centrifuged at 7500 rpm for 10 min. Following the removal of the supernatant, 1 mL Lowry solution was added on the precipitate and the precipitate was kept at room temperature for 20 min. Later on, 1 mL fooling reagent was added on the sample, which was, then, kept for 30 min. Lastly, absorbance was measured at 750 nm and results were evaluated based on a standard curve [23].

**Lipid analysis.** The total lipid content was determined using the Bligh and Dyer method (1959). 40 mL methanol and 80 mL chloroform were added on a 0.2-g sample and then 20 mL CaCl₂ (4%) was added on it. The mixture was filtered through filter paper and allowed to stand overnight in the dark. The following day, methanol and water were separated using a separatory funnel, and chloroform was evaporated in a water bath at 60 °C. The remaining part was allowed to stand for 1 h at 90 °C and the chloroform was completely evaporated and then the sample was weighed [24].

**Determination of Pigment Content.** Chlorophyll content was measured using the method developed by Strikland and Parsons (1972). A 5-mL culture was filtered through GF/C filter paper to determine the concentrations of pigment. Each filter paper was placed in 90% acetone at +4 °C, kept in the dark for 24 h, centrifuged at 3500 rpm for 5 min and absorbance was measured at 630, 645, 665 and 750 nm using a spectrophotometer [25].

Astaxanthin content was calculated using the method developed by Boussiba and Vonshak (1991).
A 5-mL sample was centrifuged for 10 min at 4000 rpm to remove the supernatant, 30% methanol and 5% KOH were added on the precipitate and kept for 15 min at 65 °C to remove the chlorophyll. The supernatant in the tubes was decanted and the tubes were washed three times with distilled water. Then, 5 mL DMSO was added onto the precipitate and sonicated for 10 min (3.0 kHz). The samples were analyzed by calculating the amount of astaxanthin at 490 nm using a spectrophotometer [26].

**RESULTS AND DISCUSSION**

Effect of different nitrogen sources on growth of *Chlorella vulgaris*. NaNO₃, NH₄NO₃ and (NH₄)₂SO₄ are the most studied nitrogen sources [2, 3, 11, 27]. This study was conducted to investigate the effect of different nitrogen sources on the growth, and pigment, protein and lipid content of *Chlorella vulgaris*. Sodium nitrate, ammonium nitrate and ammonium sulfate were used as nitrogen sources. Results show that nitrogen sources have different effects on the growth, and pigment, protein and lipid amount of *C. vulgaris*.

The results show that the number of cells, which was 11,000 cell/mL on the first day of inoculation in liquid media with different nitrogen sources, began to increase over time. The number of cells in the control medium reached 27,000 cells/mL on the first day after inoculation. The number of cells reached 32,000 cell/mL, 18,000 cell/mL and 12,000 cell/mL in media where ammonium nitrate, ammonium sulfate and sodium nitrate was used as a nitrogen source, respectively. The increase in the number of cells continued in all media until day six. The number of cells in ammonium nitrate, control, ammonium sulfate and sodium nitrate media was 96,000 cell/mL, 85,000 cell/mL, 57,000 cell/mL and 54,000 cell/mL, respectively, on day six. In subsequent days, the number of cells in all media decreased regularly (Fig. 1). The number of cells in ammonium nitrate, control, ammonium sulfate and sodium nitrate media was 87,000 cell/mL, 78,000 cell/mL, 59,000 cell/mL and 46,000 cell/mL, respectively, on the last day of the study. The highest and lowest growth rates were observed in ammonium nitrate and sodium nitrate media, respectively (Fig. 1). Many studies report that the growth rate of microalgae is better in ammonium nitrate media than in other nitrogen sources [27,28].

**FIGURE 1**

Changes in cell concentration of *Chlorella vulgaris* at different nitrogen sources
day ten. The increase in protein amount was greater in ammonium nitrate medium than in media containing other nitrogen sources. Protein amount in ammonium nitrate medium, which was 55.00 µg/mL on the day of inoculation, reached maximum (382.60 µg/mL) on day six. Protein amount in ammonium sulfate and sodium nitrate media was 264.83 µg/mL and 182.37 µg/mL, respectively, on day six. Protein amount in ammonium nitrate, ammonium sulfate and sodium nitrate media decreased regularly to 316.35 µg/mL, 217.34 µg/mL and 141.93 µg/mL, respectively, by the end of the study (Fig. 2).

**Effect of different nitrogen sources on lipid amount of** *Chlorella vulgaris*. Fig. 3 shows that ammonium nitrate supported lipid accumulation in *C. vulgaris* more than did ammonium sulphate and sodium nitrate. Lipid amount in *C. vulgaris* cultures where ammonium nitrate was used as a nitrogen source was high throughout the study. Lipid content was 15.50% in all media on the day of inoculation while it reached 41.75%, 36.41%, 26.85% and 17.75% in ammonium nitrate, control, sodium nitrate and ammonium sulfate media, respectively, on day one. The increase in lipid amount continued on day two and reached 33.45%, 42.43%, 50.56% and 60.66% in ammonium sulfate, sodium nitrate, control and ammonium nitrate media, respectively. The increase in lipid amount in *C. vulgaris* continued to increase until the end of the study and reached 64.60%, 65.59%, 72.72% and 97.62% in ammonium sulfate, sodium nitrate, control and ammonium nitrate media, respectively. The greatest increase in lipid content was observed in ammonium nitrate medium (Fig. 3).
Chlorophyll a, chlorophyll b and astaxanthin content was measured for ten days in media with different nitrogen sources. The increase in chlorophyll a content was different in four media. Chlorophyll a content reached maximum in control (0.186 mg/mL), ammonium nitrate (0.126 mg/mL), ammonium sulphate (0.112 mg/mL) and sodium nitrate (0.255 mg/mL) media on day six. Chlorophyll a content was higher in sodium nitrate medium than in other media throughout the study (Fig. 4). Chlorophyll b had a similar pattern to chlorophyll a. Chlorophyll b content in all media increased from the day of inoculation to day six. Chlorophyll b content in ammonium sulphate, ammonium nitrate and control media was 0.153 mg/mL, 0.183 mg/mL and 0.208 mg/mL, respectively. Chlorophyll b content was higher in sodium nitrate medium than in other media throughout the study and reached 0.246 mg/mL on day six (Fig. 5).

As in chlorophyll a and chlorophyll b, astaxanthin content was also higher in sodium nitrate medium than in media containing other nitrogen sources. Astaxanthin content increased from the day of inoculation to day six and reached 58.13 mg/L, 45.15 mg/L, 40.05 mg/L and 35.05 mg/L in sodium nitrate, control, ammonium nitrate and ammonium sulphate media, respectively. A decrease was observed in astaxanthin content in all media containing different nitrogen sources after day six (Fig. 6).
Microalgae have higher capacity for photosynthesis than plants. Significant changes are known to occur in their simple cell and biochemical structures under stress conditions [1,2,30]. In recent years, numerous studies have investigated the effect of physical and chemical changes in culture media on microalgae. These studies show that the biochemical composition of microalgae can be manipulated by changing the physical and chemical parameters of the culture medium [30-32].

The results of this study indicate that different nitrogen sources in culture media affect the growth, and lipid, protein and pigment content of *Chlorella vulgaris*. Ammonium nitrate is the best nitrogen source for growth, and lipid and protein content of *Chlorella vulgaris* while sodium nitrate is the best nitrogen source for chlorophyll a, chlorophyll b and astaxanthin. However, further studies are needed to optimize the culture parameters required to achieve growth, and high production of protein, total lipid and pigment in microalgae. We believe that this study will provide important insights for future research.

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ESSENTIAL AND TOXIC ELEMENTS IN RAW COW MILK COLLECTED FROM SANLIURFA, GAZIANTEP, AND MARDIN PROVINCES OF TURKEY

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ABSTRACT

The purpose of this study was to assess the concentrations of eleven elements (sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), and arsenic (As)) in raw cow milk samples collected from Sanliurfa, Gaziantep, and Mardin provinces of Turkey. Ninety-nine raw cow milk samples were analyzed by using inductively coupled plasma mass spectrometer (ICP-MS) following microwave digestion. Potassium, phosphorus, and calcium were the most abundant elements with the average concentrations of 1391.74±548.62, 1089.26±271.75, 853.87±251.27 mg L⁻¹, respectively. The concentrations of the toxic elements (lead, arsenic, and cadmium) were found under the limit of detection (<1 μgL⁻¹). When the raw milk samples were compared in terms of Na content, a statistical significance was observed between Gaziantep and Sanliurfa and Mardin (P<0.001). The P element contents showed a significant difference between Sanliurfa and Gaziantep and Mardin (P<0.001). Moreover, K and Cu element contents were statistically significantly different between the samples collected from Mardin and Sanliurfa and Gaziantep (P<0.05). Ca, Fe and Zn elements also showed a significant difference between Gaziantep and Sanliurfa and Mardin (P<0.001). In this study, the Pb, Cd and As levels were below the detection limit (<1 μgL⁻¹) indicates that there is no toxic metal pollution in these areas. These results show that raw cow milk samples are suitable for human consumption. Further studies are required on milk and dairy products to ensure food safety.

KEYWORDS:
Cow milk, essential element, toxic element, ICP-MS

INTRODUCTION

Increased social consciousness on the influence of healthy human diet have prompted to produce high quality food with health promoters. Cow milk contains a large amount of water, along with proteins, fats, carbohydrates, vitamins, and essential elements. Essential elements found in the structure of milk play a very important role in the structure of dairy products, as well as in nourishing infants for growth, development, and health [1].

On the basis of daily requirement, elements can be divided into two categories; macro elements (>100mg/day), and trace elements (<100mg/day). Milk and dairy products are significant sources of macro elements such as Calcium, Phosphorus, Sodium, and Potassium. [2]. Element content of milk varies from species to species, feeding condition, lactation stage, environmental factors, air, water, and soil composition [3]. The content of essential elements in raw cow milk may also be affected by manufacturing procedures, cow breed, and sample collection time [4]. Previous studies reported the levels of the essential and toxic elements found in cow milk in different countries worldwide [3, 5, 6, 7, 4]. The data on essential and toxic element levels in raw cow milk from Turkey is limited. Essential elements are important due to their necessity, while toxic elements are significant even at low levels when ingested over a long period [8]. For instance, Copper and Chromium are essential elements but may also become toxic at higher concentrations causing lung inflammation, fibrosis, tumors, and cardiovascular disease [9, 10]. A toxic element should be considered as a bioindicator of environmental pollution in raw milk. Presence of essential and toxic elements in raw cow milk have been widely reported worldwide, especially in industrialized and polluted areas.

In Turkey, cattle breeding stock reached 14.080.155 in 2016, which corresponds to approximately 16.786.263 tons of cow milk [11]. The cattle breeding stock of Sanliurfa, Gaziantep, and Mardin was 245.210, 153.511, 86.321 in 2016, respectively [12]. While Gaziantep is more industrialized, Sanliurfa and Mardin are generally important dairy areas of Turkey. The aim of this study was to evaluate the differences in essential and toxic element concentrations among these cities.
MATERIALS AND METHODS

A total of 99 raw cow milk samples (500 mL) were analyzed in this study. Among these, 33 milk samples were from Sanliurfa, 33 from Gaziantep, and 33 from Mardin provinces of Turkey (Fig. 1) and all were collected between 8:00-10:00 am from small farms. Raw milk samples were taken directly from the cows’ udders aseptically to sterile polypropylene bottles and kept at -20 °C until analyses. Samples of 1 mL were digested with 4 mL nitric acid (65% volume concentration HNO₃, Merck, Germany) and 2 mL hydrogen peroxide (30% volume concentration H₂O₂, Merck, Germany) in PTFE vessels. A Mars express (CEM, Turkey) microwave digestion system was used for acid digestion. Raw milk samples were digested according to the Table 1.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature(°C)</th>
<th>Time (min)</th>
<th>Power (Watt)</th>
<th>Hold (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90</td>
<td>5</td>
<td>1600</td>
<td>5</td>
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<tr>
<td>2</td>
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<td>1600</td>
<td>10</td>
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<tr>
<td>3</td>
<td>180</td>
<td>5</td>
<td>1600</td>
<td>20</td>
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</table>

All solutions were prepared with ultrapure water acquired by passing distilled water through a water purification system (MES MP Minipure, Turkey). All reagents were of analytical grade except where otherwise stated. The standard solution of elements was obtained Agilent Japan (Lot Number: 10-160YPY2). National Institute of Standards and Technology-traceable 10 mg L⁻¹ elemental standards were used for the preparation of the multi-element calibration standard. The concentrations of 11 elements (Na, Mg, P, K, Ca, Fe, Cu, Zn, Pb, Cd, and As) were analyzed in raw cow milk samples. The isotopes ²³Na⁺, ²⁵Mg⁺, ³¹P⁺, ³⁹K⁺, ⁴⁴Ca⁺, ⁵⁶Fe⁺, ⁶³Cu⁺, ⁶⁶Zn⁺, ²⁰⁸Pb⁺, ¹¹¹Cd⁺, and ⁷⁵As⁺ were detected. All samples were analyzed in triplicate and each sample was measured in triplicate by inductively coupled plasma-mass spectrometer (ICP-MS) detection.

Essential and toxic element content of the raw cow milk samples were detected with an octopole reaction system ICP-MS (Agilent 7500 ce) with an autosampler (Cetac ASX-520) and a nebulizer (Agilent, Japan) under proper conditions according to the methods described by Zhou et al. [4] (Table 2). Three of the digested milk samples were used to determine the limit of detection (LOD) and limit of quantification (LOQ). The recovery of the 11 elements (Na, Mg, P, K, Ca, Fe, Cu, Zn, Pb, Cd, and As) in raw cow milk samples were between 92.4-124.1 % as shown in Table 3.

### TABLE 1
Microwave digestion stages

<table>
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<tr>
<th>Stage</th>
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<th>Power (Watt)</th>
<th>Hold (min)</th>
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<tr>
<td>3</td>
<td>180</td>
<td>5</td>
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### TABLE 2
ICP-MS conditions

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<tr>
<td>Carrier gas flow rate (L min⁻¹)</td>
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<td>Sample depth (mm)</td>
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<td>Sample introduction flow rate (mL min⁻¹)</td>
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<td>Extract lens (V)</td>
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<tr>
<td>Number of replicates</td>
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</tr>
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</table>

### TABLE 3
Essential and toxic element concentrations in certified reference material (CWW2TM-B)

<table>
<thead>
<tr>
<th>Element</th>
<th>Certified data (mg L⁻¹)</th>
<th>Our data (mg L⁻¹)</th>
<th>Recovery (%)</th>
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<tr>
<td>Na</td>
<td>10.0</td>
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<td>99.4</td>
</tr>
<tr>
<td>Mg</td>
<td>10.0</td>
<td>9.98</td>
<td>99.8</td>
</tr>
<tr>
<td>P</td>
<td>10.0</td>
<td>9.94</td>
<td>99.4</td>
</tr>
<tr>
<td>K</td>
<td>10.0</td>
<td>10.1</td>
<td>101</td>
</tr>
<tr>
<td>Ca</td>
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<td>9.24</td>
<td>92.4</td>
</tr>
<tr>
<td>Fe</td>
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</tr>
<tr>
<td>Cu</td>
<td>10.0</td>
<td>12.4</td>
<td>124.1</td>
</tr>
<tr>
<td>Zn</td>
<td>10.0</td>
<td>9.94</td>
<td>99.4</td>
</tr>
<tr>
<td>Pb</td>
<td>10.0</td>
<td>10.0</td>
<td>100</td>
</tr>
<tr>
<td>Cd</td>
<td>10.0</td>
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<td>99.8</td>
</tr>
<tr>
<td>As</td>
<td>10.0</td>
<td>9.93</td>
<td>99.3</td>
</tr>
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</table>

The statistical analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). For multivariate analyses of variables, one-way analysis of variance (ANOVA) was performed. The Pearson correlation coefficient was used in testing the relationship between numerical variables. The analysis for mean and standard deviation in raw cow milk samples were performed using Microsoft Excel 2010 (Microsoft Corporation, USA).

---

**FIGURE 1**
Map of Sanliurfa, Gaziantep, and Mardin provinces in southeastern Turkey
### TABLE 4
Concentrations of essential and toxic elements in raw cow milk samples from Sanliurfa

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation</th>
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<tr>
<td><strong>Essential elements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na (mg L⁻¹)</td>
<td>213.95</td>
<td>1238.5</td>
<td>503.75</td>
<td>270.62</td>
</tr>
<tr>
<td>Mg (mg L⁻¹)</td>
<td>71.68</td>
<td>128.65</td>
<td>93.56</td>
<td>13.53</td>
</tr>
<tr>
<td>P (mg L⁻¹)</td>
<td>672</td>
<td>1176</td>
<td>891.84</td>
<td>132.06</td>
</tr>
<tr>
<td>K (mg L⁻¹)</td>
<td>753</td>
<td>1792</td>
<td>1218.51</td>
<td>251.57</td>
</tr>
<tr>
<td>Ca (mg L⁻¹)</td>
<td>537</td>
<td>1073</td>
<td>766.78</td>
<td>123.45</td>
</tr>
<tr>
<td>Fe (mg L⁻¹)</td>
<td>1.71</td>
<td>2.80</td>
<td>2.38</td>
<td>0.49</td>
</tr>
<tr>
<td>Cu (mg L⁻¹)</td>
<td>0.026</td>
<td>0.525</td>
<td>0.124</td>
<td>0.133</td>
</tr>
<tr>
<td>Zn (mg L⁻¹)</td>
<td>1.32</td>
<td>4.87</td>
<td>3.46</td>
<td>0.765</td>
</tr>
<tr>
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<td></td>
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<td></td>
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<tr>
<td>Pb (μg L⁻¹)</td>
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<td>&lt; 1</td>
<td>&lt; 1</td>
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</tr>
<tr>
<td>Cd (μg L⁻¹)</td>
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<td>&lt; 1</td>
<td>&lt; 1</td>
<td></td>
</tr>
<tr>
<td>As (μg L⁻¹)</td>
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<td>&lt; 1</td>
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</tbody>
</table>

### TABLE 5
Concentrations of essential and toxic elements in raw cow milk samples from Gaziantep

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
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<tr>
<td><strong>Essential elements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na (mg L⁻¹)</td>
<td>237.83</td>
<td>5657.5</td>
<td>1408.33</td>
<td>1561.49</td>
</tr>
<tr>
<td>Mg (mg L⁻¹)</td>
<td>28.93</td>
<td>181.73</td>
<td>97.05</td>
<td>42.7</td>
</tr>
<tr>
<td>P (mg L⁻¹)</td>
<td>716</td>
<td>2018</td>
<td>1298.21</td>
<td>336.07</td>
</tr>
<tr>
<td>K (mg L⁻¹)</td>
<td>204</td>
<td>3045</td>
<td>1243.11</td>
<td>806.51</td>
</tr>
<tr>
<td>Ca (mg L⁻¹)</td>
<td>420</td>
<td>1714</td>
<td>1014.12</td>
<td>357.14</td>
</tr>
<tr>
<td>Fe (mg L⁻¹)</td>
<td>1.37</td>
<td>4.24</td>
<td>2.66</td>
<td>0.618</td>
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<tr>
<td>Cu (mg L⁻¹)</td>
<td>0.020</td>
<td>0.517</td>
<td>0.110</td>
<td>0.105</td>
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<tr>
<td>Zn (mg L⁻¹)</td>
<td>1.31</td>
<td>12.06</td>
<td>4.92</td>
<td>2.236</td>
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<tr>
<td><strong>Toxic elements</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pb (μg L⁻¹)</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
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<tr>
<td>Cd (μg L⁻¹)</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td></td>
</tr>
<tr>
<td>As (μg L⁻¹)</td>
<td>&lt; 1</td>
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</tr>
</tbody>
</table>

### FIGURE 2
Hierarchical clustering results of raw cow milk samples from Sanliurfa (U), Gaziantep (G), and Mardin (M) provinces (dendrogram).

### RESULTS AND DISCUSSION

The concentrations of essential and toxic elements in raw cow milk samples are presented in Table 4, Table 5 and Table 6. The hierarchical clustering results of raw cow milk samples from Sanliurfa, Gaziantep, and Mardin provinces shown in Fig. 2.

Concentrations and correlations of essential elements in raw cow milk samples. Essential elements are involved in the physicochemical processes of the human body such as osmotic pressure, muscle contraction, bone metabolism, nerve impulses and acid-base balance [3]. Cow milk samples are generally rich in macro elements: Na, Ca, Ma, and K. Excessive or low dietary intakes of Na, Ca, Mg, and K can cause serious health problems in humans such as hypertension, cardiovascular diseases, rickets and water-electrolyte balance disorders [13].
Sodium (Na) is an important macro element of milk. The content of Na in raw cow milk samples was measured within the range of 211.5-5657.5 mg L\(^{-1}\). In this study, the mean Na level was 746.58 mg L\(^{-1}\), which is higher than previous studies that reported mean values of 600 mg kg\(^{-1}\) [3] and 512 mg L\(^{-1}\) [6]. Sodium concentrations showed a positive correlation with Mg concentrations in all samples (r: 0.720; P<0.001). Sodium is called “hydration ion” since it plays an important role in water-electrolyte balance. There is a negative correlation between Na and K cations (r: -0.736; P<0.001). There was a positive correlation between Mg concentrations and phosphate (P) concentrations (r: 0.866; P<0.001), Ca (r: 0.877; P<0.001), and K (r: 0.839; P<0.001).

Magnesium (Mg) acts as a cofactor in an average 300 enzymatic reactions. It is also responsible for nerve and muscle function [6]. The average Mg content of raw cow milk samples was 99.82 mg L\(^{-1}\), which is lower than Tibetan yak milk [6] and in parallel with Danish Holstein and Jersey milk samples [5]. The Mg concentrations in the milk samples were significantly correlated with P (r: 0.675; P<0.001), Ca (r: 0.866; P<0.001), and K (r: 0.839; P<0.001). Phosphorus (P) is an important element in energy transfer, fat and carbohydrate metabolism, and acid-base balance regulations [6]. In this study, P levels in raw cow milk samples ranged from 672 to 2018 mg L\(^{-1}\). The mean P value was 1089.26 mg L\(^{-1}\), which is higher than previous studies that reported mean values of 600 mg kg\(^{-1}\) [3] and 512 mg L\(^{-1}\) [6]. Sodium concentrations showed a positive correlation with Mg concentrations in all samples (r: 0.720; P<0.001). Sodium is called “hydration ion” since it plays an important role in water-electrolyte balance. There is a negative correlation between Na and K cations (r: -0.736; P<0.001). There was a positive correlation between Mg concentrations and phosphate (P) concentrations (r: 0.866; P<0.001), Ca (r: 0.877; P<0.001), and K (r: 0.839; P<0.001).

Phosphorus (P) is an important element in energy transfer, fat and carbohydrate metabolism, and acid-base balance regulations [6]. In this study, P levels in raw cow milk samples ranged from 672 to 2018 mg L\(^{-1}\). The mean P value was 1089.26 mg L\(^{-1}\), which is higher than previous studies that reported mean values of 600 mg kg\(^{-1}\) [3] and 512 mg L\(^{-1}\) [6]. Sodium concentrations showed a positive correlation with Mg concentrations in all samples (r: 0.720; P<0.001). Sodium is called “hydration ion” since it plays an important role in water-electrolyte balance. There is a negative correlation between Na and K cations (r: -0.736; P<0.001). There was a positive correlation between Mg concentrations and phosphate (P) concentrations (r: 0.866; P<0.001), Ca (r: 0.877; P<0.001), and K (r: 0.839; P<0.001).

Potassium (K) is an intracellular cation that plays a significant role for the nervous system, heart and kidney function. The average K level analyzed in this study was 1391.75 mg L\(^{-1}\), which was lower than the findings of Chi et al. that was 1532 mg L\(^{-1}\) [6] and Bilandžić et al. that was 2070 mg L\(^{-1}\) [3], yet in agreement with previous studies [5]. The K concentrations in milk samples were significantly correlated with Ca (r: 0.707; P<0.001), Fe (r: -0.550; P<0.001), and Zn (r: 0.582; P<0.001).

Calcium (Ca) is essential for the skeletal system and tooth formation, enzyme activity, blood coagulation and nerve conduction [6]. Concentrations of Ca in raw cow milk samples were measured in a range of 420-1714 mg L\(^{-1}\). Mean Ca concentration of Croatian milk samples was 1390 mg L\(^{-1}\) [3], which is higher than the presented findings (853.88 mg L\(^{-1}\)).

Copper (Cu) is a nutritional element which exists in the body in two oxidation states Cu\(^{+}\) and Cu\(^{2+}\) [14]. Daily intake of Cu in children varies from 0.6-0.8 mg/day [2]. The average Cu level in this study was estimated as 0.09 mg L\(^{-1}\), which was lower than other studies in Iraq (0.19 mg L\(^{-1}\)) [15] and Romania (0.17 mg L\(^{-1}\)) [16].

Zinc (Zn) is an essential element in mostly liver, muscle, kidney, and skin, which affects the activity of different enzymes in plasma membranes. Its deficiency is associated with growth failure, pellagra, dermatitis, neurophysiological abnormalities, loss of hair, and hemolytic anemia [2]. The amount of Zn in bovine milk was previously reported between 0.94-4.923 mg L\(^{-1}\) [17, 18]. The mean Zn (3.88 mg L\(^{-1}\)) values in our study were within the range of these literatures.

Concentrations and correlations of toxic elements in raw cow milk samples. Studies showed that due to its chemical similarity to Ca, Pb is considerably discarded via milk [19]. Since Pb can replace Ca, Zn and Fe, it inhibits the reactions of these elements. For instance, Pb can compete with Fe for binding to heme, thereby hampering oxygen transport [20]. With its potential neurotoxic effect, Pb may lead to kidney failure and reproduction function disorders especially in children, since it can not be degraded under environmental conditions [2, 21]. Industrial wastes and excessive usage of phosphatic fertilizer in cultivated areas create toxic levels of Cd [22]. Cd being a similar metal to Zn results in their substitution in the enzymatic reactions that Zn acts as a cofactor and therefore loss of Zn dependant biologic activity [23]. Long term As exposure leads to liver cancer.

### TABLE 6

Concentrations of essential and toxic elements in raw cow milk samples from Mardin

<table>
<thead>
<tr>
<th>Essential elements</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mg L(^{-1}))</td>
<td>211.5</td>
<td>428.25</td>
<td>327.64</td>
<td>63.22</td>
</tr>
<tr>
<td>Mg (mg L(^{-1}))</td>
<td>72.28</td>
<td>132.4</td>
<td>108.85</td>
<td>15.73</td>
</tr>
<tr>
<td>P (mg L(^{-1}))</td>
<td>849</td>
<td>1249</td>
<td>1077.72</td>
<td>91.66</td>
</tr>
<tr>
<td>K (mg L(^{-1}))</td>
<td>1106</td>
<td>1942</td>
<td>1713.62</td>
<td>183.09</td>
</tr>
<tr>
<td>Ca (mg L(^{-1}))</td>
<td>611</td>
<td>1001</td>
<td>780.72</td>
<td>89.52</td>
</tr>
<tr>
<td>Fe (mg L(^{-1}))</td>
<td>1.45</td>
<td>4.05</td>
<td>2.07</td>
<td>0.51</td>
</tr>
<tr>
<td>Cu (mg L(^{-1}))</td>
<td>0.008</td>
<td>0.210</td>
<td>0.037</td>
<td>0.049</td>
</tr>
<tr>
<td>Zn (mg L(^{-1}))</td>
<td>1.79</td>
<td>5.59</td>
<td>3.257</td>
<td>0.882</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxic elements</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb (µg L(^{-1}))</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Cd (µg L(^{-1}))</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>As (µg L(^{-1}))</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>
and various skin diseases [8] such that inorganic As is known as a group A carcinogenic in USEPA [24, 25]. In this study, the Pb, Cd and As levels being beneath the detection limit (<1 μg L⁻¹) indicates that there is no toxic metal pollution in these areas.

**Difference analysis by one-way analysis of variance (ANOVA).** When the raw milk samples were compared in terms of Na content, a statistical significance was observed between Gaziantep and, Sanliurfa and Mardin (P<0.001). The P element contents showed significant difference between Sanliurfa and, Gaziantep and Mardin (P<0.001). Moreover, K and Cu element contents were statistically significantly different between the samples collected from Mardin and, Sanliurfa and Gaziantep (P<0.05). Ca, Fe and Zn elements also showed significant difference between Gaziantep and, Sanliurfa and Mardin (P<0.001).

**CONCLUSIONS**

In this study, concentrations of 11 elements (Na, Mg, P, K, Ca, Fe, Cu, Zn, Pb, Cd, As) in raw cow milks from Sanliurfa, Gaziantep, and Mardin provinces of Turkey were assessed where K, P, and Ca were found the highest in raw cow milk samples. In this study, the Pb, Cd and As levels were below the detection limit (<1 μg L⁻¹) indicates that there is no toxic metal pollution in these areas. The results show that raw cow milk samples are suitable for human consumption and that further studies are required on milk and dairy products to ensure food safety. Thus, it would be useful if authorities routinely scan both essential and toxic elements in dairy foods.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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VISIBLE PHOTOCATALYST CdS: PREPARATION BY SLOW PRECIPITATION-WATER THERMOSETTING AND APPLICATION FOR THE DEGRADATION OF SULFAMETER

Hui Chen, Bo Gao, Huiyu Ren, Qingzhu Jia

Department of Environmental Engineering, Tianjin University of Science & Technology, Tianjin 300457, China

ABSTRACT

In this work, catalyst Cadmium sulfide (CdS) with high photo activity driven by visible light were prepared via the slow precipitation-water thermo-setting method. Also, this CdS catalyst was characterized by TG, XRD, UV-Vis means and, its optical performance was evaluated by the photo-degradation of antibiotics Sulfameter. The results show that this self-made CdS catalyst has the hexagonal crystal structure with high quality and the absorption spectrum is in the range of 250-600 nm. Comparing with the commercially available CdS, the self-made CdS catalyst is more responsive in the visible area. The photo degradation rate of the Sulfameter was above 80% with the self-made CdS, whereas the degradation rate was only about 33% with the commercial CdS. Moreover, based on HPLC/ESI-MS analysis and density functional theory, the photo-degradation pathway of Sulfameter was deduced, and main reactions as ammonia oxidation, splitting decomposition, and open loop, etc occurred during the photo-degradation of Sulfameter. In the summary, this visible-light-driven catalyst CdS was effective for the degradation of antibiotics Sulfameter, and the results could be valuable for the assessment of antibiotics transformation in the water body.

KEYWORDS:
Visible photo-catalyst, CdS, Hydrothermal synthesis, Sulfameter, Photo-catalytic

INTRODUCTION

Being as a kind of synthetic antibiotics, Sulfameter is widely used in the disease treatment and aquaculture. However, because of the high stability of antibiotics, generally, it is difficult to remove them thoroughly during the normal sewage treatment. Investigations demonstrated that the antibiotic concentration in the secondary effluent was about 4.8-1106.9 ng·L⁻¹ in the sewage treatment plant in Beijing, China [1], and also, authors suggested that Antibiotics (110 t/yr) might contribute 74% of the load of pharmaceuticals (152 t/yr) in the Yangtze [2]. Therefore, owing to the over utilization or even abuse, a great amount of antibiotics were discharged directly into the water body. Certainly, the accumulation of antibiotics in the water environment eventually affect aquatic organism [3] and/or even human health through the food chain [4]. For example, Zhang et al. [5] have argued that sulfamonomethoxine affects the cognitive and behavioral development of young rats. Consequently, it is necessary to study and assess the transformation process of antibiotics including Sulfameter in water environment.

Photochemical process plays an important role in the transformation and degradation of contaminants in water body [6, 7]. Researches states that the composition of natural water, such as ions (H₂PO₄⁻, NO₃⁻, and NH₄⁺) and other insoluble materials would influence differently the UV-photo degradation of sulfonamides [8]. Considering the economic and efficient utilization of visible light sources of the sun, the visible light-driven catalysts as SnS₅/SnO₂, AgBr–TiO₂/SiO₂@Fe₃O₄ and CdS–graphene etc. are more promising [9-11]. Among which, CdS is a n-type semiconductor with band gap of 2.4eV, and it is considered an ideal visible light-driven photo-catalyst with excellent optical performance [12, 13].

Investigations demonstrated that the optical performance of CdS is largely influenced by its morphologies, which might also be effected by the preparation method such as solve thermal method [8, 14], electrospinning method [15] and so on. The CdS microcrystals with fine particle size can be prepared by hydrothermal method and solvo-thermal method. Until now, visible light-driven CdS photo catalytic process has been widely used for the degradation of various pollutants including methylthionine chloride, bisphenol A [16], methyl orange [17] and hydrogen production [18]. However, to the best of our knowledge, there are few investigations involving CdS photo degradation of antibiotics. Moreover, authors suggested that photo catalyst CdS has some drawbacks for the degradation of sulfonamides, for example, the too larger
particle radius and the wider particle size distribution might influence the degradation efficiency of sulfonamides [19].

Our work focuses on the improving the photocatalytic degradation of Sulfameter by CdS. In the present work, CdS was prepared by the slow precipitation-water thermosetting method. The effects of various preparation conditions including hydrothermal temperature and calcination temperature on the photo-catalytic activity were studied in detail. The absorption spectra and structure of CdS was characterized by UV-Vis absorption spectra and X-ray diffraction, and its visible light-driven degradation performance for Sulfameter was discussed in detail. Results obtained in this work might be valuable for the guiding of the transformation assessment of antibiotics in water body.

**MATERIALS AND METHODS**

**Materials.** Sulfameter (>99%) was supplied by Adamas-beta. Anhydrous ethanol (C2H6O) and isopropanol (C3H8O) were purchased in China. Sodium sulfide (NaS • 9H2O) and cadmium acetate hydrate (C2H3CdO2) used during our experimental analysis grade. Mini Q water is used for all hydrothermal synthesis. First, appropriate amount of cadmium acetate and sodium sulfide dispersed in the mixed solution (80ml isopropyl alcohol and 20ml of Mini Q water) were sonicated for 30 minutes. And then sodium sulfide solution dropped-wise into the cadmium acetate solution to form cadmium sulfide solution being stirred for 1 hour. The resulting CdS solution was put into the hydrothermal reactor with temperature of 140°C, 160°C and 180°C, respectively. Then, the precipitate was separated by centrifugation, washed with water and anhydrous ethanol, then dried in an oven at 80°C for 16 h followed by calcination without air at 250°C, 300°C, 350°C and 400°C for 2 h.

**Preparation of catalyst.** In this work, CdS was obtained by the slow precipitation-hydrothermal synthesis. Firstly, appropriate amount of cadmium acetate and sodium sulfide dispersed in the mixed solution (80ml isopropyl alcohol and 20ml of Mini Q water) were sonicated for 30 minutes. And then sodium sulfide solution dropped-wise into the cadmium acetate solution to form cadmium sulfide solution being stirred for 1 hour. The resulting CdS solution was put into the hydrothermal reactor with temperature of 140°C, 160°C and 180°C, respectively. Then, the precipitate was separated by centrifugation, washed with water and anhydrous ethanol, then dried in an oven at 80°C for 16 h followed by calcination without air at 250°C, 300°C, 350°C and 400°C for 2 h.

**Characterization of CdS.** The characterization of the photo-catalyst was tested by the following equipment. X-ray diffraction patterns of nanoparticles was measured with a D/MAX-2500 X-ray diffractometer (XRD, Rigaku, Japan). Thermal stability was measured with a thermogravimetric analyzer (TG, TAQ50, America). UV-Vis absorption spectra was carried out by UV-visible spectrophotometer (UV-vis, uv-2550pc, Japan).

**Photo-catalytic experiments.** The photocatalytic performance of this self-made catalyst was tested by degradation of Sulfameter. The photolysis experiments (100 mL Sulfameter solution with initial concentration of 10 mg/L) were conducted in a jacketed photo reactor, and after dark reaction for 80 min, the lighting halogen bulb (λ> 380nm, 205 W) started to work for 2 h photolysis. Samples were taken at a given time interval. During the reaction process, samples were obtained through a 0.2 μm membrane for the HPLC (Ultimate 3000) analysis. Here, the mobile phase was 1 mL/min Mini Q water and Methanol (55:45 of V/V), and an Agela Venusil MP (C18 (2);4.6×250 mm, 5μm) column was used, and the detector wave length was set as 267nm.

**RESULTS AND DISCUSSION**

**Effect of Preparation Conditions on Catalysts. Hydrothermal temperature.** In order to study the structure of CdS nanoparticles at different hydrothermal temperatures, XRD patterns of these catalysts prepared at 140°C, 160°C and 180°C were determined as Figure 1. It described that the angle of each 2θ corresponding to each peak is 24.80 °, 26.40 °, 26.60 °, 28.12 °, 43.60 °, 44.05 °, 47.72 °, 51.7 ° and 52.20 °, which were consistent with the crystal face (100), (002), (111), (101), (110), (220), (103), (112) and (311) of the standard JCPDS cards 41-1049 CdS, indicating that the sample is CdS crystals. From the diffraction peak analysis in the Figure 1, it can be seen that the diffraction peak becomes stronger and sharp with the increasing of temperature. According to the XRD results, it could be demonstrated that the CdS prepared at the lower hydrothermal synthesis temperature is mainly cubic, and the sample prepared at the higher hydrothermal temperature is largely hexagonal. In addition, the hydrothermal synthesis of CdS is of dissolved-recrystallization process, and investigation suggested that the hexagonal crystal phase is thermodynamically stable, thus, the cubic crystal converts to hexagonal crystals with the increasing of hydrothermal temperatures. Clearly, the results shown in Fig. 1 further demonstrates the formation of CdS nanoparticles with hexagonal crystal at the higher temperature, which is consistent with those of prior work.

Fig. 2 showed the optical performance of CdS catalyst prepared under different hydrothermal temperatures. The results showed that the photodegradation efficiency of Sulfameter was also improved with the increase of hydrothermal temperature, and the photo-degradation rate increased from 71.8% at 140°C to 80.69% at 180°C.

Combining with the results of Fig. 1 and Fig. 2, the higher photo degradation efficiency at higher hydrothermal temperature might be due to the decrease of the photo-generated electron-hole recombination rate caused by the increase of the
crystallization degree. This also showed that the crystallinity of the catalysts was the major factor in the photo-catalytic experiments. Considering the experimental safety, the optimum hydrothermal temperature of the catalyst is set at 180°C.

**Calcination temperatures.** The effect of various calcination temperatures on the optical performance of CdS catalyst for the degradation of Sulfameter was investigated. Thermogravimetric analysis results (with N₂ atmosphere) of this self-made catalyst (shown as Fig. 3) described that not much change was occurred within the range of 180-350°C. However, it was obvious that some slight change occurred near the 400°C, suggesting the little change of catalyst structure. Hence, various calcination temperature was set in the range of 250°C -400°C. Fig. 4 was the XRD of CdS morphology at different calcination temperatures, and Fig. 5 showed the effect of the catalyst calcinated at different temperatures on the degradation of Sulfameter.

As shown in Fig. 4, when the temperature rises from 250°C to 400°C, the peak type gradually becomes sharp. In comparison with the CdS diffraction peaks, it was found that the (100) and (101) planes of CdS-400 were significantly higher and sharper than those of others. Moreover, the preferential orientation of CdS nanostructures along the (002) plane has the highest intensity, and the opposite (111) plane diffraction peaks become shorter. This shows that when the temperature rises to 400°C, the cubic phase-based miscible catalyst becomes into the hexagonal phase-based miscible catalyst. At the same time, the (111) plane particle sizes of Cd-300 and Cd-400 were 15.6 nm and 18.5 nm, respectively. This indicates that CdS nanoparticles are coarsened with the increasing of temperature [14]. However, this would be detrimental to the photocatalytic degradation of Sulfameter.

Results in Fig. 5 suggested that the photolysis efficiency of Sulfameter was very good for CdS with the calcination temperatures of 250°C to 350°C, and the photodegradation rate reached approximately 80% for 2 h. However, it was obvious
that the photodegradation rate of Sulfamer only got to 20% with CdS-400 catalyst. This suggested that the photo catalytic performance of CdS decreased with the increasing of calcination temperature. Additionally, it is interesting that the photocatalytic efficiency was very similar for CdS with the calcination temperatures range of 250°C to 350°C, and without calcination.

Therefore, combining the XRD pattern of CdS and the photodegradation effect for Sulfamer, the calcination temperature was set as 300°C.

**Effect of Catalyst dosage for photo degradation of Sulfamer.** Under the optimum preparation condition of CdS with the hydrothermal temperatures of 180°C and the calcination temperature of 300°C, the effect of different catalyst dosages (D: 0.6, 1.0, 1.4, 1.8 and 2.2 g•L⁻¹) on the degradation of Sulfamer(C₀=10mg/L) was investigated and the comparison results was shown as Fig. 6.

From Fig. 6, when dosages of the catalyst increased from 0.6g/L to 1.0g/L, the degradation efficiency of Sulfamer increased from 63.59% to 80.69% respectively, and the first-order kinetic constant K value was also increased to 0.0125min⁻¹. However, when catalyst dosages were greater than 1.0g/L, the photocatalytic degradation efficiency of Sulfamer was decreased to 57.46%. This may be due to the fact that the contact area of the photocatalyst with the light source was affected when the dosage of photocatalyst was too large, resulting in a reduction of the photogenerated quantum, which would lead to a reduction for the degradation efficiency of Sulfamer.

**Comparative Study on the Catalytic Effect of Commercial Cadmium Sulfide.** In order to further evaluate the photocatalytic activity of prepared catalyst (CdS-300), commercial cadmium sulfide (C-CdS) was also used for the degradation of Sulfamer, and the comparison results was showed in Table 1 and Fig. 7.

As can be seen from Table 1, the adsorption rate of Sulfamer on CdS-300 is about 21.7%, while, the adsorption rate is only 11.2% for the C-CdS. Moreover, the degradation rate of Sulfamer
TABLE 1
Comparison results of CdS-300 and C-CdS for the degradation of Sulfameter.

<table>
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<tr>
<th>Catalyst</th>
<th>Adsorption rate %</th>
<th>Degradation rate %</th>
<th>First-order kinetics constant /min⁻¹</th>
<th>The band gap energy value / eV</th>
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UV - Vis absorption spectra of CdS-300 sample and C-CdS sample

is 80.7% and 32.8% for CdS-300 and C-CdS, respectively. In addition, their kinetic constants K values are also very different. Therefore, it could be suggested that the CdS-300 prepared by the slow precipitation-water thermosetting method has a good effect for the adsorbing contaminants, and its visible light response is also better than that of the commercial C-CdS.

In order to further study the optical properties of the catalyst, we measured the UV-Vis absorption spectrum of the catalyst as shown in Fig. 7. The wavelength of the absorption region of both CdS-300 and C-CdS were about 330-550 nm, which was consistent with that of Hao Yang et al. [20]. Further, it is obvious that the visible light absorption capability CdS-300 is much stronger than that of C-CdS. Compared to the C-CdS, the CdS-300 have significant red shift. Also, according to the $E_g=1240/\lambda_0$ (eV) ($\lambda$: absorption wavelength threshold, abscissa vertical line), the band gap energy values of CdS-300 ($\lambda=517$nm) and C-CdS($\lambda=502$nm) was calculated as 2.40 eV and 2.47 eV. Therefore, with much stronger light absorption capability and much lower band gap energy value, CdS-300 prepared in this work would produce more photogenerated electrons and holes, and thus can be a good visible light-driven photo catalyst for the degradation of organic pollutants.

Analysis of Photo-catalytic Degradation Pathways. Based on the theory of density functional theory [21] and the structural formula of Sulfameter molecule showed as Fig. 8, the atomic charge values of the Sulfameter molecular were calculated and listed in Table 2. It can be seen that C16, S8, N7, O9, O10, N11, N13, N17, O18 of Sulfameter have higher frontier electron density values, and the bonds associated with these atoms may be preferentially attacked, especially N7, O9, O10, N11 have high electronegativity values, thus, these atoms can easily be adsorbed, the adjacent chemical bonds can be also attacked easily.

In order to further investigate the intermediate products produced during the photo degradation process of Sulfameter, HPLC/ESI-MS analysis was performed. The peak time of the characteristic peaks of the Ion flow chart of HPLC/ESI-MS could be shown at 3.9 min, 4.2 min, 4.6 min, 5.7 min, 6.7 min, 12.6 min and 13.5min. The corresponding intermediate product is Sulfaguanidine, 2-Amino-5-methoxypyrimidine, Sulfonamides, Sulfadiazine, p-Phenylenediamine, Hydroquinone, and Pyrimidin-2-amine. Therefore, the main reaction of the Sulfameter degradation process may be ammonia oxidation, splitting decomposition, and open loop, etc.

Hence, degradation pathway of Sulfameter can be speculated and also expressed in detail as Fig. 9. Here, two main pathways can be summarized as follows. The first, Sulfameter takes off the methoxy
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**FIGURE 9**

Possible pathways of Sulfameter oxidative degradation

group to form the sulfonamides under the attack of hydroxyl radicals. Further, it is liable to open the pyrimidine ring and then Sulfaguanidine is formed accordingly. Moreover, breakage of disulfide bond and hydroxylation of the amidogen could take place owing to the attack of the hole and/or hydroxyl radical, which further led the formation of p-Phenylenediamine and Hydroquinone. While, the second path is that the pyrimidine ring falls off under the attack of hydroxyl radicals, followed by open-loop and ammonia oxidation reaction. In addition, it is found that the location of the chemical bond may be consistent with the intermediate of the experimental process, which further proves the theoretical guidance of the density functional theory.
CONCLUSION

CdS visible light-driven photo-catalyst was prepared by the slow precipitation-water thermosetting method. Results demonstrated that both the hydrothermal temperature and the calcination temperature influence the catalyst properties, including the crystallization process and nanostructures’ intensity. The hydrothermal temperature of 180°C and the calcination temperature of 300°C were determined as the optimum conditions to form the cubic phase-based miscible catalyst. Comparing with the commercial CdS catalyst, this home-made CdS samples performed better at visible absorption capability with higher optical activity for the degradation of Sulfameter. Also, the degradation of Sulfameter by this prepared CdS catalyst is followed with the first order kinetic model. Moreover, combined with HPLC/ESI-MS analysis and density functional theory, the degradation path of Sulfameter and the possible reactions are deduced in detail.

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BIBLIOMETRIC ANALYSIS OF RESEARCH ON FISH METAL FROM 1997 TO 2016

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ABSTRACT

Bibliometric analysis based on the publications of fish metal related research from 1997-2016 was conducted to evaluate the literature patterns of publication outputs, categories, journals, corresponding authors, countries/territories, institutions, and author keywords. The results showed that fish metal related research experienced rapid growth during the past twenty years. Environmental sciences and ecology was most productive subject categories. The top 20 most productive journals were responsible for approximately 36.7% of the total publications, and Aquatic Toxicology was the most active journal. Wang from Hong Kong University of Science and Technology was most productive corresponding author in this field with 42 publications. The USA was the most productive country in fish metal research. The world wide geographic distribution of authors showed that the North America, Europe, East Asia, South Asia, and South America were the major spatial clusters of authors. National collaboration will be a main trend in this research field. The Chinese Academy of Sciences was the most productive institution. The author keywords analysis indicated that the cadmium, mercury, copper, lead, zinc, and arsenic were the most concerned metals during the past 20 years. Co-word analysis revealed that increased research interest in “cadmium”, “mercury”, “bioaccumulation”, “biomarker”, and “oxidative stress”.

KEYWORDS:
Bibliometrics, Fish, Metal, Co-word analysis

INTRODUCTION

Metals from natural and anthropogenic sources pose a high environmental risk and are of global concern in recent decades due to their persistence, toxicity, bioaccumulation and biomagnification through the food chain [1, 2]. The metals are toxic to aquatic organisms and even produce lethal or sublethal adverse effects on biota, such as fish, hydrophytes, and benthonic organisms [3-5]. Among these aquatic species, fish are particularly vulnerable because they occupy a range of higher trophic levels [6]. The accumulation of metals in fish has become a serious issue not only because of the threat to fish but also due to the human health risks from fish consumption [7, 8]. Numerous studies have been carried out from various perspectives of fish metal related research, such as the relationships between metal concentrations and fish species, tissues, length, and sex [9-12], health risk assessment for edible fish muscle [13], effects of metals on fish behavior [14], metal toxicity on fish [15], gene expression of fish under metal stress [16], as well as biomonitoring of metals in fish [17, 18].

Bibliometric analysis is a useful tool to evaluate and quantify the development and growth of literature for any specific research field [19, 20]. Bibliometric methods have been applied to obtain and analyze the information of publication outputs and patterns of authors, journals, countries/territories, institutions. They can also allow for new insights on spatial distribution of authors and collaboration networks of different groups. The variation of research topics could be reflected by performing the co-word analysis of the frequently used keywords from the publications. Bibliometric methods have been widely used in various research fields which were associated to fish. Aksnes and Brownman [21] used bibliometric indicators to characterize research activity in fisheries science from 2010 to 2013. Tao et al. [22] reviewed the global research related to productive capacity of fish habitats from 1946 to 2014. Nikolic et al. [23] explored the research trends and the evolution of publications of seven diadromous fish species from 1970 to 2010. The literatures published within the field of sturgeon sturgeons and paddlefish research have also been analyzed by using a bibliometric approach [24, 25]. However, bibliometric method has not been used previously to assess the research on fish metal.
In recent years, there has been a rapid development of the fish metal research due to the growing public awareness of metal pollution in aquatic environment and food safety. The purpose of present study was to investigate the characteristics of fish metal research from 1997-2016 by using bibliometric method, identifying general patterns of publication outputs, subject categories, journals, main authors, countries, institutions, collaborations, and author keywords. The findings will provide a better understanding of current state of fish metal research and a useful reference for future studies.

MATERIALS AND METHODS

The data used in present study were based on the database of Science Citation Index Expanded (SCI-Expanded) which was accessed from the Web of Science on September 14, 2017. “Fish” AND “metal*” were used as keywords to search titles, abstracts, and author keywords from 1997 to 2016. A total of 9013 publications in eleven document types were retrieved. There were 8094 paper articles comprising 89.8% of the total production, followed by proceedings paper (460; 5.1%), reviews (352; 3.9%), and meeting abstracts (57; 0.6%). The other seven document types accounted for less than 0.5% in each item. Publications originating from England, Scotland, North Ireland, and Wales were reclassified as being from the United Kingdom (UK) [26]. The analysis of publication outputs, authors, countries, institutes, subject categories, journals, and keywords were processed by Microsoft Excel 2007. The affiliations of authors were geocoded by CiteSpace V5.1.R6 SE [27] and the world-wide geographic distribution of authors was plotted by ArcGIS 10.4. The collaboration network among countries/territories and Co-word analysis of keywords were visualized using Ucinet 6 for Windows. The thickness of links represents the strength of collaborations, and the size of the nodes represents the amount of countries/territories and keywords. The impact factor (IF) of each journal was obtained from the Journal Citation Reports (JCR) which released in 2016.

RESULTS AND DISCUSSION

Characteristics of publication outputs. To obtain an overview of fish metal related research, the annual number of articles during 1997-2016 was showed in Table 1. The number of metal in fish publications increased from 168 in 1997 to 812 in 2016. The total number of authors increased from 630 in 1997 to 4213 in 2016, and the average number of authors per publication also showed an increased trend from 3.8 in 1997 to 5.2 in 2016, although decreased in some years. There was a slight fluctuation of average pages per publication, with an average of 9.9 pages. Additionally, 40.5 references were cited per publication in 1997, comparing to 53.0 references per publication in 2016, with slight increases during the last 20 years.

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Average 4.5 9.9 45.8

P: number of publications; AU: author number; AU/P: author number per publication; PG: page count; PG/P: page count per publication; NR: cited reference count; NR/P: cited reference count per publication.
Distribution of subject categories and journals. The metal in fish related research covered 100 subject categories in Web of Science. Among these subject categories, 66 categories contained less than 20 publications. The top 7 productive subject categories were illustrated in Fig. 1. The category of environmental sciences and ecology contributed the most with 3981 publications, followed by toxicology (2138), marine and freshwater biology (1360), and chemistry (944), biochemistry and molecular biology (663), fisheries (596), and food science and technology (590). Environmental sciences and ecology held primacy from 1997 to 2016, and were not exceeded by other subject categories. Since 2005, the number of publications in toxicology increased moderately and ranked second in 2016. The growth of publications in other five subject categories was relatively slower. It indicated that the research on fish metal mainly focused on metal concentrations of fish in aquatic environment all over the world and the metal toxicity on fish [15, 28].

FIGURE 1
Publications of the top seven productive Web of Science categories during 1997-2016

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<td>323 (3.6)</td>
</tr>
<tr>
<td>Ecotoxicology and Environmental Safety</td>
<td>263 (2.9)</td>
</tr>
<tr>
<td>Science of the Total Environment</td>
<td>260 (2.9)</td>
</tr>
<tr>
<td>Environmental Monitoring and Assessment</td>
<td>254 (2.8)</td>
</tr>
<tr>
<td>Environmental Toxicology and Chemistry</td>
<td>242 (2.7)</td>
</tr>
<tr>
<td>Bulletin of Environmental Contamination and Toxicology</td>
<td>230 (2.6)</td>
</tr>
<tr>
<td>Marine Pollution Bulletin</td>
<td>221 (2.5)</td>
</tr>
<tr>
<td>Chemosphere</td>
<td>208 (2.3)</td>
</tr>
<tr>
<td>Archives of Environmental Contamination and Toxicology</td>
<td>185 (2.1)</td>
</tr>
<tr>
<td>Environmental Science and Pollution Research</td>
<td>156 (1.7)</td>
</tr>
<tr>
<td>Fresenius Environmental Bulletin</td>
<td>139 (1.5)</td>
</tr>
<tr>
<td>Environmental Pollution</td>
<td>129 (1.4)</td>
</tr>
<tr>
<td>Comparative Biochemistry and Physiology C-Toxicology and Pharmacology</td>
<td>123 (1.4)</td>
</tr>
<tr>
<td>Environmental Science and Technology</td>
<td>106 (1.2)</td>
</tr>
<tr>
<td>Food Chemistry</td>
<td>104 (1.2)</td>
</tr>
<tr>
<td>Water Air and Soil Pollution</td>
<td>87 (1.0)</td>
</tr>
<tr>
<td>Marine Environmental Research</td>
<td>80 (0.9)</td>
</tr>
<tr>
<td>Ecotoxicology</td>
<td>78 (0.9)</td>
</tr>
<tr>
<td>Environmental Research</td>
<td>63 (0.7)</td>
</tr>
<tr>
<td>PLoS One</td>
<td>59 (0.7)</td>
</tr>
</tbody>
</table>

TP: total number of publications; %: percentage of total publication for a certain journal; IF: impact factor; TC/TP: average of citation.
The total of 9013 publications was divided into a wide range of 1528 journals in present study. Among these journals, 1383 (90.5%) journals published no more than 10 publications on metal in fish. The top 20 most productive journals with accounting for approximately 36.7% of the publications were showed in table 2. Aquatic toxicology published the most publications with 323 (3.6%) publications. The second most productive journal was Ecotoxicology and Environmental Safety with 263 (2.9%) publications, followed by Science of the Total Environment (260, 2.9%), Environmental Monitoring and Assessment (254, 2.8%), Environmental Toxicology and Chemistry (242, 2.7%), Bulletin of Environmental Contamination and Toxicology (230, 2.6%), Marine Pollution Bulletin (221, 2.5%). There were no significant differences between percentages of these top productive journals. It indicated that the research on metal in fish was carried out by various research perspectives. Although Environmental Science and Technology ranked fourteenth with 106 (1.2%) publications, it is noticeable that its IF of 6.198 ranked first. The other productive journals with relative higher IF were Environmental Pollution (5.099), Science of the Total Environment (4.900), Food Chemistry (4.529), Chemosphere (4.208), and Aquatic Toxicology (4.129), respectively. These journals not only have a high publication number but also have a high IF. It could be concluded that these journals had important influences on research related to metal in fish. Food Chemistry had the highest average of citation (44.6) among there 20 most productive journals. It may be attributable to the consideration of food safety associated with fish consumption [29, 30].

Author productivity. Table 3 shows the top 10 most productive corresponding authors with at least 14 publications. Corresponding author is usually the senior author who provides the intellectual input and designs the study protocols. These top 10 corresponding authors produced about 2.4% of total publications. Wang WX from Hong Kong University of Science and Technology was most productive corresponding author in this field with 42 publications. Handy RD from University of Plymouth has the highest average of citation (94.7), followed by Domingo JL from University of Rovira & Virgili (55.4). Sures B from University Karlsruhe has the highest h-index with 21, which indicates that more of his papers are widely recognized. These corresponding authors with outstanding performance were significant research pioneer in this field.

Country/territory and institution. There were 20 (0.22%) publications without author address information on the ISI web of Science, so 8993 publications with author address information published from 1997 to 2016 were analysis. It covered 142 countries/territories, of which 1863 (20.7%) were internationally collaborative publications covering 136 countries/territories and 7130 (79.3%) were single country/territory publications covering 96 countries/territories. The world wide geographic distribution of authors was plotted in Fig. 2. It is clearly that the North America, Europe, East Asia, South Asia, and South America were the major spatial clusters of authors. According to the publication production, the 142 countries/territories were divided into five groups. 115 countries/territories belonged to the first group with 1-100 publications; 14 countries/territories belonged to the second group with 101-250 publications; 9 countries/territories belonged to the third group with 251-500 publications; 3 countries (Canada, China, and Turkey) belonged to the fourth group with 501-1000 publications; and only the USA with 1566 publications belonged to the fifth group with more than 1000 publications.

Eight Asian countries/territories, eight European countries, two North American counties, one South American country, and one Oceania country were listed in the top 20 productive countries/territories of publications (Table 4). The USA published the most single and collaborative publications. The first authored publications of the USA was also ranked top one. Although the USA

<table>
<thead>
<tr>
<th>Corresponding author</th>
<th>Institution</th>
<th>TP</th>
<th>TC</th>
<th>TC/TP</th>
<th>h-index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang, WX</td>
<td>Hong Kong Univ Sci &amp; Technol</td>
<td>42</td>
<td>1483</td>
<td>35.3</td>
<td>20</td>
</tr>
<tr>
<td>Burger, J.</td>
<td>Rutgers State Univ</td>
<td>33</td>
<td>925</td>
<td>28.0</td>
<td>17</td>
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<tr>
<td>Sures, B.</td>
<td>Univ Karlsruhe</td>
<td>25</td>
<td>1059</td>
<td>42.4</td>
<td>21</td>
</tr>
<tr>
<td>Handy, RD.</td>
<td>Univ Plymouth</td>
<td>20</td>
<td>1894</td>
<td>94.7</td>
<td>17</td>
</tr>
<tr>
<td>Canli, M.</td>
<td>Cukurova Univ</td>
<td>18</td>
<td>609</td>
<td>33.8</td>
<td>9</td>
</tr>
<tr>
<td>Wong, MH.</td>
<td>Hong Kong Baptist Univ</td>
<td>16</td>
<td>732</td>
<td>45.8</td>
<td>14</td>
</tr>
<tr>
<td>Costa, PM.</td>
<td>Univ Nova Lisboa</td>
<td>16</td>
<td>373</td>
<td>23.3</td>
<td>11</td>
</tr>
<tr>
<td>Janz, DM.</td>
<td>Univ Saskatchewan</td>
<td>16</td>
<td>343</td>
<td>21.4</td>
<td>11</td>
</tr>
<tr>
<td>Liao, CM.</td>
<td>Natl Taiwan Univ</td>
<td>15</td>
<td>170</td>
<td>11.3</td>
<td>8</td>
</tr>
<tr>
<td>Domingo, JL.</td>
<td>Univ Rovira &amp; Virgili</td>
<td>14</td>
<td>775</td>
<td>55.4</td>
<td>10</td>
</tr>
</tbody>
</table>

TP: total number of publications; TC: total number of citations; TC/TP: average of citation.
was ranked the fifth of total publications, the percentage of internationally collaborative publication was ranked top one, indicating a higher collaboration rate than other countries. The cooperative relationships among the top 20 productive countries/territories were further analyzed by using NetDraw (Fig. 3). The size of circles represents the frequency of international cooperation and the thickness of lines represents the strength of cooperative relationships. The USA took the central position in the network, because it was the principal collaborator with other productive countries such as Canada, China, UK, Spain, France, and Germany. As the metal pollution in water has become a global issue, collaboration between different countries and territories will be a main trend in the research field which is based on metal in fish.

### TABLE 4
The top 20 most productive countries/territories during 1997-2016

<table>
<thead>
<tr>
<th>Country</th>
<th>TP</th>
<th>TP R(%)</th>
<th>SP</th>
<th>SP R(%)</th>
<th>FP</th>
<th>FP R(%)</th>
<th>CP</th>
<th>CP R(%)</th>
<th>C%</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>1566</td>
<td>(1) (17.4)</td>
<td>1039</td>
<td>(1) (14.6)</td>
<td>1242</td>
<td>(1) (13.8)</td>
<td>527</td>
<td>(1) (28.3)</td>
<td>33.7</td>
</tr>
<tr>
<td>Canada</td>
<td>834</td>
<td>(2) (9.3)</td>
<td>521</td>
<td>(2) (7.3)</td>
<td>670</td>
<td>(2) (7.5)</td>
<td>313</td>
<td>(2) (16.8)</td>
<td>37.5</td>
</tr>
<tr>
<td>China</td>
<td>672</td>
<td>(3) (7.5)</td>
<td>488</td>
<td>(3) (6.8)</td>
<td>604</td>
<td>(3) (6.7)</td>
<td>184</td>
<td>(5) (9.9)</td>
<td>27.4</td>
</tr>
<tr>
<td>Turkey</td>
<td>534</td>
<td>(4) (5.9)</td>
<td>490</td>
<td>(3) (6.9)</td>
<td>514</td>
<td>(4) (5.7)</td>
<td>44</td>
<td>24(2.4)</td>
<td>8.2</td>
</tr>
<tr>
<td>UK</td>
<td>461</td>
<td>(5) (5.1)</td>
<td>171</td>
<td>(1) (2.4)</td>
<td>288</td>
<td>(9) (3.2)</td>
<td>290</td>
<td>3(15.6)</td>
<td>62.9</td>
</tr>
<tr>
<td>India</td>
<td>453</td>
<td>(6) (5.0)</td>
<td>384</td>
<td>(5) (5.4)</td>
<td>418</td>
<td>(5) (4.6)</td>
<td>69</td>
<td>16(3.7)</td>
<td>15.2</td>
</tr>
<tr>
<td>Spain</td>
<td>449</td>
<td>(7) (5.0)</td>
<td>267</td>
<td>(7) (3.7)</td>
<td>371</td>
<td>(7) (4.1)</td>
<td>182</td>
<td>6(9.8)</td>
<td>40.5</td>
</tr>
<tr>
<td>Brazil</td>
<td>412</td>
<td>(8) (4.6)</td>
<td>324</td>
<td>(6) (4.5)</td>
<td>376</td>
<td>(6) (4.2)</td>
<td>88</td>
<td>12(4.7)</td>
<td>21.4</td>
</tr>
<tr>
<td>France</td>
<td>389</td>
<td>(9) (4.3)</td>
<td>208</td>
<td>(10) (2.9)</td>
<td>279</td>
<td>(10) (3.1)</td>
<td>181</td>
<td>7(9.7)</td>
<td>46.5</td>
</tr>
<tr>
<td>Italy</td>
<td>381</td>
<td>(10) (4.2)</td>
<td>265</td>
<td>(8) (3.7)</td>
<td>325</td>
<td>(8) (3.6)</td>
<td>116</td>
<td>9(6.2)</td>
<td>30.4</td>
</tr>
<tr>
<td>Japan</td>
<td>328</td>
<td>(11) (3.6)</td>
<td>201</td>
<td>(11) (2.8)</td>
<td>263</td>
<td>(11) (2.9)</td>
<td>127</td>
<td>8(6.8)</td>
<td>38.7</td>
</tr>
<tr>
<td>Germany</td>
<td>322</td>
<td>(12) (3.6)</td>
<td>129</td>
<td>(16) (1.8)</td>
<td>195</td>
<td>(14) (2.2)</td>
<td>193</td>
<td>4(10.4)</td>
<td>59.9</td>
</tr>
<tr>
<td>Iran</td>
<td>253</td>
<td>(13) (2.8)</td>
<td>210</td>
<td>(9) (2.9)</td>
<td>241</td>
<td>(12) (2.7)</td>
<td>43</td>
<td>25(2.3)</td>
<td>17.0</td>
</tr>
<tr>
<td>Portugal</td>
<td>235</td>
<td>(14) (2.6)</td>
<td>155</td>
<td>(14) (2.2)</td>
<td>202</td>
<td>(13) (2.2)</td>
<td>80</td>
<td>13(4.3)</td>
<td>34.0</td>
</tr>
<tr>
<td>Australia</td>
<td>216</td>
<td>(15) (2.4)</td>
<td>125</td>
<td>(17) (1.8)</td>
<td>177</td>
<td>(15) (2.0)</td>
<td>91</td>
<td>10(4.9)</td>
<td>42.1</td>
</tr>
<tr>
<td>Poland</td>
<td>191</td>
<td>(16) (2.1)</td>
<td>103</td>
<td>(20) (1.4)</td>
<td>133</td>
<td>(16) (1.5)</td>
<td>33</td>
<td>35(1.8)</td>
<td>17.3</td>
</tr>
<tr>
<td>Norway</td>
<td>191</td>
<td>(17) (2.1)</td>
<td>158</td>
<td>(13) (2.2)</td>
<td>174</td>
<td>(19) (1.9)</td>
<td>88</td>
<td>11(4.7)</td>
<td>46.1</td>
</tr>
<tr>
<td>South Korea</td>
<td>174</td>
<td>(18) (1.9)</td>
<td>132</td>
<td>(15) (1.9)</td>
<td>153</td>
<td>(17) (1.7)</td>
<td>42</td>
<td>27(2.3)</td>
<td>24.1</td>
</tr>
<tr>
<td>Pakistan</td>
<td>160</td>
<td>(19) (1.8)</td>
<td>114</td>
<td>(18) (1.6)</td>
<td>139</td>
<td>(18) (1.5)</td>
<td>46</td>
<td>22(2.5)</td>
<td>28.8</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>149</td>
<td>(20) (1.7)</td>
<td>76</td>
<td>(23) (1.1)</td>
<td>103</td>
<td>(24) (1.1)</td>
<td>73</td>
<td>15(3.9)</td>
<td>49.0</td>
</tr>
</tbody>
</table>

TP: total number of publications; SP: single country/territory publications; FP: first author publications; CP: internationally collaborative publications; R: rank; %: percentage of publications; C%: percentage of internationally collaborative publications in total publications for each country/territory.

![FIGURE 2](image-url)

Global geographic distribution of authors according to the number of publications by country/territory during 1997-2016
Among the 8924 publications with author address information, 67.3% of them involved inter-institutional collaboration. Although there were 6071 institutes devoted to the fish metal related research, 4899 (80.7%) institutes published no more than 3 publications. The top 20 most productive institutions with their outputs are shown in Table 5. Among the top 20 institutions, 5 were in Canada, 4 were in the USA, 2 were in Spain and one each in China, Portugal, Iran, Turkey, Russia, Brazil, Italy and Belgium. The Chinese Academy of Sciences (China) was the most productive institution for the total publications (215), and first authored publications (119), as well as inter-institutionally collaborative publications (199). It may be due to the Chinese Academy of Sciences has many sub-institutes in China. The McMaster University although ranked 4th in total number of publications (129), it had published the most single institute publications (44). In addition, although the University of Belgrade ranked 13th on publication outputs (75), all the publications were inter-institutionally collaboratively.

<table>
<thead>
<tr>
<th>Institution</th>
<th>TP</th>
<th>TP R(%)</th>
<th>SP</th>
<th>SP R(%)</th>
<th>FP</th>
<th>FP R(%)</th>
<th>CP</th>
<th>CP R(%)</th>
<th>C%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese Acad Sci</td>
<td>215</td>
<td>(2.4)</td>
<td>16</td>
<td>(0.5)</td>
<td>119</td>
<td>(1.3)</td>
<td>199</td>
<td>(3.3)</td>
<td>92.6</td>
</tr>
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<td>Univ Saskatchewan</td>
<td>164</td>
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<td>5</td>
<td>(0.2)</td>
<td>105</td>
<td>(0.8)</td>
<td>159</td>
<td>(2.6)</td>
<td>97.0</td>
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<tr>
<td>Univ Aveiro</td>
<td>137</td>
<td>(1.5)</td>
<td>11</td>
<td>(0.4)</td>
<td>58</td>
<td>(0.6)</td>
<td>126</td>
<td>(2.1)</td>
<td>92.0</td>
</tr>
<tr>
<td>McMaster Univ</td>
<td>129</td>
<td>(1.4)</td>
<td>44</td>
<td>(1.5)</td>
<td>77</td>
<td>(2.0)</td>
<td>85</td>
<td>(1.4)</td>
<td>65.9</td>
</tr>
<tr>
<td>Islamic Azad Univ</td>
<td>126</td>
<td>(1.4)</td>
<td>10</td>
<td>(0.3)</td>
<td>49</td>
<td>(1.0)</td>
<td>116</td>
<td>(1.9)</td>
<td>92.1</td>
</tr>
<tr>
<td>Environm Canada</td>
<td>122</td>
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<td>11</td>
<td>(0.4)</td>
<td>55</td>
<td>(1.1)</td>
<td>111</td>
<td>(1.8)</td>
<td>91.0</td>
</tr>
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<td>US Geol Survey</td>
<td>114</td>
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<td>21</td>
<td>(0.7)</td>
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<td>(0.6)</td>
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<td>(1.5)</td>
<td>81.6</td>
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<td>13</td>
<td>(0.4)</td>
<td>53</td>
<td>(0.8)</td>
<td>85</td>
<td>(1.4)</td>
<td>86.7</td>
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<tr>
<td>Cukurova Univ</td>
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<td>42</td>
<td>(1.4)</td>
<td>55</td>
<td>(0.6)</td>
<td>48</td>
<td>(0.8)</td>
<td>53.3</td>
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<td>(1.0)</td>
<td>68</td>
<td>(1.1)</td>
<td>77.3</td>
</tr>
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<td>5</td>
<td>(0.2)</td>
<td>55</td>
<td>(1.0)</td>
<td>68</td>
<td>(1.1)</td>
<td>77.3</td>
</tr>
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<td>50</td>
<td>(0.6)</td>
<td>56</td>
<td>(0.9)</td>
<td>70.0</td>
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<tr>
<td>Univ Belgrade</td>
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<td>0</td>
<td>-</td>
<td>25</td>
<td>(0.3)</td>
<td>75</td>
<td>(0.9)</td>
<td>100</td>
</tr>
<tr>
<td>Univ Sao Paulo</td>
<td>74</td>
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<td>7</td>
<td>(0.2)</td>
<td>26</td>
<td>(0.3)</td>
<td>67</td>
<td>(1.1)</td>
<td>90.5</td>
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<td>US EPA</td>
<td>70</td>
<td>(0.8)</td>
<td>11</td>
<td>(0.4)</td>
<td>33</td>
<td>(0.4)</td>
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<td>(1.0)</td>
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<td>Univ Calif Davis</td>
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<td>(0.7)</td>
<td>9</td>
<td>(0.3)</td>
<td>26</td>
<td>(0.3)</td>
<td>57</td>
<td>(0.9)</td>
<td>86.4</td>
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<tr>
<td>Univ Cadiz</td>
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<td>7</td>
<td>(0.2)</td>
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<td>(0.3)</td>
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<td>CNR</td>
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<td>8</td>
<td>(0.3)</td>
<td>35</td>
<td>(0.4)</td>
<td>58</td>
<td>(1.0)</td>
<td>87.9</td>
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<tr>
<td>Fisheries &amp; Oceans Can-</td>
<td>65</td>
<td>(0.7)</td>
<td>7</td>
<td>(0.2)</td>
<td>26</td>
<td>(0.3)</td>
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<td>(1.0)</td>
<td>89.2</td>
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<tr>
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<td>23</td>
<td>(0.8)</td>
<td>41</td>
<td>(1.0)</td>
<td>41</td>
<td>(0.7)</td>
<td>64.1</td>
</tr>
</tbody>
</table>

TP: total number of publications; SP: single institution publications; FP: first author publications; CP: inter-institutionally collaborative publications; R: rank; %: percentage of publications; C%: percentage of inter-institutionally collaborative publications in total publications for each institute.
Author keywords distribution analysis. The keywords analysis can be used to identify research trends [31]. There were 1965 publications with no author keywords, and the rest of the 7048 publications contained 40854 occurrences of 15898 unique keywords. However, 11885 (74.8%) keywords appeared in one publication, and the large number of once-only author keywords indicated that it was lack of continuity in research [32]. Only 420 (2.6%) keywords appeared in more than 10 publications, and these keywords represent the mainstream research field. The top 20 most author keywords are shown within each of 5-year intervals during 1997-2016 in Table 6. The top 20 most frequently used keywords appeared 6884 times (16.9% of total keywords occurrences) during this period. The phenomenon that a small group of keywords were widely used has also been found in other studies [33]. The keywords “fish”, “heavy metals”, “metals”, “heavy metal”, and “Trace metals” which were the search terms in the data retrieval process ranked 1st, 2nd, 5th, 9th, and 16th respectively. With the exception of “fish” and “heavy metals”, the two most frequently used keywords were “cadmium” and “mercury”. In addition, “copper”, “lead”, “zinc”, and “arsenic” were also listed among the 20 most frequently author keywords. It indicated that the cadmium, mercury, copper, lead, zinc, and arsenic were the most concerned metals during the past 20 years. It is noticed that the occurrence frequency of “cadmium” was higher than that of “mercury” during 1997-2011, while the occurrence frequency of “mercury” was higher than that of “cadmium” during 2012-2016. This change suggested that the mercury has been paid more and more attention because of its high toxicity and health hazard, especially for the methylation of mercury [34-36]. During the entire survey period, the ranking of “bioaccumulation” increased from 10th in 1997-2006 to 3rd in 2012-2016. The keyword “biomarkers” ranked 21th in 1997-2001, increased to 18th in 2002-2006, 17th in 2007-2011, and 12th in 2012-2016. Metals are considered as one of the most critical contaminants in aquatic ecosystem because of their potential to enter water bodies and also their bioaccumulation in fish. Patterns of bioaccumulation of metal in fish have been widely studied [37]. The fish are often used as biomarkers in evaluating the metal pollution status of aquatic ecosystems [38-40]. Co-word analysis of the frequently used keywords showed that there were high co-occurrence frequencies between “fish” and “cadmium”, “mercury”, “bioaccumulation”, and “biomarker” (Fig. 4), also indicating that the bioaccumulation of cadmium and mercury in fish as well as using fish as a metal biomarker in water ecosystem were the main research areas. In terms of metal toxicity in fish, the ranking of “metallothionein” decreased from 5th in 1997-2001 to 21th in 2012-2016. However, “oxidative stress” increased from 28th in 1997-2001 to 9th in 2012-2016. It suggested that recent studies associated with metal toxicity in fish focus on oxidative stress [41, 42]. The indices of oxidative stress have been considered as sensitive biomarkers in fish, which was exposed to metal pollution [43, 44].

<table>
<thead>
<tr>
<th>Author keywords</th>
<th>1997-2001(R)</th>
<th>2002-2006(R)</th>
<th>2007-2011(R)</th>
<th>2012-2016(R)</th>
<th>TP (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>123(1)</td>
<td>197(1)</td>
<td>328(1)</td>
<td>427(1)</td>
<td>1075(1)</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>81(2)</td>
<td>138(2)</td>
<td>309(2)</td>
<td>378(2)</td>
<td>906(2)</td>
</tr>
<tr>
<td>Cadmium</td>
<td>78(3)</td>
<td>104(3)</td>
<td>170(3)</td>
<td>180(6)</td>
<td>532(3)</td>
</tr>
<tr>
<td>Mercury</td>
<td>58(4)</td>
<td>91(4)</td>
<td>165(4)</td>
<td>216(3)</td>
<td>530(4)</td>
</tr>
<tr>
<td>Metals</td>
<td>42(7)</td>
<td>78(5)</td>
<td>152(5)</td>
<td>190(5)</td>
<td>462(5)</td>
</tr>
<tr>
<td>Bioaccumulation</td>
<td>29(10)</td>
<td>46(10)</td>
<td>121(6)</td>
<td>216(3)</td>
<td>412(6)</td>
</tr>
<tr>
<td>Copper</td>
<td>47(6)</td>
<td>78(5)</td>
<td>89(9)</td>
<td>117(10)</td>
<td>331(7)</td>
</tr>
<tr>
<td>Lead</td>
<td>27(12)</td>
<td>54(7)</td>
<td>95(8)</td>
<td>133(8)</td>
<td>309(8)</td>
</tr>
<tr>
<td>Heavy metal</td>
<td>13(24)</td>
<td>34(13)</td>
<td>103(7)</td>
<td>154(7)</td>
<td>304(9)</td>
</tr>
<tr>
<td>Metallothionein</td>
<td>47(5)</td>
<td>49(7)</td>
<td>78(5)</td>
<td>117(10)</td>
<td>331(7)</td>
</tr>
<tr>
<td>Zinc</td>
<td>41(8)</td>
<td>47(9)</td>
<td>68(12)</td>
<td>60(21)</td>
<td>216(11)</td>
</tr>
<tr>
<td>Toxicity</td>
<td>37(9)</td>
<td>38(11)</td>
<td>56(13)</td>
<td>75(17)</td>
<td>206(12)</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>10(28)</td>
<td>21(25)</td>
<td>52(15)</td>
<td>118(9)</td>
<td>201(13)</td>
</tr>
<tr>
<td>Sediment</td>
<td>19(16)</td>
<td>32(14)</td>
<td>72(11)</td>
<td>77(14)</td>
<td>200(14)</td>
</tr>
<tr>
<td>Pollution</td>
<td>22(13)</td>
<td>32(14)</td>
<td>55(14)</td>
<td>77(15)</td>
<td>186(15)</td>
</tr>
<tr>
<td>Trace metals</td>
<td>21(14)</td>
<td>34(13)</td>
<td>48(18)</td>
<td>69(18)</td>
<td>172(16)</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>15(21)</td>
<td>29(18)</td>
<td>49(17)</td>
<td>78(12)</td>
<td>171(17)</td>
</tr>
<tr>
<td>Arsenic</td>
<td>12(25)</td>
<td>31(16)</td>
<td>46(19)</td>
<td>77(13)</td>
<td>166(18)</td>
</tr>
<tr>
<td>Liver</td>
<td>18(17)</td>
<td>30(17)</td>
<td>29(26)</td>
<td>60(23)</td>
<td>137(19)</td>
</tr>
<tr>
<td>Trace elements</td>
<td>5(67)</td>
<td>25(20)</td>
<td>40(22)</td>
<td>63(20)</td>
<td>133(20)</td>
</tr>
</tbody>
</table>

TP: total number of publications; R: rank.
CONCLUSIONS

Based on a bibliometric analysis, an overview of the research on fish metal was obtained with the patterns of categories, journals, corresponding authors, countries/territories, institutions, and author keywords. Researches on fish metal enjoyed rapid growth during the past twenty years, with increasing number of publications, authors, and references. Environmental sciences and ecology, toxicology, marine and freshwater biology, chemistry, biochemistry and molecular biology, fisheries and food science and technology were most productive subject categories in fish metal studies. The top 20 most productive journals were responsible for approximately 36.7% of the total publications of fish metal. Aquatic Toxicology published most publications in this study area. The top 10 corresponding authors produced about 2.4% of total publications. Wang from Hong Kong University of Science and Technology was most productive corresponding author in this field with 42 publications. Sures from University Karlsruhe has the highest h-index with 21. The publications covered 142 countries/territories, of which 1863 (20.7%) were internationally collaborative publications covering 136 countries/territories and 7130 (79.3%) were single country/territory publications covering 96 countries/territories. The USA published the most single and collaborative publications. The first authored publications of the USA was also ranked top one. The world wide geographic distribution of authors showed that the North America, Europe, East Asia, South Asia, and South America were the major spatial clusters of authors. The Chinese Academy of Sciences was the most productive institution for the total publications, and first authored publications, as well as inter-institutionally collaborative publications. Collaboration between different countries/territories and institutions will be a main trend in this research field. The author keywords analysis indicated that the cadmium, mercury, copper, lead, zinc, and arsenic were the most concerned metals during the past 20 years. Co-word analysis revealed that increased research interest in “cadmium”, “mercury”, “bioaccumulation”, “biomarker”, and “oxidative stress”.

ACKNOWLEDGEMENTS

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REFERENCES


THE EVALUATION OF A LAND CONSOLIDATION PROJECT BY WATER MANAGERS IN A RURAL AREA: A CASE STUDY IN KARACABEY

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ABSTRACT

Turkish rural development policy aims to improve and maintain the working and living conditions of rural communities within a region. This goal will increase the quality of life in rural areas to keep up with the living conditions of urban areas. Land consolidation projects are the greatest opportunity and investment in rural areas to achieve this goal.

Land consolidation projects affect different target groups. The most affected target group is business owners and those who use the lands of business owners. In addition, water managers at different levels of authority within irrigation areas are also among the most important stakeholders of land consolidation projects and are also affected by land consolidation.

To further the success of land consolidation projects, evaluation of results and applying those evaluations to new projects are of great importance. This study investigates time-dependent changes in the opinions of water managers about land consolidation in rural areas that are directly affected by land consolidation. Accordingly, surveys were carried out 6 and 16 years after the completion of a land consolidation project with water managers in rural areas. The results of the survey were evaluated by statistical methods. The success and benefits of land consolidation are realized over time. Considering time-dependent changes in the opinions and suggestions of water managers will aid the success of future projects.

KEYWORDS:
Land consolidation, water managers, survey, t-test, Turkey

INTRODUCTION

Land consolidation (LC) is the joining of land parcels and shares owned by an individual or a farming family that had been fragmented for various reasons. Furthermore, land consolidation is essential for ensuring rural development and increasing land use efficiency [1].

[1, 2, 3, 4, 5] define LC as a standard tool for increasing the effectiveness of soil use. The social benefits of implementing a new policy in relation to land ownership and land management are described in the works of [6, 7, 8, 9].

Land consolidation projects have also provided solutions to structural problems in agriculture as well as in in-field development applications, irrigation network projects, improved settlement, and protection of rural environments [10]. Since land consolidation involves the re-allocation of property and re-arrangement of rural area, its content and scope of service are changing. Thus, land consolidation has emerged as a means to transform a rural area into an efficient and competitive agricultural region [11, 12].

Both the economic and landscape benefits of land consolidation must be reconciled with management by land owners (e.g., social aspects). Newly merged plots must be created with clarified ownership rights. Land owners have seen (for more than 100 years) a gradual reduction in land value that once formed the basis of their livelihood. The fragmentation of land ownership has increased from generation to generation, which is a consequence of both past and present inheritance laws. Fragmented ownership is a serious problem for many countries, both within and outside of Europe [13]. According to [14]: [15], land fragmentation can be a major barrier to agricultural development.

Agricultural development can benefit rural areas. However, rather than single-standing agricultural production areas, rural areas can take on multiple roles. They reach a multidimensional position and their scope expands to include environmental awareness and increasing use of both natural and non-agricultural areas. Therefore, planning of consolidation projects is carried out so that more efficient and versatile uses of rural areas include agriculture, landscape, nature conservation, recreation, and transportation. Land consolidation projects have contributed to the renewal of villages, development and conservation of natural landscapes, and the achievement of agricultural and rural development goals. These projects, with the goal to improve living and working conditions, include the allocation and planning of adequate space for new residential areas.
workplaces and agricultural areas. Thus, land consolidation can have significant contributions to changing rural economies and sustainable rural development [6, 16, 17]. Improving agricultural infrastructure is crucial for high quality and efficient agricultural production that can compete on the global market. One of the most important problems limiting agricultural infrastructure improvement is existing infrastructure problems and adequate provisioning of financial resources [18]. Irrigation is an important factor in the development of rural areas. Investments to irrigation within a region can promote agriculture, resulting in an increase in quality and productivity of the land, and hence in quality of life [19, 20].

The main objective of irrigation projects is to increase production and income and thus maximize the welfare of farmers. In addition to the distribution and use of water, the effective use of other factors is also necessary to increase production [21]. These factors include physical limitations of networks, lack of farmer participation in management, and agricultural infrastructure faults, which can limit the success of irrigation projects that have been built with large labor and financial resources [22].

Various studies have been carried out to evaluate the effects of land consolidation, which requires a sufficient study length that includes all phases of land consolidation. [23] divided the process of land consolidation into three phases, the construction period (CP), the restoration period (RP) and the stabilization period (SP). In this study, the focus was on effects during the SP. In general, the SP begins 3–5 years after land consolidation. [24] identified the effects of changes in agricultural activities and businesses following the adoption of a land consolidation project. The results showed that the age of the farmer (AGE), ease in crop pattern planning (CRPT), reduction in distance between farmstead center and plots (DFCP), reduction in time consumed in accessing the plots (TIMES), and improvement in working conditions in the fields (WORC) significantly affected satisfaction toward land consolidation. [25] investigated the effects of personal, physical, and socioeconomic factors in the adoption of land consolidation by farmers.

In conclusion, land consolidation projects have effects on different target groups. In land consolidation projects, the most affected target groups are land owners and those who use or work with that land. However, other manager groups that are affected by land consolidation include muhtar (the highest elected authority of villages in Turkey), azas (members of the village council), presidents and vice presidents of irrigation cooperatives, presidents and vice presidents of production cooperatives, mayors, and district governors. This study investigates time-dependent changes in opinions regarding land consolidation by water managers in rural areas that have been directly affected by land consolidation. The surveys were carried out at six and 16 years after the completion of the land consolidation project (2006 and 2016, respectively). The results of the survey were evaluated by statistical methods. In this study, those who took the survey will be referred to as the participants.

**MATERIALS AND METHODS**

The rural area used in this study was within the Bursa Karacabey Plain Irrigation Project. Land consolidation was carried out at Bursa Karacabey Lowlands between 1995 and 2000 at 10 villages covering 7776 ha. The water sources for the Karacabey Irrigation Project were Lake Manyas, Koracay, the Murveter rivers that feed Lake Manyas, and the river banks of the lake.

This study investigates those changes caused by the land consolidation project within the region over a 10-year-period as perceived by the participants of the survey. Accordingly, surveys were collected from 60 individuals in 2006 and 2016. Individuals surveyed included the president and vice president of the Bursa Karacabey Plain Irrigation Project, engineers employed in the project, and muhtars and village council members of the villages in the region. As the managers composing the main body were available and few in number, no sampling methods were needed for the number of participants in the survey, and the survey was carried out on all managers. The respondents gave Yes (1) or No (0) responses to the survey questions.

**RESULTS AND DISCUSSION**

The results of the surveys carried out in 2006 and 2016 are given below.

Table 1 presents those questions that were significantly different over time among the total 65 questions that were asked of the participants. Analysis of variance was performed on the data and LSD (least significant difference) was calculated at a significance level 0.05 to determine those variables which were significantly different. The results obtained for each year are given in Table 1.

Improvements to the irrigation system are expected to create settlements suitable for cohabitation and increase both the urban-to-rural migration and number of households. Coupled with land consolidation and correction of borders, the renewal of the irrigation system will facilitate optimal use and distribution of water to land. Road systems planned with the irrigation system are arranged to be parallel with the channels. Thus, access to both the parcels and channels is possible.

For the year 2006, participants expressed that the revised road system, along with land consolidation, facilitated access to the land (F_{1,50}=21.7241,
However, over the course of time, since land fragmentation had occurred again, the adequate maintenance of the roads was not carried out, there were no renovations, and the road system lost its appeal.

In this study, questions were asked to reveal time-based perceptions resulting from irrigation to the rural area; the topics that were significant were modern structure ($F_{1,56}=22.0429$, $P<0.0001$), a planned settlement ($F_{1,56}=12.0533$, $P=0.001$), habitability ($F_{1,56}=4.5563$, $P=0.0372$), increase in village settlement areas ($F_{1,56}=22.0429$, $P<0.0001$), increase in industrial activity ($F_{1,56}=5.0465$, $P=0.0286$), development of tourism ($F_{1,56}=11.5862$, $P=0.0012$), and construction of sports facilities ($F_{1,56}=7.8997$, $P=0.0068$). With both land consolidation and irrigation access, modern construction was occurring in the village. Consequently, habitability had increased. Over the course of time, growth, increase in industry, development of tourism, and construction of sports facilities occurred. Land consolidation had encouraged youth to stay in the village. Development projects had considered the needs of the youth.

The amount of total potential agricultural land in Turkey is 25 million hectares. Currently, 8.5 million hectares is economically available for irrigation. In Turkey, according to 2011 data, 5.61 million hectares of land is under irrigation, including irrigation by farmers. In irrigated areas, irrigation rates range from 20% to 80% while irrigation efficiency ranges from 20% to 85% [26]. Low irrigation rates and efficiency within irrigation networks are due to the building of irrigation projects without regard for land consolidation and in-field development services.

**TABLE 1**
The questions asked and the responses that significantly varied between survey years.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Survey Question</th>
<th>Abbreviation</th>
<th>Survey Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>Has the transportation from the village to the land become easier?</td>
<td>Q10</td>
<td>Does the irrigation system have an effect on the irrigation rate?</td>
</tr>
<tr>
<td>Q2</td>
<td>Were modern structures built along with the irrigation in the village?</td>
<td>Q11</td>
<td>Have the irrigation system and condition of the land affected the irrigation?</td>
</tr>
<tr>
<td>Q3</td>
<td>Does the irrigation have an effect on the formation of a planned settlement in the village?</td>
<td>Q12</td>
<td>Has the current situation led to an excessive water loss?</td>
</tr>
<tr>
<td>Q4</td>
<td>Has the irrigation contributed to the habitability of the village?</td>
<td>Q13</td>
<td>Has the land consolidation facilitated the irrigation management?</td>
</tr>
<tr>
<td>Q5</td>
<td>Has the irrigation led to an increase in the settlement areas in the village?</td>
<td>Q14</td>
<td>Has the land consolidation provided effective water use?</td>
</tr>
<tr>
<td>Q6</td>
<td>Has the irrigation led to the establishment of industrial facilities in the village?</td>
<td>Q15</td>
<td>Has the land consolidation reduced the maintenance and repair expenses of the Union?</td>
</tr>
<tr>
<td>Q7</td>
<td>Has the irrigation led to the development of tourism in the village?</td>
<td>Q16</td>
<td>Has the land consolidation contributed to the improvement of the irrigation technology?</td>
</tr>
<tr>
<td>Q8</td>
<td>Has the irrigation led to a change in the construction of a sports facility in the village?</td>
<td>Q17</td>
<td>Has the consolidation increased the collection of irrigation fees?</td>
</tr>
<tr>
<td>Q9</td>
<td>Were there any increase in the irrigation rate in recent years?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**
Year-based statistical analysis results.

<table>
<thead>
<tr>
<th></th>
<th>2006</th>
<th>2016</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of Squares</td>
<td>F Ratio</td>
<td>Prob &gt; F</td>
<td>Sum of Squares</td>
</tr>
<tr>
<td>Q1</td>
<td>2.6600985</td>
<td>21.7241</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Q2</td>
<td>3.9737274</td>
<td>22.0429</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Q3</td>
<td>2.3727422</td>
<td>12.0533</td>
<td>0.0010</td>
</tr>
<tr>
<td>Q4</td>
<td>1.0857964</td>
<td>4.5563</td>
<td>0.0372</td>
</tr>
<tr>
<td>Q5</td>
<td>2.8099343</td>
<td>14.5093</td>
<td>0.0003</td>
</tr>
<tr>
<td>Q6</td>
<td>0.2955665</td>
<td>4.8276</td>
<td>0.0322</td>
</tr>
<tr>
<td>Q7</td>
<td>3.1219212</td>
<td>25.1034</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Q8</td>
<td>4.7290640</td>
<td>38.6207</td>
<td>&lt;.0001</td>
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<tr>
<td>Q9</td>
<td>1.8472906</td>
<td>16.0920</td>
<td>0.0002</td>
</tr>
<tr>
<td>Q10</td>
<td>4.1564039</td>
<td>33.4218</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Q11</td>
<td>2.2352217</td>
<td>18.7424</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Q12</td>
<td>3.4503284</td>
<td>17.9341</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Basin water management, protection of water resources, proper management of surface and ground waters, integrated approaches, public participation, legal regulations, and proper water pricing are all important factors for the effective use of water. Water and/or irrigation management is defined as the storage, transfer, and distribution of water at the right time and the proper quantity. The efficiency of water and/or irrigation management depends largely on how well the cooperation between water users and management is maintained. The most important factor determining the success or the failure of an irrigation project is management. Expected benefits from investments made to date have not been achieved despite it being a long time since construction of the irrigation system. According to many experts, the failure of irrigation systems is due to the lack of an effective monitoring and evaluation system during the network operation phase rather than failures made during planning, project design, and construction.

In 1993, irrigation projects carried out by State Hydraulic Works were transferred to irrigation associations and cooperatives to partially eliminate problems encountered at the irrigation management phase [27].

Monitoring and evaluation activities include project promotion information, maintenance and repair information, water charge information, and budget information. Performance evaluation include irrigation efficiency, irrigation rate, and benefit-cost ratio as determinants. The evaluation of irrigation performance aims to improve system operation, determine the overall state of the system, and compare the performance of the system within itself or with other systems [28].

Benefits of land consolidation include increasing irrigation rates and irrigation efficiency for irrigation projects. Within irrigation networks, parcels can be small and irregular in shape, and may not be directly connected to irrigation canals. Therefore, water may not be distributed to every corner of the land. In this case, water supplied to the land is accumulated in pit areas and cannot reach higher elevations [29]. In public irrigation networks there are big differences between areas available for irrigation and irrigated areas. The main reason for low irrigation efficiency and low irrigation rate is, in addition to irrigation network problems, the improper management of in-field development services. The main problems that lead to low irrigation rates are the irregular and small shape of irrigated parcels and inadequate irrigation network length. Land consolidation is recommended as an alternative solution to these problems [12]. As a result, responses to the following topics were significantly different: the increase in the irrigation rates in recent years (F1,56=14.5093, P=0.0003), the role of irrigation in the low irrigation rates (F1,56=4.8276, P=0.0322), the effect of irrigation systems and land conditions (F1,56=25.1034, P<0.0001), excessive water loss due to the current situation (F1,56=38.6207, P<0.0001), facilitation of irrigation management due to land consolidation (F1,56=16.0920, P=0.0002), effective use of water (F1,56=33.4218, P<0.0001), reduction in maintenance and repair costs (F1,56=18.7424, P<0.0001), positive contribution of land consolidation to irrigation technology (F1,56=17.9341, P<0.0001), and increase in the collection of irrigation fees (F1,56=9.6552, P=0.0030). Many years must pass to appreciate the contribution of land consolidation by farmers. The consolidation project for this study area was completed in 2000. Examining the results of this study, responses to questions regarding increase in irrigation rate, the effect of irrigation system on irrigation rate, the effect of the irrigation system and land use on irrigation, water loss, irrigation management, effective use of water, and improvement of irrigation technology were found to be significantly different six years after the completion of the consolidation project (2006), however no changes were found during the 2016 survey. These effects were realized because they were 6 years after the consolidation project. After 16 years participants overooked the benefits as they had become accustomed to the consolidation. After the first 6 years of consolidation, the effects of consolidation were recognized by the water managers. It is only normal that changes became less noticeable since no changes had occurred between the two surveys and the established management system continued. This survey study revealed that recognizing the effect of land consolidation both on the reduction in the cost of union-maintenance and repair and on the increase in the collection of irrigation fees requires a long time.

Land consolidation is an approach to correct problems in agricultural infrastructure. In a broad sense, land consolidation includes methods that accelerate rural development. Hence, land consolidation is regarded as a method that can provide solutions to problems of rural areas by improving agricultural and living conditions, rearranging village and residential areas, increasing agricultural production and income, and using and protecting nature. With new agricultural irrigation lands, water use is increasing. Educational activities involving the more effective and efficient use of water in agriculture should be carried out so that those involved in agriculture can use water more economically.

[30] stated that land consolidation is used as an effective means of rural development to improve rural infrastructure, provide housing, protect the environment, and generate jobs in agricultural production for the people living in villages and improve their economic and social status. This study also found that land consolidation has become an important factor in the sustainable development of income for rural areas.

Land consolidation has a great impact on diversity and ecological functions in different areas.
through technical and biological measures as stated by [31], [23, 32, 33, 34] etc. mention methods of landscape evaluation and perception in terms of land consolidation for the development of rural tourism and politics.

Irrigation of land is important in increasing agricultural yields. Water needed to grow plants is provided directly to the root zone for maximum and rapid water uptake. Classic and old-style irrigation practices not only waste water but also result in soil pollution due to salinization and drainage defects. Excess water threatens human health by polluting ground water with salt, pesticide and residues from agricultural fertilizers. To eliminate this problem in Turkey, the main objective should be to plan irrigation systems that allow for the implementation of drip irrigation or sprinkler irrigation systems that are also closed irrigation systems. In the case of completed projects where surface irrigation is implemented, traditional flood irrigation methods can be adopted. To increase irrigation rate of the land, the disadvantages of surface irrigation should be eliminated. In addition, land consolidation projects and in-field development services have to be carried out complementarily. Land consolidation projects allow for the implementation of irrigation projects with low costs in large areas and the efficient and accurate use of irrigation water sources in agricultural lands in Turkey. As a result, farmers in the agricultural sector will be able to increase their income and carry out sustainable and efficient production to which technology can be applied.

Increasing quality of life in rural areas to keep up with the conditions of urban areas, Turkish rural development policies aim to improve and maintain the working and living conditions of rural communities within a region. Land consolidation projects and investments in rural areas are great opportunities to achieve this goal. To further the success of projects, evaluation of the results of land consolidation projects and applying the results to new projects are of great importance. As well as farmers, water managers, who manage water in irrigation areas, are also important stakeholders in land consolidation projects. For these projects to succeed, it is important to receive and evaluate the thoughts and suggestions of the water managers on the implementation of land consolidation projects. For those studies that take time-dependent changes into account, the evaluation of the opinions and suggestions of water managers can improve the success of future projects.

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BIO-INSECTICIDAL EFFICACY OF BOTANICAL EXTRACTS OF CITRONELLA AND CINNAMON AGAINST TRIBOLIUM CASTANEUM, SITOPHILUS ORYZAE AND DROSOPHILA MELANOGASTER UNDER LABORATORY CONDITIONS

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ABSTRACT

Synthetic insecticides result in environmental pollution, pest resistance and residues problems; hence, focus is now given on plant based products for pest control. This study aims to assess the efficacy of two plant extracts, Citronella and Cinnamon against the two stored grain pests, Tribolium castaneum, Sitophilus oryzae, and the fruit fly Drosophila melanogaster under laboratory conditions.

The extracts of two medicinal plants, Citronella and Cinnamon were applied separately at various concentrations (1, 2 and 3 mL/petri dish) on filter papers in petri dish containing the test insects. Mortality was recorded after one, two and three hours exposure.

The three hours exposure at 3mL/dish caused 90-99% mortality. Mortality was largely dependent on exposure time and concentrations of both the extracts. A decrease in concentration resulted in low fatality and vice versa. Mortality in control and solvent batches was less than 10% in all the experiments.

Our results confirm that the extracts of Citronella and Cinnamon are highly effective against Sitophilus oryzae, Tribolium castaneum and Drosophila melanogaster. These findings further suggest the need for molecular studies of these medicinal plants.

KEYWORDS:
Tribolium castaneum, Sitophilus oryzae, Drosophila melanogaster, Citronella, Cinnamon.

INTRODUCTION

Presence of insects in stored grains is a problem throughout the world. The red flour beetle, Tribolium castaneum and the rice weevil, Sitophilus oryzae have a long association with human stored food that are found in association with a wide range of commodities including grains, flour, peas, beans, nuts, dried fruits and spices [1]. Adult as well as larval stages of these insects feed on milled products but can also attack broken grains if present in bulk storage [2]. An estimated direct and indirect loss of grains and grain-based products caused by stored-product insects range from about 10% in temperate regions to almost 50% in humid tropical areas [1, 3]. The fruit fly, Drosophila melanogaster causes injury to different types of pulpy fruits particularly guava and banana (rendering the fruits worthless). It has also been reported to be a vector for different parasites among animals [1]. A recent survey has reported that the annual loss due to insects and rodent pests is 10 to 25% of the world harvested food. In Pakistan alone, fruit flies inflict around seven million Rupees loss to growers annually [1, 4]. The control of these flies as well as other insect pests is an important economic and health concern in the world. The synthetic products have been used heavily to control these pests, however, resistance against synthetic products (in the pests) [1, 4], negative effects on the environment as well as victimizing the non-targeted organisms have turned researchers to introduce certain new and safe alternatives posing minimum adverse effect on the environment and non-target animals [2].

Plants are rich source of bioactive molecules which may act as insecticides, antifeedants, anti-moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, as well as attractants against specific target-insects and are eco-friendly [4]. Essential oil of Eucalyptus
globules has been tested to find out its toxicity on eggs of Acanthoscelide obtectus, T. confusum, Ephesia kuehniella, C. maculates, S. oryzae and T. Castaneum [5]. Similarly, the Cinnamon (Cinnamomum verum, synonym C. zeylanicum) has been used to treat diarrhea and other problems of the digestive system. The essential oil of Cinnamon has antioxidant and antimicrobial properties as well as remarkable pharmacological effects in the treatment of type II diabetes. It is also used as a spice in food material in Asia [6]. Citronella grass (Cymbopogon nardus) is a source of essential oils as remarkable pharmacological effects in the treat-

**MATERIALS AND METHODS**

**Plant material and extract preparation.** The two plants, i.e., citronella (Cymbopogon nardus L.) and cinnamon (Cinnamomum verum Pers.) were collected from the hilly areas of district Buner (Chagharzi and Gokand). The upper leaves of the plants were washed with water, dried first at room temperature for 7 days and then at 45 °C in a hot air oven for 48 hours. The materials (500g each) were then pulverized using electric grinding machine. The resulting powder was passed through a 25-
mesh sieve to obtain a fine dust and then extracted in isopropanol using Sox test extraction apparatus (Model, SX-6 by Raypa, Spain). The resulting extracts were then subjected to solvent evaporation at 65 °C using a Rotary evaporator (RE-2010 Biobase, China) and subsequently preserved in tightly corked labeled bottles and stored in a refrigerator (Dawlance) at 4 °C until use for insect bioassays. Appropriate concentrations were prepared by mixing the solvent residues with isopropanol for subsequent experiments.

**Test Insects.** Three different species of insects were used for the experiment. Adults of T. castaneum and S. oryzae were obtained from infested stock of wheat and rice, respectively from local market in district Buner. These two insects were reared in plastic containers (20 cm height × 7.5 cm diameter) containing wheat flour mixed with yeast (10:1, w/w) for T. castaneum and rice grains (Fakhre Malakand variety) for S. oryzae covered by a fine mesh cloth for ventilation. The insects were kept at 28 ± 2 °C with a relative humidity of 70 ±5, and maintained on a standard 12:12 h light/dark cycle [1,2]. The culture of D. melanogaster was raised using rearing cages (30×30x30 cm) containing banana as a food source. Adults of T. castaneum, S. oryzae (50% of each sex) and larvae (3rd instar) of D. melanogaster were used in the experiments.

**Insect Bioassay.** To evaluate the effect of different doses (1 mL, 2 mL and 3 mL/Petri dish) of citronella and cinnamon extracts, experiments were conducted using completely randomized design (factorial).

The filter papers (Whatman No. 1) were impregnated with the test extracts at selected doses. The impregnated filter papers were then placed in petri dishes (9 ×1.3 cm) and marked with respective dose of the extracts. After drying (10 minutes), 10 adults of T. castaneum and S. oryzae, while 10 larvae of D. melanogaster were introduced in each petri dish (9 ×1.3 cm) having treated filter papers. Data were recorded for 1, 2 and 3 hours after exposure. The control batch of insects was kept under similar conditions but without any treatment. Similarly, another batch was run as solvent toxicity (alcoholic extraction). The experiment was discarded in case of counting more than 10% dead insects in control and solvent batches. Mortality was assessed by direct observation. The insects were considered dead after observing no leg or antennal movements following gentle probing with a needle. The experiments were repeated ten times for achieving accuracy.

**Statistical analysis.** Data were analyzed using a three-factor (plant extracts, dose and time duration) completely randomized design. Data collected were analyzed statistically with the help of Statistix 8.1 software and means were compared using LSD test at 0.05 level of significance.

**Ethical considerations.** The study and associated protocols were designed based on national ethical legislative rules (according to the updated version of the declaration of Helsinki) [7] and approved by Local Ethic Committee of AWKUM, Buner Campus.

**RESULTS**

In the present investigations, the extracts of citronella and cinnamon gave promising mortality of more than 90% and 80%, respectively at very low concentrations of 2 to 3 mL/cup for three hours of exposure (Tables 1-3).

**Sitophilus oryzae.** Data regarding the efficacy of citronella and cinnamon against S. oryzae is presented in Table 1. The results indicated that the
interaction of Dose*Time*Treatment was highly significant (F_{12, 72}=41.32, p<0.0000) therefore, interaction means were considered instead of simple means. It was observed that cinnamon extract incurred significantly maximum mortality (99.3%) at 3 mL dose for 2 hours of exposure as compared to the solvent (10%) and untreated control (0%). The mortality value of citronella (98.6%) was statistically higher than that of cinnamon but at 2mL/dish with three hours exposure period.

**Table 2** depicts the data regarding efficacy of citronella and cinnamon against *Drosophila melanogaster*. The results revealed that the interaction of Dose*Time*Treatment was highly significant (p<0.0000). In the course of observations, it was recorded that cinnamon extract induced significantly maximum mortality (99.0%) at 2 mL/dish and after 3 hours of exposure as compared to the solvent (06%). No mortality was recorded in the control batch. Similarly, the mortality value of citronella (99.0%) was statistically higher than the cinnamon at the dose of 2mL/dish after three hours exposure period.

**Tribolium castaneum.** The results regarding efficacy of citronella and cinnamon against *T. castaneum* are presented in table 3. Again the interaction of Dose*Time*Treatment was highly significant (p<0.0000). The citronella extract induced significantly higher mortality (99.0%) at 2 mL/dish after three hours of exposure than the solvent (5%) for the same exposure time, while no mortality was recorded in the control batches. The data further showed that mortality value for cinnamon (95.0%) was less than that of citronella. Overall, both the extracts, i.e., citronella and cinnamon showed promising ability to kill all the tested insects.
This study evaluated the efficacy of Cinnamon (Cinnamomum verum J.Presl) and Citronella (Cymbopogon nardus L.) extracts against the two stored-grain insect pest and a fruit fly where they were equally effective causing over 90% mortality to the insects. Higher doses and longer time of application resulted in higher mortality rates of insects. Citronella oil is believed to be an effective repellent for many insects such as cockroaches where it provides 70-87.8% control depending on dose and exposure time [8]. Similarly, Mekonnen et al. 2015 [8] documented the repellency of cream containing 14% citronella oil for about two hours against Aedes aegypti (L.), while another study [9] has also reported that citronella oil (at the rate of 20 μL/cm²) is responsible for the desirable repellency against mosquitoes. The present study also observed that the extract of citronella causes high mortality in all the three tested insects at a lower dose than in the study mentioned above. These findings suggest that citronella is equally effective against a variety of pest insects irrespective of the form in which it is available i.e., oil, extract or cream etc.

The previous work, on citronella, reports mainly its potential repellency, but in the present study, we have determined toxicological evaluation of this plant. Since the insects were actually killed by the extracts; hence, we can claim that citronella extract is having insecticidal property as well. Overall results suggest its wide range protection against a variety of insects irrespective of their method of application or way of extraction. It has also been reported that burning an unscented candle (as a citronella candle) had reduced mosquito biting in the field down to minimum level. Similarly, citronella oil has been tested to increase the quality of Chinese kale by repelling insect pests [10]. The product containing principal ingredients of Eucalyptus has provided significant protection from mosquito’s bites (in the field) by repelling them [8]. Many reports have mentioned that cinnamon extracts in general act as antioxidants. Moreover, in regards to its wide range application, it is also documented that cinnamon extract exhibits a protective capacity against irradiation induced lipid peroxidation in liposomes, hydroxyl radicals and hydrogen peroxide. Similarly, the aqueous extract of cinnamon has been reported to have hepatoprotective nature, and is due to antioxidant effects of its flavonoids [11, 12]. These results suggest for a study determining the potent chemicals/ingredients of these plants and their subsequent molecular study against wide range of pests.

A recent study [1] has carried out toxicological evaluation of the extract of Oostegia limbata against third instar larvae of D. melanogaster. They reported about >85% death rates. Likewise, the current study has observed 95% mortality of the same species when treated with extract of cinnamon and citronella. These finding suggest about sensitivity of D. melanogaster to the plant-derived insecticidal products. A report [13] has also documented the toxicological effects of essential oil of Perovskia abrotanoides (Lamiaceae) against S. oryzae (L.) and T. castaneum (Herbst). They observed a significant mortality, which was then improved well by increasing the concentrations (32, 161, 322, 483 and 645 μL/L air) and time of exposure (from 2 to 15 h). The current study observed quite similar results.

The methanolic extract of Duabanga grandiflora is also effective against adults of T. castaneum and S. oryzae [14]. Significant repellency against S. oryzae was reported ranging between 37 and 83% (at 5 min to 2 h) and from 60 to 100% (at 4 to 24h) after exposure. The exposure period appeared to be the most important factor affecting the repellency of crude (methanolic) extract of D. grandiflora. These results clearly indicate that the tested species are susceptible to botanicals. Although the two experi-

### TABLE 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mL/Cup)</th>
<th>Time duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citronella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20.0 I</td>
<td>98.0 A</td>
</tr>
<tr>
<td>2</td>
<td>40.0 H</td>
<td>99.0 A</td>
</tr>
<tr>
<td>3</td>
<td>60.0 F</td>
<td>99.0 A</td>
</tr>
<tr>
<td>Cinnamon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20.0 I</td>
<td>80.0 D</td>
</tr>
<tr>
<td>2</td>
<td>40.0 H</td>
<td>90.0 C</td>
</tr>
<tr>
<td>3</td>
<td>50.0 G</td>
<td>95.0 B</td>
</tr>
<tr>
<td>Solvent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0 L</td>
<td>5.0 K</td>
</tr>
<tr>
<td>2</td>
<td>0.0 L</td>
<td>7.0 JK</td>
</tr>
<tr>
<td>3</td>
<td>5.0 K</td>
<td>8.0 F</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0 L</td>
<td>0.0 L</td>
</tr>
<tr>
<td>2</td>
<td>0.0 L</td>
<td>0.0 L</td>
</tr>
<tr>
<td>3</td>
<td>0.0 L</td>
<td>0.0 L</td>
</tr>
</tbody>
</table>

**LSD Value**  
(Dose*Time*Treatment) 2.2039
ments have utilized different plant extracts and the subsequent concentrations, the species mortality remain high indicating the high efficacy of plant derived anti-pests substances. The cinnamon and citronella derived bio-pesticides are effective in controlling pests that have developed resistance to synthetic pesticides. These findings suggest about the presence of some potent constituents in the individual extracts possessing different bioactivities (toxicological activities). The isolation and identification of the bioactive compounds in the selected plant extracts are of utmost importance so that their potential application in controlling stored-product pests can be fully exploited. However, the present study has not recorded any data on the repellent effect of these chemical, therefore, considered worth to be tested in future studies. Nevertheless, the insecticidal efficacy of these two extracts are reported here unlike many previous studies.

CONCLUSIONS

The results revealed that the plant extracts have a promising efficacy against the tested insects. Citronella and cinnamon, both were highly potent in causing mortality, which may be due to the presence of some bioactive compounds in their extracts. Thus, it is concluded that citronella and cinnamon can act as effective alternatives to conventional chemicals against these insect pests and are not only repellents but insecticidal as well.

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EXPERIMENTAL STUDY ON SHRINKAGE CHARACTERISTICS OF BENTONITE–SAND MIXTURES CONSIDERING THE ASSOCIATED IMPACT OF SAND CONTENT

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ABSTRACT

Used as cushioning material for nuclear waste storage and isolation pad for refuse landfill, bentonite–sand mixture possesses strong shrinkage characteristics. Its shrinkage characteristics in the process of water loss bear remarkable significance for engineering applications. This paper carried out studies on compacted and water-saturated bentonite–sand mixtures so as to investigate the shrinkage characteristics of the mixtures with various contents of sand and the effect of dry density on shrinkage characteristics of the mixtures. Comparisons were also made among various shrinkage prediction models so as to determine the most suitable one. Considering the associated impact of sand content on the mixtures, we employed accurate mathematical formulas to conduct fitting analysis on the experimental data. As a result, a relational equation between the final void ratio of the mixture and the actual dry density of the bentonite in its original state was established. In addition, the shrinkage process of the mixture was photographed by a high-definition camera in order to obtain the morphological features of the surface cracks, which aid in the analysis of the effect of sand content and dry density on shrinkage-induced cracks in the mixtures. This study can provide some beneficial guidance in designing and calculating the engineering parameters of cushioning materials.

KEYWORDS:
Bentonite-sand mixture, shrinkage characteristics, dry density, shrinkage prediction model, crack pattern

INTRODUCTION

As the world energy crisis escalates, nuclear power, a new source of energy, is finding wider and wider application in a number of fields, with countries across the globe bent on the exploitation and utilization of this power. Accompanied by the increasing share of the nuclear power in the energy mix, the nuclear wastes resulting from nuclear power production are also on the rise. Therefore, the effective disposal of the nuclear wastes has become a pressing issue. At present, geological storage is a good way to dispose the nuclear wastes. First, an underground opening at a depth of over 500 m is constructed; second, the metal tank encapsulating the high-level radioactive wastes is to be placed in the opening; third, the cushioning materials are to be used to fill the void between the surrounding rocks and the tank. It should be noted that the cushioning materials in this application must possess the following properties: low permeability, strong ion exchange capacity, and excellent heat dissipation characteristics.

Bentonite is a kind of nonmetallic ore, which is mainly made of montmorillonite. Montmorillonite is a 2:1 crystal structure characterized as having two silica tetrahedrons sandwiching one alumina octahedral; its cation exchange capacity is due to isomorphic substitution of Mg, Na, and K for Al in the central alumina plane. The substitution of lower valence cations in such instances leaves the nearby oxygen atoms with a net negative charge that can attract cations. Therefore, bentonite possesses strong ion exchange capacity. With low permeability, strong adsorption capacity, excellent cation exchange, and great dilatancy, bentonite–sand mixtures stand out as an optimal cushioning material that can be used in the geological disposal of nuclear wastes. Therefore, it is of great theoretical and engineering significance to study the swelling and shrinkage characteristics of the bentonite–sand mixture in terms of using it to dispose nuclear wastes.

Scholars at home and abroad have carried out a great deal of studies on the drying shrinkage characteristics of the soil. Kowalski investigated the migration mechanism of pore water and concluded that the migration of water in soil results from the interaction between gas and liquid [1]. He further pointed out that during the drying process as the moisture content decreases and matric suction in soil increases the pore air pressure exceeds the pore water pressure, resulting in the migration of pore water in the soil.
under the action of gravity and negative pressure. Hillel. D deemed that three conditions are essential for maintaining the moisture content in the soil, namely, necessary supply of heat on the soil surface, necessary supply of water in the soil, and a lower vapor pressure in the external environment than that at the soil-air interface [2]. Tang carried out shrinkage experiments on the saturated and pasty soil samples, and revealed three stages in the volume shrinkage process, namely, normal shrinkage stage, residual shrinkage stage, and zero shrinkage stage [3]. Chertkov et al., found that the moisture content at the dividing point of the normal shrinkage stage and the residual shrinkage stage at and the dividing point of the residual shrinkage stage and the zero shrinkage stage separately corresponds to the point of admission and shrinkage limit [4, 5]. For aggregated soils with swell-shrink characteristics, Haines, Stirk, and Bronswijk concluded that the drying shrinkage process can be divided into four stages: structural shrinkage stage, normal shrinkage stage, residual shrinkage stage, and zero shrinkage stage [6-8]. Considering the variation of the suction and saturability in the soil’s drying shrinkage process, Liu divided the process into three stages according to the saturability, namely 100% saturability, nearly 100% saturability, and less than 100% saturability [9]. Brooks used the s-Sr curves obtained from experiments to determine the saturability at the point of admission [10]. In regard to the shrinkage mechanism of the swelling soil, scholars at home and abroad attempted to establish a suitable shrinkage model so as to describe the shrinkage process of the swelling soil. Giraldez et al., proposed a GSVC model, a cubic polynomial that reflects the effect of moisture content [11]. McGarry et al., put forward a MM-1 mathematical model describing the three stages in the shrinkage process [12]. With consideration of the zero shrinkage stage, Tariq put forth a four-stage shrinkage model for the soil: structural shrinkage stage, normal shrinkage stage, residual shrinkage stage, and zero shrinkage stage [13]. Considering the effect of dry density on soil shrinkage, Chertkov also proposed a three-stage mathematical model, which is different from the MM-1 model.

Literature review shows that thus far few studies have been carried out to probe into the shrinkage characteristics of bentonite–sand mixtures as a special-purpose engineering material. Subject to the restriction of multiple conditions, the shrinkage process of the mixtures is of great complexity, among which, sand content and dry density have a direct impact on the shrinkage characteristics of the mixtures. In addition, in actual engineering application, the shrinkage characteristics of the mixture are under the influence of various factors. Therefore, it is of great significance to launch systematic investigations into the associated impacts of sand content and dry density on the shrinkage characteristics of the bentonite–sand mixtures. Our study introduced drying shrinkage mechanism and model for the soil, experimental material and method, experimental study of the effect of sand content on the shrinkage characteristics of the bentonite-sand mixture, experimental study of the effect of dry density on the shrinkage characteristics of the bentonite-sand mixture, the associated impact of dry density and sand content on shrinkage-induced cracks in bentonite-sand mixtures in order to elaborate shrinkage characteristics of bentonite–sand mixtures considering the associated impact of sand content comprehensively.

**DRYING SHRINKAGE MECHANISM AND MODEL FOR THE SOIL**

**Drying Shrinkage Mechanism for the Soil.** As a porous and deformable material, soil shrinks with the loss of pore water, and its shrinkage characteristics are closely related to the moisture condition of the soil and the compressibility of the solid matrix. There is now a universal consensus among the researchers that drying shrinkage of the soil is induced by capillary action.

When the soil is unsaturated, the surface of the liquid water in soil pores is arc shaped. This arc shaped water surface is a kind of film that possesses surface tension. Surface tension results from the greater attraction of water molecules to each other (due to cohesion) than to the molecules in the air (due to adhesion). The net effect is an inward force at its surface that causes the water to behave as if its surface were covered with a stretched elastic membrane. Thus, the surface becomes under tension from the imbalanced forces [14].

![FIGURE 1](image.png)

**FIGURE 1**

Schematic diagram for force analysis of the shrink film (two-dimensional)

Fig. 1 illustrates the force analysis of the water surface, where $u_0$ is the air pressure acting vertically on the surface layer of the liquid water, $u_w$ is the pore water pressure, $T_s$ is the surface tension along the tangential direction of the shrink film, and $R_s$ is the radius of curvature of the shrink film. The
difference between $u_s$ and $u_w$ is termed as matric suction, denoted by $S$. According to force balance, such an equation should be satisfied:

$$S = u_s - u_w = \frac{T_z}{R_s}$$

(1)

Given that the shrink film is a three-dimensional surface in actuality, Laplace transformation is carried out on equation (1). Therefore, equation (1) is transformed to equation (2).

$$S = u_s - u_w = T_z \left( \frac{1}{R_1} + \frac{1}{R_2} \right)$$

(2)

Where, $R_1$ and $R_2$ are the radius of curvature of the normal surfaces of the shrink film. It should be noted that as water in the soil evaporates the matric suction increases. Therefore, equation (2) indicates that with the evaporation of pore water the radius of curvature of the curved liquid surface decreases, meaning that soil particles approach each other more closely.

**Drying Shrinkage Model for the Soil.** An establishment of an accurate mathematical equation that describes the drying shrinkage characteristics of the soil in accordance with the experimental data is of great help to the accurate evaluation of the basic properties of the soil and the prediction of the drying shrinkage process. Moreover, the establishment of the shrinkage model is also helpful in guiding engineering practice. Therefore, for the past several decades, various attempts have been made by the scholars to establish a suitable shrinkage model that can faithfully characterize the shrinkage process of the swelling soil.

Earlier in the middle of the 20th century, some scholars had already realized that the shrinkage process of the soil can be divided into three stages, namely, structural shrinkage stage, normal shrinkage stage, and residual shrinkage stage. At that time, McGarry et al., proposed a MM-1 mathematical model.

$$
\begin{align*}
\frac{e - e_o}{\omega_b - \omega_a} + \frac{\omega}{\omega_b} & = 0 & (0 < \omega < \omega_a) \\
\frac{e - e_o}{\omega_b} + \frac{\omega}{\omega_b} & = 0 & (\omega_b < \omega < \omega_a) \\
\frac{e - e_o}{\omega_b} + \frac{\omega}{\omega_b} & = 0 & (\omega_a < \omega < \omega_c) \\
\frac{e - e_o}{\omega_b} + \frac{\omega}{\omega_b} & = 0 & (\omega_b < \omega < \omega_c) \\
\end{align*}
$$

(3)

Where, $e_0$ is the final void ratio in the drying shrinkage process, $e_o$ is the void ratio that corresponds to porous gas, $e_s$ is the void ratio that corresponds to the midpoint of the curve in structural shrinkage stage, and $W_a$, $W_b$, and $W_c$ refers to the point of admission, limit of expansion, and maximum volume water content, respectively.

The above MM-1 model can effectively differentiate the three shrinkage stages. However, this model contains many parameters, and the acquisition of these parameters through experimentation is quite complicated. As a result, McGarry put forward a continuous mathematical function to describe the shrinkage process, which is termed as MM-2 model.

$$e = e_0 + \frac{e_v}{1 + e^{-k(\omega - \omega_c)}}$$

(4)

Where, $e_0$ is the final void ratio in the drying shrinkage process, $e_v$ is the difference between the maximum void ratio and minimum void ratio, and $k$ is the slope parameter related to the point of admission.

With consideration of the zero shrinkage stage, Tariq put forth a four-stage shrinkage model for the soil: structural shrinkage stage, normal shrinkage stage, residual shrinkage stage, and zero shrinkage stage.

$$
\begin{align*}
\frac{e - e_o}{\omega_b - \omega_a} & = 0 & (0 < \omega < \omega_a) \\
\frac{e - e_o}{\omega_b} & = 0 & (\omega_a < \omega < \omega_c) \\
\frac{e - e_o}{\omega_b} & = 0 & (\omega_b < \omega < \omega_c) \\
\frac{e - e_o}{\omega_b} + \frac{\omega}{\omega_b} & = 0 & (\omega_b < \omega < \omega_c) \\
\end{align*}
$$

(5)

Where, $e_0$ is the final void ratio in the drying shrinkage process, $e_o$ is the void ratio at the point of admission, $k_0$, $k_1$, $k_2$, $k_3$, and $k_4$ are the coefficients determined by various boundary conditions; $W_a$, $W_b$, $W_c$, and $W_d$ separately refers to the limit of shrinkage, point of admission, volume water content and maximum volume water content against the limit of expansion.

Considering the effect of dry density on soil shrinkage, Chertkov also proposed a three-stage mathematical model, which is different from the MM-1 model.

$$
\begin{align*}
\frac{e - e_o}{\omega_b - \omega_a} & = 0 & (0 < \omega < \omega_a) \\
\frac{e - e_o}{\omega_b} & = 0 & (\omega_a < \omega < \omega_b) \\
\frac{e - e_o}{\omega_b} + \frac{\omega}{\omega_b} & = 0 & (\omega_b < \omega < \omega_c) \\
\frac{e - e_o}{\omega_b} + \frac{\omega}{\omega_b} & = 0 & (\omega_b < \omega < \omega_c) \\
\end{align*}
$$

(6)

Where, $e_0$ is the final void ratio in the drying shrinkage process; $W_a$ and $W_b$ refers to the volume water content against the limit of shrinkage and point of admission, respectively; $\rho_w$ and $\rho_s$ is water density and dry density of the soil, respectively; $\alpha$ is model parameter.

Although many scholars at home and abroad have put forward various mathematical models, thus far no model is available to universally characterize...
the shrinkage process of soil with different properties. In addition, no study has been conducted to make clear the shrinkage characteristics of bentonite–sand mixtures with different sand contents under the condition of different dry densities.

EXPERIMENTAL MATRICALS AND METHODS

Experimental Material. The bentonite used as the experimental material is taken from Shouguang City, Shandong Province. The main chemical components measured by X-ray fluorescence analysis (XRF) were: SiO₂, 63.17%; Al₂O₃, 17.06%; Fe₂O₃, 4.86%. The grey white bentonite is powder-like and the physical properties and chemical components of the bentonite are given in Table 1. The quartz sand used as the experimental material is taken from Fengyang City, Anhui Province. The granular quartz sand is milk white and has greasy luster. The diameter of the quartz sand used in the experiment is 0.05 mm. The physical properties and chemical components of the quartz sand are detailed in Table 2.

Experimental Method. Experiments were carried out on compacted and water-saturated bentonite–sand mixtures. Prior to the preparation of the mixtures, bentonite and quartz sand were both placed in the 105 drying oven for 12 h. Then, they were taken out and were encapsulated by the plastic bag so as to prevent moisture ingress.

The initial dry density of the compacted and water-saturated mixtures is controlled within a range of 1.2 ~ 1.8 g/cm³. For each experimental group, the content of the quartz sand for each mixture is controlled as 0%, 10%, 20%, 30%, 40%, and 50%. In addition, considering the variation of specific weight of mixtures with various contents of bentonite, we used equation (7) to calculate the specific weight of the bentonite–sand mixtures in accordance with the specific weight of the bentonite and quartz sand.

\[
G_s = mG_{S1} + (1-m)G_{S2}
\]  

Where, \( m \) is the content of the bentonite; \( G_{S1} \) and \( G_{S2} \) separately refers to the specific weight of the bentonite (2.75) in the mixture and that of the quartz sand (2.65) in the mixture.

During the preparation of the compacted and water-saturated mixtures, a certain amount of bentonite and quartz sand were mixed up, followed by the addition of distilled water. The distilled water was carefully added into the mixture so that the target moisture content could be achieved. Secondly, the prepared mixtures were encapsulated by the plastic bag. Thirdly, they were left for 24 h so as to make the water distribute as evenly as possible. Then, a certain amount of mixture was placed in the cutting ring sample kit, followed by compaction. The compaction did not stop until the target sample size and dry density were achieved. Lastly, the mixture was placed in the vacuum cylinder for 2 days so as to ensure that the mixture was fully saturated.

RESULTS

Experimental Study of the Effect of Sand Content on the Shrinkage Characteristics of the Bentonite-sand Mixture. Drying shrinkage experiments were carried out on the compacted bentonite-sand mixtures so as to investigate the shrinkage characteristics of and the crack development in mixtures with different contents of bentonite. The mixtures are fully saturated and the controlled dry density of the mixtures is \( \rho = 1.4 \) g/cm³.

During the drying shrinkage process, measurements were directly made on the mixtures at a constant interval so as to obtain such data as diameter (d) and height (h). With these data, the radial shrinkage strain \( \varepsilon_r \), axial shrinkage strain \( \varepsilon_z \), and volumetric shrinkage strain \( \varepsilon_V \), can be calculated through equation (8).
Where, \( d \), \( h \), and \( V \) are the diameter, height, and volume of the in-test mixture at the measuring point, respectively; \( d_0 \), \( h_0 \), and \( V_0 \) are separately the initial diameter, height, and volume of the mixture.

Fig. 2 shows that the mixtures with different contents of sand exhibit pronounced anisotropic characteristics in the drying shrinkage process. The order of the magnitude of various strains is as follows: volumetric shrinkage strain > axial shrinkage strain > radial shrinkage strain. In the initial stage of drying shrinkage, each shrinkage strain linearly increases as the moisture content decreases, with the volume shrinkage strain in particular. As the moisture content decreases to a certain extent, each shrinkage strain stabilizes at a certain level.

In order to analyze the effect of sand content, we assume the moisture content where the shrinkage strain versus moisture content curve begins to level off as the shrinkage limit moisture content. Therefore, we can obtain the shrinkage limit moisture content for the mixtures with different contents of bentonite. The final radial shrinkage strain, axial shrinkage strain, and volumetric shrinkage strain are also summarized in Table 3. From Table 3, we can see that the shrinkage limit moisture content decreases with increasing sand content and the final radial, axial and volumetric shrinkage strain are on the whole inversely related to the sand content. However, when

\[
\begin{align*}
\varepsilon_r &= \frac{d_0 - d}{d_0} \\
\varepsilon_a &= \frac{h_0 - h}{h_0} \\
\varepsilon_v &= \frac{V_0 - V}{V_0}
\end{align*}
\]

\( (8) \)

![Shrinkage strain curves for mixtures with different contents of sand](image)

**FIGURE 2**

**TABLE 3**

<table>
<thead>
<tr>
<th>Content of bentonite</th>
<th>Shrinkage limit moisture content</th>
<th>Final radial shrinkage strain</th>
<th>Final axial shrinkage strain</th>
<th>Final volumetric shrinkage strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( W_s /% )</td>
<td>( \varepsilon_{rl} /% )</td>
<td>( \varepsilon_{ax} /% )</td>
<td>( \varepsilon_{vl} /% )</td>
</tr>
<tr>
<td>0%</td>
<td>14.34</td>
<td>7.57</td>
<td>11.74</td>
<td>24.63</td>
</tr>
<tr>
<td>10%</td>
<td>16</td>
<td>7.45</td>
<td>10.50</td>
<td>23.39</td>
</tr>
<tr>
<td>20%</td>
<td>16</td>
<td>7.60</td>
<td>11.72</td>
<td>24.65</td>
</tr>
<tr>
<td>30%</td>
<td>19</td>
<td>9.15</td>
<td>11.17</td>
<td>26.70</td>
</tr>
</tbody>
</table>
the mixture has a sand content of 10% and 20%, the various shrinkage strains show an apparent drop. This drop results from the significant uneven shrinkage that occurs to the mixtures. As a result, pronounced cracks are generated, rendering some errors to the measurement of the in-test mixture’s dimension. Therefore, the calculated shrinkage strain is smaller.

Experimental Study of the Effect of Dry Density on the Shrinkage Characteristics of the Bentonite-sand Mixture. The soil’s drying shrinkage process is a process for the rearrangement of the soil particles, which is always accompanied by the changes of the soil structures. Existing studies have shown that the dry density exerts a material influence on the shrinkage characteristics of the soil. In engineering practice, the method of controlling the dry density of the swelling soil is employed to limit the swell and shrink of the soil so as to mitigate or reduce engineering hazards.

In this section, tests were carried out on the mixtures with various matching ratios of bentonite and quartz sand. By controlling different dry densities, detailed comparison can be made on the drying shrinkage process among various mixtures. The comparison can assist in understanding the effect of dry density on the shrinkage and crack development characteristics of the bentonite-sand mixture. In the drying process, measurements were made on the mixtures at a constant interval so as to obtain the diameter and height of the in-test mixture. With these data, the radial shrinkage strain $\varepsilon_r$, axial shrinkage strain $\varepsilon_\lambda$, and volumetric shrinkage strain $\varepsilon_v$, can be calculated through equation (8). Then, the obtained strains were plotted against the moisture contents, which are shown in Figs. 3 ~ 5.

From Figs. 3~5, the following conclusions can be drawn:

1. The shrinkage strain curves for mixtures with different contents of sand bear some resemblance to each other. All the strain curves undergo rapid changes at the initial stage of drying shrinkage. Over 60% of the initial moisture content is lost. At this stage, the slope of the curve is defined as the shrinkage coefficient $\lambda_v$. As the moisture content reaches the shrinkage limit moisture content $W_s$, the strains change slowly and later level off.

2. For mixtures with the same sand content, the final volumetric shrinkage strains all decrease
with increasing dry density. However, similar characteristics are not observed for the axial and radial shrinkage strain when the mixture has a sand content of 10%.

(3) The increase of the dry density is effective in restricting the shrinkage, and the mixtures exhibit pronounced anisotropic characteristics in the drying shrinkage process.

In regard to the third conclusion, the authors surmise that the following three factors are at play: (1) microstructure induced anisotropy resulting from various dry densities; (2) water evaporation path induced anisotropy resulting from various dry densities; and (3) evaporation rate and suction stress induced anisotropy resulting from various dry densities.

Establishment of the shrinkage model considering the associated impact of dry density and sand content. The results obtained from drying shrinkage experiments on bentonite-sand mixtures can be used to establish the relation between the moisture content and the void ratio. However, the experimental results are discrete data, which cannot be directly used in engineering practice. Therefore, analysis is performed on existing shrinkage models and attempts are made to obtain the shrinkage curves in accordance with the experimental results under different conditions so as to select a suitable shrinkage model.

In the bentonite-sand mixture, quartz sand is an inert material. Under the dry condition, the volume of the quartz sand particle remains virtually unchanged. Therefore, in the study of the shrinkage characteristics of the bentonite-sand mixtures, the effect of volume changes of the quartz sand particle is negligible. It is safe to only consider the effect of volume changes of bentonite. As shown in Fig. 6, $V_b$, $V_p$, and $V_q$ separately stands for the volume of bentonite, quartz sand, and pores.

![Graphs showing shrinkage strain versus moisture content](attachment:image)

(a) Axial shrinkage strain versus moisture content (b) Radial shrinkage strain versus moisture content (c) Volumetric shrinkage strain versus moisture content

**FIGURE 4**
Various shrinkage strain curves for mixtures with different dry densities (sand content: 10%)
Denoted as $\rho_{bd}$, the actual dry density of the bentonite in its initial state can be expressed as:

$$\rho_{bd} = \frac{m_b}{V_b} = \frac{\rho_d (1 - \lambda)}{1 - \rho_s \lambda} \quad (9)$$

Where, $m_b$ is the mass of the bentonite, $\rho_d$ is the initial dry density of the mixture, $\lambda$ is the mass percentage of the quartz sand, and $\rho_s$ is the density of the quartz sand.

As can be seen from equation (9), the actual dry density of the bentonite is co-determined by the mass percentage of the quartz sand ($\lambda$) and the initial dry density of the mixture ($\rho_d$). Therefore, $\lambda$ and $\rho_d$ also determine to what extent the mixtures can shrink. According to the experimental data, a relational equation between the final void ratio of the mixture ($e_0$) and the actual dry density of the bentonite in its original state ($\rho_{bd}$) is established, which is shown in Fig. 7.
Fig. 7 clearly shows that the final void ratio of the mixture \( (e_0) \) diminishes with the increase of the actual dry density of the bentonite in its original state \( (\rho_{bd}) \). In addition, with the increase of the \( \rho_{bd} \), decrease of the \( e_0 \) attenuates. The reasons are as follows: 1) content of sand in the mixture reduces and content of bentonite in the mixture increase as \( \rho_{bd} \) increases; as a result, the mixture exhibits stronger shrinkage characteristics; 2) as \( \rho_{bd} \) increases to a certain level, the extent of shrinkage of the mixture is restricted and the shrinkage characteristics of the mixture weaken and are even restricted.

**The Associated Impact of Dry Density and Sand Content on Shrinkage-induced Cracks in Bentonite-sand Mixtures.** The shrinkage process of the mixture was photographed by a high-definition camera to obtain the morphological features of the surface cracks. The typical photographs at some point were selected so as to study the correlation of the shrinkage-induced cracks with dry density and sand content. The selected photographs are shown in Fig. 8.

It can be seen from Fig. 8 that the crack pattern of the mixture is directly related to the dry density and sand content.

(1) Comparisons among figures a1, b1 and c1, among figures a3, b2 and c2, and between figures d2 and e1 demonstrate that at the same dry density shrinkage-induced cracks are much less developed as the content of gold in the mixtures increases. Therefore, it can be concluded that presence of bentonite plays a critical role in the growth of the cracks in the mixtures during the drying shrinkage process.

(2) A separate comparison among figures a1, a2 and a3 and among figures b1, b2 and b3 shows that at the same sand content the increasing dry density to some extent helps restrict the growth of the cracks in the mixtures. With a low dry density, cracks penetrate deeply into the mixtures and the mixtures buckle; with a high dry density, the number of cracks increases, but in most cases these cracks are short and slender ones.

(3) Comparisons among figures c3, d2 and d3 reveal clearly that as the dry density increases to a certain level less or even no cracks are observed in the mixture. A comparison between figures d and e shows that as the content of sand in the mixtures increases to 40% virtually no cracks are generated in the drying shrinkage process.

**CONCLUSIONS**

(1) This study delves deep into the shrinkage mechanism of the bentonite-sand mixture, which provides theoretical support for microanalysis of the shrinkage of bentonite in mixtures. In addition, this study also presents a systematic analysis of the existing drying shrinkage models for soils.

(2) At the same dry density, the shrinkage strains of the bentonite-sand mixture are inversely related to the content of sand in the mixture. The shrinkage limit moisture content decreases with increasing sand content and the mixtures with different contents of sand exhibit pronounced anisotropic characteristics in the drying shrinkage process. The order of the magnitude of various strains is as follows: volumetric shrinkage strain > axial shrinkage strain > radial shrinkage strain.

(3) For mixtures with the same sand content, the final volumetric shrinkage strains all decrease with increasing dry density. However, similar characteristics are not observed for the axial and radial shrinkage strain when the mixture has a sand content of 10%.

![Graph delineating the relation between the final void ratio of the mixture and the actual dry density of the bentonite](image-url)
FIGURE 8
Typical photographs taken during the drying shrinkage process
(4) The shrinkage strain curves for mixtures with different contents of sand bear some resemblance to each other. All the strain curves undergo rapid changes at the initial stage of drying shrinkage. As the moisture content reaches the shrinkage limit moisture content $W_s$, the strains change slowly and later level off.

(5) The final void ratio of the mixture ($e_0$) diminishes with the increase of the actual dry density of the bentonite in its original state ($\rho_{bd}$). In addition, with the increase of the $\rho_{bd}$, decrease of the $e_0$ attenuates.

(6) At the same dry density shrinkage-induced cracks are much less developed as the content of gold in the mixtures increases and at the same sand content the increasing dry density to some extent helps restrict the cracks from growing in the mixtures.

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DEVELOPMENT OF SURFACE IRRIGATION USING SURGE IRRIGATION TECHNIQUE

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²Post graduate student

ABSTRACT

A field experiment was conducted at Research Farm, Faculty of Agriculture, Kafrelsheikh University, Egypt, during successful growing season 2016/2017. Its location at the Northern part of the Nile Delta, 31° 07' N Latitude and 30° 57' E Longitude and 6 meters above sea level. The experiment was included two different irrigation methods (surge with cycle ratio 1/2 and continuous irrigation), three furrow lengths were used 20, 30 and 40 m and three different inlet discharges were used, (12.24, 24 and 44.4 U/min) to irrigate the maize crop. The results can be summarizing as follows: surge irrigation caused to decrease in mean advance time about 11%, also obtained the highest mean value of water use efficiency was 70 kg/m³ at flow rate of 12.24 U/min and furrow length of 40m. The highest mean value of grain yield (3920 kg/fed) at flow rate of 12.24 U/min and furrow length of 40m under continuous irrigation, also the highest mean value of applied water was 2430.89 m³/feddan achieved at flow rate of 12.24 U/min and furrow length of 30m with continuous irrigation. The inflow rate treatment of 44.4 U/min achieved the highest value of mean water saving by 14.47 % under surge irrigation technique.

KEYWORDS:

INTRODUCTION

The traditional irrigation system caused to lose a large amount of water applied, so it has a little water use efficiency. As a result, Egypt suffers from scarcity of severe water in the present years whereas, the Nile River is the main source for fresh water, also Egypt’s agricultural sector consider one of the highest water consuming sectors. We needed to overcome the problems of irrigation and planted crops which consumed little amount of water to save more water by trying to replace the agriculture of rice which need high amounts of water requirements by agriculture of maize.

Maize is more sensitive to water and don’t need a large amount of water as rice. It is the third important plant after wheat and rice in Egypt.

So the study mentioned that the effect of irrigation system on Maize productive. This field experimental studied surge irrigation system due to save more water and got high water use efficiency in addition to study the soil moisture and soil salinity such as several studies as, the study that compared the different inflow rates between surge and continuous irrigation systems and found that the 4 l/s/m inflow rate obtained higher values of distribution efficiency than with the 3 l/s/m inflow rate [1].

Also, the surge flow improved the uniformity of soil moisture distribution better than that with the continuous flow due to less amount of irrigation water applied and less water losses by deep percolation. The surge flow on alternate furrows achieved the best irrigation water productivity (0.61 kg/m³) and reduced irrigation water use by 44% [2]. Also, surge irrigation obtained high efficiency in clay soil, reduced percolation and achieving better uniformity in soil moisture distribution in several studies, the soil moisture increased when irrigation depth increased, at the time following the irrigation increased, the relationship between soil moisture and irrigation depth decreased [3] The soil moisture content was reduced under sub-soiling treatment that obtained greater soil moisture depletion and more concentration root system than rotary-tillage and no-tillage treatments in deep soil profile. Therefore, the spatial distribution of root density, moisture content and N status has been improved by sub-soiling treatment [4].

The water salt distribution improved using surge irrigation than continuous irrigation [5]. Data studied the effect of salinity of irrigation water on maize yield using water had (0.8 and 4.55 dsm-1) of electrical conductivity and found that the treatment which had high salinity obtained maize yield lower than that obtained lower salinity of irrigation water [6]. In addition to the treatment of the study with high salinity of water obtained the lowest value of plant height, it was recorded (141.4 cm) and the lowest value of stem diameter, it was recorded (2.36 cm) while, the fresh water treatment obtained the highest value of plant height, it was recorded (144.5 cm), also obtained the highest value of stem diameter, it was recorded (2.58 cm) [7]. Surge irrigation obtained the highest water saving value compared with cutback
Factors of the experiment. The study included in the following factors:

Irrigation System. The experimental field was surge and continuous irrigation systems. The land was ploughed with plowing width was 2.5 m and 20 cm plowing depth. The furrows length was 90 m with 0.7 m distance between them.

Control unit. The pump 5hp with gasoline engine and 900 l/min inflow rate at 3600 rpm under 26 m water head and 75 mm inside diameter of water outlet.

Pipes. Main tube made of PVC with 63 mm diameter and 90 m length. The main pipe connected with three sub-main tubes with 63 mm diameter and 15 m length made from poly using T-Shape and many surge valves with 16 mm diameter connected with short poly pipe.

Estimation of water requirements. Water consumptive use is the total amount of water used in evapotranspiration using the following formula as [11]:

\[ ET_{\text{crop}} = ET_0 \times K_c \]  

Where:
- \( ET_{\text{crop}} \): Crop water consumptive use, (mm/day).
- \( ET_0 \): Reference evapotranspiration, (mm/day), and
- \( K_c \): Crop coefficient, (dimensionless).

Evapotranspiration of maize crop was calculated using FAO CROPWAT program as [12], depending on the average of climatic data according to Penman – Monteith methods as shown in Table 2. The water consumptive use was presented in Table 3.

Applied irrigation water. The applied amount of irrigation water was calculated with the following formula as [13].

\[ Q = q \times t \]  

Where:
- \( Q \): Applied irrigation water (m³/fed.); \( q \): Discharge (m³/min) and \( t \): Total irrigation time (min/fed.).

---

### TABLE 1

**Mechanical analysis of soil.**

<table>
<thead>
<tr>
<th>Soil depth, cm</th>
<th>Particle size distribution</th>
<th>Soil Texture</th>
<th>Field capacity (F.c) %</th>
<th>Permanent wilting point (pwp)%</th>
<th>Available water (Aw), %</th>
<th>Bulk density g/m³</th>
<th>Organic Matter (O.M) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>57.05</td>
<td>Clayey</td>
<td>43</td>
<td>22</td>
<td>21</td>
<td>1.14</td>
<td>1.90</td>
</tr>
<tr>
<td>20-40</td>
<td>59.04</td>
<td>Clayey</td>
<td>40</td>
<td>21</td>
<td>19</td>
<td>1.24</td>
<td>2.00</td>
</tr>
<tr>
<td>40-60</td>
<td>60.32</td>
<td>Clayey</td>
<td>39</td>
<td>21</td>
<td>18</td>
<td>1.32</td>
<td>2.04</td>
</tr>
<tr>
<td>Mean</td>
<td>58.80</td>
<td>Clayey</td>
<td>40.66</td>
<td>21.3</td>
<td>19.33</td>
<td>1.23</td>
<td>2.04</td>
</tr>
</tbody>
</table>
FIGURE 1
Layout of experimental field

TABLE 2
Monthly mean values of some meteorological data during Maize growing season 2016/2017.

<table>
<thead>
<tr>
<th>Country</th>
<th>Meteo Station</th>
<th>Altitude (metre)</th>
<th>Coordinates</th>
<th>Month</th>
<th>Max. Temp. (°C)</th>
<th>Min. Temp. (°C)</th>
<th>Humidity (%)</th>
<th>Wind speed km/day</th>
<th>Sunshine hours/day</th>
<th>Solar radiation MJ/m²/day</th>
<th>Total rainfall (mm)</th>
<th>ET₀ (mm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGYPT</td>
<td>Sakha</td>
<td>20</td>
<td>31.11 N.L</td>
<td>30.95 E.L</td>
<td>June</td>
<td>32</td>
<td>17</td>
<td>58</td>
<td>130</td>
<td>10.8</td>
<td>26.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>July</td>
<td>34</td>
<td>19</td>
<td>63</td>
<td>112</td>
<td>10.5</td>
<td>25.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aug.</td>
<td>33.5</td>
<td>18.3</td>
<td>67</td>
<td>112</td>
<td>10.2</td>
<td>24.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sep.</td>
<td>32</td>
<td>17.6</td>
<td>71</td>
<td>95</td>
<td>9.5</td>
<td>21</td>
<td>0</td>
</tr>
</tbody>
</table>

Reference evapotranspiration ET₀ according to Penman – Monteith

TABLE 3
Calculated water consumptive use for Maize crop.

<table>
<thead>
<tr>
<th>Growth stages</th>
<th>Et₀ (mm/day)</th>
<th>Kₑ</th>
<th>Et_crop (mm/day)</th>
<th>Et_crop period(mm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>5.69</td>
<td>0.3</td>
<td>1.707</td>
<td>34.14</td>
</tr>
<tr>
<td>1/6/2016- 20/6/2016</td>
<td>5.46</td>
<td>1.15</td>
<td>6.279</td>
<td>313.59</td>
</tr>
<tr>
<td>Mid-season</td>
<td>5.46</td>
<td>1.15</td>
<td>6.279</td>
<td>313.59</td>
</tr>
<tr>
<td>End / late</td>
<td>4.31</td>
<td>1.05</td>
<td>4.5</td>
<td>144</td>
</tr>
<tr>
<td>10/8/2016- 10/9/2016</td>
<td>4.31</td>
<td>1.05</td>
<td>4.5</td>
<td>144</td>
</tr>
<tr>
<td>Et_crop (mm/ season)</td>
<td>12.49</td>
<td></td>
<td>492.09</td>
<td></td>
</tr>
</tbody>
</table>
Soil moisture content. Soil moisture content was measured by the gravimetric method, as [14]. Screw auger was used to collect soil samples before and after the irrigation, from three different locations in the beginning, middle and end of each treatment at four different Soil layers. Soil samples were weighted and dried in an oven at 105°C for 24 hours and using the following equation to calculate soil moisture content:

\[ MC = \frac{W_w}{W_d} \times 100 \quad \quad (3) \]

Where:
- MC: Moisture content (%);
- \( W_w \): Water weight (g);
- \( W_d \): Dry weight (g).

Soil salinity. Soil salinity was measured before planting and after harvesting, 20 gram of soil sample put on 100 gram of distilled water to make extract 1:5 then shaken the sample for 30 minutes using shaker device and filter this extract by filtration papers, finally measured Electrical Conductivity (EC) by the conductivity meter device model 4520.

Water saving. This study aimed to calculate the water saving as a ratio to know the benefit of surge irrigation for saving the water using the following formula [10].

\[ \text{Water saving(\%)} = \frac{V_c - V_s}{V_c} \times 100 \quad \quad (4) \]

Where:
- \( V_c \): Water volume under continuous irrigation per season (m³/fed) and \( V_s \): Water volume under surge irrigation per season (m³/fed).

Grain yield. The grain yield was estimated from (samples) 10 metres of the middle of each treatment to avoid border effect. Samples from each furrow (10 m long × 0.7 m space = 7 m²). The average yield per fadden (4200 m²) was multiple the yield by per m² by 4200.

Water Use Efficiency (WUE). Water use efficiency used to describe the relationship between crop production and the total amount of water used [14].

\[ \text{WUE} = \frac{Y}{WR} \quad \quad (5) \]

Where:
- WUE: Water use efficiency (Kg/m³);
- \( Y \): Grain yield (kg/fed) and \( WR \): Total amount of water requirement (m³/fed.).

Statistical analysis. The effect of treatments on water applied, grain yield, water consumptive use efficiency and water use efficiency were analyzed using 3-Way Completely Randomized design, using three treatments with three replicates per each treatment. Collected data in this study were analyzed and examined statistically using analysis of variance (ANOVA) from the Statistical Analysis System according CoStat to program for windows. Means were compared by LSD test at 1% level of significance. The mean values of each treatment were designated by letters (a, b, c, etc.) which represent the significance degree of the difference between the means. Means represented by two letters indicate that the difference is not significant or weakly significant.

RESULTS AND DISCUSSION

Advance time (AT). Data examined the effect of all treatments on advance time as shown in Figure 2. The results indicated that the 44.4 l/min inflow rate treatment tended to decrease the advance time under surge irrigation system about of (78 and 54) % compared with (12.24 and 24) l/min inflow rate respectively. The 20 m furrow length caused reduction in mean values of advance time about 33.9 and 50.7 % compared with 30, 40 m furrow lengths respectively. Surge irrigation caused to decrease the advance time with different lengths that reduction recorded 11 % compared with continuous irrigation. This data might be occurred due to the partial seal layer which formed due to surge flow and it was led to faster water movement, in addition to cycle ratio that was 1/2, this made the off time long enough to allow to the water to infiltrate before the second surge start.

Applied irrigation water (Wa). The data of water applied for each treatment during the growing season collected from the study and acts in Table (3) and shown in Figure 3. The lowest value of water applied obtained by 20 m treatment with 44.4 l/min inflow rate it was recorded 1785.27 m³/fed/season under surge irrigation system, while, the highest value of water applied obtained by 30 m treatment with 12.24 l/min inflow rate, it was recorded 2430.893 m³/fed/season under continuous irrigation. The mean values of 30 m furrow length treatment tended to decrease the advance time as shown in Figure 2. The results indicated that the 44.4 l/min inflow rate treatment obtained the highest value of mean water saving, it was recorded 14.47 %. It was achieved water saving about 36.9, 34.4 %
compared with 12.24, 24 $\ell$/min inflow rate respectively. The 20 m furrow length treatment obtained the highest mean values of water saving, it was recorded 12.6 %, it obtained water saving about 3.5, 34.12 % than 30, 40 m furrow lengths. Surge irrigation system saved water more than continuous irrigation system. This result might be occurred due to surge flow had less infiltration and percolation than continuous flow. These results are agreement with those obtained [15].

Soil moisture content (SMC). Data of SMC collected from the soil layers of the experimental. The up surface layers obtained higher moisture content than the under layers after irrigation. This result may be occurred due to the remain time of the water was longer at the surface soil layer than under soil layers, while before irrigation the under soil layers obtained higher moisture than the surface soil layers. Similarly, with that obtained by [13], [16]. The moisture content obtained higher values in the same furrow with the first and end of the border, while on the middle of the border the moisture content obtained low value as illustrated from Figure (5) to (7), due to the water applied was more in the first of the furrow and collected on the end of the furrow after moved faster across the middle of the furrow.

![FIGURE 2](image1.png)
**FIGURE 2**
Effect of inflow rate on advance time for surge (A) and continuous (B) flow irrigation systems under 20, 30, 40 m furrow lengths.

![FIGURE 3](image2.png)
**FIGURE 3**
Effect of surge(A) and continuous irrigation systems on water applied under 20, 30, 40 m furrow lengths
TABLE 4
Effect of treatments on WUE, Y, ECU and Wa

<table>
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<tr>
<th>irrigation system</th>
<th>inflow rate, Ė/min</th>
<th>furrow length, m</th>
<th>WUE, kg/m³</th>
<th>ECU, %</th>
<th>Y, kg/fed</th>
<th>Wa, m³/fed/season</th>
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</table>

L.S.D at 5% level 0.0203 4.5565 363.96 4.2016

-Numbers within a column with the same letter are not significantly different at 0.01% level by LSD.

TABLE 5
Effect of surge irrigation system on water saving

<table>
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<th>Water saving for surge irrigation system (%)</th>
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<th>20 m furrow length</th>
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<th>40 m furrow length</th>
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<td>44.4</td>
<td>19.14</td>
<td>16.36</td>
<td>7.9</td>
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</table>

FIGURE 4
Effect of inflow rates (A) and furrow lengths (B) on water saving

Soil Salinity (SS). The 44.4 Ė/min inflow rate obtained high values of soil salinity. This data may be occurred due to the water moved faster in less time and the lower infiltration caused in lower salt leaching. The soil salinity mean values recorded 4.262, 3.81 and 4.534 dS/m with 12.24, 24 and 44.4 Ė/min inflow rate, respectively. In addition to, the values of SS differed from point to another of the furrow length where the first of 40 m furrow length obtained the lowest values of soil salinity (2.36 dS/m) under 24 Ė/min inflow rate after irrigation, while the highest value obtained from the end of 40 m furrow length under 12.24 Ė/min inflow rate after irrigation (7.29 dS/m). This data might be attributed due to using amount of water applied for leaching requirement as illustrated in Figure 8. This agreement with [17].
**FIGURE 5**
Distribution of soil moisture content along the 20, 30, 40 m furrow length in the 12.24 l/min inflow rate.

**FIGURE 6**
Distribution of soil moisture content along the 30, 40 and 20 m furrow length in the 24 l/min inflow rate.
FIGURE 7
Distribution of soil moisture content along the 20, 30 and 40 m furrow length in the 44.4 l/min inflow rate

FIGURE 8
Distribution of soil salinity before (A) and after (B) irrigation at 20, 30, 40 m furrow lengths.

FIGURE 9
Effect of surge (A) and continuous (B) irrigation systems on yield under 20, 30, 40 m furrow length
Grain yield. Data in the previous Table 4 indicated that effect of study factors on grain yield. Continuous irrigation system obtained higher value of production than another irrigation system, it was recorded 2800.37, 3073.3 kg/fed under surge and continuous irrigation respectively. This data indicated and illustrated in Figure 9. The inlet inflow rate was effected on yield whereas 12.24 l/min inflow rate recorded increasing in mean values of yield about 17.95, 26.7 % than 24, 44.4 l/min inflow rate. The 40 m furrow length obtained higher mean values about 21.15, 8.59 % than 20, 30 m furrow lengths. These results might be attributed due to soil moisture and water applied which recorded high values of these treatments. This agreement with [18].

Water use efficiency (WUE). Data of WUE investigated significantly differences among all of treatments as presented in Table 4 and illustrated in Figure 10. Surge and continuous irrigation systems were recorded 1.36 and 1.29 kg/m³ respectively. 12.24 l/min inflow rate obtained the highest value of WUE (1.43 kg/m³), it was obtained mean values more than 24, 44.4 l/min inflow rate about 11.19, 10.49 % respectively. This result was agreement with [17]. Surge irrigation system obtained the highest value of WUE (1.63 kg/m³) with 40 m furrow length under 12.24 l/min inflow rate, while the lowest value of WUE obtained by continuous irrigation system, with 20 m furrow length under 44.4 l/min inflow rate (1.05 kg/m³). This result may be attributed due to the rapidly of advance rate for the wetting front.

DISCUSSION

Surge irrigation caused reduction in advance time with different lengths about 11 % compared with continuous irrigation due to the partial seal layer which formed by surge flow, these results are agreement with those obtained by [4]. The 44.4 l/min inflow rate treatment caused reduction in mean values of water applied about 14, 3.8 % compared with 12.24, 24 l/min inflow rate treatments due to the increasing inflow rate lead to increasing in water velocity, therefore the set time under the large inflow was lower than under the little inflow, these results are agreement with those obtained [9]. Surge flow saved water more than continuous flow due to surge flow had less infiltration and percolation than continuous flow, this result agreement with those obtained by [15]. The 40 m furrow length with the 12.24 l/min inflow rate which located in the end of the field experiment recorded 7.29 ds/m of electrical conductivity and obtained little fresh grain yield than another cultivated region, that results occurred due to high salinity and the long distance due to more lost water also only little amount of water consumptive by the plant, this occurred reduction in fresh grain yield due to maize is relatively sensitive to salinity. These results are similar to the study of [7]. Surge flow obtained WUE more than continuous flow, it was recorded 1.36 and 1.29 kg/m³ respectively, this result may be attributed due to more rapid advance rate for the wetting front, this result agreement with [10].

CONCLUSIONS

It has been concluded that:
Surge irrigation can be used to reduce water consumption and obtained the highest mean value of water use efficiency. Also, the study showed that the high flow rate achieved reduction of advanced time due to the velocity of water movement, this achieved percolation little than the lower flow rate.
ACKNOWLEDGEMENTS

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REFERENCES


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STUDY ON THE CORRELATION BETWEEN OCCURRENCE CHARACTERISTICS OF MOVABLE FLUIDS AND MICROSCOPIC PORE STRUCTURE OF CHANG 8 FORMATION RESERVOIR IN CENTRAL ORDOS BASIN

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ABSTRACT

The study of movable fluids in low permeability reservoir has a very important role for oil recovery. On the basis of the rock thin section observation, scanning electron microscopy observation, constant speed mercury penetration and nuclear magnetic resonance technology, microscopic pore structure and movable fluids occurrence characteristics of Chang 8 oil layer are studied. The results indicate that the reservoir is mainly composed of fine grain feldspar sandstone and lithic arkoses sandstone, and the interstitial materials are mainly carbonate minerals and clay minerals. The pore types of the reservoir mainly include intergranular pore and feldspar corrosion pore. The reservoir can be divided into two categories: mercury injection expulsion pressure<0.5Mpa with right peak nuclear magnetic T2 spectrum; mercury injection expulsion pressure >0.5MPa with left peak nuclear magnetic T2 spectrum. The sorting coefficient, median radius and maximum pore throat radius have a positive influence on the porosity of movable fluids.

KEYWORDS:
Pore structure, Movable fluid, Low permeability reservoir, Chang 8 oil layer, Ordos Basin

INTRODUCTION

Located in central China, the Ordos Basin is predominantly a Mesozoic depressed basin developed in the Palaeozoic North China Craton with a Proterozoic crystalline basement. Affected by late tectonic movements, the basin is an irregular rectangle with an area of 25×10^4Km^2 [6-9]. The whole basin is divided into six secondary tectonic units, the Yimeng Uplift, Yishan Slope, Weibei Uplift, Jinxixi Flexural Zone, Tianhuan Depression and Western thrust Zone. Influenced by Indosinian orogeny, during the period of Yanchang Formation, the southern Ordos Basin proved to be an intracontinental foreland basin and developed a fluvial-lacustrine sedimentary system [10].
According to sedimentary cycles and lithological assemblages, the Triassic Yanchang formation is divided into ten oil layers, from the bottom to the top named as Chang10 to Chang1, showing an integrated record of progradation-aggradation-retrogradation cycle [10, 11]. The study area which is located in the middle of the Yishan slope between Jingbian city and Zhidan city. The study area is generally located in the delta front of Zhijing river delta system, with the northern provenance as the main source [12, 13]. Chang 8 deposits are mainly delta front deposits, vertical subaqueous distributary channel and interdistributary bay alternately. Its lithology is mainly gray-green siltstone.

**FIGURE 1**

*Interstitial material of Chang 8 oil layer.*

((a)9#-4,1876.6m, calcite crystals filled between particles; (b)10#-1,2001.3m, feldspar solution pore, iron calcite filling pore; (c)15#-3,1706.4m, lamellar kaolinite aggregates filled between clastic particles; (d)6#-3,1975.87m, chlorite and illite in intergranular pore; (e)12#-4,2132.3m, chlorite film filling pore, intergranular pore development; (f) 9#-4,1876.6m, feldspar solution pore; (g)13#-3,1825.35m, intercrystalline pore; (h)14#-1,2051.67m, iron calcite filling pore, iron dolomite metamorphic clastic, microfissures developed, feldspar solution pore.)
SAMPLES AND METHODS

A total of 40 samples from 15 wells were selected for the identification of casting slices. Image analysis method is used to measure pore structure. The test methods were in accordance with SY/T6103-2004. The measurement were conducted on JS-2 rock pore casting instrument.

Ten rock samples were analyzed by scanning electron microscopy. The test methods were in accordance with SY/T5162-2014 and SY/T6189-1996. Testing instrument is scanning electron microscope (JSM-5500LV) and X-ray energy spectrometer (QUANTAX400). The experiment temperature is 20 °C with 50% humidity.

For analysis of High Microscopic pore structure, 7 samples were performed pressure mercury injection experiment. The experiments were conducted on a 9505 mercury injection instrument. The sample size is 25cm×25mm×25mm. The Test methods were in accordance with GB/T29171-2012.

Nine cores were tested and analyzed by nuclear magnetic resonance (NMR) technique. The experimental equipment was Newmai-NMR. The temperature was 32°C and the field strength was 0.5T. The test methods were in accordance with SY/T 6490-2000.

RESULTS

Reservoir petrological characteristics. The core samples were analyzed by thin slice and conventional physical properties. As shown in Figure 1, the interstices are mainly carbonate minerals and clay minerals, the main types of interstitial composition are calcite, ferrocacite, illite, chlorite, kaolinite, silicous, dolomite, etc. Carbonate rock minerals are mainly calcite [Figure 1(a)] and iron calcite [Figure 1(b)], and clay minerals are mainly chlorite [Figure 1(c)], illite [Figure 1(d)] and kaolinite [Figure 1(e)]. It directly affects the movable fluid characteristics of the reservoir.

Microscopic pore structure characteristics. The difference of pore structure determines the state of fluid occurrence and percolation law [14]. The porosity is concentrated at 4-10% and permeability is 0.01-1mD, and the reservoir heterogeneity is strong.

Pore type characteristics. The pore types of Chang 8 reservoir are mainly intergranular pore [Figure 1(d) and Figure 1(e)], feldspar solution pore [Figure 1(b) and Figure 1(f)], intergranular pore [Figure 1(g)] and lithic solution pore [Figure 1(h)], occasional microcracks [Figure 1(h)]. According to the identification of elastic rock slices, the main pore types are feldspar dissolved pores and intergranular pores.

Pore throat structure characteristics. The characteristics of pore throat structure include the combination and matching of pore and throat. Because the combination and matching characteristics of pore throat determine the connectivity between pores, the connectivity between pores directly determines the validity of pores [15-17].

Pore throat quantitative characteristic parameter. The quantitative parameters of pore throat were obtained by mercury injection experiments. Pore throat characteristics parameter was quantitatively analyzed. Skewness is in the range of 0.77-2.2. Pore throat sorting coefficient is in the range of 1.25-4.91. Coefficient of variation is in the range of 0.04-0.21. Median pressure is in the range of 0.92-91.2Mpa. Median radius is in the range of 0.01-0.8μm. Replacement pressure is in the range of 0.2-2Mpa. Maximum pore throat radius is in the range of 0.06-1.52μm. Maximum mercury saturation is in the range of 57.24-99.38% and mercury removal efficiency is in the range of 27.29-33.84%.

![FIGURE 2](image)

(a) Mercury injection curves of Chang 8 oil layer. (b) Distribution of Pore Throat of Chang 8 oil layer

(According to the shape of mercury injection curve and the distribution characteristics of roar radius, the reservoir can be divided into two categories.)
Characteristics of capillary pressure curve. From the mercury injection experiment of core samples of sandstone reservoirs in the study area, capillary pressure curves are obtained [Figure 2(a)]. Combining with the distribution characteristics of reservoir pore throat radius [Figure 2(b)], the Chang 8 reservoirs in study area can be divided into two categories: I and II.

As can be seen in Table 1, for I reservoir, the capillary pressure curve of mercury injection is less than 0.5Mpa and distributes in the range of 0.2-0.3Mpa, the average value is 0.23Mpa, the average value of pore throat radius is 1.32μm, the average porosity is 7.1%. The permeability is 0.496mD and the distribution is between 0.223mD and 0.931mD. The frequency curve of pore throat distribution is single-peak, and the peak value of the curve is inclined to the right end [Figure 2(b)], indicating that the proportion of coarse pore throat is large. The maximum mercury saturation was 96.1%, between 93.16% and 99.38% (Table 1).

In Table 1, the displacement pressure of mercury capillary pressure curve of type II reservoir is higher than that of class I, and the distribution is between 0.8 and 2.0Mpa, the average value is 1.45Mpa, indicating that the maximum pore throat radius becomes smaller, the average value is 0.14μm, and the reservoir performance is degraded; The average porosity is 6.7%, the average permeability is 0.169mD, and the distribution is 0.025-0.465mD; the middle part of the curve is relatively inclined, which indicates that the reservoir rocks are dispersed; The distribution frequency curve of pore throat is single-modal, the peak of curve is larger than the left end [Figure 2(b)], and the proportion of pore throat is larger. The maximum mercury saturation is 60.6%, between 57.0% and 71.3% (Table 1).

Reservoir movable fluid variation characteristics. Fluid occurrence in reservoir consists of bound fluid and movable fluid. Bound fluid is a fluid in bound state when the radius of the pore throat is small, which is hindered by capillary pressure and viscous force. The relative free state fluid is movable fluid [18, 19]. Nuclear magnetic resonance (NMR) technique can effectively reflect the occurrence of reservoir fluid and determine the content of movable fluids. [20, 21].

<table>
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<th>Characteristic parameter</th>
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<th>Class II reservoir</th>
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FIGURE 3
(a) Distribution of interval porosity component of NMR T2 saturated water from Chang 8 oil layer.
(b) Distribution of interval porosity component of NMR T2 unsaturated water
(Compared with T2 spectrum distribution before and after centrifugation, the distribution and size of pore roar in different samples vary greatly. After centrifugation, the fluid in the roar of the large hole is easier to come out from the roar, but the fluid in the roar of the small hole is difficult to flow.)
Nuclear magnetic resonance (NMR) technique was used to examine 9 cores of Chang 8 reservoir. The saturated formation water and centrifugal core were measured by NMR $T_2$ spectrum, respectively, and $T_2$ cutoff values and movable fluid parameters were obtained. According to Figure 3(a), the sample’s $T_2$ spectrum frequency curve may be divided into three classes. Sample 1#-1, 2#-2, 3#-1, 7#-2, and 9#-3 belong to first class which has the characteristic that the bimodal left peak is higher than the right peak. Sample 4#-3 and 6#-1 is the second class in which the bimodal right peak is higher than the left peak; while sample 5#-2 and 8#-1 is the third class where the right peak develops a weak pattern.

The samples with higher left peak than right peak show poor reservoir physical properties, small pore throat radius, pore throat distribution in the corresponding pore throat range of 0.05-1000ms, mainly concentrates between 0.2-100ms, pore throat distributes in the range of pore throat corresponding to 0.05-950ms, which mainly concentrates between 0.5-900ms, the right peak develops weak type sample, and reservoir physical property is poor. The range of pore throat corresponding to 0.05-650ms distribution is mainly between 0.3-200ms.

Characteristics of movable fluid distribution. According to the peak shape of $T_2$ spectrum, relaxation time and amplitude, the movable fluid quantity in different pore types can be evaluated [21, 22]. The parameters of movable fluid are quite different and the heterogeneity is strong. The $T_2$ cutoff value of the experimental sample is in the range of 1.32-57.22ms, the distribution range of movable fluid saturation is between 10.73% and 56.62%, and the average movable fluid saturation is 32.22%. Saturation range of movable fluid is 5.28, the porosity of movable fluid is 0.33% and 7.58%, the average is 2.5%, and the difference of porosity of movable fluid is 22.97%. The change of porosity of movable fluid is much larger than that of percentage of movable fluid, and the heterogeneity is stronger.

FIGURE 4
NMR distribution of 1#-1 and 8#-1 samples for Chang 8 oil layer
(Contrast map (a) and map (b), The physical properties of the two samples are similar, and the mobile fluid saturation is basically the same, but the pore size range of fluid distribution is quite different. It can be seen that the movable fluid exists in both macropores and micropores. The distribution of fluid in reservoir is different due to the difference of micro pore structure.)

FIGURE 5
Relationship among movable fluid saturation, porosity and permeability of Chang 8 oil layer
(From Figure (a) and Figure (b), we can find that there is a positive correlation between movable fluid saturation and porosity and permeability, and the correlation with permeability is better than porosity.)
In Figure 4(a), the porosity is 5.1%, the permeability is 0.03mD, the movable fluid saturation is 18.86%, the movable fluid is mainly distributed in the pores corresponding to 0.15ms-25ms. In Figure 4(b), the porosity is 5.1%, the permeability is 0.04mD, the movable fluid saturation is 15.72%, and the movable fluid is mainly distributed in the pore corresponding to 1ms-500ms. It can be seen that the physical properties of the two samples are similar, and the movable fluid saturation is basically the same. However, the pore size range of fluid distribution varies greatly, and it can be seen that the movable fluid exists in both macropores and micropores. Most of the moveable flow rates are distributed in the meso-macropores corresponding to 1ms with T2 relaxation time, and there are a small number of movable fluids in the tiny pores.

DISCUSSION

Macroscopic physical parameter influence. The macroscopic parameters reflecting reservoir storage and permeability are porosity and permeability, but both of them are controlled by micro-pore structure and the relationship between them is not fixed [23, 24]. The sample nuclear magnetic resonance shows that the porosity distribution is between 5.1% and 8.2%. The average value is 6.5%, the permeability distribution range is 0.01-0.94mD, and the average is 0.34mD. Both porosity and permeability indicate that the reservoir has poor porosity and permeability. As shown in Figure 5(b), there is a strong positive correlation between permeability and movable fluid saturation. In contrast, the effect of permeability on movable fluid saturation is much greater than that of porosity [Figure 5(a) and Figure 5(b)]. The better the reservoir permeability is, the better the porous percolation capacity is. The more the moveable fluid is controlled by the bore channel, the greater the saturation of the movable fluid is. The product of movable fluid saturation and porosity is defined as movable fluid porosity, which can reflect both porosity and characteristics of movable fluid. There is a strong correlation between movable fluid porosity and movable fluid saturation [Figure 6].

Influence of microcosmic pore throat characteristics. By analyzing the relationship between pore structure characteristic parameters and movable fluid porosity [Figure 7], it is found that the coefficient of variation, median radius, mean pore throat radius, maximum throat radius, maximum mercury saturation and mercury removal efficiency are positively correlated with the porosity of mobile fluids. There is a negative correlation between displacement pressure, median pressure and porosity of movable fluid.

The pore throat structure parameters obtained by mercury injection are many, only reasonable selection and combination of the parameters can fully characterize the pore throat structure [25]. Thus, five parameters which can reflect the pore throat size of pore throat distribution and pore throat connectivity was selected [21]. The relationship between the selected parameters and the porosity of movable fluid is analyzed separately, and the influence of pore throat quantitative parameters on movable fluid porosity is determined.

Relationship between porosity of movable fluid and movable fluid saturation of Chang 8 oil layer
(There is a positive correlation between the movable fluid saturation and the movable fluid porosity, and the correlation coefficient is very high.)
(By analyzing the relationship between pore structure characteristic parameters and movable fluid porosity, it is found that, the coefficient of variation, median radius, mean pore throat radius, maximum throat radius, maximum mercury saturation and mercury removal efficiency are positively correlated with the porosity of mobile fluids. There is a negative correlation between displacement pressure, median pressure and porosity of movable fluid.)
Characteristic parameters of pore throat size. The median radius and the maximum radius of the pore throat can be used to characterize the size of the pore throat. The median radius of the sample in the study area is wide and the mean value is medium. As shown in Figure 7(d), the porosity of the movable fluid increases with the increase of the median radius. There is a significant positive correlation between it and the porosity of movable fluid. Compared with the median radius, the distribution range of the maximum pore throat radius is obviously larger, between 0.06μm and 1.52μm. The correlation curve between the movable fluid porosity and the maximum pore throat radius shows that there is a good correlation between the two [Figure 7(f)]. Under normal conditions, the throat control reservoir permeability, as the throat becomes larger, the physical properties of the reservoir become better. In some samples, the porosity of movable fluid does not increase strictly with the increase of pore throat radius, which is due to the heterogeneity of the reservoir and the internal pore throat configuration complex. Part of the pore throat is controlled by a small roar channel, which indirectly weakens the control effect of the maximum pore throat radius on the porosity of the movable fluid.

Characteristic parameters of pore throat distribution. The pore throat distribution can be characterized by the sorting coefficient and skewness. From Figure 7(c), it is found that the porosity of movable fluid has a strong negative correlation with the sorting coefficient. The pore throat separation coefficient of the sample is between 1.25 and 4.91, and the mean value is 3.06. When the sorting coefficient increases, the porosity of movable fluid decreases instead. This is because when the sorting coefficient of pore throat is too high, the heterogeneity of pore throat becomes stronger. Therefore, the sorting coefficient of pore throat should not be too high [26]. When the sorting coefficient is 1.25 - 2.5, the porosity of movable fluid is the highest. The effect of bias is obviously weaker than that of sorting coefficient, as shown in Figure 7(a). This is because when the skewness becomes larger, the specific gravity of the pore macropores increases and the movable flow rate also grows, but the movable fluid is still controlled by hydrophilic particles and the law of variation with the skew state is not very obvious. The porosity of movable fluid is mainly controlled by the degree of homogeneity of pore throat and it's not that the bigger the space, the better. The relationship between pore throat configuration relations is very important.

Characteristic parameters of connectivity of pore and throat. The efficiency of mercury withdrawal is selected as the characteristic parameter of pore throat connectivity. It also reflects the relationship between pore and throat configuration of reservoir. The maximum mercury withdrawal efficiency of this area is 33.84%, the minimum value is 28.29%, the average is 29.54, which shows a positive correlation with the porosity of movable fluid [Figure 7(j)]. The movable fluid porosity increases with the increase of mercury withdrawal efficiency. In the process of mercury withdrawal, the mercury that is present in the tiny howling path is first emptied, which destroys the connectivity of the entire space, leaving parts of the pore and the howling path isolated, become dead pores, and no longer emit mercury. So the mercury withdrawal efficiency can indirectly reflect the size of the movable fluid porosity, which indicates that the pore throat connectivity determines the pore structure.

CONCLUSIONS

(1) The porosity of Chang 8 formation in the study area is mainly distributed in 4-10% and permeability is in the range of 0.01-1mD, the reservoir heterogeneity is strong. The separation property is medium, and the interstices are mainly carbonate minerals and clay minerals. The clastic grain is mainly fine sandstone and siltstone, and the grading circle is sub-angled.

(2) Pore types have intergranular pore, feldspar dissolved pore, intercrystalline pore and cuttings dissolved pore. According to mercury injection curve and NMR T2 spectrum frequency curve, reservoirs can be divided into two categories: one is mercury pressure less than 0.5Mpa, the pore throat radius is between 0.1μm and 3μm, and the right peak of T2 spectrum is higher than the left peak, movable fluids mainly occur in macropores. While the other is mercury pressure > 0.5Mpa, the radius of pore throat is less than 0.16 m, and the left peak of T2 spectrum is higher than that of right peak. The cutoff value of the movable fluid mainly exists in the small pore. The cutoff value of T2 is mainly distributed in the range of 5-24ms. The distribution range of the movable fluid saturation is between 10.73% and 56.62%, and the average movable fluid saturation is 32.22%.

(3) Permeability, sorting coefficient, maximum pore throat radius and movable fluid saturation have strong correlation with movable fluid porosity. Therefore, the formation with high permeability, large porosity and moderate distribution range of pore throat has good pore throat configuration and high porosity of movable fluid, which should be the key target exploration and development formation in the oil field.
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SPACE-TEMPORAL ANALYSIS OF SOIL ORGANIC CHARACTERISTICS ON THE ECOTONE BORDER

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ABSTRACT

The paper deals with differences of characteristics in agricultural and forest soil. Study areas are in Training Forest Enterprise Masaryk Forest Křtiny (Školní lesní podnik Masarykův les Křtiny) in Brno. In TFE was choose five areas with slightly different soil type, climate conditions, height above sea level and type of land-use. In these five areas was specified 30m long line transect that crosses agriculture and forest land. The line transect includes 11 sampling points for laboratory testing soil samples for different soil characteristics, e.g. humic acid to a fulvic acid ratio (HA/FA) or maximum capillary water capacity and minimum air capacity. The line transect was perpendicular to the line of ecotone. Acquired soil data from the laboratory was processed in GIS software, specifically in Esri ArcGIS. It was created series of the maps for every sampling area for every month in vegetation season (March - October). Sampling points was interpolated by Inverse Weight Distance and Ordinary Kriging. Because line transect created interpolation line, the point surroundings was extrapolated by 3m.

KEYWORDS:
Soil, interpolation, map, GIS, land-use

INTRODUCTION

Every single ecosystem component contains some variabilities that are possible to interpret by mathematics, statistic or geography in time and space. Pedosphere variability is very high in comparison with other ecosystem segments. It is a result of the complex structure of soil and many factors that are involved in the soil-making process [1].

Define main characteristics of soil is crucial for effectiveness in agricultural business. Suitable and sustainable using of agricultural soil and take into consideration of soil degradation are principal objectives for sustainable development. Soil variability is the result of soil-making factors, where the different influence of these factors in an area leads to space-temporal soil differentiation and variable characteristics. There is indisputable influence of human activities on soil-making factors [1]. A conventional method for soil description characteristics is soil exploration in different soil layers (horizons) [2]. Soil variability (in a broad term) is a background of every soil exploration. Soil exploration is catching and interpretation soil variability in general (broad spectrum of soil characteristic) or in specific (single soil characteristic, e.g. nutrients state) level. Results from soil exploration are used for land-use management, gain strategic information, provide scope for sensor monitoring or extrapolation of local studies [1].

Properties for gain and interpretation of soil variability are soil exploration, monitoring and mapping with much powerful mathematics, statistical and geostatistical interpolation methods. Soil characteristics are measured and gaining in aimed areas by single points of sampling network [1].

Long-time development is necessary for the origin of soil. Some of the processes of soil genesis are accelerated positively or negatively by human activities, e.g. intensive forestation, agriculture, pollutants from industry and transport, waste storing, relief changing or land-use. Coexistence of these factors and time have significant participation in soil development, e.g. erosion, contamination [1], [3].

MATERIALS AND METHODS

Study area. The study area is in the Training Forest Enterprise Masaryk Forest Křtiny (Školní lesní podnik Masarykův les Křtiny) in Brno, which is special-purpose facility own by Faculty of Forestry and Wood Technology, Mendel University of Agriculture and Forestry in Brno since 1923. TFE serve not only for students as study and training area but also for research and tasks verification. TFE represent the exclusive area for the University needs. TFE has 102 650 000 m² and creates entire complex between metropolis Brno and city Blansko. Forest is located between 210 and 575 metres above sea level (EPSG:5705). Whole TFE area is very diversified and provides excellent conditions for being study area for University. In forest can be observed 116 different forest types in four forest vegetation zones. The forest is divide by 46% of coniferous and 54% broadleaf trees. Average year temperature is 7.5°C and perception is around 610mm. Granodiorite, greywacke and limestone compose geology bedrock. The area has diversity in relief too. There are deep
valleys and glens, especially near river Svitava and river Kránský potok [4].

**Sampling areas.** In TFE was chosen five representative location. Each land has different conditions, e.g. meters a.s.l, slope, aspect or agriculture and forest land. In these sampling areas were determine line transect where half of the samplings were on agriculture land, half in the forest and one point on the edge between agriculture and forest land (in ecotone). Every sampling point has spacing 3m. The samplings areas are named as Hrabůvka (1), Lesní (2A), Loučka (2B), Rudice (3) and Polesí ŠLP (4).

Sampling area 1 (Hrabůvka) have 9 131.4 m², and it is the biggest area from all five. 60.5% covers agriculture land, 16% beechwood and the rest are spruce or mix forest. Average slope is 2.7°, with maximum 16.3° and general aspect is south-west. Height a.s.l is between 524.3m, and 529.3m. 

Sampling area 2A (Lesní) have 6 572.1 m². Agriculture land covers 42%, and 58% covers forest. Average slope is 2.3° and aspect is north-east. Height a.s.l is between 556.7m and 560.1m. 

Sampling area 2B (Loučka) have 3 893.8 m². The ratio between agriculture and forest is 40 / 60%. Average slope is 3.3° and aspect is in north-west part oriented to the south-west; in south-east part is oriented to north-east. Height a.s.l is between 518.5m and 521.9m. 

Sampling area 3 (Rudice) have 4 255.8m². Agriculture land covers 48%, the rest is a forest (beechwood). Average slope is 3° and aspect is from north-west to north-east. Height a.s.l is between 496.8m and 500.8m. 

The last sampling area 4 (Podlesí) is the smallest one with 3 278.1m². Agriculture land converts 73%, the rest is beechwood forest. Average slope is 12° and aspect is east-west. Height a.s.l is between 456.8m and 465.6m.

In Figure 1 is illustrated the samplings points, where Z values are for agriculture land, the L values are for forest land, and E point is ecotone.

**Land use changes analyses.** Analyses in this study have been done using standard methods of environmental geography under applying GIS tools [5]. Usable input data for modeling and analysis soil is very limited amount. Even accessible spatial data is not completely applicable, predominantly because their regional character. Hence field sampling is obligatory. Many analysis technics for modeling soil characteristics was already studied e.g. soil depth, soil horizons, color, pH, amount of carbon, phosphorus, humus. For space distribution of required soil characteristic was developed many approaches based on statistical or geostatistical methods, that take advantages of spatial correlation of soil characteristics. Simple geostatistical methods, like linear regression or simple kriging are not convenient for so complex analyses as spatial distribution of soil characteristics. More advanced methods like kriging with drift, universal and regression kriging are more suitable for this type of analyses. Appropriate advanced methods for analyses are often dependent on expert experience and affordable software [6]. Interpolation methods used in this study was Inverse Distance Weighting (IDW) and Ordinary Kriging included in Esri ArcGIS 10.5. Soil characteristics was measured in specialised laboratory that is part of Mendel University in Brno. Measurements provide information about soil organic matter (SOM), humic acid and fluvic acid ratio (HA/FA), maximum capillary water capacity, minimum air capacity, soil—active reaction (pH/H₂O) and potential soil reaction (pH/KCl).
During the vegetational year (9 months; March - November) of 2015 was collected almost 1000 points with field measurement in five separate locations. All soil characteristics were gain in the professional laboratory in Mendel University in Brno. Data from the laboratory was receiving in a tabular structure. Hence, it was necessary adjust data structure to be valid for use in GIS software. After that, import into Esri ArcGIS happened commonly into Esri File geodatabase. It can be used a unique tool for import soil data usage Import Tool from research project MOSESO available at http://gislib.upol.cz/moseso/. Because spatial information about sampling location was gathered separately by a device with GPS (Garmin SCx60), also spatial layer for GIS was created separately. Hence, after importing tabular data into GIS was necessary to join spatial layer with soil characteristic. Next, it was created two types of spatial interpolations, specifically IDW and Ordinary Kriging. Their setup was based on the results of similar studies for interpolation of soil characteristics [7, 8, 9]. For create multiple automatic Ordinary Kriging interpolations for soil data, it can be used SoilVar toolbox for Esri ArcGIS created under MOSESO project. It is essential to create only interpolation because GIS software does not always respect the inner boundary of interpolated points and calculate result raster with extrapolated pixels. In Esri ArcGIS, it can be set up a mask for interpolation to respect minimum boundary or it can be used separate polygon layer as a boundary. If the points in line transect were precisely in a straight line the interpolation will be done as a line. For better visualisation and understanding of soil characteristics behaviour was set as minimum surroundings for sampling points 3m, especially for relatively stable soil characteristics (ratio humic acid to fluvic acid (HA/FA), maximum capillary water capacity, minimum air capacity). It respects 3m between sampling points. As fieldwork was done every month, the analysis created a time series, where are immediately see the changes through the year at the same location. Map series was created based on interpolation of sampling points for every vegetation month of the year 2015.

**RESULTS**

Ratio values HA/FA are influenced by humification process that is dependent on climate factors, water and vegetation conditions. It is a fundamental indicator of organic soil matter quality where the higher ratio HA/FA indicate higher quality are humus substances. At tracked transects was valued HA/FA in 5cm soil horizon (A horizon) up to 1 (Figure 2) which is very standard in the Czech Republic [10]. Generally, it can be observed that in agriculture land is HA/FA ratio slighter higher, then in forest land. It is caused by humification of agriculture land. On the other hand, in forest land is HA/FA much more stable, then in agriculture land (Figure 5).

Maximum capillary water capacity and minimum air capacity. Values of MCWC and MAC are affected by soil granularity, amount of organic substances, climatic condition and agriculture processes, primarily by strengthen soil by heavy
agriculture machinery. Graphs interpretation of MCWC is that agriculture land has much more stable values and there are much smaller differences between sampling points instead of forest land, where MCWC values have much higher variance (Figure 3). MAC values have a high variance in both lands, agriculture and forest (Figure 4). However, generally, in agriculture land is MAC slightly lower than in forest land. The main reason can be strengthened soil by heavy agriculture machinery.

**DISCUSSION AND CONCLUSION**

This study provided space-time distribution analyses of soil characteristics in the Training Forest Enterprise Masaryk Forest Křtiny (Školní lesní podnik Masarykův les Křtiny) in Brno. For precise measurement was done field point sampling as line transect, long 30m with five points in agriculture land, five points in forest land and one point in the ecotone. Sampling was done every vegetation month (March - October) of 2015 once in current month. Measurements just once by month can be used for primordial long-term research, where is discovered general characteristics of soil. Nowadays, for more detailed and more often taking of sampling is more suitable to use automated machine collecting methods. Manual sampling and laboratory appraisals are very time and money consuming. The wireless sensor network should acquire future evaluating of soil characteristics [11]. There are many solutions for automatic gaining, processing and analysing data, especially for agriculture, soil and weather. (Libelium company, Vo-Ta logger - MOSESO). Worldwide are available wireless networks for transport data from sensors to数据中心(Sigfox, LoRaWAN, NB-IoT) and application gateway for processing and visualisation data, e.g. AGISED from MOSESO project [12] or a sensor web [13]. For manual sampling was not ideal, but enough to store data in tabular form. Every month was once measured 110 samplings (five locations, 11 points, two soil horizons) and in the laboratory, some number of soil characteristics was calculated. If the soil and weather characteristics were measured by a sensor, e.g. every 15 minutes, then the number of records will be much higher (almost 300× more). For easy access, robust and secure storage of data is database ideal solution. Because data contain location and time, there are some spatially enabled database management systems, e.g. PostgreSQL with PostGIS. PostgreSQL provides a robust open-source solution for spatial data storage. PostGIS extension is capable for spatial analyses. To secure high availability for many (hundreds) queries to data, it is suitable to use replication mechanisms, e.g. Slony-I or PgPool-II [14].

In this study was proved that an agriculture land is more stable than a forest land that has the higher amplitude of values of samplings measured in the laboratory. Crucial sampling point is ecotone, where start main differences in values between agriculture and forest land. With the higher frequency of samplings of soil characteristics using sensors (streaming data) will be reached much more detailed results. More detailed soil measurements will help more precision decision-making scenarios for agriculture or forest land.

![Maximum capillary water capacity (MCWC) transect 3, horizon 5cm, 2015](image)

**FIGURE 3**

Variability of maximum capillary water capacity during the year 2015 on transect 3
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FIRST OBSERVATION OF THE EMBRYOS OF SPINY BUTTERFLY RAY, *GYMNURA ALTAVELA* (LINNAEUS, 1758) (CHONDRICHTHYES: GYMNRURIDAE) FROM EASTERN MEDITERRANEAN, A SPECIES CRITICALLY ENDANGERED

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ABSTRACT

In this study, the morphometric identification of a pregnant *Gymnura altavela* and its three embryos, which is incidentally captured by fishermen with trammel nets on April 2, 2018, in the Gulf of Izmir in the Aegean Sea, Turkey, was carried out. In the study, 18 morphometric measurements of the pregnant specimen and three embryos were compared with each other. The disc width (DW), total length (TL), and weight (W) of the pregnant specimen were 135.5 cm, 90.0 cm, and 20,200 g, respectively. Of the embryos obtained from the right uterus of the female specimen, 1 was male and 2 were female. The disc width of the embryos varied between 28.6 cm and 30.9 cm, total length of the embryos varied between 22.2 and 22.9 cm, and the weight of the embryos varied between 289.51 and 296.13 g. The results obtained with the morphometric measurements were evaluated using discriminant analysis and the results revealed that female and male embryos and the adult specimen had a 100% discrimination ability.

KEYWORDS: *Gymnura altavela*, Spiny butterfly ray, Embryos, Morphometry, Endangered

INTRODUCTION

The Gymnuridae family is represented by 16 species worldwide and the only member of the family in the Mediterranean Sea is the *Gymnura altavela* species. The species is the largest member of the Gymnuridae family, which can grow up to a disc width (DW) of 4000 mm [1-5]. A wide-ranging butterfly ray from tropical and warm temperate continental shelf waters on the eastern (Portugal to Angola) and western (Massachusetts State, USA to Buenos Aires Province, Argentina) sides of the Atlantic Ocean, including the Mediterranean Sea, the

Black Sea and the Madeira and Canary Islands [1, 6, 7]. It is rarely reported from the Gulf of Mexico [8].

Historically, *G. altavela* was not uncommon in the catch of demersal fisheries (trawl and set nets) throughout the Mediterranean and the southern shores in particular. However, it is absent from the whole of the Mediterranean International Trawl Survey (MEDITS) records (i.e., since 1994). Only occasional specimens have been caught in demersal fisheries to testify that it is not extirpated from the region. Occasional specimens turn up serendipitously in the catch of demersal fisheries, for example one adult male was captured in recent years near Anzio, Italy, now deposited in the collection of the University of Naples [9] and one specimen captured in the Southern Adriatic in 2000 [3] thus testifying that the species is not extirpated from the region. Given that its occurrence in the Mediterranean today is so rare (despite some comprehensive survey work throughout its historical range) it must have declined massively in the past 20 years, and since its previously known habitat and area of occurrence continue to face fishing pressure and degradation from coastal development, this species is assessed as Critically Endangered in the Mediterranean on the basis of a suspected past decline of >80% [10]. The Turkish seas are among the natural habitats of the species in the Mediterranean Basin [11].

The biological findings of the species have been obtained from the central Mediterranean Sea [12, 13], the Adriatic Sea [3] the Atlantic Ocean [14, 15, 4], the Aegean Sea [16, 17], the East Mediterranean Sea [18-22]. Moreover, although there are studies investigating the reproductive traits of the populations distributed in the Atlantic Ocean [14, 15, 23, 12, 24, 21], no literature have been found about the reproductive traits of the species in the Turkish coasts. The studies investigating the species found in the Turkish coasts of the East Mediterranean Sea have mostly focused on the length-weight relationship in some cartilaginous fish species and presented the results for the species together [16-20, 22]. Also, coastal development, pollution and anthropogenic

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disturbance through tourism activities are also a threat to its shallow coastal habitat in the Mediterranean. Hence, the preliminary findings obtained with this study investigating the reproductive traits of the critically endangered species will contribute to the protection and sustainability of the stocks distributed in the East Mediterranean Sea and development of the management plans for the species.

As G. altavela has a critically endangered population in the Mediterranean, this study has a great importance for conservation actions.

MATERIALS AND METHODS

In the study, a pregnant Gymnura altavela and three embryos obtained from the pregnant specimen that was captured on 2 April 2, 2018, by the fishing boats hunting with trammel nets in the Gulf of İzmir in the coasts of the Aegean Sea, Turkey were examined. The 18 measurements taken (Table 1) were: disc width (DW), total length (TL), weight (W), disk length (DL), head length (HL), interorbital distance (IOD), spiracle width (SW), spiracle length (SL), head width (HW), precloacal distance (PCD), preoral length (PORL), internarial distance (IND), mouth width (MW), first gill transverse distance (DG1), gill width1 (GW1), interbranchial distance 5 (ID5), interbranchial distance 1 (ID1), anterior pelvic length (LAPV), pelvic fin length (PL) [25, 26]. The discriminant analysis was applied to the data obtained with the morphometric measurements of the adult female and male and female embryos [27].

RESULTS

The disc width (DW), total length, and weight (W) values of the adult female were 135.5 cm, 90.0 cm, and 20.200 g, respectively. Of the 3 embryos detected in the right uterus of the female specimen, 1 was male and 2 were female and the disc width of the embryos varied between 28.6 cm and 30.9 cm, total length of the embryos varied between 22.2 cm and 22.9 cm, and weight values of the embryos varied between 289.51 g and 296.13 g. As the case for adults, the tails of the embryos were short, while their discs were considerably large. The specimens were gray on the dorsal surface and had small blotches (Figure 1).
Table 1 shows the morphometric measurements for the pregnant female and three embryos. The body ratios (DW%) of the embryos were higher than those of the adult specimen. Furthermore, the male embryo was determined to be larger than the female specimens. The clasper length of the male embryo was calculated to be 1.0 cm (DW%: 3.24) (Table 1).

The discriminant analysis performed on the data obtained with the morphometric measurements revealed that female embryos and male embryo and
the adult specimen had a 100% discrimination ability (Figure 2).

**DISCUSSION AND CONCLUSIONS**

*G. altavela* is a critically endangered species and information on its reproductive traits is of great importance regarding the conservation of the species and the stock sustainability [10]. However, there are only a limited number of studies investigating the biology of the species both in the Turkish seas and seas worldwide.

*G. altavela* exhibits ovoviviparity and has an annual reproductive cycle with a gestation period of six months producing 2-7 young [1] and [28]. *G. altavela* is an aplacental viviparous species which produces eggs included in a case [15]. Aplacental yolksac viviparous reproduction with litter size varying from 2 to 8 pups depending on geographic location (four pups/litter reported by [14]; 2-6 by [12] and 1-3 by [29] for the Mediterranean; up to five per litter in southern Brazil (Vooren unpub. data); and up to eight by Musick et. al. (unpub. data) for the Northwest Atlantic). *G. altavela* exhibits ovoviviparity and has an annual reproductive cycle with a gestation period of six months producing 2-7 pups [1,28]. Although [30] reported a fecundity ranging from 2 to 7 for the species, 3 embryos were detected in the study.

In a study carried out in Syria, disc width and weight of the 6 *G. altavela* specimens were determined to vary between 340 and 390 mm and 280 and 465 g, respectively. A scar detected on the ventral surface showed that the internal yolk sac was reabsorbed (exhibited on the ventral surface, a scar showing the place of the resorbed umbilical stalk and remains of internal vitelline vesicle) [21]. In the study, the embryos were found in the right uterus of the mother and the absorption of their yolk sacs had not yet occurred. The disc widths of the embryos were between 28.6 and 30.9 cm (286-309 mm) and their weights were between 289.51 and 296.13 g. With the absorption of the yolk sac, the length and weight values of the embryos examined in this study would have reached levels close to those determined in the study by Alkusairy et al. (2014) [21].

On the other hand, in another study in which the morphometric measurements of 5 embryos obtained from 2 adult females captured in Rio de Janeiro were given, the disc widths of the embryos varied between 22.2 cm and 28.9 cm and weight values of the embryos varied between 119.6 g and 267.7 g [25]. Moreover, according to the findings, yolk sacs were fully absorbed in all embryos. Although the results obtained for the pregnant female specimens were close to the results obtained in this study, there is a distinct difference due to the considerably smaller embryos compared with the results obtained in the Mediterranean coasts [21, this study]. Compared with embryos obtained in other regions, obtaining smaller embryos with fully absorbed yolk sacs from similarly sized adult specimens can be associated with the water temperatures.

The water temperature in the Atlantic is also greatly affected by currents, most notably the Gulf Stream. The average annual water temperature in Brazil is within the range of 19.7-24.3 °C [31]. Temperature is the most important abiotic factor affecting the growth period of fish. In general, both the incubation period and the overall growth period last shorter at higher temperatures compared with those at lower temperatures. Energy consumption for metabolism is lower at lower temperatures and hence, the nutritive material of the eggs, i.e. vitellus, leads to higher meristic element formation [32]. Correspondingly, due to the faster metabolism of fish in waters with higher annual water temperatures, the embryos more rapidly consume the yolk material, which leads to reduced embryo development. On the other hand, in colder waters, the yolk material is consumed in a longer period and thus, the embryos have the chance to grow more. In other words, the consumption of the yolk in longer periods results in increased growth. The higher annual water temperatures of Brazil compared with that of Turkey results in the faster consumption of the yolk material and naturally, the formation of smaller embryos. In addition to the two studies, in a previous study carried out in the western Atlantic Ocean by [14], the disc width was recorded to be 382-444 mm, while in another study carried out in the Mediterranean Sea by [12], it was recorded to be 294 mm. According to the results obtained in the studies carried out in the Mediterranean Sea, the disc width values of the specimens were close to each other, while the specimens found in the western Atlantic Ocean had the highest disc width and the specimens found in the southern Atlantic Ocean had the lowest disc width (Table 2).

### TABLE 2

<table>
<thead>
<tr>
<th>Localities</th>
<th>Size at birth (mm) recorded for <em>Gymnura altavela</em> from different localities</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Atlantic</td>
<td>382-444</td>
<td>Bigelow and Schroeder (1953) [14]</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>294</td>
<td>Capapé et. al. (1992) [12]</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>267-281</td>
<td>Alkusairy et. al. (2014) [21]</td>
</tr>
<tr>
<td>Southern Atlantic</td>
<td>222-289</td>
<td>Paiva et. al. (2018) [25]</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>286-309*</td>
<td>This study</td>
</tr>
</tbody>
</table>

*unborn*
The *G. altavela* specimen examined in this study was incidentally captured by the fishing boats hunting near the coast. In previous studies, [33] and [34] determined that, especially among the adult specimens, female specimens were more dominant than the male specimens in the coastal waters. The researchers attributed the dominance of the females to approach coastal waters for birth, which allow more suitable hydrobiological conditions, to keep their newborn away from intraspecies and interspecies competition for food, in addition to avoid segregation for sex, which is an unavoidable phenomenon for viviparous elasmobranchs during the postnatal period, and cannibalism.

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SPATIAL DISTRIBUTION AND DYNAMIC CHANGES IN SOIL ORGANIC CARBON DENSITY IN A RESTORED GRASSLAND CONVERTED FROM FARMLAND

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ABSTRACT

Soil is the most important organic carbon pool of the terrestrial ecosystem. However, studies on the long-term dynamic changes in soil organic carbon after farmland conversion have rarely been reported. This study investigated the spatial distribution and dynamic changes in soil organic carbon (SOC) density in grasslands converted from farmlands from 2003 to 2016. In 2003, three plots were established in restored grasslands of the Taowan watershed, Henan, China: a control plot (P1) where planted crabgrass (Digitaria sanguinalis (L.) Scop.) naturally grew, a crabgrass plot where plant litter was removed (P0), and a crabgrass plot with twice the litter amount (P2). The SOC densities at 5-cm intervals from the surface to 30 cm depth were measured at the end of February each year. In 2003, the three plots did not show significant differences in SOC density. In 2016, the SOC densities of P1, P0, and P2 were 5303 ± 438 g m⁻², 4685 ± 335 g m⁻², and 4341 ± 347 g m⁻², respectively, showing significant differences among groups (P<0.05). The SOC densities in P1 and P0 increased annually, whereas that in P2 first decreased then increased. Plant species and litter mass primarily affected the SOC density in the 0–20-cm layer. During the farmland-grassland restoration process, the SOC density reached a new balance after 12 years. The annual SOC sequestration rate was 277 g m⁻². This study may provide a basis for the assessment of “carbon sink” functions of restored grasslands.

KEYWORDS:

INTRODUCTION

Soil represents the largest organic carbon pool of the terrestrial ecosystem, and the stability of organic carbon is higher in soil than that in plants; even a minor variation in soil organic carbon may cause a relatively large fluctuation in the CO₂ concentration in the atmosphere, which may break the global carbon balance and drive climate change [1–4]. Furthermore, soil organic carbon is an important index of soil fertility; the change in land use acts as an important factor that influences the changes in the soil carbon pool [5, 6].

After the conversion of land from farmland to grassland, the patterns of land use changes and soil organic carbon (SOC) density become unbalanced, which produces the so-called “carbon source” or “carbon sink” effect [7, 8]. During the restoration of farmland to grassland, both plant diversity and soil biomass increase [8, 9]. The accumulation exceeds the consumption of soil carbon, creating a carbon sink. In addition, the spatial distribution patterns of SOC change [2, 10, 11]. This process continues until a new balance is established. Accurate determination of the intervals between the two dynamic SOC balances is the key to calculating the SOC fixation rate [8–10].

At present, numerous studies on SOC reserves after land use changes in different areas and under different vegetation types have been released [12]. Eclesia et al. [13] found that the change in the SOC content was determined by the quality of both the original and replacing vegetation forms after a change in land use. Rahman et al. [14] investigated the SOC contents in different soil layers after farmland was transformed to woodland and found that the SOC content in the 0-5 cm soil layer continuously increased with increasing stand age, whereas that in the 5-15 cm layers remained stable. Deng et al. [5] found that the soil carbon reserve first decreased then increased during the 1–2 years after farmland abandonment. Tang et al. [6] investigated the influence of the transformation from farmland to artificial vegetation land on the carbon gain and found that the addition of exogenous organic carbon had a noticeable effect on the mineralization of background soil organic carbon, whereas the addition of excessive plant debris and litter caused the decomposition of the original organic carbon, i.e., priming effect. However, all of these abovementioned studies focused on short-term observations and long-term changes in the dynamic balance and vertical spatial distribution of SOC.

In 2000, the Chinese government proposed the “returning cultivated land to original forest
growth environments, altitudes and slopes. The site was farmland for many years, providing a scientific reference for effective implementation of management practices.

MATERIALS AND METHODS

Overview of the study area. The Taowan watershed is located in the middle of the Funiu Mountain area, Henan Province, China (110°24′57″E, 33°51′12″N), which is located in a warm temperate monsoon zone. The average annual rainfall is 879 mm, 71% of which occurs from May to August, although the annual rainfall totals vary widely. The research field has an area of 4000 m² with a slope of < 20° and an elevation of approximately 950 m above sea level. The site was farmland for many years, producing corn (Zea mays L.), peanuts (Arachis hypogaea L.), sweet potato (Ipomoea batatas (L.) Lam.), and more. In 2003, the zone underwent natural afforestation. Since then, it has been restored grassland, where crabgrass (Digitaria sanguinalis (L.) Scop) is the most frequent plant species.

Experimental design. In January 2003, three study areas, designated P1, P0, and P2, were set in the research field. Each experimental plot was separated from the surroundings with hard tape. Five 5 × 5 m experimental plots were set for each group. P1 was a control area, where D. sanguinalis was planted in March 2003. P0 was a litter-free area with the same plant as P1, but the aboveground plant debris and litter were removed in November each year. P2 was a crabgrass area with twice the natural litter volume, which was achieved by adding the plant debris and litter from P0. All investigated areas had similar growth environments, altitudes and slopes.

Determination of organic carbon density. In February 2003, prior to the planting of crabgrass, the diagonal process was applied to select three sample points within each plot to obtain soil samples. Three simple cross-sections were taken from representative locations of P1, P0, and P2 following the methods of Romkens et al. [10]. The inside diameter of the soil auger was 5 cm, and the sample depth was 30 cm. A cutting ring was used to obtain soil profiles, which were brought back to the laboratory for soil bulk density measurement. The soil columns were segmented from the top down, and every 5 cm was designated a layer. Thus, the layers were S1 (0–5 cm), S2 (5–10 cm), S3 (11–15 cm), S4 (15–20 cm), S5 (20–25 cm), and S6 (25–30 cm). The three soil samples from the same layers from the same experimental plot were thoroughly mixed to obtain a total of 90 soil samples (six layers in each of the five replicates in three areas). Sampled points were sealed and marked to prevent repeated sampling of the same locations. The mean value of each layer was taken as the soil bulk density value of the layer.

At the end of February from 2004 to 2016, the triangle sampling method was used to obtain three soil columns from each area as described above. Thus, 90 soil samples were obtained each year. Soil samples were air-dried for three days. Root material, gravel, and other non-soil components were removed. The samples were ground and sieved through a 1.5-mm sieve. The SOC content was measured by the potassium dichromate–external heating method [17].

The SOC density was calculated as follows:

$$
\rho_k = \frac{C_k \times D_k \times E_k \times (1 - G_k)}{100}
$$

where $k$ is the soil layer (S1–S6), $\rho_k$ is the SOC density (kg m⁻²) of layer $k$, $C_k$ is the SOC content (g kg⁻¹) of layer $k$, $D_k$ is the soil density of layer $k$ (g cm⁻³), $E_k$ is the soil thickness of layer $k$ (cm), and $G_k$ is the percentage (%) of gravel >2 mm in diameter in layer $k$.

SOC accumulation rate. In February 2016, a natural grassland (G0) with environmental conditions similar to that of the experimental field was chosen. Five sampling areas were chosen as above for SOC content measurements and SOC density calculations. The SOC densities in P1 and G0 were compared. The rate of SOC accumulation after grassland restoration was calculated spatially instead of temporally based on the following equation:

$$
V = \frac{G_i - F_{in}}{t}
$$

where $V$ is the annual carbon sequestration rate of the 0–30-cm layer in the restored grassland (P1), $F_{in}$ is the SOC density (g m⁻²) of the 0–30-cm layer in the farmland in 2003, $G_i$ is the carbon content (g m⁻²) of the 0–30-cm layer in the restored grassland (i.e., P1, P0, P2) after SOC density has reached a new balance,
and \( t \) is the transition time from farmland to grassland.

**Statistical analysis.** Data are presented as the means ± standard deviations and were processed by Excel 2003 and SPSS 23.0. One-way ANOVA was used for the comparisons among the different treatment groups. SigmaPlot 12.0 was used for plotting. P<0.05 was considered significantly different.

**RESULTS**

**Annual trends in SOC density.** Figure 1 shows the annual trends in the total SOC densities of the 0–30-cm soil depths in areas P1, P0, and P2. The differences in SOC density among P1 (1964 ± 110 g m\(^{-2}\)), P0 (1975 ± 98 g m\(^{-2}\)), and P2 (1979 ±105 g m\(^{-2}\)) were not statistically significant in 2003 (\( P > 0.05 \)); however, from 2004 to 2016, the differences across plots were statistically significant (\( P < 0.05 \)). The SOC densities of P1 and P0 steadily increased from 2003 to 2015, whereas the SOC density in P2 fluctuated. In P2 in 2005, the SOC density was lowest and then increased annually until 2015. In 2004 and 2005, the SOC density relationship was P0 > P1 > P2. However, the relationship was P1 > P0 > P2 from 2006 to 2016. This phenomenon indicated that the control area (P1) was most suitable for the accumulation of SOC.

Figure 2 shows the annual changes in SOC density. The changes in SOC density in all three treatments mainly occurred from 2004 to 2011. The changes in SOC density were concentrated in layers S1–S5, with significant differences year-to-year (\( P < 0.05 \)). The amount of change in layer S6 was small throughout the study period. The SOC density in layer S1 increased annually before 2015. The largest increase was 191 g m\(^{-2}\) and occurred in 2006, which was the third year after farmland conversion. In layer S2, the variation was similar to that in layer S1. The largest increase was 223 g m\(^{-2}\) and occurred in 2006. The magnitude of this yearly increase was even smaller in layers S3, S4, and S5.

From 2004 to 2016, the total increase in SOC density in layer S1 was 790 g m\(^{-2}\), with an average annual increase of 60.8 g m\(^{-2}\). The SOC density in layer S2 increased by a total of 848 g m\(^{-2}\), with an average annual increase of 65.3 g m\(^{-2}\). The total increase in SOC density in layers S1–S4 accounted for 89.4% of the increase in layers S1–S6.

**Dynamics of SOC density in six soil layers.** Figure 3 shows the SOC density dynamics in each layer of P1 from 2004 to 2016. The changes in SOC density were concentrated in layers S1–S5, with significant differences year-to-year (\( P < 0.05 \)). The amount of change in layer S6 was small throughout the study period. The SOC density in layer S1 increased annually before 2015. The largest increase was 191 g m\(^{-2}\) and occurred in 2006, which was the third year after farmland conversion. In layer S2, the variation was similar to that in layer S1. The largest increase was 223 g m\(^{-2}\) and occurred in 2006. The magnitude of the change in layer S2 was larger than that in S1. The magnitudes of this yearly increase were even smaller in layers S3, S4, and S5.

From 2004 to 2016, the total increase in SOC density in layer S1 was 790 g m\(^{-2}\), with an average annual increase of 60.8 g m\(^{-2}\). The SOC density in layer S2 increased by a total of 848 g m\(^{-2}\), with an average annual increase of 65.3 g m\(^{-2}\). The total increase in SOC density in layers S1–S4 accounted for 89.4% of the increase in layers S1–S6.
Figure 4 shows the SOC density dynamics in each layer of area P0 from 2004 to 2016. The changes in SOC density were also concentrated in layers S1–S4, with significant differences year-to-year ($P < 0.05$). The SOC density in layer S1 increased annually throughout the study period, and the largest increase of 115 g m$^{-2}$ occurred in 2004, which was the first year after farmland conversion. The changes in layer S2 mostly occurred prior to 2008. The largest increase was 240 g m$^{-2}$ and occurred in 2004. The magnitude of the change in layer S2 was larger than that in S1 in the first two years after returning farmland to grass. Layer S3 showed a slight increasing trend over time. Layers S4, S5, and S6 showed irregular fluctuations in the late stage of the experiment. From 2004 to 2016, the SOC density in layer S1 increased by a total of 658 g m$^{-2}$, with an average annual increase of 50.7 g m$^{-2}$. The SOC density in layer S2 increased by a total of 652 g m$^{-2}$, with an average annual increase of 50.2 g m$^{-2}$.

Figure 5 shows the SOC density dynamics in each layer of area P2 from 2004 to 2016. The changes in SOC density were again concentrated in layers S1–S4, with significant differences year-to-year ($P < 0.05$), while the amount of interannual variation was relatively small in layers S5 and S6. In area P2, the pattern of the change in SOC density differed from that in P1 and P0. The SOC density first decreased and then increased in all layers. The SOC density in layer S1 suddenly decreased in 2004 and reached the lowest value of -52.5 g m$^{-2}$. However, SOC density increased dramatically in 2006, which is the third year after farmland was converted to grassland. Moreover, the SOC density reached a maximum value of 94 g m$^{-2}$ in 2007. The change in SOC density in layer S2 was consistent with that in S1, but the range of the change was higher than that in the S1 layer. The total change in SOC density in layer S1 was 289 g m$^{-2}$, with an average annual increase of 22.2 g m$^{-2}$. The SOC density in layer S2 decreased and then increased with a total increase of 600.1 g m$^{-2}$ and an average annual increase of 46.2 g m$^{-2}$. In layer S3, the increase in SOC density was higher than that in S1 and S2. The annual average increase in S3 was 56.7 g m$^{-2}$. In layer S4, the initial rate of increase fluctuated and trended up before it decreased and reached a plateau. The SOC density in layers S5 and S6 showed smaller changes throughout the study.

In the thirteen years of the study, the total SOC density in layer S1 in areas P1, P0, and P2 increased by 790 g m$^{-2}$, 658 g m$^{-2}$, and 288 g m$^{-2}$, respectively. The difference was statistically significant ($P < 0.01$). In layer S2, the total SOC density in areas P1, P0,
and P2 increased by 848 g m⁻², 652 g m⁻², and 600 g m⁻², respectively. The difference was statistically significant (P < 0.01). In layer S3, the total SOC density in areas P1, P0, and P2 increased by 706 g m⁻², 578 g m⁻², and 736 g m⁻², respectively. The difference between P1 and P2 was not significant, but the differences among P1, P2, and P0 were highly significant (P < 0.001). The SOC densities in layer S4 were the same as those in S3. The changes in SOC density in layers S5 and S6 were not obvious, and the observed differences were not significant.

![FIGURE 4](image)

**FIGURE 4**

Changes in SOC density by layer in area P0 from 2004 to 2016.
S1, 0–5 cm; S2, 5–10 cm; S3, 11–15 cm; S4, 15–20 cm; S5, 20–25 cm; and S6, 25–30 cm.

![FIGURE 5](image)

**FIGURE 5**

Changes in SOC density by layer in area P2 from 2004 to 2016.
S1, 0–5 cm; S2, 5–10 cm; S3, 11–15 cm; S4, 15–20 cm; S5, 20–25 cm; and S6, 25–30 cm.

![FIGURE 6](image)

**FIGURE 6**

Spatial distributions of SOC densities in farmland, grassland, and experimental areas in February 2016; Different letters indicate significant differences between plot types within a layer. F0, the original SOC density of the farmland. P1, the SOC density of the control. P0, the SOC density of the plant-litter-free area. P2, the SOC density of the area with twice the amount of plant litter.
In the initial stage of returning farmland to grassland, SOC density changed dramatically in all three treatments. This change mainly occurred at a depth of 0–20 cm and continued until 2014, when it reached a steady state. This finding indicated that the organic carbon density in each layer of soil had reached a new dynamic equilibrium.

Spatial distribution and rate of SOC accumulation. The total SOC densities in layers S1–S6 (0–30 cm) in areas P1, P0, P2, and G0 were 5303 g m$^{-2}$, 4685 g m$^{-2}$, 4341 g m$^{-2}$, and 5348 g m$^{-2}$, respectively, and the initial SOC density F$_{ini}$ (at the beginning time of farmland reuse) was 1975 g m$^{-2}$. There was no significant difference between areas P1 and G0 in 2016 ($P > 0.05$), whereas the differences between P1 and P0 and P2 were significant ($P < 0.01$). The SOC densities of all layers in F0, P1, P0, P2, and G0 are shown in Fig. 6. In farmland area F0, there were no significant differences in SOC density among layers S1 to S6 ($P > 0.05$). The average SOC density of each layer was 322 g m$^{-2}$. The vertical spatial distribution of SOC density in grassland G0 showed that the top layer had a high SOC density that decreased rapidly with increasing depth. The difference across layers S1 to S6 in G0 was significant ($P < 0.05$), with an average SOC density of 891.3 g m$^{-2}$ per layer. The SOC distribution pattern of P1 was similar to that of G0, and the differences of S1–S6 were significant ($P < 0.05$), with an average SOC density of 883.8 g m$^{-2}$ per layer. In layers S1–S6, the difference between areas G0 and P1 was not significant ($P > 0.05$), which indicated that the SOC density of area P1 had reached the local grassland dynamic equilibrium state.

P1 began undergoing restoration in 2003, and the SOC densities in all layers of P1 in 2015 were not significantly different from those in 2016; thus, the transition time between farmland and grassland balances was 12 years (from 2003 to 2015). If the SOC density of farmland (F0) was used, then after the farmland-grassland conversion, the annual SOC sequestration rate would be 277 g m$^{-2}$ in P1. Similarly, the SOC sequestration rates of P0 and P2 were 226 and 197 g m$^{-2}$, respectively.

In 2016, the total SOC density in P1 was much higher than that in areas P0 (litter-free) and P2 (double litter) (Fig. 6). P1, P0, and P2 had completely different vertical spatial distribution patterns. The results indicated that vegetation species and litter volume affected the dynamic balance of SOC.

**DISCUSSION**

SOC density is the balance between "new carbon" input into the soil by exogenous organic matter and the decomposition of "old carbon" already present in the soil. Estimation of the SOC sequestration rate of restored grassland is an important part of the evaluation of the ecological benefits of grassland restoration projects. After land use changes, the time required for the SOC density to reach a new dynamic balance is an important source of uncertainty in determining the SOC sequestration rate. Previous reports have suggested that this process can require approximately 100 years, whereas others have indicated that it may take only 25 years. Wu et al. [18] reported that after grazing exclusion in grasslands (dominated by Leymus chinensis (Trin.) Tzvelev) in Inner Mongolia, it took 20 years for the soil carbon to reach a new balance. In this study, after the cessation of tillage for 12 years in the research field, no significant difference was detected between the SOC density of the research area and that of a nearby natural grassland, which indicates that the farmland-grassland conversion process can take as little as 12 years in our study area. Presumably, this inconsistency was caused by differences in study objectives, investigated plants, and study designs between our study and previous studies. Following a change in vegetation species and litter volume, changes also occurred to soil carbon sources, movements, conversions, and microbial relationships. Further studies are required to investigate whether SOC density has reached a new balance in the experimental plots.

After the cessation of tillage, the land use pattern changed. As weeds replace the single-crop pattern of farmlands, the vegetation diversity increases. Thus, the accumulation of litter and root material can provide a large quantity of carbon to the soil, leading to an increase in SOC accumulation and density. Eventually, this phenomenon forms a vertical spatial distribution pattern of high SOC density in the top layer and a rapid decrease with the increase in soil depth.

The material basis of SOC density accumulation is plant litter and plant root systems. Studies on plant litter mostly focus on the decomposition process and nutrient dynamics. Relatively few studies have focused on the migration and transformation mechanisms of litter. The chemical composition of litter is complex, and litter includes easily decomposable components, such as sugar, starch, and fat, as well as stable components, such as lignin and polyphenols. Studies on the impact of root exudates and shed substances on SOC accumulation are even rarer. It is generally believed that plant roots have long storage periods with stable chemical properties and that they can gradually transform into SOC. The effect of roots on SOC accumulation is even more stable. With the cessation of tillage, root biomass increases, which leads to an increase in the SOC accumulation rate. Even without litter as an additional carbon source, SOC continues to increase, which indicates the importance of root systems on SOC accumulation.

Soil carbon dynamics are also affected by the amount of litter. Classical ecology believes that with an increase in the amount of litter, SOC...
density also increases. The results of this study indicate that a large amount of litter does not increase the fixation of SOC but instead promotes the decomposition of "old carbon" in the soil, which results in the so-called "priming effect" of soil [26-28]. In this study, litter treatments were divided into no litter (litter-free) and twice-normal (double) litter treatments; the latter of which produced an obvious priming effect. The priming effect rapidly reduced the amount of SOC within a layer and then maintained it at a certain level. From the third year onwards, the total SOC continued to increase steadily. It is difficult to explain the cause of the SOC density increase in the normal litter (control) area P1, as it increased steadily and even exceeded that of the litter-free area in the fourth year. This result suggests that the SOC density to reach a new dynamic balance in farmland-grassland conversion areas. The change in the spatial distribution pattern of SOC also changes. It takes 12 years for the SOC density to reach a new dynamic balance in farmland-grassland conversion areas. The change in the amount of plant litter also causes a change in the distribution pattern of SOC. However, the priming effect cannot provide a complete explanation for the dynamic change in SOC after farmland use change.

In conclusion, after farmland is converted to grassland, the accumulation of SOC primarily occurs within the 1-15 cm layer, and the spatial distribution of SOC also changes. It takes 12 years for the SOC density to reach a new dynamic balance in farmland-grassland conversion areas. The change in the amount of plant litter also causes a change in the distribution pattern of SOC. However, the priming effect cannot provide a complete explanation for the dynamic change in SOC after farmland use change.

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**BOTRYTIS CINEREA STRUCTURAL DEVELOPMENT AND HOST RESPONSES IN RESISTANT AND SUSCEPTIBLE GRAPEVINE CULTIVARS: A PHYSIOLOGICAL AND STRUCTURAL STUDY**

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**ABSTRACT**

*Botrytis cinerea* is a devastating necrotrophic fungal phytopathogen. Two grape cultivars were evaluated for leaf resistance to *B. cinerea*. Detached leaf assays revealed stronger resistance in Marselan (*Vitis vinifera*) than in Blue French (*V. vinifera*). Explanations behind *B. cinerea* resistance in Marselan and susceptibility in Blue French were further investigated by comparing the fungal growth, reactive oxygen species (ROS) responses and anti-oxidative system changes between the two cultivars after infection with *B. cinerea*. Our results confirmed that the high-level resistance of ‘Marselan’ can be attributed to insignificant fungal development, low ROS production and timely elevation of anti-oxidative functions. Moreover, ‘Blue French’ suffered massive infection and sustained ROS production because of its relatively, inactive anti-oxidant. However, this finding needs to be resolved by further elucidating the molecular mechanisms. This study provides understanding of *B. cinerea* infection of grapes and thus could help breeder’s select suitable germplasm for future research.

**KEYWORDS:**
Grapevine, *Botrytis cinerea*, biotic stress, resistant cultivar, physiology, fungus structural development, SEM

**INTRODUCTION**

*Botrytis cinerea*, causal agent of grey mold, is the second most important phyto-pathogen responsible for pre-and postharvest decay and fruit quality deterioration in greenhouses, open fields, and during storage, including cold storage (0–10 °C) [1]. Grey mold is harmful to grape growing areas, where duration of relative humidity (>90%) and cold temperature (14–28 °C) persist for a long time and coincide with bloom and ripening periods [2].

China is a major wine-consuming country with a huge potential for rising viticulture and its wine industry [3]. The grape production in this country is economically very important [4], which is currently affected by *B. cinerea* [5]. The traditional control of *B. cinerea* includes strong fungicide treatments during the seasonal crop cycle. Moreover, the risk of fungal resistance to fungicides has limited the use of chemicals for crop protection [6].

Reactive oxygen species (ROS) play important role in plant physiology, such as in plant development, cellular signaling, biotic and abiotic stress tolerance. Thus, ROS production needs to be firmly regulated to balance its physiological functions [7]. Stressful environments disrupt the stability of ROS production and exclusion. This phenomenon damages essential cellular compartments and plant membranes, leading to plant cell death. Plants possess a sophisticated array of anti-oxidative defense system that scavenge harmful ROS and protect cells from oxidative damage [8]. Substantial evidence indicated that *B. cinerea* invasion can overthrow the effects of ROS stress on plants [9, 10]. Multiple cellular signaling actions mostly rely on redox reactions. Thus, ROS may be straightly involved in the cellular redox metabolism. ROS regulate the redox regulative network in each cell, gene expression, translation, and metabolism [17]. The hyper oxidant state is momentary and uncertain, causing ROS production to exceed the antioxidant capacity of the cell; moreover, insufficiency in antioxidant enzymes increases ROS levels [18].

In the present study, we observed phenotypic, microscopic and histochemical disease signs and symptoms of *Vitis–B. cinerea* interactions. Furthermore, we investigated the critical levels of ROS, its responsible enzymes in leaves of two grape cultivars namely Marselan and Blue French to reveal the resistant level.
MATERIALS AND METHODS

**Plant and Fungal Material.** Grape leaves were taken from the grape germplasm depository at the Northwest A&F University, Yangling, Shaanxi, China, from two grape cultivars, “Marselan” and “Blue French”. *B. cinerea* spores were isolated from “Gold finger” (*V. vinifera*) in a field located at north campus of Northwest A&F University, and were cultured on potato dextrose agar (PDA) medium at 22°C. 20 days later, the conidia were collected and a concentration of 1.5x 10^6 spores mL^-1 was made in sterilized water [4]. Prior to application of *B. cinerea*, a pre-germination test was conducted. For this purpose a 100 μL of *B. cinerea* suspension was poured in three Petri dishes, containing PDA media. All the Petri dishes were incubated in 22-25°C temperature. After 16 to 24 hours a 95% germination of spores were confirmed. Leaves of same size and age (from the shoot at nodes 3 and 4) were arbitrarily selected from both grape cultivars. The detached leaves were washed with distilled water three times. For laboratory assessment, total 48 leaves from three replicates of each cultivar were evaluated. Leaves were quickly transferred to trays having agar (0.8%) containing 50 mM sodium phosphate buffer (PBS) (pH 7.0), 75 μM riboflavin, 13 mM methionine and 100 μM NBT, 0.1 mM EDTA, 2 μM riboflavin, 13 mM methionine and 100 μM NBT, 0.1 mM EDTA, 2 μM NBT, 0.1 mM EDTA, 2 μM riboflavin, 13 mM methionine and 100 μM guaiacol and was added to the mixture and incubated at 25°C for 20 min. The final solution was mixed with 225 μL ether, and the absorbance of the pink phase was measured at 415 nm.

**Disease incidence (%).** Grape leaves were regularly monitored for any disease infection throughout the experiment. The percent disease incidence was calculated by the following formula.

\[
\text{Percent disease incidence} = \frac{\text{Number of leaves infected}}{\text{Total number of leaves}} \times 100
\]

**Light Microscopy.** *B. cinerea* colonization on leaves was examined at 96 hours post-inoculation (hpi) of both cultivars. Leaves were cut into segments of 2-3 cm² pieces, fixed and decolorized in ethanol (100%) and in saturated chloral hydrate. Subsequently, samples were stored in 50% glycerol and stained with blue aniline solution at the time of observation with an Olympus BX-51 microscope [5].

**Scanning Electron Microscopy (SEM).** Infected leaves were cut into pieces (1-1.5 cm³) at 96 hpi and kept in the solution of 4% glutaraldehyde. After vacuumized for 30 min, the samples were rinsed five times with 0.1M PBS (pH 6.8) 5, 10, 15, 20, and 20 minutes for each time. Segments were dehydrated with gradient alcohols: 30%, 50%, 70% alcohol each for 15min; 80%, 90% alcohols each for 20min; and 100% alcohol, twice each for 30min. Finally, samples were kept in acetone for 30 min and isoamyl acetate for 15 and then 30min. Segments were critical point desiccated by CO₂ and then coated with gold in a sputter coater, and observed with scanning electron microscope at 15 kV [5].

**Reactive Oxygen Species Measurement.**

**Measurement of H₂O₂.** The H₂O₂ content was calculated at different time points i.e.; 0, 4, 12, 24, and 48 hpi, according to Moloi and Westhuizen [24]. H₂O₂ was extracted in cold acetone, and the extract was centrifuged (5930×g) at 4°C for 15 min. The supernatant (1 ml) was mixed with 0.2 ml ammonia and 0.1 ml 95% (v/v) hydrochloric acid containing 20% (v/v) titanium tetrachloride. After centrifugation at 5930×g and 4°C for 5 min, the sediment was repeatedly washed with cold acetone and centrifuged at 5930×g and 4°C, following which the pellet was dissolved in 3 mL 1 mol L⁻¹ H₂SO₄. The absorbance of the resulting solution was measured at 415 nm.

**O₂⁻ Measurement.** The rate of O₂⁻ production was calculated at different time points i.e.; 0, 4, 12, 24, and 48 hpi, by following the method of [25], with slight modifications. The SOD extract (750 μL) was added to 65 mmol L⁻¹ phosphate buffer (750 μL; pH 7.8) containing 1 mmol L⁻¹ hydroxylamine chlorohydrate and incubated at 25°C for 1 h. Sulfanilamide (375 μL of 17 mmol L⁻¹ solution) and α-naphthylamine (375 μL of 7 mmol L⁻¹ solution) were added to the mixture and incubated at 25°C for 20 min. The final solution was mixed with 225 μL ether, and the absorbance of the pink phase was measured at 530 nm.

**Enzyme Extraction and Activity Assay.** Superoxide dismutase (SOD) was measured at different time points i.e.; 0, 4, 12, 24, 48 hpi, according to [26]. In brief, 3.4 mL reaction mixture containing 50 mM sodium phosphate buffer (PBS) (pH 7.0), 75 μM NBT, 0.1 mM EDTA, 2 μM riboflavin, 13 mM methionine and 100 μl crude protein extract was irradiated for 20 min at 4,000 Lux and then observed at 560 nm. Peroxidase (POD) activities were measured at different time points i.e.; 0, 4, 12, 24, 48, hpi, according to [46]. 0.5 g leaves were used [27]. 600 μl crude protein extract was added to a 3mL reaction mixture comprising 2% H₂O₂ and 0.05M guaiacol and was observed at 470 nm.

Catalase (CAT) activity was measured at various time points i.e.; 0, 4, 12, 24, 48, hpi, from about 2.5 g leaves that were crushed in 25 mL 0.2 M PBS buffer (pH 7.8) in a tray having ice. The mixture of 2.5 mL 10% H₂SO₄ and 2.5 mL 0.1 M H₂O₂ and 3 mL crude protein extract was incubated for 10 minutes at 30°C and then titrated with 0.1 M KMnO₄. Samples with three mL boiled extract in the reaction mixtures were used as controls. The consumption of 1.7 mL 0.1 M KMnO₄ was supposed to be equal to 1.7 mg H₂O₂. The KMnO₄ solution of 0.1
M was critically determined by 0.1 M oxalic acid GR [28].

**Statistical Analysis.** Experiments were performed using three replicates by using completely randomized design. Means and standard errors were computed from independent replicates by using SPSS 13.0 program, and for significant differences LSD 0.05 was used. All pictures were coupled with Adobe Photoshop. All graphs were made by using Origin pro. 2016 32 bit software.

**RESULTS**

*B. cinerea* Growth on the Marselan and Blue French. The detached leaf assay was used to evaluate *B. cinerea* infection in vitro [31]. The spread of leaf lesions caused by *B. cinerea* was measured at 96 hpi (4 days). LSD analysis showed similarity among the repeats, and the average disease incidence was significantly different (P ≤ 0.05) between the two cultivars (Table 1).

Disease incidence was calculated post botrytis infection on leaves surface. More disease incidence (Fig. 1) was observed on the leaves of Blue French cultivar with significant mycelium growth and new sporulation (Table 1) while no/less disease incidence (Fig.1) was observed on Marselan leaves (Table 1).

Samples from both, Marselan and Blue French cultivars were chosen for phenotypic, macroscopic, and SEM evaluation of fungal growth at 4 days post-inoculation (Fig. 2). The leaves of Blue French (Fig.2 B) were completely moldy and were roofed by mycelium along with sporulation (Table 1). However, the un-germinated fungus spores were found on the surface of Marselan (Fig.2 A). Microscopic (Fig. 2 C) and SEM (Fig.2 E) observation at 96 hpi also confirmed that spore germination was clearly delayed and fungal growth was mostly blocked on Marselan leaves which indicating the restricted *B. cinerea* proliferation. While, on other hand, Blue French leaves were augmented progressively until 96 hpi (Fig.2 D, F) respectively. Hyphae were branched with apparent lesion spread and showed sporulation on Blue French leaves surface which was clear indication of its susceptibility.

**TABLE 1**

**Disease incidence percentages, mycelium and new sporulation on the grape leaves cultivars infected with* B. cinerea.***

<table>
<thead>
<tr>
<th>Species</th>
<th>Name of cultivars</th>
<th>Disease Incidence</th>
<th>Microscopic a mycelium</th>
<th>New b sporulation</th>
<th>P ≤ 0.05*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. vinifera</em> L.</td>
<td>Marselan</td>
<td>10.38 ± 1.21</td>
<td>×</td>
<td>×</td>
<td>A</td>
</tr>
<tr>
<td><em>V. vinifera</em> L.</td>
<td>Blue French</td>
<td>80.67 ± 2.39</td>
<td>✓</td>
<td>✓</td>
<td>B</td>
</tr>
</tbody>
</table>

*Significance at P ≤ 0.05. Different letters associated with each level of disease severity indicates significant differences at P ≤ 0.05.

a✓Mycelium or sporulation were observed by the naked eye on leaf surface.
b✓No mycelium or sporulation was observed by the naked eye on leaf surfaces.

**FIGURE 1**

Disease incidence percentage of HR “Marselan” and HS “Blue French” leaves at 96 hpi.
Leaves were observed 96 hpi. Experiment was repeated three times.

**Phenotypic**

**Microscopic**

**SEM**

**FIGURE 2**

Comparison of *B. cinerea* spore development on Marselan and Blue French leaves. Progression of *B. cinerea* colonization, on Marselan (A, C, E) and Blue French (B, D, F) respectively.

Leaves were observed 96 hpi. Experiment was repeated three times.

Activities of Peroxidase (POD), Superoxide Dismutase (SOD) and Catalase (CAT) in Marselan and Blue French cultivars Infected with *B. cinerea*. The SOD activities in control and inoculated samples of both Marselan and Blue French cultivars were approximately the same at 0 and 4 hpi (Fig. 3). The activity in the inoculated Marselan was approximately the same as that in the control throughout the experiment. The activity of Blue French was relatively the same as that of Marselan at 4 hpi. However, a sudden increase was observed at 12 hpi, which dropped gradually until 48 hpi.

The POD activities in Marselan and Blue French leaves were tested to evaluate the robustness of the antioxidant system during *B. cinerea* infection. The control samples of both cultivars displayed the same POD background activities within the all-time series (Fig. 4). However, in inoculated Marselan leaves, POD activity slowly increased from 4 hpi to 12 hpi and then slightly decreased at 24 hpi while the highest peak was observed at 48 hpi. The POD activities in the inoculated Blue French were relatively the same as that in the control Marselan and Blue French throughout the experiment.
CAT protein extracts from the leaves of control Marselan and inoculated and control Blue French exhibited similar CAT background activities with basic invariants (Fig.5). However, in the inoculated Marselan leaves, the CAT activity gradually increased from 4 hpi to 12 hpi, with a gradual increase at 24 hpi. The highest peak was observed at 48 hpi. The CAT activity in inoculated Blue French was approximately similar to both, Marselan and Blue French control (Fig. 5).

H₂O₂ Accumulation in *B. cinerea* inoculated cultivars Marselan and Blue French. Graphical values of data showed that *B. cinerea* significantly influenced H₂O₂ production post infection. The maximum H₂O₂ content (Fig 6) was noted in grape cultivar Blue French, while minimum H₂O₂ was observed in grape cultivar Marselan at various time points (0, 4, 12, 24, 48 dpi). Under stress condition at 0 hpi, the H₂O₂ level in Marselan and Blue French was nearly the same. H₂O₂ production increased in Blue French from 4 hpi to 12 hpi with a small decrease at 24 and remained till 48 hpi. While there was no significant increase observed in Marselan at different time points. Thus, significant differences in H₂O₂ production existed between Marselan and Blue French.
O$_2^-$ Accumulation in the Interactions of B. cinerea with Marselan and Blue French. It is evident from the graph values that B. cinerea significantly affected the O$_2^-$ concentration post infection. The higher O$_2^-$ (Fig 7) was noted in Blue French grape cultivar at different time points (0, 4, 12, 24 and 48, dpi). While lower O$_2^-$ was observed in Marselan cultivar which statistically varies from each other. In our study, low O$_2^-$ production levels were observed in both Marselan and Blue French at 0 hpi. A sudden increase was observed at 4 hpi in Blue French, followed by a slight decrease at 12 hpi and continued up to 24 hpi. At 48 hpi, the O$_2^-$ production decreased again.

**DISCUSSION**

Grape cultivar Marselan was found to be highly resistant to Botrytis, while low or no resistance was observed in Blue French cultivar (Table 1). Grape genotypes vary in terms of their infection resistance, degree of fungal colonization, and disease severity.
Discrete colonization of *B. cinerea* on the grape leaves were studied phenotypically, microscopically and by SEM at 96 hpi. In Blue French, the pathogen built an initial limited infection then *B. cinerea* induced a substantial development and sporulation. Meanwhile, during the initial infection steps on Marselan expressed a significant postponement, as evident from the considerably inferior germination and infection rates. The majority of conidia on Marselan leaves had unable to develop into infection pegs compared with those on Blue French leaves. Similar results were reported by [4], [36] reported that the sporulation densities of *V. dividdii* var. Langao-5 and *V. pseudoreticulata* var. Baihe-35-1 are significantly lower than those of HS *V. vinifera* cv. Pinot noir. At initial stages, the conidia/colonization of *B. cinerea* was stopped in Marselan probably because of its physical and chemical barriers, such as cell wall strengthening and phytoalexin production [37, 5].

Considering the present finding that Marselan can virtually block *B. cinerea* and that Blue French is a favorable host, we evaluated the possible fundamental mechanisms in Marselan and Blue French. Overall, high levels of ROS were accumulated in Blue French. [9] Reported that in host–pathogen interactions where the pathogen is a necrotroph, the pathogen-induced cell death and ROS accumulation promote pathogen growth and disease development. Thus, ROS facilitate colonization on the leaves by the necrotrophic fungus *B. cinerea* [38]. By contrast, low ROS level was observed post-inoculation on the Marselan cultivar. The ROS level after *B. cinerea* infection is low because the anti-oxidative system maintains redox equilibrium [26, 39] and protects cells from ROS damage [40].

Oxidative stress disturbs the redox equilibrium in the infected tissue, thereby promoting disease development [11]. In the present study, ROS detection after inoculation was recorded on both grapevine leaves. High ROS level was detected in Blue French than in Marselan. Thus, Blue French infected significantly from constant ROS detection. Moreover, Marselan did not contend with substantial oxidative stress because of its high and timely raised anti-oxidative capacity. H$_2$O$_2$ increases either the resistance or susceptibility toward *B. cinerea*. Meanwhile, O$_2^-$ serves as a first substrate for H$_2$O$_2$ formation [9, 11, 41]. Some reports have suggested that O$_2^-$ plays a role in supporting *B. cinerea* invasion [42, 43].

H$_2$O$_2$ is induced in plant cells, accompanied by O$_2^-$ generation, which can raise programmed cell death and disease lesion development to promote *B. cinerea* infection [38]. Therefore, the high and low levels of ROS production in Blue French and Marselan are accountable for their susceptibility and resistance to *B. cinerea* infection, respectively [44]. ROS respond to pathogen attack in plants [45, 39]. Thus, we evaluated ROS accumulation and its possible outcome of antioxidant enzymes during the interactions with *B. cinerea* [43].

[4] reported that low ROS production and timely increase in anti-oxidative enzymes are associated with the strong pathogen resistance of Pingli-5. Meanwhile, HS cultivar Red Glob massively suffers from infection and sustained ROS production due to comparatively unchanged anti-oxidative activities. These results suggest the significance of ROS response in the timely detection and defense to *B. cinerea*. Considering enzyme activities, we discovered that the post-inoculated Blue French leaves manifested slight variation in POD activities with lesion development. However, they showed increased...
SOD, which corresponds well to H$_2$O$_2$ production and O$_2^-$ reduction. However, the POD activities in Marselan increased during the experiment, and no significant change was observed in SOD activity. Low levels of ROS accumulation are necessary for the anti-oxidative system to sustain redox equilibrium [39]. Similar to the results above, we observed that the infected Blue French certainly encountered the effects of an insufficient anti-oxidative system, resulting in consistently elevated ROS levels. Meanwhile, Marselan quickly synchronized its anti-oxidative functions, especially the POD activities, following the inoculation. Thus, Marselan experienced less ROS-induced stress. Given that substantial ROS was induced by Blue French but not in Marselan, the particular coordination of ROS production and scavenging mechanisms related to the anti-oxidative system during the consolidated abiotic and biotic stress [46] may be relevant for Marselan to shield itself against $B$. *cinerea*.

In this study, we investigated the resistance of Marselan to $B$. *cinerea*. While, Blue French cultivar was susceptible by detached leaf assay. The results were further investigated by comparing the fungal growth, ROS responses and antioxidative system changes between Blue French and Marselan after $B$. *cinerea* inoculation. Our results confirmed that low ROS production and timely elevation in anti-oxidative function were associated with fungal resistance in Marselan. Meanwhile, Blue French got substantial infection and sustained ROS production due to relatively unchanged anti-oxidative activities, which may have caused its susceptibility to $B$. *cinerea*. However, this finding needs to be resolved by further elucidating ROS based molecular mechanisms. This study provides deep understanding of $B$. *cinerea* infection of grapes and thus could help breeder’s select suitable germplasm for future research.

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A FEASIBLE METHOD FOR THE PREPARATION AND CHARACTERIZATION OF A SEMI-GRAphitized CARbon ELECTRODE MATERIAL CATALYZED BY NICKEL

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ABSTRACT

Activated carbon (AC) from pepper straw was used as a carbon source to prepare a semi-graphitized carbon electrode material (GCM) via catalytic graphitization with a nickel catalyst. The influence of the Ni/AC and graphitization temperature on the properties (phase composition and microstructure) of the GCM was investigated. The morphology and surface properties of the samples were characterized by means of a specific surface area and aperture analyzer, scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction, and Raman spectroscopy. The cyclic voltammetry characteristics of the samples were examined using an electrochemical workstation. The results showed that the GCM was successfully prepared via the catalytic graphitization method, and it had a large surface area (743.429 m²/g), when the quantity of Ni/AC was 0.2 mol%, the heat treatment temperature was 700 °C, and the soaking time was 1 h. The specific capacitance of the GCM was 262.595 F/g, and a number of microporous and mesoporous structures existed in the material. The GCM had a three-dimensional structure (d₀₀₂~0.340 nm, Lc~11.523 nm). The d₀₀₂ was close to that of ideal graphite, indicating the formation of a well-graphitized structure. The degree of graphitization was 0.46, and the value of I₀/Iₓ was 0.65. The results are significant for improving the electrochemical performance of activated carbon electrodes. A new approach was also established for the treatment and disposal of pepper straw.

KEYWORDS:
Catalyst graphitization, activated carbon, electrosorption electrode

INTRODUCTION

As a new water treatment technology, electrosorption desalination has features of convenient operation, no secondary pollution and low energy consumption. It has attracted interest for the applications of seawater desalination and industrial wastewater treatment. The development of efficient electrode materials with good absorption and desorption capacity is vital [1]. Carbon-based materials are widely used as electrosorption electrodes due to their large specific surface area, low cost [2] and high efficiency [3]. Electrosorption deionization technology has attracted the attention of international academic researchers. It has been reported that the removal and recovery of 3.5 mmol Br⁻ were achieved by a working electrode with 1 g of activated carbon cloth [4]. A mesoporous carbon aerogel was used as the active material [5], and carbon aerogel-based electrodes showed a high adsorption capacity of NaCl of 8.4 mg/g. Activated carbon felt (ACF) was used as an electrosorbent material to manufacture circulating capacitive deionization (CDI) devices for the desalination of brine wastewater [6]. Large-scale Li-ion-doped graphene/carbon nanofiber porous architectures were prepared via homogeneous electrospinning with LiNO₃/graphene oxide/polyacrylonitrile nanofibers [7], and the architectures showed excellent electroadsorption. ACF-based flow-through CDI exhibits good stability. However, the conductivity of this kind of carbon material is still low even if it has a large specific surface area because the microstructure of carbon-based materials is not dense or the arrangement of the carbon crystals is irregular, these factors lead to a large difference between the theoretical value and the specific capacitance in practical applications and an average electrosorption performance.

Furthermore, carbon materials can be converted into graphitized carbon. Moreover, the growth of three-dimensional graphite is accompanied by a decrease in the porosity and the surface area, which impacts the electrical conductivity. Graphite is considered a very promising candidate in practical applications because of its high reversible capacity, excellent conductivity, perfect charge-discharge potential profile and relatively low cost [8-10]. However, common methods for graphitization, such as arc-discharge [11], chemical vapor deposition [12, 13] and the use of molten salt [14, 15], consume a large amount of energy [16] and have a high cost and complex processing steps [17]. High-temperature routes are the conventional way to obtain highly graphitized carbon materials, but
inevitably, this treatment leads to very poorly-ordered mesoporous structures [18]. Therefore, inexpensive and environmentally friendly methods for graphitization of carbon materials are urgently needed. In this study, a semi-graphitized carbon electrode material (GCM) was obtained by graphitization with an added catalyst, which could reduce energy consumption and costs. Compared to the nongraphitized material, the graphitized carbon materials obtained from mesoporous carbon materials after catalytic treatment have higher conductivity, higher chemical stability and better electrochemical performance [19].

In this paper, Ni(NO$_3$)$_2$·6H$_2$O was used as a catalyst to transform an activated carbon material (AC) to graphitized carbon. The physical structures and electrochemical properties of the GCM were characterized. Chemical catalysis can not only convert a large amount of carbon materials into graphitized carbon but also meet the needs of industrial production. This study provides excellent electrode materials for electroadsorption deionization technology and promotes the development of water treatment methods.

MATERIALS AND METHODS

Materials. The preparation of the AC pepper straw (BET specific surface area of 1637.793 m$^2$/g) included K$_2$C$_3$O$_4$ as the activator, an impregnation ratio of 1.5:1, a dipping time of 18 h, an activation temperature of 850 °C and an activation time of 120 min. All the chemicals used were of analytical reagent grade.

Preparation. (1) The Ni(NO$_3$)$_2$·6H$_2$O was dissolved in water. The AC was dispersed in the solution during stirring according to the molar percent of Ni (AC) until it was dissolved and well distributed. Then, the coordination compound of Ni/AC was obtained by sonicating for 2 h, drying for 12 h at 110 °C in an electric oven and mixing with a batch agitator.

(2) The complex was placed in the middle of an electric resistance tube furnace and heated to the required temperature under a pure N$_2$ atmosphere with a heating rate of 5 °C/min. The temperature was then held for 1 h. After the reaction was completed, it was cooled to the ambient temperature. Composite materials of graphitized carbon and metal were obtained.

(3) Finally, the metal was removed by 3 M HCl and dried after it was washed to a neutral pH with deionized water. The graphitized carbon electrode material (GCM) was obtained.

Characterization. The GCM was prepared via an OTF-1200X tube furnace. A V-Sorb 2800 specific surface area and aperture analyzer was utilized to calculate the specific surface area and pore structure. The morphology and internal pore structure of AC and GCM were examined by field emission scanning electron microscopy (SEM) on a Quanta-450-FEG instrument and high-resolution transmission electron microscopy (TEM) on a JEM-3010 instrument. X-ray diffraction (XRD) patterns at low (10°) and high angles (90°) were collected on an XRD-7000S diffractometer, and the radiation source was Cu Kα (λ=0.154178 nm). First-order Raman spectra of samples were obtained with a LabRAM HR Evolution Laser Raman spectrometer [20]. A CHI610D electrochemical workstation was used to obtain the cyclic voltammetry characteristics of the samples.

RESULTS AND DISCUSSION

Experimental results and analysis of GCM preparation. In this paper, the influences of the Ni/AC molar percent and graphitization temperature on the specific surface area and specific capacitance of GCM were investigated, and the optimum preparation and processing parameters of GCM were obtained. The experimental results are shown in Table 1. The GCM was successfully prepared from the AC, and the BET specific surface area decreased. The catalytic graphitization changed the internal structure of the AC, which led to a decrease in the specific surface area. The specific capacitance value was approximately 200 F/g, indicating that the GCM had good electroadsorption properties. According to the extreme difference analysis, when the BET specific surface area was used as the evaluation index, the graphitization temperature in the preparation process led to k(K) > m. That is the influence of the heat treatment temperature during the preparation of the GCM was greater than the mass ratio of the Ni(NO$_3$)$_2$·6H$_2$O to the AC, and the optimum level was M$_1$N$_1$. When the specific capacitance was used as the evaluation index, k(K) > m was the same as in the former case, but the optimum process parameter was M$_2$N$_2$. Therefore, it was necessary to consider the effect of Ni/AC and the graphitization temperature on the optimization of the GCM preparation process. The various influencing factors that correspond to the k(K) value are shown in Fig. 1.

According to Fig. 1, the BET specific surface area decreases with increasing Ni/AC and graphitization temperature. The bonds between carbon atoms broke during the catalysis of the Ni(NO$_3$)$_2$·6H$_2$O, causing the collapse and penetration of the original pores in the AC. The increasing reaction temperature accelerated the reaction, which led to a further decrease in the specific surface area. Fig. 1(b) shows that the specific capacitance increases and then decreases with the increase of these two factors. When the Ni/AC is 0.2 mol%
and the graphitization temperature is 800 °C, the capacitance reaches its maximum. The purpose of catalytic graphitization is not only to shape the channel that can provide ion transmission but also to retain its relatively high specific surface area.

Therefore, the optimum preparation process parameters for the GCM are a Ni/AC amount equal to 0.2 mol%, a graphitization temperature of 700 °C, and a heat-retaining time of 1 h (level 8); this condition is referred to as GCM-5.

**TABLE 1**

Experimental results obtained for the semi-graphitized carbon electrode material (GCM)

<table>
<thead>
<tr>
<th>Levels</th>
<th>Ni/AC (M)</th>
<th>Graphitization temperature (N)</th>
<th>BET specific surface area /mg·g⁻¹</th>
<th>Specific capacitance/F·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1 mol%</td>
<td>500</td>
<td>1079.217</td>
<td>198.160</td>
</tr>
<tr>
<td>2</td>
<td>0.1 mol%</td>
<td>600</td>
<td>874.688</td>
<td>238.335</td>
</tr>
<tr>
<td>3</td>
<td>0.2 mol%</td>
<td>700</td>
<td>819.701</td>
<td>234.504</td>
</tr>
<tr>
<td>4</td>
<td>0.2 mol%</td>
<td>800</td>
<td>719.163</td>
<td>217.901</td>
</tr>
<tr>
<td>5</td>
<td>0.2 mol%</td>
<td>900</td>
<td>557.751</td>
<td>231.613</td>
</tr>
<tr>
<td>6</td>
<td>0.2 mol%</td>
<td>500</td>
<td>958.407</td>
<td>239.003</td>
</tr>
<tr>
<td>7</td>
<td>0.2 mol%</td>
<td>600</td>
<td>826.572</td>
<td>239.003</td>
</tr>
<tr>
<td>8</td>
<td>0.2 mol%</td>
<td>700</td>
<td>743.429</td>
<td>262.595</td>
</tr>
<tr>
<td>9</td>
<td>0.3 mol%</td>
<td>800</td>
<td>669.647</td>
<td>271.698</td>
</tr>
<tr>
<td>10</td>
<td>0.3 mol%</td>
<td>900</td>
<td>558.571</td>
<td>200.200</td>
</tr>
<tr>
<td>11</td>
<td>0.3 mol%</td>
<td>500</td>
<td>944.667</td>
<td>217.197</td>
</tr>
<tr>
<td>12</td>
<td>0.3 mol%</td>
<td>600</td>
<td>829.598</td>
<td>236.152</td>
</tr>
<tr>
<td>13</td>
<td>0.3 mol%</td>
<td>700</td>
<td>757.329</td>
<td>201.870</td>
</tr>
<tr>
<td>14</td>
<td>0.3 mol%</td>
<td>800</td>
<td>494.847</td>
<td>239.745</td>
</tr>
<tr>
<td>15</td>
<td>0.3 mol%</td>
<td>900</td>
<td>336.645</td>
<td>187.932</td>
</tr>
</tbody>
</table>

Note: k(K) represents the average value of the 5 experimental results at each level; r(R) indicates range, the larger the r(R), the greater the influence.

**FIGURE 1**

Effect diagram
Fig. 2 shows the N\textsubscript{2} adsorption isotherms of the AC and GCM. According to the IUPAC classification, the GCM adsorption curve is a type IV isotherm. Similar to the type I isotherm for AC, a rapid increase in the adsorption volume occurs in the low-pressure zone. However, in the range of 0.45–1 relative pressure, there is an obvious hysteresis loop due to adsorption condensation, which indicates that microporous and mesoporous structures exist simultaneously in the GCM. At the same time, the amount of N\textsubscript{2} adsorbed by the GCM is less than that of AC, and the specific surface area of the AC decreases after being modified by catalytic graphitization, which is consistent with the BET analysis results. To further determine the effect of pore structure changes, the pore structure parameters of the AC were compared with those of GCM. The pore structure parameters are shown in Table 2.

The specific surface area and total pore volume of the AC are 1637.793 m\textsuperscript{2}/g and 1.085 cm\textsuperscript{3}/g, respectively (see Fig. 2). The micropore volume accounts for 60% of the total pore volume, with a small average pore diameter, indicating that the AC structure is mainly microporous. With increasing Ni(NO\textsubscript{3})\textsubscript{2}·6H\textsubscript{2}O, the BET specific surface area and total pore volume of the GCM decrease, but the mesopore volume and the average pore diameter increase continuously. With increasing graphitization temperature, the mesopore volume and the average pore diameter increase at the same time. The mesopore volume decreases and the pore size increases at 900 °C because the graphitization temperature forces the pores and micropores to collapse and form a large pore structure, which leads to a decrease in the specific surface area. Although a high temperature increases the degree of graphitization of materials, the specific capacitance of the material decreases when the specific capacity is too small. Therefore, when a balance in the amount of catalyst and the temperature is achieved, which occurs when a balance in the surface area of the material and the degree of graphitization is achieved, the electroadsorption process is efficient.

**Surface morphology analysis.** After catalytic graphitization, the AC still maintained its abundant and developed pore structure. However, compared with AC, the GCM has a rough surface and a relatively low density (Fig. 3), and fine pores with different sizes are distributed on the surface. Ni(NO\textsubscript{3})\textsubscript{2}·6H\textsubscript{2}O is involved in the process of catalytic active carbon graphitization and is first decomposed into NiO, which needs to continuously consume active C atoms during reduction to metal Ni. The consumption of C causes a reduction in the micropores and generation of mesoporous holes, which increases the surface roughness of the AC. The structure of the graphitized electrode materials was analyzed by TEM at the atomic level, as shown in Fig. 3 (e, f, g, h). From the comparison in Fig. 3 (a) and Fig. 3 (e, f, g, h), it can be clearly observed that the porous surface structure of the AC changed upon being transformed to GCM. Fig. 3 (e) shows the overall morphology of the graphitized electrode materials. It is clear that the sample consists of a winding structure of graphene, as shown in Fig. 3 (f), and overlapping and bending carbon nanowires.

<table>
<thead>
<tr>
<th>Sample</th>
<th>S\textsubscript{BET} (m\textsuperscript{2}/g)</th>
<th>V\textsubscript{total} (cm\textsuperscript{3}/g)</th>
<th>V\textsubscript{mic} (cm\textsuperscript{3}/g)</th>
<th>V\textsubscript{meso} (cm\textsuperscript{3}/g)</th>
<th>D (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>1637.793</td>
<td>1.058</td>
<td>0.637</td>
<td>0.457</td>
<td>2.654</td>
</tr>
<tr>
<td>GCM-1</td>
<td>819.701</td>
<td>0.566</td>
<td>0.314</td>
<td>0.226</td>
<td>2.762</td>
</tr>
<tr>
<td>GCM-2</td>
<td>757.329</td>
<td>0.600</td>
<td>0.260</td>
<td>0.313</td>
<td>3.171</td>
</tr>
<tr>
<td>GCM-3</td>
<td>958.407</td>
<td>0.663</td>
<td>0.355</td>
<td>0.125</td>
<td>2.410</td>
</tr>
<tr>
<td>GCM-4</td>
<td>826.572</td>
<td>0.552</td>
<td>0.334</td>
<td>0.270</td>
<td>2.769</td>
</tr>
<tr>
<td>GCM-5</td>
<td>743.429</td>
<td>0.547</td>
<td>0.272</td>
<td>0.240</td>
<td>2.941</td>
</tr>
<tr>
<td>GCM-6</td>
<td>669.647</td>
<td>0.498</td>
<td>0.244</td>
<td>0.277</td>
<td>3.295</td>
</tr>
<tr>
<td>GCM-7</td>
<td>558.571</td>
<td>0.365</td>
<td>0.185</td>
<td>0.148</td>
<td>3.614</td>
</tr>
</tbody>
</table>
with a diameter of 10 nm, as shown in Fig. 3 (g); the images show a three-dimensional network structure in which the holes are interconnected and have good transmittance. These results show that the GCM has a low-density porous structure, which is conducive to electron and ion transport. The microporous and mesoporous structure of the GCM can be seen in Fig. 3 (h), and the lattice fringes of graphite can be seen on the edge that have a very low interlayer distance d_{002}, which further indicates that the GCM has a certain degree of graphitization.

**X-ray diffraction analysis.** Fig. 4 shows the GCM XRD patterns after catalytic graphitization, where the degree of graphitization changed. For the GCM, the XRD pattern exhibits three diffraction peaks at approximately 26°, 44° and 77°, which correspond to the peaks for the (002), (101) and (110) planes of graphite, respectively, indicative of the existence of some microcrystalline graphitic structure in the GCM. However, there are no sharp and intense diffraction peaks in AC diffraction pattern, indicating that the AC comprised an amorphous structure. When comparing the diffraction peak at 26° for AC and GCM, the intensity of the (002) diffraction peak for the GCM is much higher than that for the AC, indicating that the GCM obtained by catalytic treatment of Ni(NO₃)₂·6H₂O has a certain degree of graphitization. At the same time, there are no diffraction peaks at 44.5°, 51.8° and 76.4° for the GCM. This indicates that the NiO and Ni elements formed during the reaction of the Ni(NO₃)₂·6H₂O catalyst were removed after the sample was treated with hydrochloric acid. The Ni had no effect on the performance of carbon materials. The (002) crystal plane spacing represents the stacking thickness (Lc) of the graphite formed inside the carbon material. According to the Bragg formula (interlayer separation (d_{002})=λ/(2sinθ)) and the Scherrer equation (Lc=Kλ/β_{002}cosθ), a d_{002}=0.340 nm and microcrystalline size Lc=11.523 nm were obtained for the GCM. The d_{002} value is between the value of 0.3354 nm of completely ungraphitized carbon and 0.344 nm of ideal graphitized carbon, which indicates that a graphite crystal structure exists. According to the equation to calculate the degree of graphitization, the GCM had a value of 0.46, indicating that it had a high degree of graphitization.

\[
g = \frac{0.3440 - d_{002}}{0.3440 - 0.3354}
\]

**FIGURE 3**
(a) SEM images of activated charcoal (AC), (b, c, d) the semi-graphitized carbon electrode material (GCM), and (e, f, g, h) TEM images of the semi-graphitized carbon electrode material (GCM)

**FIGURE 4**
XRD patterns of the semi-graphitized carbon electrode material (GCM)
Raman spectrum analysis. Raman curves for the AC and GCM are shown in Fig. 5. The degree of graphitization of the samples was further determined by Raman spectroscopy. The AC and GCM have two distinct peaks in the Raman spectra: the D band at approximately 1350 cm⁻¹ and the G band at approximately 1600 cm⁻¹. The D band peak in a typical amorphous carbon structure, which is caused by a graphite lattice defect, indicates a disordered graphitization structure. The G band peak is the telescopic vibration of the sp² bond in C in the hexagonal base of the graphite, indicating an ordered graphite lattice. The higher the D peak intensity is, the higher the disorder of the material. In contrast, the higher the G peak intensity is, the better the crystallinity and the higher the degree of graphitization. The intensity ratio of the D band and the G band (I_D/I_G) is widely used to estimate the degree of graphitization [21], and it is known that a smaller ratio of I_D/I_G produces a more graphitized structure [22, 23]. The GCM G band is much higher than that of AC, and the half peak width is narrower, which indicates that GCM had a high graphitization degree. The calculated I_D/I_G ratios for the AC and GCM are 0.69 and 0.65, respectively, which are consistent with the above analysis. This result shows that the GCM contained graphitized crystalline carbon and had a high degree of graphitization.

Electrochemical analyses. AC and GCM electrodes were prepared by a vapor coating method with a mass ratio of AC, PVDF and graphene crystallite of 8:1:1. The electrochemical performance of the AC and GCM was measured using cyclic voltammetry with an electrochemical workstation comprising a three-electrode system and 0.5 M NaCl electrolyte, and the results are compared in Fig. 6. Platinum wire was used as the counter electrode, and the AC or GCM electrode formed the third electrode in the three-electrode system.

As shown in Fig. 6 (a), the cyclic voltammetry curves (CV), with shapes resembling a willow to a rectangular shape, have good symmetry, and do not indicate an obvious redox reaction; this result shows a rapid charging and discharging process, low internal resistance [24,25], excellent electrolyte accessibility throughout the electrode and evidence that the AC and GCM electrodes mainly operated on the principle of a double electron layer. The area of the CV curve for the GCM electrode is obviously larger than that for the AC electrode, which further proves that the specific surface area of the GCM is relatively small; however, its active area ratio is high and can provide more adsorption sites than the AC. Therefore, the GCM electrode has a high specific capacitance of 271.698 F/g, which is 44.13% higher than the specific capacitance of 188.515 F/g of the AC electrode. Fig. 6 (b) shows the CV curves of the GCM electrode at different scanning rates (5, 10, 20, 50 mV/s), all of which show a tendency to change from leaf-like to rectangular, and the shape is symmetrical. The curve area gradually decreases with increasing scanning rate, but the attenuation is slow at the same time, indicating that the cyclic voltammetry of GCM was performed. The GCM has the advantage of cycling stability for double electrode materials, which can be attributed to the existence of a considerable number of mesopores.
that facilitate the rapid transmission and infiltration of electrolyte ions in the pore channels, thus improving the utilization of the specific surface area.

CONCLUSIONS

(1) The optimized parameters to prepare the GCM with Ni(NO$_3$)$_2$·6H$_2$O as the catalyst via the graphitization method were a Ni/AC amount of 0.2 mol%, a graphitization temperature of 700°C, and a heat-retaining time of 1 h. The BET specific surface area of the prepared GCM was 743.429 m$^2$/g, and the specific capacitance was 262.595 F/g.

(2) The GCM contained microporous structures and a considerable number of mesoporous structures. There was a high degree of graphitization; the GCM (002) crystal plane spacing d$_{002}$ was 0.340 nm, and the crystallite size L$_c$ was 11.523 nm. The degree of graphitization was 0.46, and the I$_D$/I$_G$ value was 0.65.

(3) The CV area of the GCM electrode was substantially larger than that of the AC electrode, and the specific capacitance of the GCM electrode was 44.13% higher than that of the AC electrode (188.515 F/g). The GCM was stable as an electric double layer electrode material.

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In this study, it has been aimed to investigate the effects of the plant *Tribulus terrestris* whether it is effective as a liver protective agent with an anti-inflammatory effect against carbon tetrachloride (CCl4), which is hepatotoxic when used chronically, and it has been aimed to examine the degree of its effectiveness when such an effect has been observed.

In the study, 35 Wistar-Albino male rats aged 8-12 weeks were used. The rats were assigned to five groups of seven rats each. The rats were fed with a standard diet in all groups. Excluding the control group, all rats in the other groups received CCl4 at a dose of 0.8 ml/kg administered intraperitoneally for 14 days. CCl4 was administered to the rats in the groups 3, 4, and 5 and this period was followed by the administration of *Tribulus terrestris* plant extract for a period of 8 weeks. The serum levels of NO, TNF-α, IL-6, and IL-1β were tested in the rats. It was observed that all study parameters increased significantly in the CCl4 group compared to the control group (p<0.01). Compared to the CCl4 group, the study parameters decreased in all treatment groups (p<0.01) and the extent of decrease was the highest in the group 5, where a high dose of the plant extract was administered. While the levels of NO, TNF-α, IL-6, and IL-1β decreased significantly in the treatment groups compared to the CCl4 group, the reduction in the NO and IL-6 groups approached to the levels observed in the control group. This study has demonstrated that the plant has a liver protective effect against CCl4 which is known with its toxic effects and which induces inflammation in the rat liver. More effective results were observed especially with the higher doses of this plant’s extract. We are of the opinion that the extract of *Tribulus terrestris* can be beneficial in human beings when used either for preventive purposes or for its anti-inflammatory effects and it can be considered as a supplement in the clinical treatment of liver diseases, becoming one of the agents in adjuvant treatments.

**KEYWORDS:** Carbon tetrachloride, *Tribulus terrestris*, Inflammation, interleukin
against carbon tetrachloride (CCl4), which is hepatotoxic when used chronically, and it has been aimed to examine the degree of its effectiveness when such an effect has been observed.

MATERIALS AND METHODS

In the study, 35 Wistar-Albino male rats aged 8-12 weeks were used. The rats were assigned to five groups of seven rats each. The rats in the control group were fed with a standard diet for 14 days. The rats in the groups 2 and 3 were fed with a standard diet and they were administered 0.8 ml/kg CCl4 intraperitoneally for 14 days. The rats in the groups 3, 4, and 5 received the extract of Tribulus terrestris plant for a period of 8 weeks following the intraperitoneal administration of 0.8 ml/kg CCl4 for a period of 14 days. The body weights of the rats were measured at the beginning and at the end of the study. All rats were decapitated in an appropriate environment under appropriate conditions. The rats in group 1 and 2 were decapitated 2 weeks after the baseline and the rats in group 3, 4, and 5 were decapitated 10 weeks after the baseline. After decapitation, the blood samples were collected to investigate the study parameters. After collecting the blood samples in gel tubes for biochemical tests, they were centrifuged at 4000 rpm for 5-10 minutes, eliciting the serum. The local ethics committee on animal research of Firat University (FÜHADYEK) approved the study (31.03.2017/2017/07, decision no: 81, Protocol no: 2017-39) and the study was conducted in compliance with the ethical principles of standard experimental animal research.

All interventions to be applied to the animals were performed in compliance with the principles announced by the local ethics committee on animal research of Firat University (FÜHADYEK). The care of the animals took place at Firat University Experimental Animal Application and Research Center. Carbon tetrachloride, which was used in this study to induce toxicity, was provided by the company Merck.

A total of 5 groups were made, assigning 7 rats to each, using random sampling. The groups and the summary of the procedures applied in each group are listed below:

- Group 1: Control group
- Group 2: CCl4 group
- Group 3: CCl4 + 0.5 g/kg Tribulus terrestris
- Group 4: CCl4 + 1 g/kg of Tribulus terrestris
- Group 5: CCl4 + 2 g/kg Tribulus terrestris

Analysis of The Levels of Cytokines. In the present study, the levels of inflammatory cytokines including TNF-α, IL-1β, and IL-6 in the plasma and liver tissue homogenates were analyzed in an ELISA device (Biotek ELx800) using a rat-specific kit (e-Bioscience, Vienna, Austria) in compliance with the product protocols. For the purpose of generating the calibration curve required to automatically determine the concentrations of the study samples using their absorbances during the ELISA analyses, 8 standards were prepared by dissolving and then diluting the standards supplied with the kits.

**Determination of NO.** The measurements have been based on the diazotisation of nitrite with an aromatic amine sulphonamide in an acidic environment and then forming an azo derivative with N-(1-Naphthyl)ethylenediamine (NEDD). This reaction is called as The Gries Reaction, which is the conversion of nitrate to nitrite by Vanadium chloride in a 37°C environment [7].

**Tribulus terrestris Administration.** The extract of Tribulus terrestris was prepared by dissolving in water. The doses of the extract administered orally by group were 0.5, 1.0, and 2.0 mg/kg for group 3, 4, and 5, respectively.

**CCl4 Administration.** Dissolving 0.8 ml/kg CCl4 in 0.8 ml/kg liquid oil, all rats except the ones in the control group received an intraperitoneal administration of 0.8 ml/kg CCl4 daily for 14 days in addition to the standard feed.

**Termination of The Study.** 24 hours after the end of the period when Tribulus terrestris administrations were made, blood samples were collected from all of the animals under sodium pentobarbital anesthesia (pentobarbital sodium, 40 mg/kg, i.p.) [12] and then the blood samples were prepared for performing the laboratory analyses. All data were described as mean ± standard error (SE). A one-way analysis of variance (ANOVA) was used to evaluate the data.

RESULTS

The serum levels of nitric oxide (Figure 1), tumor necrosis factor-α (Figure 2), interleukin-6 (Figure 3), and interleukin-1β (Figure 4) were determined in the rats with CCl4-induced liver injury and statistically significant differences were observed in inter-group comparisons (p<0.01). The serum levels of NO, TNF-α, IL-6, and IL-1β were tabulated (Table 1). Significant increases were found in the levels of all parameters in group 2 compared to group 1 (p<0.01). CCl4 resulted in significantly increased levels of NO, TNF-α, IL-6, and IL-1β (Table 1). Compared to the CCl4 group, the study parameters decreased in all treatment groups (p<0.01) and the
extent of decrease was the highest in the group 5, where a high dose of Tribulus terrestris extract was administered. While the levels of NO, TNF-α, IL-6, and IL-1β decreased significantly in the treatment groups compared to the CCl4 group, the reduction in the NO and IL-6 groups approached to the levels observed in the control group.

**DISCUSSION**

Several studies have demonstrated that increased oxidative stress in liver diseases is associated with liver damage, inflammation, and fibrosis [8-11]. Oxidative stress increases lipid peroxidation by its negative effects on the antioxidant mechanism, leading to inflammation and fibrosis in the liver [12]. Carbon tetrachloride (CCl4) is a hepatotoxin and causes the production of free radicals, consequently leading to tissue damage [13]. It is known that oxidative stress plays an important role in the formation of liver toxicity due to carbon tetrachloride [14]. Inflammation is closely associated with oxidation in biological systems and it is actively involved in the development of acute hepatic injury induced by APAP [15]. Inflammation develops with the release of pro-inflammatory mediators, such as NO, TNF-α, IL-6, and IL-1β. Huang et al. observed that the levels of NO, TNF-α, IL-6, and IL-1β were elevated in the rats, which Acetaminophen (APAP) was administered. The administration of Ziziphus jujuba (ZJF)
decreased the expression of these pro-inflammatory mediators significantly. NF-κB p65 is a key transcription factor for the up regulating inflammatory cytokines. These results suggest that ZJF inhibits the inflammatory responses induced by the APAP. Furthermore, the ZJF plant attenuated the production of APAP-induced inflammatory mediators such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) [16]. Therefore, the elevated levels of NO, TNF-α, IL-6, and IL-1β, occurring due to the use of another chemical (CCl4) in our study to induce liver toxicity, are consistent with the results of that study. The decreased levels of the study parameters observed in the groups treated with the extract of Tribulus terrestris, which has an antioxidant content rich in flavonoids and saponins, support our study.

Nitric oxide (NO) is a highly reactive oxidant produced in response to inflammatory stimuli in the liver and plays an important role in hepatic injury [17]. In an animal study, several results have been obtained suggesting that increased iNOS and decreased eNOS activities may be responsible for fibrosis [18]. Furthermore, liver necrosis and fibrosis were found to decrease in the iNOS-gene knocked out rats, in which cirrhosis was induced by carbon tetrachloride. It was suggested that NO expressed by iNOS contributed to the development of fibrogenesis by inducing collagen synthesis. It was further suggested that this process was mediated by ONOO which was produced via the interaction of NO with SOR [19]. It was demonstrated in the rats with carbon tetrachloride-induced cirrhosis that the administration of simvastatin was followed by the decrease in the portal pressure in association with the increased levels of NO at the sinusoidal level. It was also demonstrated that simvastatin leads to eNOS expression and NOS activation increasing the levels of NO quantitatively [20]. In our study, the increased levels of NO in association with the effect of CCl4 present similarities with that study. The inducible nitric oxide synthase (iNOS) is related to the pathophysiology of several disorders including the overproduction of NOS, inflammatory disorders, hepatocarcinoma, and autoimmune diseases [21, 22]. TNF-α may induce iNOS and accelerate the production of nitric oxide, contributing to nitrosative stress [23]. In another study, the increased iNOS levels in the serum of CCl4 treated rats showed that the production of NO and nitrosative stress increased in response to liver damage. It was also demonstrated that iNOS mediated the CCl4 induced liver damage and the administration of the total amount of saponin in the Actinidia valvata plant to the rats produced favorable effects in the prevention of acute liver damage [24]. The results of these studies support the results of our study as the increased levels of NO induced by the administration of CCl4 has decreased with the administration of Tribulus terrestris in our study. Tumor necrosis factor, α (TNF-α), Interleukin-6 (IL-6), and Interleukin-1β (IL-1β) are the major pro-inflammatory cytokines involved in the progression of liver injury induced after the administration of acetaminophen [25]. The inflammation of the liver is discriminative in differentiating hepatic fibrosis in chronic diseases of the liver in terms of liver immunity. TNF-α, IFN-γ, and IL-6 are the critical mediators of fibrosis. TNF-α promotes the development of hepatic fibrosis caused by various pathogens [26-29]. IL-6 induces hepatic inflammation and collagen synthesis in vivo [30]. CCl4 is demonstrated to induce hepatic inflammation and fibrosis in the given rat models. In this study, the levels of ALT, AST, TNF-α, and IL-6 increased significantly in the model group compared to the normal group [30]. Abdel-Moneim et al. investigated the hepatoprotective effect of taurine (TAU) alone or in combination with silymarin (SIL) in CCl4-induced liver injury. The result was a significant increase in the hepatic levels of TBARS, NO, and NOS compared to the control group. Taurin and silymarin administration resulted in significant reductions in the NO levels. The levels of IL-6 and TNF-α were elevated with the administration of CCl4 and decreased significantly with the administration of antioxidants silymarin and taurine [31]. In individuals treated with the powder of the Chinese herbal extract Fuzheng Huayu, it was demonstrated that the extract led to significant improvements in the hepatic function, ameliorated the inflammatory and fibrotic changes, and decreased the hydroxyproline content in the liver after the exposure to CCl4 [32]. In the present study, it was observed that liver injury and inflammation developed in the CCl4 administered groups in alignment with the data in the literature. A remarkably significant increase occurred in the levels of NO, TNF-α, IL-6, and IL-1β in the CCl4 administered group. The inflammation was ameliorated and the levels of NO, TNF-α, IL-6, and IL-1β decreased in the groups treated with various doses of Tribulus terrestris plant extract after the administration of CCl4.

**CONCLUSION**

This study has demonstrated that the Tribulus terrestris plant has a liver protective effect against CCl4 which is known for its toxic effects and which induces inflammation in the rat liver. More effective results were observed especially with the higher doses of this plant's extract. **We are of the opinion that the extract of Tribulus terrestris can be beneficial in human beings when used either for preventive purposes or for its anti-inflammatory effects and it can be considered as a supplement in the clinical treatment of liver diseases, becoming one of the agents in adjunctive treatments.**
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EVOLOTION BARLEY GENOTYPES IN MULTI-ENVIRONMENT TRIALS BY AMMI MODEL AND GGE BIPLOT ANALYSIS

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ABSTRACT

The uniformity of genotypes are significant for crop breeding program decisions to improve new varieties. The AMMI (Additive main effects and multiplicative interaction) analysis and Genotype x Environment Interaction (GEI) is make to estimation grain yield and understands GxE interaction patterns by researches as differential ranking of variety yields in multi-environment trials. Therefore, fifteen barley advanced line and six national cultivars and four foreign varieties (registered in abroad) were used in the study. The experiments were performed according to a complete randomized block design with four replications at five environments during two years. The stability and superiority of genotypes for yield and other traits were determined using AMMI and GGE biplot analysis. Factors (G, GE, and GEI) were found to be highly significant (P < 0.01) for grain yield. AMMI analysis indicated that the major contributions to treatment sum of squares were environments (98.52%), GE (0.45%) and genotypes (1.02%), respectively, suggesting that grain yield of genotypes were effected environmental conditions. The GGE biplot indicated that PCA 1 axes (Principal component) was significant as P<0.01 and supplied to 49.36% of complete GxE interaction. The AMMI indicated that G8 and G23 desirable and stable genotypes for grain yield in multi-environment. Moreover, E2 and E5 (irrigated environments) were high yielding, while E3 (drought stress) low yielding as forecast. On the other hand, GGE biplot indicated that three group were occurred among traits, first group (GY: grain yield, CC: crude cellulose, CD: cold damage), second group (PC: , HW: hectoliter weight, TGW: thousand grain weight, SH: seed humidity), third group (LOD: lodging, PH: plant height, HT: heading time). Moreover: the study showed that G3, G6, G7, G8, G13 and G21 were the best genotypes both grain yield and other traits. The results of AMMI model and GGE biplot indicated that G8 is suitable to recommend for release and G23 desirable origin for yield stability and G7 valuable source for quality to use in barley breeding program.

INTRODUCTION

Turkey is important for barley grain, because it ranks 6th barley production in in the world. In fact, Turkey has more barley production potential, but there are more limiting factors. Some of these limiting factors are lack of varieties with high yield potential and agronomic practices, fluctuations in product prices policy and etc. On the other hand, the environment conditions is changing from region to region and year by year. Therefore, it is an essential to improve modern varieties, which is stable and favorable under environmental and other factors. Barley has been cultivated for many years and has a wide range of adaptation, growing best on fertile, well-drained soils in Shout –Eastern Anatolia of Turkey. It is also grown mainly on rainfall conditions, but genotype x environment interaction (GEI) restricts the progress in yield improvement under rain fed and unpredictable climatic conditions [1]. Modern barley breeding is largely directed towards the development of genotypes characterized with increased yield potential, wide adaptation and high responses to agronomic inputs [2]. Some agronomic and technological traits such as lodging (LOG), plant height (PH), thousand kernel weight (TKW), hectoliter mass (HM) and grain protein content (GPC) have significant influence on barley grain yield and quality. The yield and other traits of each variety in any environment is a sum of environment (E) main effect, genotype (G) main effect and genotype by environment interaction (GE or GEI) [3]. Farmers need varieties that show high performance in terms of yield and other essential agronomic traits. Their superiority should be reliable over a wide range of environmental conditions and years. Different statistical analysis, such as correlation, path coefficient and principal component analysis (PCA) can be used to reveal associations between yield and other agronomic traits. The impact of AMMI and GGE Biplot methods has been clearly showed by different researchers using
multi-environment. These methods; provide the correlative size and significant effects of GEI and its interaction [4]. This method enables better understanding of genotypes performance over several environments, and selection of stable and high yielding genotypes [5]. Also it is important for testing promising lines under across environments to estimate stability and performance [6], and thus, it is useful for breeders and supporting breeding program decisions. The major objective of study reveal adaptation of barley genotypes using AMMI and GGE Biplot analysis to estimate the importance of GE interaction on yield, define the correlations among traits and estimate performance of genotypes and recommend lines to release in breeding program.

**MATERIALS AND METHODS**

**Plant genetic materials.** The experimental material comprising sixteen lines, five regional and four international spring barley varieties (Table 1) were evaluated in seven rain-fed environments in different growing season (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pedigree of genotypes</th>
</tr>
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<tr>
<td>G1</td>
<td>Hml02/ArabiAbiad//ER/Arpm/3/Belford..TR-0AP-5AP-0AP-4AP-8AP-0AP</td>
</tr>
<tr>
<td>G2</td>
<td>Lignee527/Chn-01/Lignee527/As4ICB93-0813-0AP-5AP-0AP</td>
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### Table 2

<p>| Years, sites, codes, coordinate status of environment long term of precipitation. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
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<th>Latitude</th>
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The experiment was conducted in a randomized block design with four replications at five environments (rainfall and irrigated) during 2004-2005-06 growing seasons. Totally, nearly 150 mm supported in per irrigated environments. The seeding rate was used 450 seeds m⁻². Plot size was 7.2 m² (1.2 × 6 m) consisting of 6 rows spaced 20 cm apart. Sowing was done by Wintersteiger drill. The fertilization rates for all plots were used 60 kg N ha⁻¹ and 60 kg P ha⁻¹ with sowing time and 60 kg N ha⁻¹ was applied to plots at the early stem elongation. Harvest was done using Hege 140 harvester up on 6 m².

**Statistical analysis.** The data grain yields of twelve (25) genotypes in five (7) environments were evaluated by AMMI analysis [7]. The AMMI and GGE biplots were used to identify the mega-environments and superior genotypes for grain yield and other traits. All statistical analyses were performed using GenStat Release 14.1 (Copyright 2011, VSN International Ltd.) and GGE biplot software programs.

The data were graphically analyzed for interpreting GE interaction using the GGE biplot software [8]. GGE biplot methodology is composed of the biplot concept [9] and GGE concept [28]. The graphs generated based on (1) The AMMI 1 model showing Genotype x Environment means, (2) The relationship genotype by five environments (3) Mega environments “which-won-where” pattern to identify the best genotypes in each environment, (4) The relationship genotype by trait, (5) “which-won-where” pattern to identify the best genotypes for traits, (6) Ranking genotypes based on traits by mean and stability, (7) Comparison of genotypes based on traits by ideal genotype.

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<th>F Ratio</th>
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df, degrees of freedom; **, p<0.01; *, p<0.1; G, Genotypes; E, Environments.

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**TABLE 3**
The variance of AMMI analysis on grain yield of barley

**TABLE 4**
The average yield performance at each E and over environments (kg ha⁻¹)
RESULTS AND ANALYSIS

The variance of AMMI Analyses in grain yield: The variance of AMMI analysis showed that as p<0.01, all factors had significant effect on barley grain yield of twenty five genotypes tested in five environments and total sum of squares explained 98.52% for environmental effects, 1.02% for genotypic effects and only 0.45% GEI effects (Table 3). The high addition of environment effects showed that there were important differences among environments for grain yield. On the other hand, the genotype effect were high than GEI effect. The results of AMMI analysis showed similar results of [14], [15], [16], showed 89.6%, 8.6% and 1.8%. Moreover, [17] and [18], reported that the environment effect had the highest effect than other factors on barley and soybean grain yield respectively. The results of Environment, Genotype and G x E effects obtained from this study illustrated similar results of the studies described above and the effect of environment >genotypes>GEI. The existence interaction of grain yield displayed by GGE biplot, especially when the interaction portioned between two interaction principal component axis (PCA) (Table 3). This status of GGE biplot made it establish and the biplot calculate effects of genotype and environment. The results of mean square of the interaction axis PCA 1 was significant (p<0.01), while PCA 2 was not significant ([2], [11]. Results of GGE biplot analysis also indicated that the PCA 1 axis accounted 74.32%, PCA2 accounted for 24.68% (Figure 2). GGE biplot showed existence interactions of G x E, so it was portioned between first and second IPCA (Interaction Principal Component Axes).

The barley grain yield variation is depending on genotypic and environment factors as shown Table 1 & Table 2. [6], [17], suggested that the AMMI model is the most accurate a model because it can predict using the first two IPCAs. The closer the IPCAs scores to zero are meaning that genotypes are the most stable across their environments. Actually, these biplots is removed two types, model of AMMI 1 and model of GGE biplot [19], [20]. In AMMI 1, the genotype and environments means are plotted on coordinate, the IPCA scores of same genotypes and environments, which are on the ordinate.

### TABLE 5
AMMI selections the first four genotypes for per environment and PCA scores.

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<th>Score 4</th>
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### TABLE 6
The traits value of average for twelve genotypes.

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<th>Protein content (%)</th>
<th>Crude Cellulose (%)</th>
<th>Seed Humidity (%)</th>
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<td>11.8</td>
<td>4.7</td>
<td>10.3</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

Mean 111 96 39.7 63.2 12.3 4.9 10.7 28.5 3.9
For interpretation of AMMI, size and signal scores of the IPCA1 were observed, score near to zero were typical of genotypes and environments, which contribute little to the interaction that is they are stable [21].

The AMMI model showing Genotype x Environment means of grain yield. In the AMMI model, x-axis represents the genotypes and environment main effect and y axis represents the effects of interaction (Figure 1).

The environment and genotypes indicated much more variability in both main effect and interaction. According to AMMI, majority of genotypes (G2, G3, G7, G8, G9, G11, G13, G17, G18, G20, G23 and G25) and majority environments (without E3) showed good performance, because of they took place above on axis (mean yield). It is believed that these genotypes and environments were high yielding and desirable. On the other hand, G4, G5, G6, G12, G10, G15, G16 and E3 demonstrated low performance, because they located under axis (mean yield). So, these cultivars and environments, which located under axis (mean yield) were low yielding and undesirable. Additionally, E4 and E5had both high yield potential and positive IPCA1 scores; it means that this two environment are desirable. While G8 and G23 had highest grain yield amongst genotypes, G17 was very stable with low and positive IPCA scores (Table 5).

According to [5], the genotypes have small IPCA1 values are more stable, [22] the basic static concept of stability shows minimal variance of stable genotype across different environments. Therefore, G17 can be recommended to all environments, while G8 and G23 for high yield potential environments (special environment). Similar outputs were recorded by [23], in barley.
The relationship genotype by five environments. The GGE biplot analysis was used for estimation of discriminating power and representativeness of an environment as a test one for assessing genotypes. GGE biplot allows visualizing environment vectors lengths, which are proportional to standard deviations of genotype yields in corresponding environment (Fig. 2).

If the marker of a test environment is close to the biplot centre, i.e. has a short vector, all genotypes in it are similar, and this environment is not informative about their differentiation. In the study, all environments had nearly same long vectors, so they had same discriminating power and characterized by same discriminating power. The cosine of the angle between environment vectors is used for assessment of approximation between them: the smaller the angle between environment vectors is the larger correlation between them [21]. Correspondingly, there is a strong correlation between environments E1, E2 E4 and E5, while there is no correlation between E3 and other environments.

Mega environments “which-won-where” pattern to identify the best genotypes in each environment. Dividing the target environment into meaningful mega-environments and deploying different cultivars for different mega-environments is the only way to utilize positive GE and avoid negative GE and the sole purpose for genotype by environment interaction analysis [11]. A mega-environment is defined as a group of environments that consistently share the same best cultivar(s) [18]. This definition explain the following biplot based on the multi-environment trials (MET) data of barley yield illustrates two points: 1) A mega-environment may have more than one winning genotypes (Sector 2), and 2) even if there exists a universal winner (G23, G8, G15), it is still possible, and beneficial, to divide the target environments into meaningful mega-environments (Figure3).

Mainly, these six lines divide the biplot into six sectors. The environments located only three sectors; other sector did not related with any environment just covered special genotypes. On the other hand, E2, E4, E5, took places in the same sector consist of G11, G17 G23, G25 and G23 was high yielding and represented of vertex these there environments. The second sector consists of E1 with G3, G8, G21 and G21 took places of vertex of this sector. The third sector consists of E3 with G5, G10, G12, G15 and also G15 located the vertex of sector. Consequently, G23 had high yielding at three environments (E2, E4, E5), while G8 for E1 and G15 for E3 [24], reported that there is a strong correlations between environments, which located in same sector [25], the large variation due to location indicated strong influence of environments and existence of mega-environment among trial conducting locations, this suggests the usefulness of GGE biplot technique for identifying mega-environments among barley growing locations. Same [12], reported that the GGE biplot graphic analysis complements the AMMI biplot stratification, defining mega-environments and the cultivars that optimize performance in such mega-environments.

The GGE Bipplot Analysis of genotypes by traits. In this analysis, the results of traits were examined by GGE Bipplot analysis using different figures. The biplot of the principal component analysis illustrates relationships between the studied barley traits and genotypes at five environments (Figure 4- Figure 7). First PCA explained 27.53% of total variation, while second PCA explained 25.52%. Together, both axes accounted for 53.7% of the total variation in the data. According to the biplot figures, the relations between genotype and traits by traits were examined. The GGE Bipplot showed that the breeders could select best genotypes for all traits and specific genotype for specific trait in breeding program.
The relationship between genotype and trait. Both the genotype vectors and the traits vectors are drawn in Figure 4, so that the specific interactions between a genotype and a trait (i.e., the performance of each genotype in each environment) can be visualized (Fig. 4).

Figure 4 can be used (1) to rank the genotypes based on performance in any trait, and (2) to rank traits on the relative performance of any genotype. The interpretation of performance a genotype in a trait is better than average if the angle between its vector and the trait’s vector is <90°; it is poorer than average if the angle is >90°; and it is near average if the angle is about 90° [8]. When the angle between two genotypes is >90°, then these two genotypes are different as genetic. Therefore, the results of traits showed that there is high variation among genotypes. According to results, G3, G4, G6, G7, G13 and G21 are desirable for all traits, and so they can be recommended for release. On the other hands, some national cultivars (G5, G10, G15), G12 and G18 had been late maturity time, very tall and lodging scores, so these genotypes is not recommend for these environments. The cultivar which located near to the biplot center have less contribution to G or GE, while genotypes having longer vectors show the most contribution of G and/or GE [8]. So, G7 with the longest vectors is the best genotypes, while some other genotypes (G11, G18 and G20) have short vector. The genotypes are far from center of Biplot graphs, are specific genotypes for specific environment. There are negative correlation between two traits, are opposite to each other on graph and the angles of vector is
>90°. Therefore, there is major contribution of trait to traits; because of they have opposite direction, so they can make up different genetic contribution [26], [5].

"which-won-where" pattern to identify the best genotypes for traits. The polygon view of a biplot is the best way to visualize the interaction patterns between genotypes and traits [27] to show the presence or absence of cross over GT interaction which is helpful in estimating the possible existence of different mega environments [28], [29]. Visualization of the "which won where" pattern of MET data is necessary for studying the possible existence of different more traits in the target traits [28].

Fig. 5, represents a polygon view of barley genotype MET data in this investigation. In this biplot, a polygon was formed by connecting the vertex genotypes with straight lines and the rest of the genotypes placed within the polygon. The partitioning of GT interaction divided into six sector. The traits (CC and CD) took place in sector 1 and related with G1; GY in sector 2 and related with G23; the three traits (HW, TGW and PC) in sector 3 and related with G3, G6, G7, G8, G13 and G21; SH and PH in sector 4 and related with G5, G10, G15, G19 and G24; HT in sector 5 and related with G12 and G25; LOD in sector 6 and related with more genotypes (G2, G9, G11, G14,G16, G17 and G22). On the other hand, there were correlation amongs sector 4 (PH), sector 5 (HT) and sector 6 (LOD), it means that the genotypes, have late heading time, are very tall and have lodging features as well as. The vertex genotypes in this study were G1, G7, G5 and G16. These genotypes were the best or the poorest genotypes in some or all of the traits, because they were farthest from the origin of the biplot [27]. From the polygon view of biplot analysis of MET data, the genotypes fell in six sections and the traits fell in six sections. The first section contains G1(resistance to cold damage), second section contains G23 (best yielding), third section contains 6 genotypes especially G7 (very quality), and other sections (4, 5 and 6) contains poor genotypes, which have tall plant height, late maturudy and lodging and low yielding features. They were located far away from all of quality traits and GY, reflecting the fact that they yielded poorly. Also, those genotypes within the polygon (for example G5 and G16) were less responsive to quality and grain yield than the G23 and G7, have vertex genotypes [26], [10].

Comparison genotypes based on traits mean and instability. The genotype has both high traits mean and high stability is called a favorable genotype. It should have both high mean performance and high stability for all traits (Figure 5, Figure 6). The center of the concentric circles (ideal) is a point on the AEA ("absolutely stable") in the positive direction and has a vector length equal to the longest vectors of the traits on the positive side of AEA ("highest mean performance"). Therefore, genotypes located closer to the stable line and has high mean values of traits are meaning that it is more favorable than others ([8]; [3]). Thus, any genotype was not located center of AEA ("absolutely stable"), but; G7, G13 and G24 took place of near center of AEA and high mean of traits. So these genotypes are favorable than others. According to Figure 6, the G20, G24 and G7 are high stable and moderate favorable, while G7 and G13 are moderate "stable" and more favorable, because these two genotypes have high mean value of traits. From this example, we can recommend G7 and G13 to study for more traits.
Ranking of genotypes based on traits. The genotype has both high traits mean and high stability is called an ideal genotype (Figure 7).

The center of the concentric circles is a point on the AEA (“absolutely ideal”) in the positive direction and has a vector length equal to the longest vectors of the traits on the positive side of AEA (“highest mean performance”). Therefore, genotypes located closer to the ideal circle are meaning that it is ideal genotype than others [8]. In the study, any genotype was not located center of AEA (“absolutely stable”), but; G5, G7, G3 and G13 took place of near center of AEA. So these genotypes are ideal than other genotypes. Consequently, G7 and G13 are close to ideal genotype, so, these genotypes can be recommended for release in terms of all traits. The researchers reported that the biplot show excellent discriminating to select genotypes for all traits and to recommendation for release [29].

CONCLUSION

The AMMI results indicated that yield performance of barley genotypes were highly influenced by environment followed G effect and GE interaction with the least effects. The genotype G8 and G23 showed best performance among genotypes tested across environments, while the oldest cultivar (Tokak) had least grain yield and adaptability. Therefore, the G8 was desirable in terms of high mean yield and stability; this means that the study provided an indication of the genetic progress. According to the results, the specific genotypes were appropriate for specific traits (G7 for quality, G8 for GY). The AMMI method and GGE biplot analysis allowed a meaningful and useful summary of GE interaction data and assisted in examining the natural relationships and variations in genotype performance across test environments. As a result indicated that G8 and G23 are suitable to recommend for release and G7 valuable source for quality to use in barley breeding program.

ABBREVIATIONS

Abbreviations and Symbols. AMMI, Additive main effects and multiplicative interaction; AEA, average-environment axis; AEC, average-environment coordination; GE, genotype by environment interaction; GGE, G + GE; MET, multi-environment trials; PC, principal component; PCA, principal component analysis; E, environment; G, genotype; TGW, thousand grain yield; Hw, hectoliter weight; PC, protein content; SA, sieving above; LS, lower sieving.

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STUDY OF THE INSECTICIDAL ACTIVITY OF TAXUS BACCATA OF THE IFRANE VALLEY ON SITOPHILUS ORYZAE (L.) (COLEOPTERA: CURCULIONIDAE)

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Laboratory of Molecular Chemistry and Natural Substance, Moulay Ismail University, Faculty of Science, B.P. 11201 Zitoune, Meknès, Morocco

ABSTRACT

The aim of this work is to propose a new alternative method based on plants to fight against synthetic insecticides. The powder and the ethanolic extract, prepared from the leaves of Taxus baccata of Ifrane Val, were tested on Sitophilus oryzae (L.) with different concentrations and for various durations of exposure.

On the first hand, the toxicity of the prepared powder and ethanolic extract increases with the concentration. On the second hand, it is noted that this toxicity increases with the duration of exposure of Sitophilus oryzae (L.). Given these results, the powder and ethanolic extract of the leaves of Taxus baccata can be recommended as a bioinsecticide candidate against Sitophilus oryzae (L.) and as an alternative to synthetic agriculture pesticides.

KEYWORDS:
Taxus baccata, Sitophilus oryzae (L.), Bioinsecticides, Ifrane

INTRODUCTION

Since the 1970s, environmental problems have been one of the biggest and most formidable challenges in the world. They are manifested by the threats that result, among other things, in climate change, loss of biodiversity, the rapid degradation of soils, the upheavals in crop production through the inadequacy of the technical itineraries, limited access to water where 40% of the world's inhabitants will be under water stress in 2025 [1], and damage to human health.

Pests are a serious problem during storage of grain and its derived industry [2]. In the world, between 5 and 15% of the total weight of cereals, oilseeds and legumes are lost after harvest [3], and between 5 and 10% of these losses are due to the presence of parasites [4]. Wheat weevil is one of the pests of infestation and destruction of stored grain. Because of its high incidence, synthetic insecticides were used to control it, but the potential damage to human health and the environment caused by these insecticides is now considered a real problem.

Methyl bromide and phosphine are toxic to a broad spectrum of insect pests and have been commonly used to control Coleoptera which is harmful to stored products [5]. Although these fumigants are effective and economical, they have led to unexpected side effects such as ozone depletion, environmental pollution, pest resistance, and harmfulness to the organism [6, 7]. The problems of resistance and harmfulness of synthetic insecticides have led to the need to find more effective and healthier alternatives. Thus, plant products are the most tested products currently [8-15]. These natural insecticides, also called plant insecticides, have several advantages over synthetic compounds because of their rapid biodegradation and the reduction of environmental risks. For this, we thought to test the powder and the ethanolic extract of the plant Taxus baccata also called the “If” on the same pests mentioned above. In fact, Taxus baccata, also called the “If”, is a plant found in the Ifrane region in the middle atlas of Morocco. It is a plant that contains mainly a molecule of taxol, a molecule used in medicine as a precursor against breast cancer disease [16].

Our laboratory has carried out insecticidal tests of the essential oils of certain medicinal plants from the Middle Atlas region [12, 13]. We continued our work by studying the insecticidal activity of the powder and the ethanolic extract of the leaves of Taxus baccata on the wheat weevil (Sitophilus oryzae (L.)) when storing durum wheat (Triticum turgidum L. subsp. (Desl.) Husn.).

MATERIALS AND METHODS

Plant used. The leaves of Taxus baccata were harvested in February (2014) in the Ifrane region (31°42'07"N 6°20'57"O) located in the middle atlas of Morocco.

Sitophilus oryzae (L.) strain. Insects come from a strain isolated from wheat grains infested with Sitophilus oryzae (L.). These grains were taken from a farmer in the region of Meknes. This strain is
cultured in the laboratory, in an airy room where the temperature varies between 24 and 28 °C and a humidity of 70%. Mass rearing is carried out in glass jars, with a grid lid, filled with durum wheat grains, in which are added a sufficient number of insects Sitophilus oryzae (L.), of indeterminate sex. The jars are then left at room temperature. After one or two weeks of infestation, the adults are removed from the kernels. These infested grains are incubated until new adults appear. The adults used for the tests are obtained from these mass rearing.

**Drying of plant material.** The leaves of Taxus baccata collected are dried in the laboratory for seven days in the dark. These sheets are spread in thin layers on crafts paper and returned frequently to room temperature 25 °C.

**Crushing plant matter.** After drying, the leaves were crushed using an electronic mixer (Moulinex) until it was reduced to powder. The crushed materials were screened using a sieve with a mesh size of 100 μm.

**Fine powder formulation.** The fine powders obtained will be used in biotests by coating the durum seeds. To determine the powdered dose required coating a durum wheat seed, five small, medium and large durum wheat seeds were weighed, powder coated and weighed again using a precision scale. The difference between seed weight before and after sprinkling will correspond to the normal powdered dose (Dn). The fine powder was stored at -4 °C until use.

**Ethanolic extract.** 100 g of powder of the leaves of Taxus baccata were placed in a container for 48 hours and at ambient temperature in contact with 300 mL of ethanol, the whole mixture being stirred magnetically. After maceration, the resulting mixture was filtered using a Whatman type filter paper. Then, the solvent contained in the filtrate was evaporated until a pasty extract was obtained. To calculate the normal concentration (Dn) we apply the following relation:

\[
D_n = 10\% \text{ of the yield of ethanolic extract (for 10 mL of ethanol)}
\]

Let \( D_n = 8.4\times10\% \) for 300mL of ethanol

Where \( D_n = 0.028g \) for 10mL of ethanol

**Effect of leaf powder of Taxus baccata on adults of Sitophilus oryzae (L.).** 10 wheat burrows are introduced into petri dishes containing 50 of wheat seeds mixed with the powder of the leaves of Taxus baccata at five selected doses (0; Dn/2; Dn; 2Dn and 4Dn) of the weight of the powder per 50 durum wheat seeds), a powder weight of 0g; 0.014g; 0.028g; 0.056g and 0.112g respectively. The tests were carried out under the conditions of breeding Sitophilus oryzae (L.). Mortality control was done by enumerating dead insects from the first day of treatment to the death of all individuals. For each dose the experiments are repeated three times.

**Effect of ethanolic extract.** 10 wheat burrows are introduced into petri dishes containing 50 durum wheat seeds mixed with the ethanolic extract of the leaves of Taxus baccata at five doses selected (0; Dn/2; Dn; 2Dn and 4Dn), either an extract weight of 0g; 0.014g; 0.028g; 0.056g and 0.112g respectively. The tests were carried out under the conditions of breeding Sitophilus oryzae (L.). Mortality control was done by enumerating dead insects from the first day of treatment to the death of all individuals. For each dose the experiments are repeated three times.

**Data analysis.** To compare the effects of the powder and the ethanolic extract of the tested Taxus baccata leaves on the modality measured, an analysis of variance followed by the Scheffe test at 5% was performed using Excel version 2007. LC50 and CL99 were determined by Finney’s probit’s method [17]. Mortalities were corrected by Abbott formula [18]. The lethal times required for the 50% (LT50) and 99% (LT99) mortality of adults exposed to different concentrations of the powder and the ethanolic extract of the leaves of Taxus baccata were estimated.

**RESULTS AND DISCUSSION**

**Effect of leaf powder of Taxus baccata on adults of Sitophilus oryzae (L.).** The leaf powder of Taxus baccata significantly affects adult survival of Sitophilus oryzae (L.). In treated batches, weevil survival ranges between two and twenty-nine days, whereas in the control group, this varies between three and more than thirty-two days. The toxicity of the leaf powder of Taxus baccata depends on the concentration and duration of exposure. Adult survival decreases as the concentration of the leaf powder of Taxus baccata and the duration of exposure increase (Fig. 1). TL50 and TL99 are negatively correlated with tested powder concentrations (Table 1).

Toxicity parameters of the powder of the leaves of Taxus baccata are summarized in Table 2, the calculated LC50 and CL99 lethal concentrations reveal that Sitophilus oryzae (L.) adults are more sensitive to this powder. The extreme values of LC50 and CL99 vary depending on the duration of exposure; they ranged from 0.69 to 0.30 g/50 seeds and 1.63 to 2.89 g/50 seeds respectively between the 4th and 6th days.
Survivors affected by the same lowercase letter do not differ statistically from one another (Scheffé test, $p \leq 0.05$) while the others are clearly different.

Effect of ethanolic extract. The ethanolic extract of the leaves of *Taxus baccata* significantly affects adult survival of *Sitophilus oryzae* (L.). In the treated batches, the survival of the weevils ranges between two and twenty-three days, whereas in the control group, this parameter oscillates between three and more than thirty two days. The toxicity of the ethanolic extract of the leaves of *Taxus baccata* depends on the concentration and duration of exposure. Adult survival declines as the concentration of the leaf powder of *Taxus baccata* and the duration of exposure increases (Fig. 2). TL50 and TL99 are negatively correlated with the tested powder concentrations (Table 3).

The toxicity parameters of the ethanolic extract of the leaves of *Taxus baccata* are summarized in Table 4, the calculated LC50 and CL99 lethal concentrations reveal that *Sitophilus oryzae* (L.) adults are more sensitive to this powder. The extreme values of LC50 and LC99 vary depending on the duration of exposure; they ranged from 0.03 to 0.02 g/50 seeds and 0.19 to 0.10 g/50 seeds respectively between the 4th and 6th days.

All the results we found showed that *Sitophilus oryzae* (L.) was more sensitive to the powder and ethanolic extract of *Taxus baccata*. The literature [19] also showed that essential oils extracted from other plants showed toxicity on *Sitophilus oryzae* (L.). This toxicity was influenced by the chemical composition of the oil which depends mainly on the source, the season, the ecological conditions, the extraction method, the extraction time and the part.
The survivors affected by the same lowercase letter do not differ statistically from one another (Scheffé test, $p \leq 0.05$) while the others are clearly different.

**TABLE 3**

**LT$_{50}$ and LT$_{99}$ of Sitophilus oryzae (L.) adults treated with the ethanolic extract of the leaves of Taxus baccata**

<table>
<thead>
<tr>
<th>Concentrations (g/50 seeds)</th>
<th>LT$_{50}$</th>
<th>$r&gt;r(0.05 ; 2)$</th>
<th>LT$_{99}$</th>
<th>$r&gt;r(0.05 ; 2)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.18</td>
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<td>14.78</td>
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<td>0,014</td>
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<td>11.61</td>
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<tr>
<td>0,028</td>
<td>5.03</td>
<td>-0.90</td>
<td>10.82</td>
<td>-0.92</td>
</tr>
<tr>
<td>0,056</td>
<td>3.14</td>
<td></td>
<td>5.40</td>
<td></td>
</tr>
<tr>
<td>0,112</td>
<td>2.48</td>
<td></td>
<td>4.48</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4**

**Toxicity parameters of the ethanolic extract of Taxus baccata leaves on Sitophilus oryzae (L.)**

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Slope ±SE$^{(1)}$</th>
<th>$\chi^2$ (calculated)</th>
<th>$\chi^2$ (0.05 ; 2)</th>
<th>LC$_{50}$ (g/50 seeds)$^{(2)}$ [Confidence interval]</th>
<th>LC$_{99}$ (g/50 seeds)$^{(2)}$ [Confidence interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3,09±0,70</td>
<td>0,72</td>
<td></td>
<td>0,03 [0,02 ; 0,04]</td>
<td>0,19 [0,12 ; 0,60]</td>
</tr>
<tr>
<td>5</td>
<td>3,36±0,84</td>
<td>3,59</td>
<td>5,99</td>
<td>0,02 [0,01 ; 0,03]</td>
<td>0,11 [0,07 ; 0,35]</td>
</tr>
<tr>
<td>6</td>
<td>3,62±0,98</td>
<td>1,47</td>
<td></td>
<td>0,02 [0,01 ; 0,03]</td>
<td>0,10 [0,06 ; 0,35]</td>
</tr>
</tbody>
</table>

$^{(1)}$SE: Standard Error;  
$^{(2)}$LC$_{50}$ et LC$_{99}$ : Lethal concentrations, respectively, for 50% and 99% of the individuals used.

of the plant used. Another work was done by Kucukboyaci and Bilge [20], showed that the compounds that participated in this synergistic toxicity are: lignans, larciresinol, taxiresinol, 3'-demethylisolariciresinol-9'-hydroxyisopropyether, isolariciresinol, 3-demethylisolariciresinol and taxoids.

**CONCLUSION**

The powder and the ethanolic extract obtained from the leaves of Taxus baccata of the region of high middle atlas of Morocco show a very important insecticidal effect on Sitophilus oryzae (L.). Comparing with the literature, the effectiveness of powder and ethanol extract is mainly related to their concentrations and the duration of exposure of the species. This approach can be beneficial because it is environmentally friendly and socially acceptable. However, further studies must be conducted to evaluate the active ingredient responsible for this toxicity.

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PROXIMATE AND ELEMENTAL ANALYSIS OF DIFFERENT DAILY CONSUMING FRUIT PLANTS

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ABSTRACT

The biochemical study of 8 different types of fruits has been done namely mango, melon (Plain), muskmelon (Serda and Germa), watermelon, apricot, plum and dates. The proximate composition, level of vitamins and minerals of dried fruits have been reported. Results of the present study indicate that fruits, mango, dates and apricot contain high carbohydrate concentration while the carbohydrate content of other fruits analyzed is considerable (10.3-57.8 g percent). Fat is lowest constituent (0.1-0.42 g percent) of the macro-nutrients and there is less variation in the fat content of different types of fruits. Protein content (1.2-4.25 g percent) is also low as compared to carbohydrate but in higher proportion than fat content. Fruits analyzed contain a higher amount of minerals (Ca, Mg, F, P, Na and K) as compared to other conventional foods. Vitamin content is also high as compared to other conventional foodstuffs. β-carotene in mango (0.0875 g percent), apricot (2.78×10^-2 g percent) and dates (3.85×10^-2 g percent) is present in considerable amount.

KEYWORDS:
Fruits, district Sind, nutritional analysis, proximate analysis, mineral analysis

INTRODUCTION

Various types of fruits are cultivated in different areas of Pakistan and are available from season to season and some are available throughout the year. The fruit production in Pakistan is very high. The total fruit production in Pakistan was 204.48 million kilogram in the year 1979-80 [1]. Fruits as a food class serve primarily as a source of sugar, organic acids, vitamins and minerals [2]. Fruits are the good source of carbohydrate and provide enough amounts of carbohydrates when present in the diet. Mango is an important fruit of Indo-Pak subcontinent. It ranks prominent position in the fruit processing industry of Pakistan. It has a pleasant taste, aroma and also contributes significantly to diet. It is a good source of energy and contains vitamins A, C and minerals like iron and phosphorus [3].

Cucumis melo belongs to the cucurbitaceae family. Muskmelon (Cucumis melon.), a short duration fruit crop belongs to family cucurbitaceae. Its fruits are used as a dessert and also eaten alone. Muskmelon is a rich source of vitamin C, β-carotene, vitamin A, carbohydrates, sugars, protein and also traces of vitamin B, vitamin K, niacin and vitamin B. Muskmelon contains more than 90 percent water.

Watermelon (Citrullus lanatus) is a crop which is grown in warm season and is grown worldwide, usually in regions that have a long warm growing season [4]. The fruits are very juicy, with a moisture content of over 90%. Although watermelons are not a rich source of vitamin C, the level of this nutrient in the fruit is comparable to that in imported pears and grapes. The red flesh of watermelon also contains some vitamin A.

Plum is a temperate zone fruit crop which belongs to the genus Prunus of subfamily prunoideae [5]. Plum is originated from five centers; these include Western Asia for Prunus domestica Linn (Damson plum), Europe for Prunus domestica (European plum), Western and Central Asia for Prunus cerasifera Linn (Cheery plum), North America for Prunus americana (American plum) and China for Prunus salicina (Japanese plum) [5]. Plums are an excellent resource of nutrients and contribute significantly to human nutrition [6]. It contains organic acids such as malic and citric acids, carbohydrates (glucose, sucrose and fructose), fibres (pectins), aromatic substances, tannins and enzymes. These substances are vital components of taste and nutritive value of fruits [7].

Apricot (Prunus armeniaca Linn.), is grown in temperate regions of the world including USA, Spain, France, Italy, Turkey, Morocco, Iran, Africa and Australia. Apricot has an important place in human nutrition and can be used as fresh, dried or processed fruit. As known, the fruit of apricot is not
only consumed fresh but also used to produce dried apricot, frozen apricot, jam, jelly, marmalade, pulp, juice, extrusion products, etc. Moreover, the apricot kernel is used in the production of oils, benzaldehyde, cosmetics, active carbon and aroma perfume [8].

Date Palm belongs to the genus of palms, the most important species is the common date palm. Dates contain a high amount of carbohydrate, fat comprising 14 types of fatty acids, 15 salts and minerals, a protein with 23 different amino acids and proteins, six vitamins and a high amount of dietary fiber [9]. This study was aimed to evaluate the proximate composition, vitamins and minerals among different fruits viz mango (Langra and Sindri), muskmelon (Germa and Serda), watermelon, plum, apricot and date palm. These fruits grow abundantly in Sind district of Pakistan.

MATERIALS AND METHODS

Sample collection. Samples of eight fruits such as mango (Langra and Sindri), muskmelon (Germa and Serda), watermelon, plum, apricot and date palm were collected from Impress Market, Karachi. For each species, three plant samples were collected randomly at three different spots and mixed to form composite samples, kept in a labelled polythene bag and taken to the laboratory. The samples were air-dried, crushed into powdered form and kept for chemical analysis.

Proximate composition. Proximate analyses were carried out according to the procedure [10]. Briefly, ash content was determined by dry ashing method i.e., placing the sample in the furnace. Fats were extracted from powdered samples in Soxhlet extractor. The extract obtained was left overnight drying at 80 °C and the contents of fat were determined gravimetrically. Kjeldhal apparatus was used for the estimation of nitrogen content and protein content was calculated as N× 5.7.

Vitamins determination. Vitamin C was determined by 2,6-dichlorophenol indophenol method [11]. Of the sample, 5 ml was taken in 100 ml of the conical flask and 5 ml of metaphosphoric acid solution (4%) was added and titrated with 2,6-dichlorophenol indophenol dye till the endpoint appears as light pink colour.

Riboflavin was estimated by fluorometric assay [12]. Briefly, proteins were precipitated with 40 % T.C.A and bounded flavin was converted to free from of acetic acid. Fluorescence was enhanced by the help of pyridine, interfering pigments were removed by KMnO₄ and excess of KMnO₄ was oxidized by H₂O₂. Interfering fluorescent substances were removed by Na₂S₂O₃. Butanol was used to separate the riboflavin from inert matter and the fluorescence intensity of the solution is read by spectrofluorimeter.

Beta-carotene estimation. Beta-carotene was estimated by the method of [13].

Mineral analysis. For mineral analysis, the plant samples were dry ashed at 550°C. The ash was boiled with 10mL of 20% hydrochloric acid in a beaker and then filtered into a 100mL standard flask. Atomic absorption spectroscopy was used for mineral analysis. Phosphorus was determined by yellow phosphorus method [14].

Data analysis. The results were expressed as means ± SD. Data obtained was analyzed by One Way ANOVA and different group means were compared with LSD test. P < 0.05 was considered significant in all cases. The software Package Statistica was used for analysis of data.

RESULTS AND DISCUSSION

The results of the proximate analysis of fruits are significantly (P< 0.05) different from each other and are shown in Table 1. In mango langra, moisture is found to be 75.4 g %. In mango sindri 73.58 g % dry weight of the sample. In melon (Kharbuza), muskmelon (Germa and Serda) and watermelon (Tarbuz), moisture content ranges from 80.4 to 91.38 g % dry weight of the sample. The moisture content of plum has been found to be 88.4 g %. Apricot moisture content is 61.78 g % and that of dates is 13.8 g %. Some of the fruit parts have a relatively high moisture content which is typical when fresh fruit is at maturity while some have low moisture contents. The relatively low moisture content is an indication that the parts of fruits will have a high shelf life when properly packed against external conditions [15].

In mango langra and sindri ash content is 4.2 to 4.05 g % dry weight of the sample. In melon (Kharbuza), muskmelon (Germa and Serda), watermelon (Tarbuz) ash content ranges from 1.012-2.6 g %. Ash content of plum is 2.3 g % and that of apricot and plum is 2.1 and 4.9 g % respectively. Those samples which have a high percentage of ash are expected to be rich in mineral elements which are expected to speed up the metabolic process.

Carbohydrate content of mango (langra) is 10.12 and Sindri is 10.25 g %. Carbohydrate content of Melon (Kharbuza), muskmelon (Germa and Serda) and watermelon range from 3.15 to 6.4 g % dry weight of the sample. Carbohydrate content of plum, apricot and dates are 3.75. 5.2 and 57.8 g % dry weight of the sample respectively. Carbohydrate content of dates is highest among the fruits analyzed. The carbohydrate content of mango was reported 14.1 % by other studies [16]. The main
function of carbohydrate is to furnish energy to the body. Fruits contain a considerable amount of carbohydrate i.e. starch, sucrose, glucose and cellulose etc. Samples in which carbohydrates are low are ideal for diabetic and hypertensive patients requiring low sugar diets.

Fat contents of fruits are less as compared to the other macro-nutrients. In mango langra and Sindri, fat content is 0.42 and 0.40 g % dry weight of the sample respectively. Melon (Kharbuza) contains 0.3 and 0.2 g % of fat. The fat content of muskmelon (Germa and Serda) is 0.3 and 0.2 g % respectively. The fat content of fruits is less as compared to other conventional food groups; therefore fruits may be utilized as food for those patients who require less fat or fat free diet especially in heart diseases. Fila [17] reported the proximate composition of watermelon as moisture (91.8 g/100 g), ash (0.21 g/100 g), crude protein (0.44 g/100 g), fat (0.15 g/100 g) and carbohydrate (71.19 g/100 g).

The high lipid contents are comparable with those of soybean oil, locust bean and cottonseed; 19.10 g/100g, 20.30 g/100g and 14.05 g/100g crude fat respectively. This showed that some of these fruits are rich in oil and could be a source of edible vegetable oil hence could complement conventional vegetable oils, which are very expensive.

Total crude protein has been determined by Macro-Kjeldahl apparatus. Very little amount of protein has been found in the fruit sample analyzed. Protein deficiency results in retardation of growth, wasting of muscles, edema, abnormal swelling of the belly and accumulation of fluids in the body [18]. Therefore, fruits are not a good source of protein. In melon family, Kharbuza has the highest amount of protein i.e. 2.3 g % while the Germa, Serda and Tarbuz have 1.23, 1.4 and 0.65 g % of protein respectively. Other fruits e.g. dates, apricot and plum have 3.9, 1.2 and 0.89 g % of protein respectively. Achinewu [19] reported 33.8 g/100 g of crude protein in the melon seed. The dates were rich in carbohydrate (71.2–81.4% dry weight), while ash was found 1.68–3.94%; they contained low quantities of protein and lipid (1.72–4.73% and 0.12–0.72%, respectively). The main mineral was potassium, and the glucose and fructose were the main sugars [20].

The vitamin content of fruits is significantly (P< 0.05) different from each other given in Table 2. Vitamin C is required for the synthesis of collagen, the formation of teeth and bones and also required for the synthesis of neurotransmitters [21]. Vitamin content of plum is high as compared to other fruits analyzed. Plum vitamin C is 24 mg % while the vitamin C content of mango (Sindri) is 22 mg % fresh weight of the sample. Melon (Kharbuza plain), muskmelon (Germa and Serda), watermelon (Tarbuz) and dates contain a low amount of vitamin C. Apricot showed 10 mg/100 g, mango showed 28 mg/100 g, plum showed 8–10 mg/100 g and watermelon showed 10 mg/100 g of ascorbic acid.

### Table 1
Nutritive value of some fruits cultivated and consumed in Sind.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Moisture</th>
<th>Ash</th>
<th>Carbohydrate</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango (Langra)</td>
<td>75.4±3.1 a</td>
<td>4.2±0.9 a</td>
<td>10.12±1.1 a</td>
<td>0.42±0.05 a</td>
<td>4.10±0.1 a</td>
</tr>
<tr>
<td>Mango (Sindri)</td>
<td>73.58±2.9 b</td>
<td>4.05±0.8 b</td>
<td>10.25±2 b</td>
<td>0.40±0.01 b</td>
<td>4.25±0.2 b</td>
</tr>
<tr>
<td>Melon</td>
<td>80.4±2.9 c</td>
<td>1.02±0.7 c</td>
<td>3.15±1 c</td>
<td>0.2±0.03 c</td>
<td>2.3±0.21 c</td>
</tr>
<tr>
<td>Musk Melon (Germa)</td>
<td>83.5±2.7 d</td>
<td>1.01±0.5 d</td>
<td>5.75±0.9 d</td>
<td>0.3±0.01 d</td>
<td>1.23±0.02 d</td>
</tr>
<tr>
<td>Musk melon (Serda)</td>
<td>86.78±1.8 e</td>
<td>2.75±0.1 d</td>
<td>6.4±0.8 e</td>
<td>0.2±0.003 e</td>
<td>1.4±0.01 e</td>
</tr>
<tr>
<td>Water melon</td>
<td>91.38±1.1 f</td>
<td>2.6±0.2 f</td>
<td>3.3±0.1 f</td>
<td>0.1±0.000 e</td>
<td>0.65±0.04 f</td>
</tr>
<tr>
<td>Plum</td>
<td>88.4±2.1 g</td>
<td>2.3±0.1 e</td>
<td>3.75±0.12 g</td>
<td>0.3±0.01 d</td>
<td>0.89±0.02 g</td>
</tr>
<tr>
<td>Apricot</td>
<td>61.7±1.3 h</td>
<td>2.1±0.14 f</td>
<td>5.2±0.1 h</td>
<td>0.3±0.01 d</td>
<td>1.2±0.001 h</td>
</tr>
<tr>
<td>Dates</td>
<td>13.8±1.1 i</td>
<td>4.9±0.13 g</td>
<td>57.8±0.2 i</td>
<td>0.8±0.01 i</td>
<td>3.9±0.1 i</td>
</tr>
</tbody>
</table>

Results are expressed as g % dry weight of the sample

The values in a column having different superscripts are significantly (P< 0.05) different from each other by LSD test

### Table 2
Vitamins content of some fruits cultivated and consumed in Sind.

<table>
<thead>
<tr>
<th>No.</th>
<th>Systematic name</th>
<th>Common name</th>
<th>Vitamin C</th>
<th>β-carotene</th>
<th>Riboflavin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mangiferaindica</td>
<td>Mango (Langra)</td>
<td>2.2×10⁻² a</td>
<td>0.0875 a</td>
<td>1.76×10⁻⁴ a</td>
</tr>
<tr>
<td>2</td>
<td>Mangiferaindica</td>
<td>Mango (Sindri)</td>
<td>2.3×10⁻³ b</td>
<td>0.0918 b</td>
<td>1.65×10⁻⁴ b</td>
</tr>
<tr>
<td>3</td>
<td>Cucumis melo</td>
<td>Melon</td>
<td>3.0×10⁻² c</td>
<td>1.5×10⁻² c</td>
<td>1.075×10⁻⁴ c</td>
</tr>
<tr>
<td>4</td>
<td>Cucumis melo</td>
<td>Musk Melon (Germa)</td>
<td>9.4×10⁻⁴ d</td>
<td>1.5×10⁻³ d</td>
<td>n.d</td>
</tr>
<tr>
<td>5</td>
<td>Cucumis melo</td>
<td>Musk melon (Serda)</td>
<td>8.17×10⁻¹ e</td>
<td>2.135×10⁻³ e</td>
<td>n.d</td>
</tr>
<tr>
<td>6</td>
<td>Citrullus vulgari</td>
<td>Water melon</td>
<td>5×10⁻³ f</td>
<td>1.02×10⁻² f</td>
<td>2.5×10⁻⁵ d</td>
</tr>
<tr>
<td>7</td>
<td>Prunus communis</td>
<td>Plum</td>
<td>2.4×10⁻² g</td>
<td>1.2×10⁻³ g</td>
<td>n.d</td>
</tr>
<tr>
<td>8</td>
<td>Prunus armeniaca</td>
<td>Apricot</td>
<td>1.25×10⁻¹ h</td>
<td>2.78×10⁻³ h</td>
<td>n.d</td>
</tr>
<tr>
<td>9</td>
<td>Phoenicidactylifer</td>
<td>Dates</td>
<td>34×10⁻³ i</td>
<td>3.85×10⁻³ i</td>
<td>2.5×10⁻⁵ d</td>
</tr>
</tbody>
</table>

Results are expressed as g % dry weight of the sample

n.d = denotes that the constituent was not detected in the concern species due to lack of sample
Riboflavin is involved in the metabolism of carbohydrate and protein. It is a constituent of many important coenzymes (FMN & FAD) in the body [22]. The riboflavin content of analyzed fruits ranges from $2.5 \times 10^{-5}$ g % to $1.76 \times 10^{-4}$ g % of the fresh sample. Among the analyzed fruits riboflavin content of mango is higher as compared to watermelon and dates. The riboflavin content of fruit has been determined first time in Sind (Pakistan).

β-carotene is a precursor of vitamin A. Vitamin A is important for vision, for the normal bone and teeth formation and for normal health epithelial tissue. The deficiency causes night blindness, stunted growth, the β-carotene content of mango is higher than the β-carotene content of dates and plum. The β-carotene content of dates is higher than the β-carotene content of dates and plum. The β-carotene content of dates is higher than the melon (plain), watermelon (Tarbuz) and muskmelon (Germa and Serda). The theβ-carotene content of the fruit analyzed ranges from $1.02 \times 10^{-4}$ to $0.0875 \times 10^{-4}$ g % of fresh weight of the sample. The theβ-carotene content of mango is almost equal to the β-carotene content of spinach. β-carotene content of these fruits have been estimated the first time in our lab in Pakistan. These fruits contain a considerable amount of β-carotene so their utilization may be useful in the prevention and cure of different Vitamin A deficiency ailments, especially in children. Lester [23] reported the β-carotene content of muskmelon, 5.3-33.8 μg/g.

The mineral analysis of fruits is significantly different from each other. Table 3. Inorganic elements which are composed of body tissues, body fluids and some serve as a cofactor for enzymes, hormones and vitamins and some maintain the acid-base balance etc. Fruits have mineral content therefore in balance diet these would appear to be an excellent mineral source. The Mineral content of fruits is shown in Table 3.

Calcium is required for the healthy bone and teeth formation, transmission of nerve impulse and it is also an activator of some enzymes, also required in blood clotting [21]. Calcium content of dates is higher than other fruits i.e. mango, muskmelon, watermelon and plum. Calcium content of apricot is less than dates but higher than melon, Tarbuz and Plum. The Ca content of mango of Sind is higher than the Ca content of mango of Punjab. This variation may due to the different seasonal condition. Vunchi [24] reported the mineral content (mg/100g) of plum as potassium (16.5), sodium (10.40), calcium (30.27), Iron (5.20), magnesium (20.10) and phosphorus (16.50).

**TABLE 3**

Elemental analysis of some fruits consumed in Sind Pakistan.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Na</th>
<th>K</th>
<th>Mg</th>
<th>Ca</th>
<th>P</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango (Langra)</td>
<td>0.314±0.1⁴a</td>
<td>0.126±0.01⁴a</td>
<td>0.73±0.1⁴a</td>
<td>0.041±0.001⁴a</td>
<td>0.0011±0⁴a</td>
<td>0.0021±0.0003⁴a</td>
</tr>
<tr>
<td>Mango (Sindri)</td>
<td>0.350±0.1²b</td>
<td>0.127±0.01²b</td>
<td>0.77±0.2⁴b</td>
<td>0.185±0.001²b</td>
<td>0.0012±0²b</td>
<td>0.0035±0.00002²b</td>
</tr>
<tr>
<td>Melon</td>
<td>0.102±0.01⁴c</td>
<td>0.21±0.01⁴c</td>
<td>0.18±0.01⁴c</td>
<td>0.53±0.1⁴c</td>
<td>0.158±0.01⁴b</td>
<td>0.000158±0c</td>
</tr>
<tr>
<td>Musk Melon (Germa)</td>
<td>0.138±0.01¹d</td>
<td>0.18±0.021 d</td>
<td>0.37±0.03³d</td>
<td>0.015±0.003³d</td>
<td>0.0155±0.0002¹d</td>
<td>0.015±0.0001¹d</td>
</tr>
<tr>
<td>Musk melon (Serda)</td>
<td>0.147±0.01¹e</td>
<td>0.15±0.03³e</td>
<td>0.39±0.04³e</td>
<td>0.28±0.07³e</td>
<td>0.038±0.001¹d</td>
<td>0.078±0.002³e</td>
</tr>
<tr>
<td>Watermelon</td>
<td>0.80±0.02d</td>
<td>0.48±0.031f</td>
<td>0.14±0.05f</td>
<td>0.4±0.02f</td>
<td>0.185±0.003³f</td>
<td>0.275±0.01f</td>
</tr>
<tr>
<td>Plum</td>
<td>0.18±0.012g</td>
<td>0.118±0.012g</td>
<td>0.35±0.012g</td>
<td>0.4±0.021f</td>
<td>0.85±0.1f</td>
<td>0.0187±0.008⁶g</td>
</tr>
<tr>
<td>Apricot</td>
<td>0.15±0.002c</td>
<td>0.25±0.011c</td>
<td>0.79±0.12c</td>
<td>1.15±0.012b</td>
<td>2.6±0.3c</td>
<td>0.62±0.1b</td>
</tr>
<tr>
<td>Dates</td>
<td>0.201±0.003c</td>
<td>0.18±0.02d</td>
<td>0.85±0.21c</td>
<td>2.5±0.013c</td>
<td>1.05±0.1b</td>
<td>0.31±0.12c</td>
</tr>
</tbody>
</table>

Results are expressed as g % of dry weight of the sample. The values in a column having different superscripts are significantly (P < 0.05) different from each other by LSD test.

Iron is an important constituent of haemoglobin. Deficiency of iron leads to anaemia [25]. The iron content of apricot and watermelon is higher than other fruits i.e. mango, melon, muskmelon. The phosphorus content of dates is higher than mango, melon, muskmelon and watermelon but less than apricot. Individuals who suffer from chronic kidney disease are at risk for excessive levels of phosphorus and potassium in the blood, which can result in complications such as loss of bone density and irregular heart function. To prevent this, these individuals need to follow a strict diet and eliminate foods high in potassium and phosphorus.
fruits studied. Magnesium content of mango is higher as compared to melon, watermelon, muskmelon and plum. Magnesium content of dates is 0.85 gram percent of dry weight of the sample. Magnesium content of mango, melon, watermelon, muskmelon and plum ranges from 0.14 gram percent to 0.85 gram percent dry weight of the sample. Magnesium content of the fruits has not been estimated earlier in the country.

Sodium and potassium are important for the electrolyte balance. Sodium is present in extracellular fluid while K is present in Intracellular fluid [26]. People who have a high intake of sodium have a high incidence of hypertension and stroke. High blood pressure is rarely seen in those who consume less than 1.2 grams (1200 milligrams) of sodium per day. The sodium content of watermelon is higher as compared to other fruits analyzed during the study. The sodium content of watermelon is 0.80 g % dry weight of the sample. Plum and muskmelon contain less amount of sodium. The sodium content of mango is higher than melon, muskmelon, plum and dates but less than the sodium content of watermelon. Tomato, orange juice, bananas, apricots and currants are a rich source of potassium. Studies suggest boosting your potassium intake and curbing salt and sodium can slash your stroke risk by 21% and may also lower your odds of developing heart disease. Potassium, a mineral, works by protecting blood vessels from oxidative damage and keeps vessel walls from thickening. Potassium content of watermelon is high in sodium content as compared to other fruits analyzed. K content of apricot is less than K content of watermelon but more than other varieties i.e. mango, melon, muskmelon, plum and dates. [27] reported the high content of micro and macro elements among different varieties of date palm.

These results justify that the fruits collected from different areas of Sind are the rich source of nutritional constituents. In general, the variation among biochemicals shows that the genetic variability can be used to develop cultivars with enhanced health benefits.

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EFFECTS OF 28-HOMOBRASSINOLIDE ON THE ACCUMULATION AND UPTAKE OF CADMIUM, LEAD AND ZINC BY PADDY RICE

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ABSTRACT

Heavy metals in farmland soil have negative effects on rice production and pose a great threat to human health. The effects of different concentrations of 28-homobrassinolide (Br28: 0, 0.02, 0.06, 0.18, 0.54, 1.62, 4.86, 14.58 μM) on the accumulation and uptake of cadmium (Cd), lead (Pb), and zinc (Zn) in two genotypes (Donglian5 and Teyou009) of rice seedlings, grown in Cd-Pb-Zn contaminated soil, were investigated using pot experiments. The results showed that Br28 can increase the iron (Fe) content in the iron plaque formed on the rice seedlings of Donglian5, but not on Teyou009. The Fe content in the roots and shoots of Teyou009 can be increased by Br28, but not in Donglian5. The contents of Cd in iron plaque were barely affected by Br28 in both the genotypes of rice seedlings. Br28 significantly decreased the Cd content in the shoots and roots of Teyou009, but not in Donglian5. The highest proportion (60%) of Cd observed was concentrated in the shoots of Teyou009, and reduced by 24% when 0.02 μM of Br28 was used. However, Br28 did not significantly affect the accumulation and uptake of Pb and Zn in the shoots, roots, and iron plaque for both genotypes of rice plants. Our results indicate that Br28 can effectively reduce Cd uptake in rice seedlings, but these results are genotype-dependent.

KEYWORDS:
Iron plaque, Brassinosteroids, Heavy metals, Oryza sativa

INTRODUCTION

Rice (Oryza sativa) is one of the main food crops feeding more than 50% of the world’s population [1]. China is the largest producer of rice in the world, and has encountered numerous environmental problems like heavy metal pollution during rice production. The intensification of heavy metal pollution in farmlands is a direct threat to rice production and human health [2]. Reducing the uptake and accumulation of heavy metals in rice has become the focus of our attention, because the intake of excessive heavy metals is causing a variety of diseases [3, 4, 5].

In recent years, the application of plant hormones has become an effective means of reducing the heavy metal uptake in plants [6, 7, 8]. Brassinosteroids (Brs) are plant hormones discovered in the last half century [9] that can promote organ growth and regulate plant developmental and physiological responses [10]. Recent studies have reported that Brs demonstrated positive effects in terms of decreasing the heavy metal uptake (Cu, Cr, Ni, Zn, Pb, and Cd) in plants by activating different defense mechanisms [11, 12, 13, 14, 15, 16]. However, limited information is available on Br effects pertaining to heavy metal accumulation in rice plants. This information is crucial when exploring the effective method to decrease heavy metal uptake in crops.

In addition, Brs can promote photosynthesis in plants [17] that affects root radial oxygen loss determining the formation of iron plaque [18, 19]. The iron plaque is formed by oxygen or oxidizing substances secreted by the root, and Fe2+ or Mn2+ in the rhizosphere [20, 21]. Numerous studies have shown that iron plaque can adsorb heavy metals and hinder their translocation to plants [20, 22]. Therefore, the effect of Brs on the formation of iron plaque in rice plants was also investigated in this study. Based on the above considerations, the effects of Brs on the accumulation and uptake of Cd, Pb, and Zn, as well as iron plaque formation were investigated in rice plants grown in contaminated soil around a Pb/Zn smelter.

MATERIALS AND METHODS

Soil samples were collected from the surface (0–20 cms) of a paddy field near a smelter (26°15’28.49”N and 118°15’12.78”E) in Youxi County, Fujian Province, China. The details of the methods used for soil sample treatments, and the physical and chemical properties of the soil are
provided in our previous paper [23]. The concentrations of Cd, Pb, and Zn in the soil samples reached 5.88 mg kg\(^{-1}\), 1602 mg kg\(^{-1}\), and 2132 mg kg\(^{-1}\), respectively.

Rice seeds (Donglian5 and Teyou009, local cultivars from the Fujian Province, China) were sterilized using 30% H\(_2\)O\(_2\) (v/v) for 15 min and then germinated in moist quartz sand for 15 days, after washing the seeds thoroughly with deionized water. Uniform seedlings were chosen and transferred into pots with flooded soil. After 15 days, the foliage of the rice seedlings was sprayed using a solution with different concentrations of Br\(_2\) (0, 0.02, 0.06, 0.18, 0.54, 1.62, 4.86, 14.58 μM) three times during the night. Each treatment had three replicates for a total of 48 pots. The experiment was conducted in a greenhouse at 25-35 °C, and the light exposure time was 12 to 14 h every day.

After 30 days, the rice seedlings were carefully taken out of the soil and washed thoroughly using tap water. Then the rice plants, after washing with deionized water, were divided into shoots and roots. The fresh roots were soaked in a DCB (Dithionite-Citrate-Bicarbonate) solution for one hour at 25 °C for iron plaque extraction. The roots were placed in a 100 ml beaker with a mixture (50 ml) containing 0.03 mol L\(^{-1}\) Na\(_5\)C\(_6\)H\(_5\)O\(_7•2\)H\(_2\)O, 0.125 mol L\(^{-1}\) NaHCO\(_3\), and 1 g Na\(_2\)S\(_2\)O\(_4\). Next, the extracts were transferred into a 100 ml volumetric flask and filtered using a 0.45 μm filter before analyzing the concentrations of Fe, Cd, Pb, and Zn.

The samples obtained of the shoots and roots after the extraction were oven-dried at 65 °C for 72 h and weighed. Grated plant samples (about 0.2 g) were put into 100 ml Teflon tubes for digestion. During the digestion procedure, 8 ml of HNO\(_3\) was added into the tubes for 12 h, followed by 1 ml of H\(_2\)O\(_2\) (30%; v/v) at 100 °C for 4 h. The digestion solution was then transferred into a 100 ml volumetric flask and filtered using a 0.45 μm filter to determine the concentrations of Fe, Cd, Pb, and Zn using inductively coupled plasma-mass spectrometry (ICP-MS, NexION 300X, Perkin Elmer, Norwalk, USA).

Statistical analysis, a one-way analysis of variance (ANOVA), was performed using SPSS software (v.19.0, Chicago, IL, USA). The data presented are means ± standard errors (SE) (\(n = 3\)), and they were also analyzed using least significant difference (LSD) at the 5% level to detect the differences in rice biomass, and the concentrations of Fe, Cd, Pb, and Zn in rice plants. The relationships between the concentrations of Fe, Cd, Pb, and Zn in rice plants (shoot, root, and iron plaque) were investigated using correlation coefficient analysis, and all figures were created using the SigmaPlot 12 software.

![FIGURE 1](image)

The effects of Br on biomass of shoot and root of rice seedling of Teyou009 and Donglian5. Different letters indicate significant differences at \(p < 0.05\) in biomass of shoot and root of rice seedlings. (Means ± SE, \(n = 3\)).
RESULTS

Although an increasing trend was found when the biomass of the shoots and roots of Teyou009 were analyzed, Br28 did not significantly affect rice growth (Figure 1). The shoot biomass of Donglian5 rice plants was also not affected by Br28, but root biomass was reduced at Br28 concentrations of 1.62 and 4.86 μM.

The Fe content in the shoots of Teyou009 significantly increased when the Br28 concentration was 0.18 and 4.86 μM (Figure 2). Br28 noticeably increased Fe content in the roots of Teyou009 rice plants, except during the 0.02 and 4.86 μM treatments. This did not affect Fe content in the iron plaque. Br28 did not affect Fe content in the shoots and roots of Donglian5, but significantly increased Fe content in the iron plaque under the 0.54, 1.62, and 14.58 μM treatments (Figure 2).

FIGURE 2
The effects of Br on the contents of Fe, Cd, Pb and Zn in shoot, root and plaque of rice seedling of Teyou009 and Donglian5.

Different letters indicate significant differences at $p < 0.05$ among different treatments of shoot, root and iron plaque of rice seedlings. (Means ± SE, $n = 3$).
Br28 markedly decreased Cd content in the shoots of Teyou009, but did not affect that of Donglian5 (Figure 2). Cd content in the roots of Teyou009 was also significantly reduced by Br28 under the 0.18, 1.62, 4.86, and 14.58 μM treatments (Figure 2). Cd content in the plaque formed in Teyou009 plants was reduced by Br28 during the 0.18, 1.62, and 14.58 μM treatments. Br28 did not significantly affect Cd content in rice seedlings of Donglian5, with the exception of decreasing Cd content in iron plaque under the 1.62 μM treatment. Pb contents in the shoots of the two cultivars were not affected by Br28 (Figure 2). Pb content in the roots of Teyou009 increased markedly under the 0.06 and 0.18 μM Br28 treatments. Br28 at 1.62 μM significantly decreased Pb content in the plaque of Teyou009, but showed an increase in Donglian5. Zn content in the shoots of Teyou009 was significantly increased by Br28 (0.54 μM). Br28 at 0.18 μM significantly increased Zn content in the roots of Teyou009, but showed a decrease in the plaque (Figure 2). Br28 did not affect Zn content in the shoots of Donglian5, but increased Zn content in the roots under the 1.62 μM treatment. The Zn content in the iron plaque in Donglian5 was significantly increased by the Br28 1.62 and 4.86 μM treatments (Figure 2).

The proportion of Cd in the control group for Teyou009 was 60% in the shoot, 32% in the root, and 8% in iron plaque (Figure 3). However, in the presence of Br28, a decrease in the proportion of Cd in the shoots (36-57%) of Teyou009 was observed, along with an increase in the proportion of Cd in the roots (36-51%). On the other hand, Br28 did not have any effect on the proportion of Cd in the Donglian5 rice plants, when compared to the control. In the control group for Teyou009, the proportion of Pb was 23% in the shoot, 59% in the root and 18% in the iron plaque (Figure 3). Br28 decreased the proportion of Pb in the shoots (8-19%) and increased the proportion in the roots (68-88%) of Teyou009, but did not significantly affect the proportion of Pb in Donglian5 rice seedlings. The proportion of Zn in the shoot, root, and iron plaque in the Teyou009 control group was 44%, 12%, and 44%, respectively (Figure 3). The Zn proportion in the shoots of Teyou009 was reduced by Br28 during the 0.02, 0.06, 0.18, and 4.58 μM treatments. Br28 treatments (except 0.02 μM) increased the proportion in the root and decreased the proportion in the iron plaque. However, the proportion of Zn in the iron plaque in Donglian5 increased with an increase in Br28 concentration. More than 95% of Fe was concentrated in the iron plaque in the two rice cultivars (Figure 3).

FIGURE 3
The proportion of Fe, Cd, Pb and Zn in shoot, root and plaque of rice seedling of Teyou009 and Donglian5 with different levels of Br28.
The effects of Br on Cu, Co, Mn and B in shoot and root of rice seedling of Teyou009 and Donglian5. Different letters indicate significant differences at $p < 0.05$ in shoot and root of rice seedlings. (Means ± SE, $n = 3$).

### TABLE 1

Correlations between concentrations of Fe, Cd, Pb and Zn in iron plaque, root and shoot of rice seedling of Teyou009

<table>
<thead>
<tr>
<th></th>
<th>Iron plaque</th>
<th>Root</th>
<th>Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron</strong></td>
<td>Fe</td>
<td>Cd</td>
<td>Pb</td>
</tr>
<tr>
<td><strong>Fe</strong></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cd</strong></td>
<td>0.54**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Pb</strong></td>
<td>0.61**</td>
<td>0.81**</td>
<td>1</td>
</tr>
<tr>
<td><strong>Zn</strong></td>
<td>0.58**</td>
<td>0.80**</td>
<td>0.77**</td>
</tr>
<tr>
<td><strong>Root</strong></td>
<td>Fe</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fe</strong></td>
<td>-0.39</td>
<td>-0.47*</td>
<td>-0.26</td>
</tr>
<tr>
<td><strong>Cd</strong></td>
<td>0.39</td>
<td>0.75**</td>
<td>0.70**</td>
</tr>
<tr>
<td><strong>Pb</strong></td>
<td>-0.18</td>
<td>-0.44*</td>
<td>-0.18</td>
</tr>
<tr>
<td><strong>Zn</strong></td>
<td>-0.40</td>
<td>-0.48*</td>
<td>-0.28</td>
</tr>
<tr>
<td><strong>Shoot</strong></td>
<td>Fe</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fe</strong></td>
<td>-0.08</td>
<td>-0.37</td>
<td>-0.35</td>
</tr>
<tr>
<td><strong>Cd</strong></td>
<td>-0.13</td>
<td>0.35</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Pb</strong></td>
<td>-0.29</td>
<td>-0.58**</td>
<td>-0.54**</td>
</tr>
<tr>
<td><strong>Zn</strong></td>
<td>0.02</td>
<td>-0.13</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

Note: * Significant at $p < 0.05$, ** Significant at $p < 0.01$. (Means ± SE, $n = 3$).
A significant positive relationship was observed between Fe content in plaque and Cd, Pb, and Zn content in the plaque in Teyou009 (Table 1), but not in Donglian5 (Table 2). The content of Cd, Pb, and Zn in the plaque was negatively correlated with Pb content in the shoots of Donglian5 (Table 1). The Fe content in the roots showed a significant negative correlation with Cd content in the roots, but was positively correlated with the content of Pb and Zn in the roots (Table 1 and 2). The Cd content in the roots was negatively correlated with the content of Pb and Zn in the roots, as well as the content of Fe and Pb in the shoots of Teyou009 (Table 1). The Fe content in the shoots was positively correlated with Pb and Zn content in the shoots of Donglian5 (Table 2).

A few of the Br28 treatments (0.18, 4.86 and 14.58 μM) significantly decreased Manganese (Mn) content in the shoots of Teyou009, but increased the content during the 0.02, 0.06, 0.18, 0.54, and 1.62 μM treatments (Figure 4). Boron (B) content in the roots of Donglian5 was markedly reduced by Br28, and was clearly lower than that in the roots of Teyou009.

**DISCUSSION**

Our previous study showed that Br28 does not affect Fe content in rice seedlings grown in nutrient solution, under As and Cd treatments [24]. In this study, Fe content in the iron plaque in Teyou009 was also not affected by Br28, but significantly increased in Donglian5. Interestingly, our results show that the Fe content was markedly increased by Br28 in the roots of Teyou009, but not in the roots of Donglian5. Xu et al. [25] showed that Fe content in yellow flag seedlings with 5 mg L⁻¹ Cr exposure was not affected by 0.1 mg L⁻¹ Br28. However, a significant increase in Fe content was observed in *Vicia faba* L. under 0.1 and 0.5 mg L⁻¹ Br28, but not under 1 mg L⁻¹ Br28 [26]. It was reported that Br24 can significantly decrease Fe content in the shoots and roots of rice seedlings as well as the translocation of Fe from the root to the shoot [27]. However, in peanut seedlings, an increase in Fe content and Fe translocation from the root to the shoot was found due to the application of Br24 [28]. Thus, we recognize that the dissimilarity in these results can probably be attributed to the different varieties and species of plants studied as well as the Br concentrations and types used.

Br28 can protect plants from Cd toxicity, but no evidence is available to show a decrease in Cd content in these plants [29, 30, 31, 32, 33]. Our previous study showed that Cd content in the shoots of rice seedlings grown in nutrient solution, without iron plaque induction, was significantly reduced by Br28 [24]. This lines up partly with the results of our current study, revealing that Br28 clearly decreases Cd content in Teyou009 rice plants. Rady [14] also found that Br24 significantly decreased the Cd content in Phaseolus vulgaris L. However, Cd content in *Brassica juncea* L. seedlings were increased by castasterone, a six-ketone type active Br [34]. Br28 is known to reduce the content of Pb and Zn in spring wheat (*Triticum aestivum* L.), radish seedlings and *Brassica juncea* L. [13, 35, 36]. However, according to our results, Br28 does not affect the accumulation and uptake of Pb and Zn in these two genotypes of rice plants. Currently, there are no other studies that report the effects of Br28 on Pb or Zn stress in rice plants. Our previous work showed that Br28 and Br24 can improve Cr uptake and accumulation in *Iris pseudacorus* [25]. Br24 caused an increase in Cr content in the L-genotype tobacco and a decrease in the M- and H-genotype tobacco [37]. Br28 and Br24 can also significantly decrease Ni content in *Brassica juncea* [12, 38]. These results indicate that the effect of Br on the accumulation and uptake of metals in plants is influenced by the differences in plant species, geno-
types of the same plant species, types of heavy metals and Brs studied.

Our results indicate that Fe can inhibit the uptake of Cd to a certain extent in rice plants (Figure 2, Table 1). This was confirmed by He et al. [39] who found that Cd accumulation in Arabidopsis was decreased by the supply of Fe, partly due to the competition between Fe and Cd for uptake. The application of Fe fertilizers is considered to be an effective way to increase Fe content and reduce Cd accumulation in rice [40, 41]. The elevated Fe supply that decreases Cd uptake in plants is attributed to the competition for transporters [42]. In this study, a significant positive relationship between Fe content and Pb and Zn in the roots and shoots indicated that Fe supply can promote the uptake of Zn and Pb in rice plants (Table 1 and 2). This confirms the results of Zhong et al. [43] showing that iron plaque cannot affect Pb content in the shoot, but significantly increases that in the root tissue. Zhang et al. [44] reported that iron plaque with 10 and 20 mg L$^{-1}$ Fe supply can increase Zn content in rice shoots. This indicates that Fe supply or Fe plaque cannot inhibit the accumulation and uptake of Pb and Zn in these plants. Transporters shared by Fe and Zn were also found in Arabidopsis thaliana and rice plants [45, 46]. However, the effects of the competition between Fe and Zn were not obvious due to the high Zn content in the soil during this study. These results were confirmed by Rosen et al. [47] who observed that the Zn content in the leaves of corn was significantly decreased by increasing Fe supply, when they were exposed to lower concentrations of zinc. This was not the case when corn was exposed to higher Zn concentrations. Lai et al. [48] showed that iron oxides present around the rhizosphere, and not the iron plaque, determine the fate of Pb in rice. These results indicate that the presence of Fe in plants or in the environment can play a competitive role with heavy metals, to a certain extent, and lead to blocking their translocation and uptake in plants. Recent studies have shown that Cr, Ni, and Cu uptake in plants was significantly decreased by soaking their seeds in Brs [15, 38, 49]. It is pointed out that spraying Br at different stages of plant growth will also affect the accumulation and uptake of Cd and Pb in plants [13]. Therefore, the choice of method and timing of Br application may affect the response of plants to heavy metals.

CONCLUSIONS

This study provides clear evidence that plant hormones can help reduce the heavy metal uptake in rice plants. Our work shows that Br28 increases the amount of iron plaque formed in rice plants, but this effect was genotype-dependent. Br28 can decrease the accumulation and uptake of Cd in the Teyou009 rice genotype, but not in Donglian5. The accumulation and uptake of Pb and Zn was not affected by Br28 in both the genotypes of rice seedlings. Further studies need to be conducted to understand the extent to which Br28 can effectively reduce heavy metal uptake in rice plants.

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EFFECTS OF SITE PREPARATION TIME ON SEED GERMINATION AND SEEDLING SURVIVAL RATE IN MIXED SPECIES CONIFER FOREST STANDS IN GIRESUN, TURKEY

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²Forest Regional Directorate of Giresun, 28000 Giresun, Turkey

ABSTRACT

This study was undertaken to investigate the effects of the year of site preparation relative to that of large-scale seed dispersal on seed germination and seedling survival success for natural regeneration of oriental spruce in a mixed forest of oriental spruce (Picea orientalis) and Caucasian fir (Abies nordmanniana) in the Paşakonagi region of Giresun province, Turkey. Two ecologically similar sites, both 120 years of age with an area of 12 ha (site 20) or 15 ha (site 21) were selected. Seed-tree cutting, site preparation and soil tillage activities were performed in “site 20” in 2014, which was a low seed year, and in “site 21” in 2015, which was an abundant seed year, respectively. Oriental spruce trees at both sites dispersed an average of 363 seeds/m² in the abundant seed year of 2015, and 92,840 and 143,704 oriental spruce seedlings per ha germinated from these seeds in 2016 in sites 20 and 21, respectively. Seedling number decreased significantly until May 2018 by 34.04% and 49.82% in “site 20” and 21, respectively (P<0.05). The greatest significant decrease took place during the first winter germination occurred. Taller and stockier seedlings occurred at “site 21” where site preparation and soil tillage was carried out in the same year as seed dispersal (P<0.05).

KEYWORDS:
Oriental spruce, Caucasian fir, seed dispersal, seedling, germination, survival

INTRODUCTION

Natural forests that are old, unstable and have lost canopy closure can be regenerated by sustainable management of the forests to produce wood for industrial use. Spruce forests are the most challenging in terms of natural regeneration studies in Turkey. A uniform shelterwood management method is generally used to regenerate such forests but the success rate remains low [1, 2]. High precipitation and humidity in the region are important factors for the low success rate of natural regeneration [3, 4]. In addition, dense ground vegetation (which can compete with tree seedlings) and the very slow growth of oriental spruce at the seedling stage are other important factors contributing to the low success rate of natural regeneration.

Oriental spruce (Picea orientalis (L.) Link) is a relict forest tree species native to northeast Turkey and the Caucasus. It forms mostly pure stands in mountains above 1,000 m with north-facing slopes. In its native range, the oriental spruce forms mixed stands with Caucasian fir (Abies nordmanniana Spach), oriental beech (Fagus orientalis Lipsky) and Scots pine (Pinus sylvestris L.) [5, 6, 7]. Annual precipitation exceeds 1,500 mm in the native range of this species, and high humidity is characteristic of its growth environment [3].

The seeds of oriental spruce can mature over a short period of only 6 months. Seeds begin to detach from the cones in September and continue to disperse throughout the winter [8, 9]. The definitive determination of an abundant seed year takes place by July or August and results in delays in site preparation procedures and the cuts for seed-tree cutting which are required prior to seed dispersal. It also leads to underutilization of seeds produced in an abundant seed year. It is considered useful to carry out the scheduled cuts to prevent the interruption of regeneration procedures regardless of whether the season is an abundant seed year. However, there has been no study addressing the impact of cuts and site preparation procedures performed in a low seed year on the regeneration success.

In this study, the effects of site preparation time (conducted in 1 year with abundant and 1 year with less-abundant seed production) on seedling germination and seedling survival success were investigated in a mixed forest of oriental spruce and Caucasian fir, which was planned for regeneration under the uniform shelterwood management method.
Number and volume of trees per hectare at each study site before and after seed-tree cutting

<table>
<thead>
<tr>
<th>Species</th>
<th>Count</th>
<th>Volume (m³)</th>
<th>Site 21 Count</th>
<th>Volume (m³)</th>
<th>Site 20 Count</th>
<th>Volume (m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian fir</td>
<td>77</td>
<td>125,006</td>
<td>30</td>
<td>53,000</td>
<td>31</td>
<td>59,900</td>
</tr>
<tr>
<td>Oriental spruce</td>
<td>217</td>
<td>278,648</td>
<td>86</td>
<td>186,830</td>
<td>88</td>
<td>180,160</td>
</tr>
<tr>
<td>Total</td>
<td>294</td>
<td>403,654</td>
<td>116</td>
<td>239,830</td>
<td>119</td>
<td>240,060</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Description of the Study Area. The study site was located in the Pasakonagi region of Bulancak district of Giresun province in the Black Sea region of Turkey (41°30′22″–41°30′22″ N; 41°30′22″–41°30′22″ E; 1450 m, southerly aspect). The site had forest communities primarily composed of oriental spruce (69%) and Caucasian fir (31%). Sites 20 (12 ha) and 21 (15 ha) in the Pasakonagi Forest Management Office were reserved for regeneration and were selected as the study areas. The whole site had a density of 294 trees per ha, totaling 403,654 m³ of wood per ha, according to the management plan data of the region (Table 1). On average, 217 trees per ha were oriental spruce (278,648 m³ per ha) and 77 trees per ha (125,006 m³ per ha) were Caucasian fir. Precipitation occurred throughout the year with mild winters and cool summers. The high precipitation meant that there was no water deficit or drought at either site.

Seed-tree Cutting and Site Preparation. Seed-tree cutting, ground vegetation removal and soil tillage were performed on “site 20” in 2014, which was not an abundant seed year for oriental spruce at this site, while seed-tree cutting, ground vegetation removal and soil tillage were carried out prior to seed dispersal in “site 21” in 2015; this was an abundant seed year for oriental spruce at “site 21”. Of the total tree stock in sites 20 and 21, 40% and 41%, respectively, were harvested during seed-tree cutting. The number and volume of trees remaining at the site after seed-tree cutting are shown in Table 1. Dense ground vegetation, such as Rhododendron ponticum, Rubus sp. Pteridium sp. Ilex colchica and some types of grasses were found at both sites, and branch and root debris remaining after cutting were removed from the sites by mechanized operations, using a bulldozer. Soil was tilled to a depth of 30–40 cm before seed dispersal in “site 20” in 2014 and in “site 21” in 2015.

Determination of Seed Quantity and Seed Characteristics. Nine rectangular seed traps with dimensions 0.5 m × 0.6 m were installed randomly in each site before seeds detached from the cones in the seed year of 2015. Traps were kept at the sites from September 2015 to March 2016. Seeds in the traps were collected periodically and used to determine the total number of seeds dispersed at the site. The empty/full ratio, 1,000-seed weight and number of seeds per kg of the collected oriental spruce seeds were determined [10]. The number of Caucasian fir seeds was recorded but no characteristic determination was performed since the number of Caucasian fir seeds collected was very low.

Seedling Counts. Nine plots, each 1.5 m × 1.5 m, were established randomly, using wooden sticks, in April 2016 (immediately before germination started) at each of the two study sites to determine germination percentage of oriental spruce seeds, seedling survival percentage and development conditions of seedlings. Seedlings at both sites were counted in July 2016 and germination percentage was determined. Seedlings were recounted in September 2016, May and September 2017, and May 2018 and the percentage survival rates of seedlings over time were determined. For Caucasian fir, only the number of seedlings that germinated alongside oriental spruce seeds was reported but the data were not used in calculations or assessments.

Diameter and height of oriental spruce seedlings. The diameter and height of each of 100 randomly selected oriental spruce seedlings were measured, to the nearest mm, at each site in May 2018 to determine the effect of site preparation time on the growth of oriental spruce seedlings. The sturdiness index (SI) of each oriental spruce seedling at each site in May 2018 was also calculated [11]:

\[ SI = \frac{\text{height (cm)}}{\text{diameter (cm)}} \]

Statistical analysis. Student’s “t” test was used to analyze the change in the number of oriental spruce seedlings at the sites over time, the degree of decrease in the number of seedlings at the sites across time periods as well as the change in the diameter and height values of seedlings at each site and their sturdiness indices in 2018. The change in the decrease percentage of seedlings (death) across time periods was analyzed using analysis of variance (ANOVA), with a multiple comparison test being used to compare individual treatment means where the ANOVA had been shown to be significant. Comparison of two treatments was carried out using the t-test.
RESULTS AND DISCUSSION

Number of Dispersed Seeds and Seed Characteristics. There was an average of 363 oriental spruce seeds/m² (361,6667 seeds/ha), 17 Caucasian fir seeds/m² (166667/ha) and total of 380 seeds/m² (378,3334 seeds/ha) collected by the seed traps installed in sites 20 and 21 in the abundant seed year of 2015 (Table 2).

<table>
<thead>
<tr>
<th>No</th>
<th>Oriental Spruce</th>
<th>Caucasian Fir</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>17</td>
<td>137</td>
</tr>
<tr>
<td>2</td>
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<td>13</td>
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</tr>
<tr>
<td>5</td>
<td>413</td>
<td>40</td>
<td>453</td>
</tr>
<tr>
<td>6</td>
<td>363</td>
<td>17</td>
<td>380</td>
</tr>
<tr>
<td>7</td>
<td>913</td>
<td>10</td>
<td>923</td>
</tr>
<tr>
<td>8</td>
<td>553</td>
<td>13</td>
<td>567</td>
</tr>
<tr>
<td>9</td>
<td>163</td>
<td>10</td>
<td>173</td>
</tr>
<tr>
<td>Mean</td>
<td>363</td>
<td>17</td>
<td>380</td>
</tr>
</tbody>
</table>

Based on the assessments conducted on the oriental spruce seeds dispersed in 2015, the fullness ratio of seeds was 70.08%, weight per 1,000 seeds was 4.09 g and there were 226,808 seeds per kg. Seed trees that were present in the stand during seed-tree cutting dispersed 15.94 kg seeds/ha.

Seedling germination and survival percentage. Based on data from the survey conducted in May 2016, 92,840 oriental spruce seedlings/ha germinated in “site 20”, where site preparation and seed-tree cutting had been carried out in 2014, compared with 143,704 oriental spruce seedlings/ha which germinated in “site 21”, which underwent seed-tree cutting and site preparation in 2015. The seedlings that could not tolerate the environmental stresses (biotic or abiotic) at the site died over time. Oriental spruce seedling mortality over the 2 years from the first survey conducted in May 2016 to May 2018 was 34.04% and 49.82% in sites 20 and 21, respectively (Fig. 1).

The percentage mortality of oriental spruce seedlings between survey dates are given in Table 4. The greatest significant seedling mortality after the germination in 2016 was observed between September 2016 and May 2017 at both sites, where 22.62% and 38.98% of the seedlings in sites 20 and 21, respectively, did not survive the first winter after germination.

The difference percentage decrease in seedling number between “site 20” and 21 was also significant between September 2016 and May 2017. The percentage decreases during the other intervals between the sites were not significantly different (Table 3).

![Image showing change in number of oriental spruce seedlings per hectare in two sites through time](image_url)

FIGURE 1

Change in the number of oriental spruce seedlings per hectare in two sites through time

<table>
<thead>
<tr>
<th>Period</th>
<th>N</th>
<th>Site 20</th>
<th>Site 21</th>
<th>Significant LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2016- September 2016</td>
<td>9</td>
<td>13.72a</td>
<td>8.73ab</td>
<td>0.39</td>
</tr>
<tr>
<td>September 2016- May 2017</td>
<td>9</td>
<td>22.62a</td>
<td>38.98b</td>
<td>0.02</td>
</tr>
<tr>
<td>May 2017–September 2017</td>
<td>9</td>
<td>13.05ab</td>
<td>7.99a</td>
<td>0.32</td>
</tr>
<tr>
<td>September 2017- May 2018</td>
<td>9</td>
<td>3.27a</td>
<td>4.02a</td>
<td>0.77</td>
</tr>
<tr>
<td>Significant LSD (P value)</td>
<td></td>
<td>0.01</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>
Any two dates within a site with a common letter superscript were not significantly different (P>0.05). If two sites at the same date have a common number superscript, the % mortality was not significantly different (P>0.05). The analysis was carried out using ANOVA. The multiple pairwise comparison test used was Fisher’s Protected Least Significant Difference (LSD).

Seedling Growth. The oriental spruce seedlings in “site 21” were significantly taller and of higher quality (i.e., stockier) than the ones in “site 20” according to the measurements and assessments conducted in May 2018 (P<0.05). The difference in the seedling diameter between the sites was not significant (Table 4).

In this study, the 86–88 oriental spruce trees remaining per hectare (Table 1), following seed-tree cutting in a mixed stand of oriental spruce and Caucasian fir, produced an average of 363 seeds/m^2 in the abundant seed year of 2015. Atasoy [12] reported that 1,170 seeds/m^2 were collected in an oriental spruce site with a density of 203 trees/ha in the Meryemana forest of Trabzon in the abundant seed year of 1985. The number of seeds collected per tree in that study was greater than the one in the current study when the number of trees at the two sites was taken into account. This difference could be attributed to the differences in the number of trees and in growth conditions. Hofgaard [13] found that Norway spruce with a density of 215 trees/ha produced 475 seeds/m^2 in a study conducted in the Scandes mountains of Sweden in the abundant seed year of 1984. When the number of trees at the sites was taken into account, seed production by Norway spruce [13] seemed to be lower than the seed production of oriental spruce according to the results from both the current study and the ones reported by Atasoy [12]. The lower seed production of Norwegian spruce might be the result of a more northerly range and colder climate. In a study conducted in the black pine forests of the Dursunbey-Alaçam region of Turkey, Boydak et al. [14] determined that an average of 162 seeds/m^2 were collected in the abundant seed year of 1972 and that 30% of these were empty. In another study conducted to assess seed production in Scots pine, Boydak et al. [15] found that 253 seeds/m^2 and 246 seeds/m^2 were produced in 1971 and 1975, respectively and 26%–28% of these were empty. The emptiness ratio of 29.2% determined in our study was similar to the emptiness ratio values reported for black and Scots pines [15].

The weight of seeds dispersed in the abundant seed year of 2015 at the Paşakonağı sites was 15.94 kg/ha. Boydak [16] reported that 20-30 kg/ha pure seeds would be sufficient for regeneration by seed-tree cutting in Lebanese cedar stands on karstic sites that were regenerated via the uniform shelterwood method. The weight per 1,000 seeds dispersed at the site in 2015 was 4.09 g in the current study. Atasoy [12] measured 1,000-seed weights for oriental spruce seeds collected at different elevations and reported that it was 8.2 g in Meryemana located at 1,600 m, 7.5 g in Bicik at 1,550 m, 9.4 g in Ardanuç at 1,500 m, 9.1 g in Maçka at 1,100 m and 10.4 g in Hamsiköy at 1,450 m. The 1,000-seed weight in the present study was lower than those reported in these other sites.

Three percent of the dispersed seeds germinated in “site 20”, which underwent seed-tree cutting before the seed dispersal year, in comparison with 4% of the dispersed seeds germinating in “site 21”, which underwent seed-tree cutting in the seed year. The remainder of the seeds at both sites either decayed or were consumed by birds, mammals or insects or carried away by the wind. Only 1% of the Norway spruce seeds dispersed germinated in a study carried out by Hofgaard [13], while Grombone-Guaratini and Rodrigues [17] reported that 16% of the seeds collected in a mixed deciduous forest germinated. Similarly, Du et al. [18] determined that only 11% of the collected seeds germinated in *Castanopsis fargesii* stands. The differences between our results (low but not the lowest germination) and other published studies could be attributed to the growth environment conditions, species differences and seed quantity and quality.

The seedlings which germinated from the dispersed seeds following seed-tree cutting were lost to biotic and abiotic stresses (competition with ground vegetation, predation by insects, mammals and birds, and inclement weather) over time. Of the seeds which germinated for the first time in 2016, 34.04% and 49.82% were lost after 2 years in sites 20 and 21, respectively. Erkuloğlu et al. [19] and Ata [1] reported that 70%–80% of the seedlings that germinated in the first year were lost within 5 years in oriental spruce forests, while 53% of the cedar of Lebanon seedlings died within 5 years after germination in naturally regenerated forests. In a regeneration study of *Pičea abies*, Hanssen [20] found that

### TABLE 4

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Site</th>
<th>N</th>
<th>Mean</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>20</td>
<td>100</td>
<td>6.09^a</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>100</td>
<td>6.79^b</td>
<td>0.00</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>20</td>
<td>100</td>
<td>4.42^a</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>100</td>
<td>4.54^a</td>
<td>0.00</td>
</tr>
<tr>
<td>SI (eight/diameter)</td>
<td>20</td>
<td>100</td>
<td>1.38^a</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>100</td>
<td>1.50^b</td>
<td>0.00</td>
</tr>
</tbody>
</table>
only 123 (32%) of 382 seedlings/m² that germinated 1 year after the abundant seed year of 1995 survived five years, as of September 2000. We expect the seedling loss percentage after five years from germination at our site to be around 70%–80% as reported by Erkuluğlu et al. [19] and Ata [1]. Üçler et al. [21] found that there were an average of 16,920 oriental spruce trees/ha in a 14–20-year-old oriental spruce stand that had reached the thicket stage. We predict that the number of seedlings at our site to be similar to this value when the stand reaches that age.

This study proved the importance of timing of site preparation and soil tillage prior to seed-tree cutting on the number of seedlings germinating, seedling survival percentage and the growth of the seedlings. Seedlings which germinated at the site where soil was tilled in the abundant seed year developed better than the ones which germinated at the site which was tilled 1 year before the abundant seed year. Different soil structures and germination conditions have important effects on germination of seeds [22, 23, 24]. For example, phenolic compounds obtained from two different forest soils in Aspromonte (southern Italy) were reported to have significant effects on germination of Pinus laricio seeds [25].

However, tillage changes the structure of the soil. Iijima and Shibuya [26], reported higher seedling germination in tilled areas compared to untilled areas in Picea jezoensis stands. In a regeneration study conducted in black pine forests, seedling loss was lower in sites where soil was tilled using ripper, hoe and plough compared to reference sites [27]. Likewise, Celma et al. [28] showed that soil tillage and the timing of soil tillage, as in our study, had a significant and positive effect on the development of seedling roots and stems. However, Esen and Zedaker [29] and Yildiz et al. [30] pointed out that losses of soil carbon and nutrient occurred as a result of soil tillage, in particular during the removal of thick ground vegetation cover like rhododendrons, even though these procedures improved seedling germination and survival success.

CONCLUSIONS

Oriental spruce trees at the study site dispersed an average of 363 seeds/m² in the abundant seed year of 2015. From these seeds, 92,840 seedlings/ha germinated in “site 20”, where seed-tree cutting, site preparation and soil tillage were performed in 2014 while 143,704 seedlings/ha germinated in “site 21”, where seed-tree cutting, site preparation and soil tillage were carried out in the abundant seed year of 2015. Of these seedlings, 34.04% in “site 20” and 49.82% in “site 21” had died 2 years after the germination that occurred in 2016. The greatest number of deaths occurred in the winter immediately after germination. Of the seedlings that survived 2 years, those in “site 21” developed better than the ones in “site 20”.

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Sinan Guner and Zuhre Kutlu conceived and designed the experiments; and analysed the data, and Sinan Guner wrote the paper.

The authors declare no conflicts of interest.

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SYNTHESIS OF MOO$_2$ AND MOO$_2$/CO BY LASER PYROLYSIS AND ITS PHOTOCATALYTIC ACTIVITY

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ABSTRACT

Typically, molybdenum dioxide (MoO$_2$), as a promising anode material for LIBs, has triggered much attention owing to its high theoretical capacity, low electrical resistivity, high density, affordable cost and it has excellent physico-chemical properties. This report presents a bibliographic study on the synthesis of MoO$_2$ and some results concerning a preliminary study of catalytic MoO$_2$/Co materials synthesis by laser pyrolysis. The objective was to demonstrate the possibility to obtain molybdenum oxide nanoparticles from the laser pyrolysis method. In addition, the photocatalytic activity of the MoO$_2$ nanospheres is investigated.

KEYWORDS: MoO$_2$, Cluster model, Laser Pyrolysis, Nanoparticles, Catalytic performance

INTRODUCTION

Molybdenum (Mo) is widely used as an alloying agent for manufacturing steel, cast iron, structural and electronic materials, and superalloys due to its high temperature strength, creep resistance, low coefficient of thermal expansion, and high thermal conductivity. For this reason, the fabrication of molybdenum powders with suitable morphology, size, and purity has been extensively studied in an effort to obtain materials with better performance. In recent years, nano-sized Mo powder has been reported to exhibit attractive properties due to its ultrahigh specific surface area and instability of free surface [1-4].

Molybdenum dioxide (MoO$_2$), with good electronic conductivity, has promise in applications ranging of catalysis, sensing, electrochemical supercapacitors, etc. It is a promising anode material for lithium ion batteries that showed relatively large capacity (400-600 mA h g$^{-1}$). The use of nanomaterials is a popular path to improve the rate capabilities of solid-state electrodes used in batteries because of the small diffusion lengths [5] and the large surface area.

Molybdenum compounds display a plethora of physical properties [6-9]. This is related to the wide range of oxidation states – from Mo$^{2+}$ to Mo$^{6+}$ – in which Mo ions appear in different systems [10]. More specifically, molybdenum oxides have attracted the attention of the scientific community for quite some time. At room temperature, molybdenum dioxide (MoO$_2$) is a weakly paramagnetic conductor, which crystallizes in a distorted rutile structure (monoclinic). Because of its asymmetric structure, with Mo-Mo bonds (dimerization) along monoclinic a axis, this compound presents an anisotropic conductivity [11]. The conduction across a specific axis is also facilitated by dimerization in other Mo oxides, such as KxMoO$_2$-$\delta$[12] and MoOy. It was shown that they actually present superconducting behavior at different values of Tc, and with a direct correlation between the anomalous behavior and the anisotropic electron doping observed in these compounds.

In the past decade, MoO$_2$ nanorods or nanowires, nanospheres, and nanoparticles with irregular morphology have been prepared by reducing MoO$_3$ with hydrogen or ethanol vapour [13] at high temperature, by volumetric flame synthesis [14], with electro chemical deposition [15] and hydrothermal synthesis etc. A ordered mesoporous metallic MoO$_2$ materials with the cell parameter of 23.3 nm was reported, which were synthesized by using phosphomolybdic acid as a precursor and mesoporous silica KIT-6 as a hard template via nanocasting strategy, and shows a capacity up to 750 mA h g$^{-1}$ [16].

However, the smallest dimensions of most of these reported MoO$_2$ phase is greater than 10 nm. Recently, we have synthesized the MoO$_2$ nanoparticles with the dimension less than 8 nm by laser pyrolysis. To our knowledge, we are the first to report the synthesis of MoO$_2$ nanoparticles less than 8 nm which can promisingly improve the catalytic property and rate capability of MoO$_2$ nanomaterials.

EXPERIMENTAL

We had chosen molybdenum(VI) oxide bis(2,4-pentanedionat)(MOP) [Alfa Aesar, 100%] as the precursor for its good solubility in ethanol. Cobalt(II) acetylacetonate [Co(C$_2$H$_5$O)$_2$ Aldrich Chemical, 99.97%] was used for cobalt source.
In a typical synthesis of MoO$_2$, 16.3 g MOP precursor was dissolved in 400ml ethanol. After stirring with an electromagnetic stirrer, the bottle of solution was introduced in the reactor by pyrosol. We obtained the gray or black powder by decomposition of the precursor under laser beam and Argon atmosphere.

RESULTS AND DISCUSSION

**X-ray diffraction.** Figure 1 shows the X-ray diffraction patterns of the samples OMo1-OMo5, respectively. All diffraction peaks were indexed to monoclinic MoO$_2$ as confirmed from the reported data (JCPDS no. 32-0671). The samples OMo1 and OMo2 were well crystallized because they were synthesized with higher laser puissance. However the samples OMo4 and OMo5 were not well crystallized because of the large peak and the noise important.

We can estimate the crystalline size of the powder according to Scherrer equation. We obtained the size of 7.6 and 5.3 nm for the samples OMo1 and OMo2, respectively.
Figure 2 represents the XRD patterns of the samples OMoCo1-OMoCo4, the cobalt doped molybdenum dioxide (Co/MoO$_2$). The peaks were indexed to monoclinic MoO$_2$ according to the data JCPDS no. 32-0671. And there wasn't the peak corresponding CoO phase.

Transmission electron microscopy. The morphology and structure of the MoO$_2$ and Co/MoO$_2$ nanoparticles were studied by TEM measurement. Figure 3 shows that MoO$_2$ nanoparticles were spherical shape and the size was ~8 nm described in the table synthesis and characterization.

For the nanoparticles Co doped MoO$_2$, there were two parts of the size distribution, the smaller nanoparticles with dimension ~5 nm and the bigger nanoparticles with dimension ~8 nm. We observed the small nanoparticles ~5nm because of the insertion of the cobalt in the maille of MoO$_2$.

Catalytic performance. Methylene blue is a commonly used heterocyclic aromatic hydrocarbon organic dye and redox indicator. In recent years, heterocyclic aromatic hydrocarbon organic dyes such as methylene blue are often used as research objects for photocatalytic degradation. This experiment studies the photocatalytic activity of synthesis of MoO$_2$ under different light conditions.

Figure 4 shows TEM images of a) OMo1, b) OMo2, c) OMo3, d) OMo4.

Figure 5 shows the effect of different temperature on the photocatalytic activity of synthesis of MoO$_2$. The decolorization rate of MB (%)

[Graph showing decolorization rate at different temperatures over reaction time]
The experiment was carried out with sunlight and high-pressure mercury lamp as the light source. After 5 h of irradiation, the MB dye solution and the original solution after photocatalytic degradation were analyzed (Fig.5-7). According to the measurement results of the absorbance, the decolorization rate formula can be used to calculate the MoO$_2$ nanosphere pair. The decolorization rate of MB dye was 88.0%, 87.3%, which indicates that MoO$_2$ nanospheres have better photocatalytic degradation effect on MB dye. This is mainly because the small particle size structure of nanospheres makes it large. Band gap width, and the redox ability of photogenerated electron holes is enhanced, thereby increasing the photocatalytic activity of the nanospheres.

The light source is the reaction power of MB photocatalytic degradation. The sunlight continues from the near ultraviolet region to the near infrared region, and the energy is concentrated in the visible region. The wavelength of the high pressure mercury lamp is concentrated in the ultraviolet region of about 254 nm. With sunlight as the light source, it can be fully the use of solar energy, energy conservation, and mild reaction conditions, non-toxic, non-corrosive equipment, has certain application prospects and environmental protection significance in photocatalytic treatment of organic wastewater. In the past, photocatalytic materials, if the ultraviolet light does not reach sufficient strength, catalysis or the effect of sterilization is not ideal. The MoO$_2$ nanosphere, as a photocatalyst for MB degradation, is sensitive to both ultraviolet and visible light, and can achieve a good catalytic effect, so it is more applicable.

The dye is relatively stable under normal conditions. When no catalyst is irradiated with sunlight, the decolorization rate of the dye MB by
MoO₂ is 0. When the MoO₂ is added, the MB has a certain decolorization because the dye is adsorbed on the surface of the photocatalyst. Photodegradation reaction occurred on the surface of the photocatalyst.

The used MoO₂ was thoroughly washed with distilled water and absolute ethanol, and then filtered to be naturally dried. The treated MoO₂ was subjected to degradation by catalysis for 5 hours under sunlight, and the sample was analyzed by a sample. The decolorization rate was 88.9%, its catalytic activity is basically unchanged, which shows that MoO₂ as a photocatalyst has stable performance and can be reused.

CONCLUSION

In conclusion, highly dispersed crystallized MoO₂ and Co/MoO₂ nanoparticles with the dimension less than 8 nm have been successfully synthesized by pyrolyse laser in on step process. The precursor, which is soluble in ethanol, can be decomposed easily under the laser beam. We obtained gram quantity of grey powder in one hour continuously and the dimension of MoO₂ nanoparticles is less than 8 nm which can be promisingly used as catalytic materials and anode for lithium ion batteries.

In addition, the photocatalytic activity of the MoO₂ nanospheres is investigated. According to the measurement results of the absorbance, the decolorization rate formula can be used to calculate the MoO₂ nanosphere pair. The decolorization rate of MB dye was 88.0%, 87.3%, which indicates that MoO₂ nanospheres have better photocatalytic degradation effect on MB dye.

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A RESEARCH ON DETERMINATION OF VULNERABLE LANDSCAPES IN TERMS OF GROUNDWATER: DUZCE CASE, TURKEY

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Duzce University, Faculty of Forest, Department of Landscape Architecture, Turkey

ABSTRACT

Water is one of the main sources of life and its continuation depends on the availability of usable water. This study was carried out to determine the vulnerable landscape in terms of groundwater resources. The study was conducted in Duzce district which has quite rich water resources, and provides water to Istanbul, Turkey's most populous city, but is threatened by settlement, industry and agricultural development. Water process analysis method was used in the study. Water process analysis aims to reveal the movement of precipitation water in vegetation cover, soil and rock layers and convert them into spatial data. Within the scope of this method, slope, soil, rock and vegetation cover criteria were evaluated, and water infiltration status was mapped according to five criteria (very high, high, moderate, low, very low). It was revealed that %0.4 of the water infiltration map area has very high, %13.1 high, %70.2 moderate, %14.6 low and %1.8 very low infiltration value. These findings show that %83.7 of the area (at infiltration, very high, high and moderate values) are vulnerable to feeding and protection of groundwater resources. Protection and improvement of green areas for feeding groundwater resources; avoiding structural surfaces changes; preventing or reducing the area usage of agriculture, industry, settlement, solid waste storage etc. activities to prevent pollution; and switching to clean production/eco-efficiency, ecotourism, ecological farming etc. is important in these vulnerable landscapes. As a result, the water infiltration map obtained in the study is a practical data that can contribute to many studies, especially in the upper scale spatial plans.

KEYWORDS:
Water infiltration, water process analysis, water pollution, landscape vulnerability, landscape sensitivity, ecological sensitivity.

INTRODUCTION

Water is the most important resource for all life forms in the world and is their building block. At the same time, the world runs on water [1, 2]. In other words, many activities in the world, for example, continuation of ecosystems, conservation of biodiversity, protection of climate and microclimate, food production, energy production, transportation service, etc. are all related to water [3-6]. For this reason, water-related decisions touch many areas of life (agriculture, forest, industry, energy, etc.) [7].

Increasing population and changing living conditions are increasing the pressure on water resources on each passing day [8, 9]. The main sources of water pollution are unplanned, faulty and/or dense settlements, industrialization and agricultural activities. In this context, %80 of municipal wastewater on a global scale is discharged to water bodies without treatment. In addition, large quantities of chemical and organic matter, pesticide residues, sediment and saline from agricultural areas are discharged into the water springs. It also stresses that the %70 of world water use (water abstractions) is for agricultural purposes [10]. In addition, global climate change and water management beyond its natural boundaries (watershed) and away from ecological principles further increase these problems. In studies on the future of water resources, it is predicted that approximately 1.8 billion people will live in regions or countries with absolute water scarcity by 2025; %40 of the world's water demand and supply will be not be met by 2030; agricultural production to feed 9 billion people in the world will consume %15 of the remaining water by 2050 [11]. This situation refers to only a small part of water-related problems in today's world, but it also shows its importance. Awareness of this importance is the subject of various scientific studies.

This study aims to determine vulnerable areas in terms of groundwater resources. Groundwater is the most important water resource that feeds water sources such as wells, rivers, lakes and seas and is fed by precipitation. In this study, the areas where precipitation water can reach underground are considered as vulnerable areas. In this context, it is important to describe the water cycle as the source of precipitation water so as to define the study process. Water is constantly moving between the
Earth's atmosphere, hydrosphere (ocean, sea, river, etc.) and the lithosphere (soil and rock) layers. At the same time, water is stored in solid, liquid and gaseous states inside these layers and constantly changes states. This state of change and the process of transformation is called the hydrologic cycle [12-14]. The water cycle consists of "precipitation, evaporation, transpiration, evapotranspiration, interception, surface runoff, condensation, infiltration, groundwater base flow, sublimation" processes [15]. At this point, it is clear that the hydrologic cycle or the movement of water is a stochastic process [16]. Moreover, the process is transformed into a more complex system [22] by interacting with the basin as the natural border of water sources [17], natural and cultural landscape characteristics of the basin [18], and the ecological processes in the basin [19-21].

In the study, the motion of the precipitation water in soil and rock layer was evaluated in determining the vulnerable landscape. Groundwater resources are found between and above the layers of impermeable rock under permeable soils and rocks. In other words, it is located in the lithosphere layer (earth and rock layers). Some features in this layer affect the precipitation water and the feeding of groundwater resources, in other words, infiltration. These features are as follows:

- The slope of the topography affects the flow of precipitation water on the earth surface, soil and rock layers. In this context, as the slope decreases, the chance of infiltration and permeability of rainfall increases [23].
- Current land use is important in terms of precipitation water's surface flow and infiltration. That earth is covered with building structures prevents infiltration but increases runoff. It also causes the evaporation of water. That it is covered with vegetation affects infiltration, runoff, evaporation, evapotranspiration and other processes in different ways [22, 23].
- The presence of vegetation on the soil increases the soil's infiltration of precipitation water. In addition, vegetation density increases the infiltration capacity [22-24]. In addition, the nature of the plant species constituting the vegetation also affects the infiltration. For example, an area with conifers can infiltrate more rain compared to areas with broad-leaved trees. For, the broad-leaved trees shed their leaves in winter and the existence effect of vegetation decreases [25]. In this context, infiltration occurs mostly in forests, followed by bushes and lumpy formations, the least grass-covered areas and less naked areas respectively [26]. In addition, the grass patches on the ground surface are also important for drainage network (runoff). If these patches consist of perennial grasses, drainage network increases but runoff speed will decrease. This will increase infiltration. Viewed from this perspective, plants can be seen accepted as not water consumers, but as water as water recyclers [23].
- The state of infiltration in the soil can vary depending on soil texture, porosity, weight, mineralogy, the chemistry of the soil and how the layer is formed. For example, as the amount of clay and silt expressing the soil texture increases, the infiltration decreases; but, as the amount of sand and gravel in soil decreases, infiltration increases. As the porosity in soil increases, infiltration increases. As the moisture in soil increases, the infiltration rate decreases [25, 27-29]. In addition, erosion, sediment transport and deposition effect infiltration [22].
- The movement of infiltrated rain among rock layers depends on porosity and permeability of the rock. Porosity refers to the ratio of the space formed by fracture in the rock layers, cracks and faults to the whole volume; permeability refers to rock layer's ability to transmit liquid due to the pressure difference. In this context, magmatic and metamorphic rocks have low porosity. Also, they are hard to break and crack and have low permeability. Rocks with high dissolve properties such as limestone have porosity due to melting and fracturing. Clay or silty sediments are more porous than gravel or sand. However, the pores between the grains are small and, thus permeability is low. Sand and gravel deposits are the layers with most permeability. Furthermore, cementing between grains decreases the porosity and restricts the movement of water [30].

In this study, the movement of precipitation water in vegetation cover, soil and rock layers (in the lithosphere layer) was evaluated to determine the vulnerable landscape in terms of groundwater resources and this movement was converted into a spatial data (map). The data obtained have been evaluated and some recommendations have been developed to contribute to spatial planning.

MATERIALS AND METHODS

Materials. The study was conducted in Duzce district located in the west Black Sea region in Turkey. Primary and secondary data were used in the study. The primary data consists of Duzce Digital Topographic Map (2000) in 1/25000 scale [31], Digital Geological Map (1984) in 1/25000 scale [32], Duzce Digital Soil Map (2002) in 1/25000 scale [33], Duzce Digital Forest Management Map (2000) in 1/25000 scale [34], and literature about the field (institutional report, article, thesis, project, etc.). Secondary data consists of literature on the theoretical framework (water resources, infiltration, water process, etc.). ArcGIS 9.3 software was used in the evaluation and analysis process.
Study area. Duzce is located between 40° 37' and 41° 07' north latitudes and 30° 49' and 31° 50' east longitudes. It is surrounded by Bolu and Zonguldak in the east, Sakarya in the west, Bolu in the south and the Black Sea in the north (30 km coastal length) (Figure 1). The district covers approximately 2487 km², and this area constitutes %0.3 of Turkey [35, 36].

Within the scope of the study, some natural and cultural landscape features of the area were investigated. In this context, it was observed that the Duzce plain covers approximately % 14.5 and mountainous and rough terrain covers %85.5 of the study area. The plain areas except Duzce plain are on the Black Sea coast. The study area is surrounded by the northern part of Elmacik Mountains in the south, the north-western part of Bolu mountains in the east, Kaplande and Orhan mountains located between Black sea coast and Duzce plain in the north. The highest points in the study area are the heights of 1830-1388 m which are located on Elmacik and Bolu mountains. Duzce Plain is located in the middle of the study area. Duzce city is located in the plain [35]. %11.8 of the study area has %0-2 slope, %2.6 has %4.2, %6.8 has %6-12, %13 has %12-20, %18.7 has %20-30 and %45.5 has more than %30 slope. %11.3 of the area has flat aspect, %9 has east, %9.6 has west, %28.1 has south and %42 has north aspect [31]. The main rivers in the study area are Büyük Melen, Aksu Creek, Küçük Melen, Asar Water, UğurSuuyu and Büyük Creek. Melen basin provides water to Istanbul, Turkey's largest city. The most important lake in the area is Efteni Lake, and there are also small lakes such as Kuru Lake, Topuk Plateau Lake, Islak Lake, Karagöl, Silüklü Lake and Salik Lake. Hasanlar Dam which is located on Küçük Melen river was completed in 1992 and is within the boundaries of the study area. There are 4 source water reservoirs as groundwater source which are used for commercial purposes. The area has Efteni and Derdin thermal springs. In addition, there are 3 licensed geothermal resource exploration activities, as well as 2 geothermal resource operation licenses and 2 natural mineral water operation licenses [36]. There are 22 different formation types composed of different rock characters in the study area. These rock types are sandstone, shale, conglomerate, sandstone, conglomerate, siltstone, limestone, ophiolitic rocks, granite, gabbro, tuff, andesite, basalt, conglomerate, gneiss, metagranite, amphibolite, migmatite, marble, phyllite, etc. There are two important points to be emphasized here. The first one is that Duzce plain is filled with thick and loose alluvial deposits; the second is that the area is located within the 1st-degree earthquake zone and Kuzey Anadolu, Hendek, Çilimli and Duzce fault lines are in the area [32, 35]. %39.2 of the area is covered with Non-calcareous Brown Forest Soil, %21.8 with Podzolic Soils, %17.7 with Brown Forest Soils, %8 Gray Brown Podzolic Soil, %8 Alluvial Soils, %2.7 Colluvial Soil, %0.6 with Hydromorphic Soil (%1.2 of the area soil type could not be identified). In addition, %11.1 of the study area has I. degree land skill class, %2.6 has II. degree, %1.8 has III. degree, %5.4 has IV. degree, %0.78 has VI., VII. and VIII. degree [33]. The study area is within the Euxine and Xsero-Euxine transition belt in terms of its flora. The Euxine belt is characterized with mixed broad-leaved forests from the mountainous part of the coast to submountainous parts. The study area hosts 6 vegetation types including lake and swamp, riparian, remnant scrubs, rupicolias, forest and subalpine [36]. Approximately %32.4 of the area is leafy, %2.5 is coniferous, %16.4 is mixed forest, %7.2 is hazelnut and %0.8 is pasture [36]. The study area is under the influence of the Black Sea and Marmara climate. Summers are hot, winters are partly warm and partly cold. The excessive forest and surface waters and the lack of high mountains on the Black Sea coast provide an increase in precipitation. The average annual temperature is 3.2 °C, the coldest month is January (3.7°C), the hottest month is July (22.6 °C) and precipitation is 87.7 mm. In winter and autumn, precipitation is quite high. The average
annual humidity is 77.5%. The average number of snowy days is 6 days, duration of snow cover is 5 days and the frosts are 44 days. The fog is mostly seen in November and for 23 days a year [37]. 50% of the area is forest and shrubbery, %30 is agricultural area, %9 is other areas (settlement, industry, etc.) and %1 is meadows and pastures. The forests in the study area are in the 15th place in ranking in Turkey [36].

Duzce became a province with Decree 190 published in the Official Gazette numbered 23901 dated 09.12.1999 [35]. Duzce province has 7 districts, 279 villages and 2 towns. The population according to the 2018 census is 377610. Its population has increased by 65.4% from the day it became a province in 2000 (130632) [38]. The economic structure the area is based on tourism, agriculture and industry. In the study area, tourism developed with natural landscaping elements, industry developed after Incentive Law in 2004, and agriculture developed when Duzce plain was included in agriculturally protected areas in 2017 [36].

**Method.** Water process analysis method was used in the study. The method was developed based on Buuren's (1994) water infiltration analysis [17, 39, 40]. The basic philosophy of the method is the movement of the precipitation water horizontally and vertically with the effect of gravity. The vertical movement of precipitation water is: the water reaches the soil through the plants; a portion of it passes to the surface flow, the other part feeds soil moisture by being infiltrated by the soil; the water reaches the rock layers after the soil is saturated with moisture; it is percolated in the rock layer until it reaches to impermeable layers or underground water source repositories. The horizontal movement of the precipitation water is its flow on the surface due to topographical slopes [12-15]. Within the scope of the method, the water's movement in soil and rock layers are evaluated in a holistic manner, and its infiltration and permeability status has been mapped as "very high (vh), high (h), moderate (m), low (l), very low (vl)." Within the scope of the method, vegetation cover, soil layers, rock layers and slope criteria affecting the process were evaluated. Evaluation tables in the method were developed based on previous studies [17, 40] and 3 expert opinions. Multi data were holistic evaluated with overlay analysis in Geographic Information Systems (GIS) software. The method was applied in 4 stages (Figure 2).

The studies carried out in these stages are given below.

1. The first stage consists of two phases. In the first step, soil map was evaluated according to the infiltration ability of the land capability classes and soil infiltration data consisting of 5 classes were formed. According to land capability classes, I. class lands are considered "very high", II. class "high", III. class "moderate", IV., VI., VII., VIII. classes "low" and V. class "very low". In the second step, the effect of the slope on soil infiltration was considered. In this context, soil infiltration and slope data were subjected to overlay analysis. The data obtained are evaluated according to Table 1, and the soil infiltration (+slope) data generated.

The second stage consists of two steps. In the first step, the permeability of the rock layer in the geological map was evaluated based on expert opinions, and rock permeability data consisting of 5 classes were formed. The rock layer were evaluated based on expert opinions, and it was found out that unallocated quaternary, alluvium, alluvial spectrum, slope debris have "high" value, terrestrial crumbs, carbonate and crumbs, limestone, metaflysh, shale, sandstone, neritic limestone, unallocated volcanic have "moderate" value, and metagranite, metabatro, unallocated gneiss, schist, amphibolite, marble have "low" value. In the second step, the effect of the slope on rock permeability was considered. In this context, rock permeability and slope data

![Diagram of water process analysis stages](image-url)
2. were subjected to overlay analysis. The data obtained are evaluated according to Table 2 and the rock permeability (+slope) data is generated.

3. In the third stage, soil infiltration (+slope) and rock permeability (+slope) data were subjected to overlay analysis. This evaluation was developed to evaluate the infiltration of the lithosphere layer. The data obtained were evaluated according to Table 3 and soil+rock (+slope) infiltration data were generated.

4. The fourth stage consists of two steps. In the first step, vegetation cover type in the forest management map was evaluated according to infiltration status to determine the water’s level of reaching soil layer, and a vegetation infiltration data consisting of 5 levels was formed. It was observed that according to the vegetation cover type, leafy, coniferous and mixed forests are considered to be "very high infiltration", shrubbery and areas with sparse vegetation cover and irrigated agricultural areas "high", grassy vegetation cover and non-irrigable agricultural areas "medium", bare areas and rocks "low" and constructed areas are "very low". In the second step, soil+rock (+slope) infiltration and vegetation infiltration data were subjected to overlay analysis. The data obtained were evaluated according to Table 4, and water infiltration data were generated.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Soil infiltration according to the slope value (1. stage) [17].</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil Infiltration Value</td>
<td>0-2</td>
</tr>
<tr>
<td>Very high</td>
<td>vh</td>
</tr>
<tr>
<td>High</td>
<td>vh</td>
</tr>
<tr>
<td>Moderate</td>
<td>vh</td>
</tr>
<tr>
<td>Low</td>
<td>l</td>
</tr>
<tr>
<td>Very low</td>
<td>vl</td>
</tr>
</tbody>
</table>

| very high (vh), high (h), moderate (m), low (l), very low (vl) |

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Rock permeability according to the slope value (2. stage) [17].</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rock Permeability Value</td>
<td>0-2</td>
</tr>
<tr>
<td>High</td>
<td>vh</td>
</tr>
<tr>
<td>Moderate</td>
<td>vh</td>
</tr>
<tr>
<td>Low</td>
<td>l</td>
</tr>
</tbody>
</table>

| very high (vh), high (h), moderate (m), low (l), very low (vl) |

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Soil+rock (+slope) infiltration value (3. stage) [17, 40].</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rock Permeability (+Slope) Value</td>
<td>Soil Infiltration (+Slope) Value</td>
</tr>
<tr>
<td>Very high</td>
<td>Very high</td>
</tr>
<tr>
<td>Very high</td>
<td>vh</td>
</tr>
<tr>
<td>High</td>
<td>vh</td>
</tr>
<tr>
<td>Moderate</td>
<td>h</td>
</tr>
<tr>
<td>Low</td>
<td>h</td>
</tr>
<tr>
<td>Very low</td>
<td>h</td>
</tr>
</tbody>
</table>

| very high (vh), high (h), moderate (m), low (l), very low (vl) |

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Water infiltration value (4. stage) [40].</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rock+Soil Infiltration (+Slope) Value</td>
<td>Vegetation Infiltration Value</td>
</tr>
<tr>
<td>Very high</td>
<td>Very high</td>
</tr>
<tr>
<td>Very high</td>
<td>vh</td>
</tr>
<tr>
<td>High</td>
<td>vh</td>
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<tr>
<td>Moderate</td>
<td>h</td>
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<tr>
<td>Low</td>
<td>m</td>
</tr>
<tr>
<td>Verylow</td>
<td>m</td>
</tr>
</tbody>
</table>

| very high (vh), high (h), moderate (m), low (l), very low (vl) |

1: Forest areas: Coniferous, Mixed, Leafy
2: Shrub areas, irrigated areas, sparse vegetation
3: Herbaceous plants, irrigated agricultural areas
4: Bare areas, cliffs
5: Artificial areas: built areas, hard pen.
FINDINGS AND DISCUSSION

When the soil layer of the area was examined, it was seen that %11.1 of this soil had very high infiltration, %2.6 high, %1.8 moderate and %84.4 low. When the effect of slope on soil infiltration was evaluated, it was determined that %13.3 of the soil infiltration in the area had very high infiltration, %1.4 high, %0.9 moderate and %84.4 low (Figure 3). When Figure 3 was examined, it was observed that the soil layer in Duzce Plain had quite high infiltration and the rest of the area had low infiltration. The soil layer in the plain is important in terms of feeding groundwater resources, because this area is the area where infiltration has the highest value.

When the rock layer permeability of the area was examined, it was seen that %22.7 of this layer
had high infiltration, %66.8 had moderate and %10.5 had low. When the effect of slope on the rock permeability was evaluated, %15.8 of the rock layer permeability in the area was found to have very high infiltration, %20.3 had high, %53.1 had moderate, %0.3 had low and %10.4 had very low infiltration (Figure 4). When Figure 4 was examined, it was seen that the rocks layer in Duzce Plain had high permeability levels while the rest had high and moderate permeability levels. This suggests that contaminated precipitation water can pass through the soil layer and spread to rock layers.

In order to determine the effect of soil infiltration on the permeability in the rock's layer, a holistic assessment was made, and it was determined that %13.7 soil + rock layers (lithosphere) in the area had very high infiltration, %0.9 had high, %3.1 had moderate, %71.8 had low and %10.4 had very
When the infiltration status of the vegetation cover in the area was examined, it was observed that %48.9 of the vegetation cover had a very high infiltration, %9.8 had high, %34.5 had moderate, %9 had low, %4.8 had very low infiltration. Based on these findings, it can be said that the vegetation cover in the area has a high and moderate infiltration in general. When the water infiltration map (Figure 6) as the resulting map of this study was examined in order to evaluate the effect of vegetation cover on the permeability of soil+rock layers, it was observed that %0.4 of the area had very high permeability, %13.1 had high, %70.2 had moderate, %14.6 had low, and %1.8 had very low permeability. When Figure 6 was evaluated, it was observed that the infiltration value in the plain is not very but high due to low infiltration of vegetation cover in Duzce Plain area. Due to the high infiltration ability of the vegetation cover in the remaining area, it was observed that the infiltration ability was at a moderate level, not low. The water infiltration map shows that the area is vulnerable to groundwater. Aydin et al. (2018) emphasized the similar result in their study on the integrated of ecological risks in Düzce [47].

When the soil infiltration, rock permeability and vegetation cover infiltration maps were examined, it was seen that the infiltration data obtained from each map had different properties. This confirms the literature. For example, soil characteristics on the area changed the soil infiltration status as stated by Çepel (1986) [29], Öztekin and Öztekin (2007) [27], Yurtseven and Zengin (2013) [25], Haghnavazari (2015) [28], ve Connor et al. (2018) [22]. In the same way, as stated by Dirik (2018) [30], the rock characteristics differentiated the rock infiltration in the area. The change of slope in the land topography affected the infiltration level in soil and rock layer. This case was also expressed by Tongway and Hindley (2004) in their studies [23]. They also evaluated vegetation cover data which may completely affect the infiltration in soil and rock. The result changes depending on the presence and type of vegetation cover and the infiltration in the areas as stated by Tongway and Hindley (2004) [23], Yurtseven and Zengin (2013) [25], Yuksel and Avcı (2015) [47], Yılmaz and Usta (2015) [48], Connor et al. (2018) [22], Fidan et al. (2018) [26], and Okkan (2018) [24].

Water process analysis method utilized in the study was used in various studies by Sahin (1996) [39], Uzun et al. (2012) [17], Uzun and Gültekin (2011) [41], Uzun et al. (2015) [40], Karadağ and Yıldız (2013) [42], Aytaş et al. (2013) [4], Demiroğlu (2014), Demiroğlu et al. (2017) [44], Ateş (2017) [45] and Aydın et al. (2018) [46] Demiroğlu (2016) [49]. These studies contributed to the production of guiding data on landscape planning.

CONCLUSION

The main purpose of determining the vulnerable areas in terms of groundwater resources is to contribute to spatial planning in order to protect groundwater resources as the most important water sources. Spatial planning is a systematic process which produces decision on national, regional, urban and local scale based on many criteria such as natural, cultural, economic, and political. One of the basic principles of this process is the conservation of water resources for the continuation of life.

The water infiltration map obtained in the study provides the identification of the areas covered by the precipitation water and its coverage that feed the underground water. This definition defined four criteria (slope, soil infiltration, rock permeability, vegetation cover infiltration,) as spatial data and infiltration of the area is described as very high, high, moderate, low, and very low. This classification provides information on how precipitation water is protected and transferred to underground water resources.

The water infiltration map shows that the area has medium and high infiltration. The area where infiltration has high value is Duzce Plain where Duzce city centre is located and thus, where the population is the most dense, and industrial and agricultural areas are developed. However, the study area is rich in surface and underground water resources. The high annual rainfall and the rich vegetation cover has made these resources even more valuable. However, the increase in industry after the incentive law, the development of agriculture with the protection of lands, the population increase as a result, and the independent development of these spatial features threaten the water resources in Duzce. This situation also threatens the drinking water of Istanbul. For these reasons, the water infiltration situation should be evaluated especially in the spatial planning of drinking water basins.

In the water infiltration map, the areas where the water is very infiltration refer to the areas where the precipitation water is most accessible to the groundwater resources and thus, the vulnerable areas. Covering these areas with concrete surfaces such as settlement and industry will prevent the feeding of groundwater resources. For this reason, in Duzce Plain, green spaces should be protected and developed; concrete covers such as industry, settlement, etc. should be avoided.

Precipitation water rinses the layers through
which it goes down into groundwater. Therefore, the presence of pollution sources where the infiltration occurs leads washing of the pollution into the underground water resources. Therefore, preventing or limiting settlement, industry, agriculture, solid waste disposal, graveyards etc. in areas with permeability, improving existing usages to protect water sources, developing nature-based solutions and promoting their use (eco-efficiency, ecotourism, ecological agriculture etc.) is quite important. In the water infiltration map, areas with moderate permeability are other areas that need to be approached with caution as well. The infiltration level across the study area is moderate in general. Thus, spatial planning applications should include ecology- and nature-based solutions (watershed management, green infrastructure, greenway planning, storm water management, etc.). In the water infiltration map, areas, where infiltration is low and very low, are the areas where the risk of underground water pollution is at the least. For this reason, pollution producing areas (such as solid waste storage, cemetery, etc.) might be located in these areas. These areas are very small in number in the study area and can be divided into uses that may be a source of pollution during the spatial planning process.

Water process analysis used in the study can also be used in different areas. Moreover, more sensitive results can be obtained by increasing the data (criteria affecting infiltration) in the analysis process. In addition, the expert opinions used in the analysis process can be increased and they can be converted to quantitative data. These data can be included in the analysis after being evaluated with statistical methods (e.g., analytic hierarchy, Delphi technique), and more accurate and quality map can be obtained.

To conclude, the protection of water resources, especially groundwater resources, should be one of the basic principles of all kinds of work in order to maintain a healthy life. In this context, the method used in the study and the water infiltration map obtained is practical data that can contribute to many studies, especially to upper-scale spatial plans. In this context, water infiltration maps can contribute to many studies such as basin planning, determination of landscape vulnerability areas, green infrastructure plans, urban open green area plans, rainwater management, etc.

REFERENCES


ENHANCING ELECTROKINETIC REMEDIATION OF NICKEL-CONTAMINATED SOILS UTILIZING THE APPROACHING ANODES TECHNIQUE

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2Key Laboratory of Heavy Metal Pollution Control and Resources Comprehensive Utilization of Guangdong Polytechnic of Environmental Protection Engineering, Guangdong Engineering and Technology Research Center of Solid Waste Resource Recovery and Heavy Metal Pollution Control, Guangdong Polytechnic of Environmental Protection Engineering, Foshan 528216, China
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ABSTRACT

The soils contaminated by Ni near an electroplating factory were remedied via an enhancing electrokinetical method utilizing approaching anodes (AAs). The variations of the removal efficiency and the soil pH as a function of the treatment time were determined. The maximum Ni removal efficiency was found to be as high as 67.2% under a voltage gradient of 1 V/cm for 48 hours; in contrast to 31.5% when the conventional electrokinetic remediation with one fixed anode (FA) was employed. Several anodes were inserted as AAs in the treated soils. They were switched from the anode towards the cathode allowing for high H+ ions concentrations and high redox potentials to migrate fast towards the cathode. As a result, the soil remediation is accelerated. The mechanism of Ni electromigration behavior in soils during an enhanced EK method is described as the elution in an electrokinetically driven chromatogram.

KEYWORDS:
Electrokinetic remediation, approaching anodes (AAs), Ni contamination, soil remediation, mechanism.

INTRODUCTION

Soils contaminated by heavy metals become more and more serious in the recent years in many areas across the world [1-4]. Heavy metals, especially Nickel (Ni) in the soil, have recently been found to be present near the electroplating factory in the Pearl River Delta region of China. Ni is a common contaminant in the soil near the electroplating factory, accumulated Ni in soil is harmful to plant, human health and the environment [5, 6], resulting in the output of plant decreasing.

Over the past few decades, electrokinetic (EK) remediation has been demonstrated to be one of the most effective methods for in situ or ex situ soil decontamination. Numerous EK remediation investigations have shown success in degrading soil contaminants and removing heavy metals [7-9].

In the EK remediation process, electrode reactions take place on the surface to generate protons (H+) and hydroxyl (OH-) at anode and cathode, respectively. The concentration of these ions near the electrodes creates an acid front that moves from anode to cathode and a basic front that moves the other way [10, 11]. At the same time, the generation of OH- ions at the cathode leads to the precipitation of the heavy metals called the “focusing effect” [12]. This is the main barrier to electrokinetic remediation of heavy metal contaminated soils [13].

Many studies have been performed with the aim to control the soil pH and enhance the capability of electrokinetic remediation for heavy metal removal. Measures include adding strong complexing agents, such as EDTA (Ethylene Diamine Tetraacetic Acid) into soil [14] and using ion exchange membranes (IEM) to control the pH and zeta potential [15]. These modified techniques are complicated and the use of additional chemicals or devices results in secondary contamination [16, 17]. To enhance the electrokinetic remediation of heavy metals-contaminated soil, the distribution and mobility of H+ ions and heavy metals in soils were investigated in this study. Usually, the EK process is operated with one fixed anode (FA). An enhancing EK method with approaching anodes (AAs) is believed to strengthen the remediation effect. Compared with other remediation methods, we speculate that if the area of the “focusing effect” can be migrated towards the cathode in a step-by-step manner, more Ni would be precipitated in a narrow area and extracted from the contaminated soil. Therefore, to determine EK parameters suitable for the soil contaminated by Ni near an electroplating factory in the Pearl River Delta region of China were picked up and examined with the approaching anode method.
MATERIALS AND METHODS

Soil preparation. Soil samples were collected near an electroplating factory in the Pearl River Delta region of China. The measured concentration of Ni was 571.1 mg/kg for the collected soil samples and the moisture content was approximately 9.1%. The initial soil pH was 4.7. For each electrokinetic test, approximately 1000 g of dry soil sample was loaded into the electrokinetic cell. Distilled water was used as the electrolysis solution.

Electrokinetic cell. EK remediation experiments were carried out in a rectangular translucent plexiglas test cell with the following dimensions: length = 26.0 cm, width = 10.0 cm, and height = 10.0 cm, as depicted in Fig. 1. The soil was filled into the cell up to a length of 20 cm. A constant voltage of 20 V (1 V cm⁻¹) was applied with a DC power source. The filter paper and an O-ring were used between the electrode chambers and the soil cell to avoid any possible leakage. Graphite was used for both anode and cathode and was inserted into each electrode chamber and connected with DC power. To provide uniform electric current, the whole soil cross-section was covered with graphite electrode with a surface area of 54 cm² (3x9x2 cm). The thickness of the soil was 2 cm in anode compartment or cathode compartment. Electrode chambers were filled with distilled water, which was cycled by pumps to avoid concentration gradients within the compartments.

Methodology. The soil sample was divided into five sections within the cell, named S1–S5 moving from anode to cathode. EK remediation with AAs was operated in the same apparatus with the same intensity of electric field, except that five graphite electrodes were inserted as AAs in the treated soil. AAs were placed at distance of 3 cm, 6 cm, 9 cm, 12 cm, and 15 cm from anode. They were sequentially switched on at 5 h, 10 h, 15 h, 25 h and 36 h after the EK process started, and at the same time, the solution was refilled. Analysis was carried out in the same way with EK remediation based on FA. Soil pH was measured in the five different sections by a pH meter (soil/water = 1/2.5), the pH and Cu concentration were measured after the remediation at different sections S1-S5 respectively.

For total Ni analysis, 0.2 g samples, in duplicate, were digested with HNO₃-HF-HClO₄ in 25 ml Teflon beakers for soil clearing up. An inductively coupled plasma-optical emission spectroscopy (ICP-OES, Agilent) was used to determine the concentration of total Ni. The pH and Ni concentration were measured for two samples from each section, and two standard soil samples (i.e., soil with a controlled concentration of heavy metals) were analyzed for quality control. The EK remediation experiments were repeated three times.

RESULTS AND DISCUSSION

Soil characteristics. Major physico-chemical characterizations of the experimented soil with respect to the soil pH, texture, organic carbon, cation exchange capacity, zero point charge (ZPC) and metallic contaminant content are tabulated in Table 1. The soil was composed of a number of minerals where 3MgO·4SiO₂·H₂O dominates and accounts for 58.5% in weight. The tested soil of the coastal plain showed a sandy texture, which can be attributed to the silt loam according to the USDA classification system. The soil was slightly alkaline, a typical feature in Southern China.

The charge characteristic of the clay minerals in the soil is that most of the experimented soils carry negative charges on the surface, and the negative charges increase with the pH value, indicating that the negative charge is variable. The positive charge was very low in the experimented soil [18]. The point of zero charge (ZPC) of the experimented soil, determined by potentiometric titration, is around 3.0-3.5. The ZPC of the soil can be a better indicator to evaluate the surface chemical property of the soil, which is the pH when the charge of grain surface is zero. It is necessary to keep the pH in soils to be low enough when most heavy metals
are to be removed by EK remediation. With AAs method, the distance between anodes and the cathode is gradually shortened, migration distances of H⁺ ions decrease. Therefore, ZPC indicates the efficiency of AAs remediation process. The low cation exchange capacity (about 15.3 cmol.kg⁻¹ due to low organic matter and clay contents) suggests that the Ni ions are not highly absorbed onto the soil particles, which is propitious to the migration of Ni ions in the soil [19].

**TABLE 1**

<table>
<thead>
<tr>
<th>Property Value</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Texture (%)</td>
<td>Sand</td>
</tr>
<tr>
<td></td>
<td>Silt</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
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<tr>
<td>Minerals (%)</td>
<td>Chlorite</td>
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<tr>
<td></td>
<td>Mica</td>
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<td></td>
<td>Smectite</td>
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<tr>
<td></td>
<td>Kaolinite</td>
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<tr>
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<td></td>
<td>Feldspar</td>
</tr>
<tr>
<td></td>
<td>Picrite</td>
</tr>
<tr>
<td></td>
<td>Initial pH</td>
</tr>
<tr>
<td>Cation exchange capacity (cmol.kg⁻¹)</td>
<td>15.3</td>
</tr>
<tr>
<td>Total organic carbon (g.kg⁻¹)</td>
<td>10.9</td>
</tr>
<tr>
<td>Moisture content (m%)</td>
<td>9.1</td>
</tr>
<tr>
<td>conductivity (us cm⁻¹)</td>
<td>167.0</td>
</tr>
<tr>
<td>zero point charge (ZPC)</td>
<td>3.0-3.5</td>
</tr>
<tr>
<td>Zn (mg/Kg)</td>
<td>265.5</td>
</tr>
<tr>
<td>Ni (mg/Kg)</td>
<td>571.1</td>
</tr>
</tbody>
</table>

**Current changes during the experiments.**
The electric current is an indicator of the amount of ion electro-migration. The changes in the electric current during the treatments are shown in Fig. 2. During the EK remediation with AAs treatment, higher current density passed through the system than what was observed during the EK remediation with FA, and the current intensity fluctuated regularly. The current density of the EK remediation with AAs started from 3.25 mA/cm² and increased to the maximum value of 15.18 mA/cm². The maximum value for the EK remediation with FA treatment was 6.43 mA/cm², indicating the accelerated charge transport. Upon reaching the maximum value, the current density for the EK remediation with AAs began to decrease until it increased again to 6.9 mA/cm² after 14 hours because the precipitates re-dissolve and thus provide more ions for current transport. Finally, the current density decreased and reached a value of 4.87 mA/cm² for 48 hours. The decay in current intensity was due to the combination of the OH⁻ and H⁺ ions yielding H₂O thereby removing ions that transport charges to the electrode chambers. In addition, the resistance in the interface between electrodes and electrolyte might increase due to the concentration polarization and water dissociation [20]. Ions with positive or negative charges move to both ends of the electric cell, as in electrodialysis, and result in the drop of ionic strength in soils and the current.

In the EK remediation with AAs, the electrolytic distance between working electrodes decreased gradually when relay anodes were switched on one by one in direction of the cathode. The electric current in EK remediation with AAs is higher than that with FA. The results obtained in this study suggested that the AAs method could maintain more mobile ions in the system. This phenomenon partially explained the possible mechanism of enhanced Ni and removal in the AAs tests.

**FIGURE 2**

Variations of electric current intensity profile for the EK remediation with FA and with AAs.

**FIGURE 3**

Soil pH profile for the EK remediation with FA and with AAs.

**pH variation.** Low pH in soil is necessary when most heavy metals are to be removed by means of EK remediation [21]. Fig. 3 shows the pH variations in the soil profiles during EK remediation with one FA and AAs. The soil pH was 4.7 before EK remediation. The pH of the soil close to the anode was 2.9 after EK remediation with AAs. This is significantly lower than that of other soil parts. It can be seen that the pH values in the soil bed drop evidently faster with AAs than with FA.
During EK with AAs, when distance between electrodes is shortened step by step, migration distances of H⁺ ions decrease. Therefore, H⁺ ions can approach the cathode sooner.

**Redox potential variation.** Redox potential greatly influences chemical association of Nickel on soil particles. High redox potential near the anode indicates highly oxidizing conditions, while low redox potential near the cathode indicates reducing conditions. Redox potentials in the soil bed before and after EK remediation are depicted in Fig.4. Before EK remediation treatment, the redox potential of the soils is 20.0 V. After 48 hours of EK remediation with FA, redox potentials in treated soils decrease linearly from 19.6 V to 10.5 V along sites from the anode to the cathode. This is due to the electric potential of DC (Direct Current) and its redox reactions at electrodes. In reductive environments, almost all particulate Nickel is complexed by insoluble organic matter or bonded to sulfide minerals. In contrast, Nickel tends to be set off from soil particulates in oxidative environments because of the effect mechanisms of redox potential on heavy metals [22, 23]. After EK remediation with AAs, high redox potentials become offset to the cathode and are higher near the anode than after EK remediation with FA. Therefore, Ni migration capability is enhanced in EK remediation with AAs.

![Graph showing changes of redox potential in soil profiles](Image)

**Total Ni concentration variation.** The changes of total Ni concentration in the soil bed during EK remediation are displayed in Fig.5, where C/Co represents the ratio between Ni concentrations after and before EK remediation. Before the treatment the concentration of Ni was 571.1 mg/kg and Ni is removed from sections near the anode and accumulated near the cathode. After 48 hours, the average concentration of Ni for S1 to S5 region is 187.3 mg/kg using EK with AAs, compared to 390.8 mg/kg using EK with FA. Consequently, 67.2% of total Ni was removed using EK with AAs. This is in stark contrast with the 31.5% of total Ni removed via EK with FA. Therefore, the removal efficiency was enhanced and it shows great improvement of electro-migration velocity. The removal velocity of Ni concentration is the largest near the anode as shown by the increase in slope of the graph. During the EK process with AAs, soil turns to be in a more acidic condition, in which Ni exists in a free form. It can be presumed that the removal efficacy increased as more Ni ions desorbed from the soil particles as a consequence of pH decline.

![Graph showing Ni²⁺ distribution in soil after EK remediation](Image)

**Electrical conductivity.** The results of the electrical conductivity (EC) characterization are presented in Fig. 6. The EC profile of the conventional electrokinetic remediation with FA after 48 h was higher for S1 and S3 regions near the electrode chambers, and lower in S2, S4, S5 regions. This
trend was because there were more $H^+$ species near the anode and the precipitates re-dissolved due to higher concentration of Ni ions [24]. The EC for EK remediation with AAs was similar. Compared to the EC of electrokinetic remediation with FA, the EC of electrokinetic remediation with AAs was higher due to the increase of $H^+$ ion formation at the anodes as well as increased Ni ions desorption from the particles. Therefore, the EC for EK remediation with AAs coincided with the pH and Ni distribution profiles of Fig. 3 and 5.

**Mechanism.** The pH values and redox potentials in treated soils vary linearly after 48 h of EK remediation with AAs. Its mechanism lies in that OH$^-$ ions are confined in the cathode compartment. Consequently, acidic fronts steadily move towards the cathode. Thereafter, $H^+$ ions have to react with negative groups in the soil solution and on soil particles when moving forward. The chemical reaction behavior of $H^+$ ions in soils during EK remediation with AAs process is similar to that in an electrokinetically driven chromatogram [25]. After EK remediation with AAs, the distance between the anodes and the cathode is gradually shortened. Thus the soil pH was lowered and Ni precipitated and re-dissolved before migrating towards the cathode. $H^+$ ions that were produced at the anode and continuously supplied into the treated soil are similar to a mobile phase. Soil particles react as a stationary phase. When the mobile phase pH decreases, heavy metals with positive charge will accelerate electromigration towards cathode like elution. Many types of reactions occur during the remediation, including desorption, ion exchange, decomplexation, dissolution, destruction of the active sites on the soil surface, and diffusion from the inner sites of the crystal lattices. However, the most important aspect in electrochemical soil remediation is soil pH [14]. Often it is an acidic front, which is developing in the soil from the anode towards the cathode during EK remediation, used for mobilizing many heavy metals. When met with an acidic front, non-charged Ni fractions can be ion-exchanged by hydrogen ions according to the following equation.

$$PNi + 2H^+ = PH_2 + Ni^{2+} \quad \text{Eq. (1)}$$

The lower the soil pH becomes, the greater the positive charge Ni fractions. Ni with net positive charges will expedite to electro-migrate towards the cathode. Linear model pH may be the main mechanism that can account for the linear Ni removal velocity in EK remediation with AAs process. Low pH aids the release of heavy metals from the soil particles and reinforces the electroremediation effect [22], especially near the anode. This result illustrates why EK with AAs may be an effective treatment approach.

**CONCLUSIONS**

The application of conventional electrokinetic remediation with one fixed anode (FA) for 48 hours led to a Ni removal efficiency of about 31.5% from the soil near an electroplating factory. An enhancing EK method with approaching anodes (AAs) improved the efficiency to 67.2% under the same operation time. The improvement was attributed to an increased production of $H^+$ ions at the implemented anodes via water electrolysis and a greater desorption of Ni ions from the soil particles. Additionally, oxygen produced at the anodes changes the redox potential in the soil and results in Ni reorganization from oxidizable and stable forms to more dissolveable ones. More Ni can be thus removed from the soil than the conventional treatment does. Our method in decontaminating-polluted soils is associated with improved performances and low costs.

**ACKNOWLEDGEMENTS**

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SOILLESS CULTURE: MANAGEMENT OF GROWING SUBSTRATE, WATER, NUTRIENT, SALINITY, MICROORGANISM AND PRODUCT QUALITY

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\textbf{ABSTRACT}

The present article reviews the scientific literature on soilless culture and provided a critical examination of the micro-environmental factors that can affect horticultural crop growth and productivity, including, soilless substrate, fertigation solution, salinity, nutrient content and pH and plant-microorganism interaction. In addition, the role of precision agriculture such as, remote sensing in soilless system performance is reviewed. Soilless substrate source (organic and inorganic) and particle size can affect mineral content, oxygen level, salinity and pH of the nutrient solution, as well as the microorganism diversity and abundance. Organic substrates had more microorganism communities compared to inorganic substrates. Microorganism inoculation (e.g. fungi) of inorganic substrate to increase microorganism abundance is completely ineffective. However, environmentalists do not recommend the use of peat-based organic substrates because it leads to loss of soil organic carbon and destruction of wetlands. Agricultural demands for sustainable, environmental friendly soilless system highlights the benefits of using inorganic substrate (rock-wool and volcanic tuff) and the re-use of nutrient solution (closed systems) to avoid water and nutrient losses. Interestingly, biological control of root rot pathogens in closed soilless systems is more effective than open or conventional due to the lower variety of microorganism community in closed structures. However, substrate salinity is the main challenge in closed soilless systems. The use of salt-tolerance cultivars, large volume tanks to minimize electrical conductivity and pH changes and sub irrigation with low concentration nutrient solution are the main approaches to reduce salinity in growing substrates. Several precision agricultural techniques based on advanced sensing technologies such as smart irrigation systems and surface reflectance data from crop canopy may increase soilless system efficiency. These techniques are based on crop water demands, not fixed watering schedules and have the potential to optimize water and nutrient use efficiency.

\textbf{KEYWORDS:}
Hydroponics, Salt stress, Nutrient solution, Pathogen, Irrigation systems, Remote sensing.

\textbf{INTRODUCTION}

Soilless culture system has been used widely in protected agriculture to improve the growing environment and provide optimal water and nutrient supply for cultivated crops [1]. Soilless culture can potentially improve cropping systems by optimizing the use of inputs (nutrient, pesticides and water), controlling diseases more efficiently and make it possible to increase crop production regardless of the climatic conditions [2]. Soilless culture could eliminate the dependency on methyl bromide and increase total yields and facilitate the off-season production of several crops such as, strawberry [3]. Vegetables grown in soilless culture can be biofortified [5]. Increased the calcium (Ca\textsuperscript{2+}) content of baby leaf vegetables (basil, mizuna, tatsoi and endive) grown in soilless culture was increased without affecting crop growth and marketable quality [5]. Soilless culture can be used to successfully introduce new crops into a region as was done by Al-Karaki and Othman [6] who showed that medicinal and aromatic herb species can be successfully grown in an arid region (Gulf countries).

The composition of soilless substrates significantly affects plant physiology, yield and fruit quality [7]. The ideal soilless substrate has great total porosity, low bulk density, adequate aeration and high water holding capacity to facilitate root penetration and increase nutrient availability to the plants for multi-season applications [8]. The use of different soilless substrates sources (organic and inorganic), substrate particle size, N-forms and the control of single nutrients, such as potassium (K), Ca\textsuperscript{2+}, and sulphate in the nutrient solution, can also influence soilless product quality and related characteristics (e.g. yield, flower size and number, and fruit sugar and phenolic compounds) variably [9, 10, 11, 12, 13]. For example, the addition of organic substrate
Air porosity and the available water are an essential prerequisite to successfully control plant growth and development in soilless growing media, such as aggregated perlite, rockwool, and coconut coir [8]. Ultra-fine substrate (perlite, 0.3 mm) held excessive water and caused oxygen deficiency in the substrate air zone [21]. Poorly aerated substrate might create oxygen deficiency in root rhizosphere, concentrate root systems in the top layer of the media profile, and increase the susceptibility to root rot pathogens [22, 20]. However, particle size of 0–4-mm of volcanic tuff improved shoot growth and flower quality of Asiatic hybrid lily (Lilium × elegans cvs. Fangio and Ercolano) compared with other tuff sizes 0–2, 0–8, 2–4, and 4–8 mm [11]. Carrots (Daucus carota L. cv. Dr. Carotene) grown in 1.2 mm-perlite produced about 28% higher root yield than those plants grown in 5.0 mm with same nutrient solution concentration (75% of full strength solution) [21]. Overall, research studies showed that substrate particle size range of 1 to 4 mm is suitable for soilless culture.

**Substrate Composition.** Soilless substrate composition have a considerable effects on plant growth, development, shoot nutrient content, flower quality and fruit yield [9, 14, 11]. The ideal substrate should have fundamental physical, chemical, and biological properties that will improve nutrient availability and plant growth and provide an anchor for the plants. These properties include high substrate stability, sufficient air-water balance around root system, and proper pH and cation exchange capacity [23, 24]. The main physical and chemical characteristics of hazelnut husk (0–2 mm) such as pH, electrical conductivity (EC), carbon (C)/nitrogen (N), and nutrients were within acceptable ranges for the ideal soilless substrates characteristics [24]. Commonly, the main drivers for the selection of the soilless substrate are largely based on performance and economic considerations [25]. In response to the necessity for researchers to better recognize promising new materials, Barrett et al. [25] concluded that an effective substrate must perform well in two key areas; first, the physical, chemical and biological properties that are essentially required to support the root system and second, the growing media must meet the practical requirements of the production system in which it is being utilized. In addition, plant physiology, the container size and geometry and the mechanical strength of considered substrate is an essential prerequisite to successfully optimize the engineered growing substrate environment and creating stress-free growth environments [8].

Currently, two soilless substrates sources are available commercially; organic and inorganic (mineral). Organic substrates contain higher microorganism communities compared to inorganic soilless substrates which hardly contain any [26]. Several microorganism communities, such as arbuscular mycorrhizal fungi are likely to be useful for plant health and thus they can supply part of plants nutritional needs fungi [27, 26]. Tomato plants grown in cocopeat coir substrate had higher vegetative growth than perlite [28]. In addition, strawberry (Fragaria × ananassa cv. Elsanta) yield was higher when grown in coconut fiber substrate than in perlite in both closed and open soilless culture system [3]. The addition of shredded maize stems to perlite substrate

**GROWING MEDIA PARTICLE SIZE, COMPOSITION AND SUSTAINABILITY**

**Substrate Particle Size.** Physical properties of soilless culture media greatly affects plant growth and productivity [18]. Substrate particle size can substantially improve plant growth and flower quality [11, 19]. Engineering soilless growing substrates with very distinct water storage and aeration characteristics by altering substrates mixing ratios or inter- and intra-aggregate porosities and pore-size distributions to enhance plant growth conditions can conserve water [8]. Fine particle size substrate has higher water holding capacity and less aeration when compared with well-aerated, coarse substrate [20]. Air porosity and the available water are an essential prerequisite to successfully control plant growth and development in soilless growing media, such as aggregated perlite, rockwool, and coconut coir [8]. Ultra-fine substrate (perlite, 0.3 mm) held excessive water and caused oxygen deficiency in the substrate air zone [21]. Poorly aerated substrate might create oxygen deficiency in root rhizosphere, concentrate

(shredded maize stems) to inorganic soilless substrates (perlite and pumice) improved growing medium properties over inorganic substrates alone and increased tomato (Lycopersicum esculentum Mill. cv. Belladona) growth and yield [14].

The biggest challenges in soilless systems, especially in closed recirculating systems, are nutrient management, plant/microorganism interactions and pathogen control in the root environment, taste and health related compounds in the product as well as salinity levels [13]. Soilless culture type (open vs. closed) and growing substrate have altered the microorganisms dynamic within the rhizosphere [15]. In open soilless culture, water and nutrients are delivered as in conventional system and the excess nutrient and water is allowed to run to waste. Conversely, the surplus nutrient and water is recirculated in closed soilless systems [1]. Root rot was less severe in closed soilless system compared to open culture due to the higher amount of bacteria (biocontrol agent) in the closed system [16]. Biocontrol agent bacterial competes with Pythium root rot for space and nutrient [16, 17]. Overall, management strategies of soilless cultivation systems to optimize nutrient supply, root and shoot micro-climate, substrate type and microorganism content are prerequisite to developing environmentally friendly and profitably-grown products of high quality [12, 13]. This review, will discuss research findings on soilless culture systems. The discussion topics will focus on soilless substrate particle size, fertigation solution salinity, nutrient content and pH, product quality and plant and microorganism interaction. Additionally, we will discuss the use of precision agriculture in soilless culture systems performance.
resulted in a higher tomato yield, indicating there are beneficial effects of adding organic substances to inorganic substrates in soilless culture systems [14]. Water holding capacity of soilless substrate increased with decreasing the percentage proportion (100%, 75%, 50%, 25% and 0% by volume) of vermiculite to pine bark [29]. In addition, germination of radish (Raphanus sativus L.) decreased with increasing vermiculite at 3-4 days after sowing [29]. Low germination attributed to the fact that water holding capacity decreased with increasing vermiculite proportion.

Plant response to organic growing media also depends on organic substrate source. Tuber weight of Christmas bells (Sandersonia aurantiaca) in the bark soilless substrate were on average twice lower than in the peat/pumice medium. In addition, tuber weight at 100 g N m$^{-2}$ and 4.4 pH was higher than those at higher N (N 500 g N m$^{-2}$) and pH (5.9) [30]. Growing strawberry (Fragaria × ananassa) cultivars (Camarosa, Candonga and Festival) in different soilless substrates showed that productivity and berry composition were mainly affected by differences between cultivars rather than by the nature of the different growing substrates. However, regardless of the cultivar, plants grown on agrotextile-type substrate produced significantly more fruit (1018.2 g plant$^{-1}$) compared to average other substrates, 892.3 g plant$^{-1}$ and less concentration of the anthocyanins (0.74-fold) than those grown in coir fiber, perlite and rock wool [31].

Several renewable experimental inorganic substrates hold promise for soilless culture growth. However, few have been widely used [25]. Rockwool is an important and sustainable horticultural growing substrate [32]. The high processing temperatures used in manufacturing of rockwool ensure that the substrate is chemically and biologically inert and sterile from pathogens and weed seeds [32]. Rockwool slabs can be watered and reused frequently and do not modify or restrict nutrient availability. In fact, more than 98% of the applied fertigation solution is readily available for uptake by the crop [32]. Ions distribution in rockwool were associated with its moisture content, the fertigation volume and the vertical position in the rockwool slab [33]. However, as the rockwool slabs became more saturated, flow patterns were spread more horizontally [33].

Volcanic tuff substrate is widely used in Mediterranean region for soilless culture such as, Jordan [11]. Tuff substrate is characterized by good porosity, acidity resistance and cation exchange capacity [11, 34]. Soilless cultivation of cherry tomato (Solanum lycopersicum) in volcanic tuff resulted in higher marketable yield (11.9 kg m$^{-2}$) than perlite + sand (2:1, 7.67 kg m$^{-2}$) substrate [9]. However, fruit protein, P, N, and Mn were statistically similar across the studied substrates; sand, perlite and volcanic tuff [9]. The use of volcanic tuff as a soilless substrate led to higher lettuce (Lactuca sativa var. capitata) growth, higher N and K contents in plant tissues and to lower K leaching compared to perlite growing media [35]. The addition of 2% zeolite (90% clinoptilolite) + hydrogel to perlite (w:w) improved physico-chemical properties of substrate and enhanced water retention capacity and led to higher yield of greenhouse cucumber (Cucumis sativus) [36]. Zeolite + hydrogel application effect was more pronounced in plants grown at partial root-zone drying than deficit irrigation or full irrigation treatments [36]. However, the microorganism community abundance is expected to be lower in volcanic tuff than in organic substrates. Overall, we believe that volcanic tuff holds promise as a soilless substrate.

The combination of organic and inorganic substrates mixtures is a promising way to improve yield and fruit quality. Strawberry (cv. BG4.370 and Splendor) grown in cocopeat + perlite (4:1, by volume) had the highest photosynthesis, transpiration, radical scavenging activity and fruit firmness while those grown in peatmoss + perlite (4:1, by volume) had the highest yield, total phenolics and nitrate (NO$_3^-$) concentration compared to volcanic tuff (0-4 mm), peatmoss + perlite (4:1, by volume), volcanic tuff (0-4 mm), + cocopeat (4:1, by volume) and volcanic tuff (0-4 mm), + peatmoss (4:1, by volume) [7]. Perlite: compost (1:1, by volume) ratio increased the actual water content and decrease the actual air content [37].

**Substrate Sustainability.** The factors influencing material selection can be broadly divided into performance, economic and environmental reasons [25]. Growing alarm over the environmental effects of some commercially used substrates has led researchers to identify and evaluate more environmental-friendly alternatives such as those derived from agricultural, industrial and municipal waste streams [25]. Peat-based growing substrate is not recommended by environmentalist because of its negative environmental impacts such as, loss of soil organic carbon [10, 7, 38]. Although peat is renewable, it has a very slow regrowth rate and its harvest destroys wetlands [39, 38]. The frequent threats to ecologically important peat areas worldwide has forced the horticultural industry to consider alternatives such as, agricultural and industrial by-products [39, 38]. Many renewable materials, agricultural and industrial by-products such as, coconut (Cocos nucifera) coir, grape (Vitis vinifera) bagasse, composted pine (Pinus taeda) bark, and volcanic tuff have been evaluated to be used as growing substrate for soilless system [12, 6, 39]. In addition, composted olive mill waste (COMW) has been shown to be a successful replacement for the commonly used and expensive peat moss (Altieri et al., 2014). In fact, COMW produced an economically sustainable and fit-for-purpose substrate for soilless culture of strawberries. The relatively high amount of nutrients (N, K, Ca$^{2+}$, Mg$^{2+}$, Na, Fe$^{2+}$, Mn, Zn and Cu) left over in the
growing media (COMW) at the end of the study period proposed that it can be recycled as organic matter in different agricultural practices [40]. Ground reed canary grass (Phalaris arundinacea) straw had lower water holding capacity in comparison to peat, but could probably be improved by decreasing its particle size in processing [39]. Although vegetative growth of the strawberry (cv. Elsanta) plants was higher in peat, the total yield, berry size, and sugar to acid ratio in berries were similar to coir and ground reed canary grass straw substrates [39]. Tomato plants grown in non-washed shredded rubber substrate had higher shoot Zn concentration than peat: perlite soilless growing media but acid washing of rubber significantly reduced shoot Zn toxicity for plants and made it suitable for soilless culture [41].

WATER, SALINITY, PH AND NUTRIENT MANAGEMENT

Water and Salinity. Agriculture demands for a sustainable and environmental friendly culture system, especially in soilless cultivation systems highlights the benefits of using closed recirculating systems and the re-use of nutrient solution to avoid nutrient losses [13]. Closed cycle soilless systems have been recommended in commercial production because they can minimize water and fertilizer losses [42]. However, salt accumulation in the growing substrate is the main challenge in recirculating systems. Open cycle systems are normally adopted to reduce salinity level in the substrate. But, higher water use efficiency is obtained with closed cycle systems [42]. Under open soilless systems, the volume of nutrient solution supplied can be reduced to limit the amount of nutrient loss, but there must be at least 30% of leaching to avoid salt accumulation in the substrate [43]. No significant differences were found in tomato yields between open and closed soilless systems, the closed system accumulated more nutrient; Ca$^{2+}$, chloride (Cl), and Zn in the root environment. Additionally, tomato leaves from the closed system had higher content of Ca$^{2+}$, Mg$^{2+}$, and Zn [44]. In terms of water and nutrient use efficiency, the closed soilless system saved 42.5% of water, 42.1% N- ammonium (NH$_4^+$), 56.0% NO$_3^-$, 31.4% phosphorus (P), 52.1% K, 63.5% Ca$^{2+}$, 47.9% Mg$^{2+}$, 49.4% sulfur (S)- sulfate (SO$_4^{2-}$), 51.9% Cl, 50.9% iron (Fe), 47.9% Zn, 24.6% manganese (Mn), 53.3% copper (Cu) and 47.2% boron (B) compared to open non-recirculating nutrient solution system [44].

However, the substrate salinity level is expected to build up under closed soilless systems. Closed soilless systems tend to increase EC in the recycled system and consequently decreases total yield and fruit size [45]. In closed soilless systems, long-term recirculation of nutrient solution results in accumulation of ions, such as sodium (Na) and Cl. The accumulation of ballast ions; Na and Cl in the recycled nutrient solution is a significant limiting factor in closed soilless systems, specifically when irrigation water is of poor quality [46]. Salinity reduces plant fresh weight, leaf number and chlorophyll b [47]. The response of sweet pepper cultivars (Capsicum annum L., cvs. Flaviano, Sonar, Alzado) to different levels of salinity (EC 1.8, 4 and 8 dS m$^{-1}$) under closed soilless system (volcanic tuff substrate) showed that marketable fruit yield, fruit number and single fruit weight decreased in all tested cultivars with increasing salinity level [48]. High EC of nutrient solution in the closed soilless culture system resulted in a reduction of flowers number of tomato (Lycopersicum esculentum Mill. cv. Belladona) [14]. When basic nutrient solution of 1.5 dS m$^{-1}$ was supplemented with either NaCl or macronutrients to give an EC of 9 dS m$^{-1}$, shoots and roots fresh and dry weight, total root length, number of adventitious roots of all tap root laterals of tomato plants decreased with increasing nutrient solution EC. Interestingly, using NaCl to increase EC of nutrient solution had a lower impact on root fresh matter, tap root diameter, total root length and number of adventitious roots compared with the macronutrient treatment [49].

At higher concentrations of NaCl (150 or 200 mM), growth parameters (shoot length and number, leaf number, and dry weight) and nutrient acquisition of tomato (Lycopersicon esculentum L. cv. Amani) were adversely affected and seedlings died thereafter when seedlings were directly exposed to NaCl stress in Hoagland’s solution [50]. Elevated salinity significantly also reduced crude protein and fiber in shoots and roots.

To optimize nutrient availability for crops cultivated in closed soilless systems, the process of salinity build-up has to be understood [46]. In fact, several cultural approaches have been suggested to mitigate salt stress in closed recirculating systems such as, cultivar screening and nutrient management [48, 42]. Under high levels of salinity (e.g. 8 dS m$^{-1}$), genotypic variation for fruit yield and shoot and fruit nutrient content might be noticed, such as, in sweet pepper cultivars [48]. Increasing nutrient solution salinity to 10.0 dS m$^{-1}$ in hydroponically grown celery (Apium graveolens) had no significant effect on plant growth, water relations, and the tissue concentration of macronutrients, but it increased Na and Cl accumulation in the mature leaves [51]. Salinization improved the yield quality by reducing the uptake of NO$_3^-$ and the incidence of ‘blackheart’ in young leaves [51]. The NaCl-salinity of up to 5 mM in the nutrient solution had no significant effect on the uptake concentrations of macronutrients (i.e., N, P, K, Ca$^{2+}$ and Mg$^{2+}$) of melon (Cucumis melo L.) cultivated in closed-loop hydroponic systems [46]. High mixing rates of nutrient solutions or large volume tank lead to stable EC development and minimal pH
changes [52]. Alternatively, closed cycle sub-irrigation with low concentration nutrient solution can be used. For example, tomato was grown successfully in a closed cycle sub-irrigation system using saline water (3 dS m⁻¹) and with 30% less fertilizer than normally used with traditional open cycle systems [42]. Growing strawberry in peat resulted in lower EC than composted cork thin waste mixed with rice hulls and coir fiber soilless substrates [15].

Shortage of water resources coupled with high demand for water for residential and agricultural uses are critical around the world, especially in arid and semi-arid region. One possible approach to overcome this problem is using alternative water resources such as, treated wastewater for agriculture. Wastewater often contains high levels of heavy metals which might cause health risks. In fact, no solid research we aware of guarantee the direct use of wastewater to support soilless crop production, safely. However, the re-use of wastewater in soilless and hydroponic culture research studies are promising, specifically those conducted on forage and ornamental plants [53, 54]. During one year of exposure to wastewater in soilless culture (substrate, coconut fibers), rose (Rosa hybrida) growth and flower quality (visible appearance, size and vase life) were not affected by the irrigation treatments [54]. In addition, contents of all the examined micro- (Mn, Cu, B, Fe, Zn, Mo, Al) and macro-elements (N, P, K, Ca+2, Mg+2) in the leaf tissues were within the range accepted for proper plant function [54]. A wastewater re-use study for Croton (Codiaeum variegatum Blume cv. Petra) grown under different soilless substrates revealed that amendment of the substrate with zeolitic tuff is recommended to reduce the adverse effect of salinity associated with wastewater [55]. Overall, wastewater can be securely to support.

**Soilless pH.** Growing substrate pH affect nutrient availability, plant growth, and yield [56, 13]. Regulation of soilless system pH, NO3-/NH4⁺ ratio and the additional supply of silicon, and Ca+2 may reduce the pathogenic activity in soilless systems [13]. High pH in the root zone reduces nutrient (Mn, P and Fe) available to plant and reduces yield, especially in closed soilless systems [57]. The number of Christmas bells stems harvested at 6.5 pH level was lower than at growing media pH of 6.0. Leaf concentrations of S, Mn, and Zn declined whereas Mg+2 increased with increasing pH level (4.5, 5.0, 5.5, 6.0, 6.5) [56]. Kale (Brassica oleracea) and cherry tomato (Solium lycopersicum) grown in high EC~ low pH nutrient solution (EC 1-3 dS m⁻¹, and pH 5.5-6) provided higher potential gross returns compared to low EC–high pH (EC less than 1 dS m⁻¹, and pH near 7.2) system [58]. Geranium (Pelargonium×hortorum) grown at very low substrate-pH, 3.8 had lower cation content, higher anion and plants appeared healthier than at pH 4.3–5.2. At pH 4.3–5.2, geranium plants were stunted and had high tissue levels of Fe3⁺ and Mn. At pH 5.5 or 6.3, plants appeared healthy. However, shoot Fe3⁺ and Mn decreased at pH above 6.3 [59].

An acceptable pH range in soilless substrate was influenced by the applied fertilizer concentration [59]. For example, fertilizer concentration affected the pH range at which interveinal chlorosis occurred; increasing toxicity symptoms at pH 4.3–5.2, and ameliorating deficiency at pH > 6.3 [59]. A combined use of urea and NO3⁻ in nutrient solution is useful to maintain a stable solution pH and guarantee adequate plant growth without a reduction of the cation absorption [60]. Because leaf nutrients concentration were reduced by the increased pH in the root zone when recycling was applied. Karam et al. [57] suggested to supply additional K and N–NH4⁺ than those recommended for open systems to prevent nutrient imbalances in closed soilless systems.

**Nutrient Balance.** Nutrient management practices of soilless systems, particularly, controlling fertigation solution volume and nutritional composition to match plant requirements is essential and perquisite step for flower yield enhancement, fertilizer use efficiency and reduction of pollution [43]. High salinity levels of nutrient solution lead to negative impact on nutrient uptake such as, NO3⁻, K, P and Ca+2 and shoot and root nutrient content [47]. Plant growth in soilless culture system either be controlled by the pH of the medium, or, in high-gravel-content media, by the pool size of available nutrient [61]. Under a closed soilless system, the reuse of nutrient solution led to a profound decline (~ 60 %) of NH4⁺ concentration in the drainage solution, increased pH in the root environment and restricted supply of Mn to the plants [57]. The calculated mineral balances of the crop in open soilless system showed that more than 60% of the supplied nutrients were lost in the leachate [43]. However, after 76 days of nutrient solution recycling in soilless substrate (peat: pumice, 1:2 volume ratio), the variation of nutrients (N, P, K, Mg+2 and Na) in the solution was lower with the sub-irrigation than with the drip-irrigation soilless system [62]. Applying the nutrient solution containing 8.9 mmol N l⁻¹ and 3.2 mmol K l⁻¹ with N–NH4⁺/N–NO3⁻ ratio of 0.37 resulted in a shorter vegetative period and more and larger flower production of Anthuriums (Anthurium andraeanum) in tropical soilless conditions [43].

Oxygen level through soilless substrate has an immediate impact on both water and nutrient uptake and the yield of the whole plant [63]. Under oxygen deficit condition through soilless substrate, K is the nutrient most sensitive to oxygen deprivation while NO3⁻ uptake is the least affected by oxygen levels [63]. Oxygen deficiency in the nutrient solution induced the presence of nitrite ions and caused a 42% reduction of water consumption by tomato roots [63]. In addition, the lack of oxygen in the nutrient
solution induced an efflux of K, Mg$^{2+}$, P and sulfate from the root into the nutrient solution [64]. However, the application of potassium peroxide (1 g l$^{-1}$) through nutrient solution led to increase oxygen content in soilless substrate (perlite and rockwool) and resulted in higher pepper and melon yield than the control [65].

In soilless culture systems, fine roots, specifically those in the root hair zone, are the most essential for nutrient uptake and the most susceptible to high nutrient solution levels and temperature and root pathogens [66, 49]. High nutrient level (N) in growing substrate can negatively impact root growth especially at early growth stages [67]. The temperature of nutrient solution can affect the number of leaves per plant, water uptake and the functionality of root system [66]. Root temperatures are essential to stimulate plants growth, flowering and yield [68]. In tomato, root-zone heating increased root dry weight, relative growth rate, mineral nutrient uptake and xylem exudation compared with those of the control [69]. Root dry weight was minimum when temperature of nutrient solution was 20ºC [66].

**PLANT/MICROORGANISM AND PATHOGENS INTERACTION**

**Beneficial Microorganisms.** Microorganisms enhance plant growth and productivity [67] and reduce the impact of pathogens in soilless substrate [2]. Structure and activity of spontaneous fungal communities in organic substrates could have specific effects on organic matter evolution and disease control in soilless crops [2]. However, microbial activity in soilless systems strongly depends on substrate origin (organic vs. inorganic-mineral substrate) and manufacturing process [2]. Microorganism population differ significantly between soilless culture systems and the indigenous microorganism type in each soilless system is unique. Organic (coconut-fiber) soilless substrate system had the highest amount of fungi and *Fusarium* spp., whereas the inorganic substrate (rockwool) contained the highest amount of fluorescent pseudomonads. In addition, aerobic bacteria could be dominant over fungi in inorganic (rockwool) substrate [70].

Soilless substrates lack the diverse biological and microbial communities found in conventional agriculture soil [71]. For soilless culture, a few studies have assessed the usefulness of inoculating plant with microorganisms at early stages but none of them showed a significant positive impact on cultivated crop [72, 73]. Growing *Pelargonium peltatum* in inoculated substrate (mainly fungal) revealed that fungal communities were highly variable between the plants in the soilless substrate (spruce bark compost and finely milled dolomite). In addition, they found that fungal inoculation was completely ineffective as a factor that affected the total indigenous microbial communities in the substrate [72]. Arbuscular mycorrhiza inoculation at the seedling stage has limited effects on yield and quality of tomatoes grown on sawdust or coir as a soilless growing medium [73]. However, biological control in soilless culture could be more effective than in conventional soil culture because the variety of microorganisms in soilless culture would be less than conventional, therefore suitable condition for beneficial microorganisms can be relatively easily set up [71].

**Pathogens.** Soilless systems are not always free of disease. This is because the plants are genetically identical, and can be uniformly susceptible in soilless greenhouse systems. High planting density in soilless system can stimulate the movement of pathogens from infected plants to healthy ones. In fact, the physical environment of soilless system such as, temperature and moisture level, can be ideal place for the pathogen. In closed soilless systems, nutrient solution recirculate across the system and enhance pathogens to spread easily from one plant to the other. As a result, small amount of infection can lead to significant loss [71].

The significant and negative impact of root pathogen (e.g. *Pythium aphanidermatum*) in soilless systems increased with time (after infection) and increased number of pathogen oospores [49]. Root pathogens normally reduce the number and the length of fine roots and increase their diameter, especially young roots [49]. Growth conditions, soilless system type (open or closed), substrate, and the microbiome altogether impacted on the protection of crops against the soil-borne pathogens [74, 26]. Biological control would seem to be ideally suited for closed soilless systems due to the lower diversity of microorganisms community in closed structures (compared to conventional soil) and consequently, suitable condition for the biocontrol agents to easily control and flourish [71]. *Pythium* root rot diseases infection progress was nearly three-times slower in closed soilless rockwool as compared to open rockwool culture system [16]. In strawberry, open soilless system had higher fungal pathogens population than closed and closed with slow sand filtration systems [15]. Strawberry grown in closed and open soilless systems (coir fiber substrate) had the same dispersion of inoculated *Phytophthora cactorum* propagules. But, sand filtration of closed system reduced *P. cactorum* propagules in the water drained from the growing media [74]. In addition to soilless type (open and closed), growing substrate also influenced root rot diseases infestation. For example, wood fiber increased protection against the soil-borne pathogen *Fusarium oxysporum* at the end of the first assay, but did not during the second assay compared to coir fiber and peat [26]. Although soilless type (e.g. open and closed systems) and substrate (e.g. peat and coir) can significantly affect microbial activity and their total number [74, 16], the sole determination of
microbial activity in soilless substrates is not sufficient to predict protection against the soil-borne pathogen such as, *Fusarium oxysporum* [26]. This is because, seasonal variation of plant growth and plant density may have impacted crop physiology and may have indirectly modified rhizosphere fungal community structure [26].

**PRODUCT QUALITY**

The physical characteristics and mineral composition of soilless grown crops are significantly different than those under organic and conventional cultivation [75]. Cultural systems (conventional and soilless) have different nutrient and fruit quality [76]. Soilless technique enables growers to produce vegetables without quality losses compared to traditional culture system [77]. For example, tomatoes grown in rockwool soilless substrate had higher sugar, vitamin C content and firmness than those grown in soil [78]. Favorable fruit quality (total sugar, total soluble solids, glutamic acid, aspartic acid, alanine, and volatile acetate components) of muskmelon (*Cucumis melo*, cv. reticulatus Naud.) in soilless medium culture were achieved when K level was adjusted to near 240 mg l$^{-1}$ in nutrient solution [79].

Medicinal and aromatic plants have different phytochemical components such as, volatile oil tannins, and flavonoids, which play a key role in cosmetics and healthcare systems [80]. Soilless cultivation of medicinal plants has gained attention recently due to potential yields and quality and year round production, with minimum consumption of water resources [6]. Flower head volatile oil chemical composition of soilless culture-grown *Chrysanthemum balsamita* was comparable with previously reported wild- and field-grown plants of *C. balsamita* [80]. In arid and semiarid regions which are characterized by limited water resources, crop production including medicinal and aromatic plants is a major challenge [6]. Soilless cultivation of five medicinal and aromatic herb species; sage (*Salvia officinalis*), German (*Matricaria chamomilla*) and Jordanian (*Matricaria aurea*) chamomiles, thyme (*Thymus vulgaris*) and mint (*Mentha piperita*) under arid conditions (Arabian Gulf region) showed that all studied species were well adapted to the soilless system [6]. Soilless cultivated sage out-yielded the soil by 264%, German chamomile by 313%, Jordanian chamomile by 220%, thyme by 247, and mint by 243%. Flower head yield of soilless grown German and Jordanian chamomile out-yielded the soil cultivated by 199 and 334%, respectively. In addition, water use efficiency of soilless system (closed system, zeolite substrate) was about 70% higher than conventional production [6].

Although soilless culture systems are based on environmentally friendly technologies, soilless technique does not guarantee the production of high-quality vegetables [77]. A short-cycle, high-density (122 plant m$^{-2}$) greenhouse hydroponic (floating raft system) culture stimulated plant growth and root production in *Echinacea angustifolia*, but it did not ensure sufficient caffeic acid derivatives (caftaric acid, chlorogenic acid, echinacoside, caffeic acid, cyanarin, p-coumaric acid, ferulic acid and cichoric acid) accumulation in dried roots [81]. Strawberries cultivated in soilless systems (open and closed system) using peat, composted cork and coconut fiber had lower values of pH, Ca$^{2+}$, Zn, Fe, P, N, reducing sugars, total soluble solids and reducing sugars/acidity ratio than conventional [76]. Soilless cultivation of two different pepper (*Capsicum annuum* cvs. Almuden and Quito) led to higher fruit NO$_3$ and lower Ca$^{2+}$ concentrations and less greenish (lower hue) than those grown in conventional and organic soil [75].

**ADVANCED WATER MANAGEMENT AND SENSING TECHNOLOGIES TO OPTIMIZE SOILLESS SYSTEM EFFICIENCY**

The irrigation system is an essential component of production cost and crop productivity in soilless cultures [82]. The introduction and advancement of automated fertigation soilless systems has gained more attention recently for the effective use of fertilizers and water resources [83]. Automated irrigation is wildly used in soilless culture systems to reduce labor costs. However, automatic irrigation based on fixed schedules does not necessarily mean efficient irrigation because watering schedules is not designed based on crop water requirements [83]. Precision agriculture based on advanced sensing technologies such as smart irrigation systems and remote sensing has been used recently to detect water [83, 84] and chlorophyll status in plants [85]. Elvanidi et al. [86] used a hyperspectral machine vision as a non-destructive technique for detecting changes in spectral reflectance of a soilless tomato crop grown under different irrigation regimes. The detected crop surface reflectance was within the wavelength between 400 nm and 1000 nm. They found that canopy spectral reflectance increased with increasing water deficit stress. In addition, when the modified red simple ratio index value increased by more than 2.5%, the substrate volumetric water content decreased by more than 3%. They also found that when the Transformed Chlorophyll Absorption Reflectance Index value increased by about 16%, the leaf chlorophyll content of tomato plants decreased by about 3% [87]. Smart irrigation systems that use wireless sensor network for real-time sensing of substrate water status are effective tool for precision irrigation management of greenhouse soilless crops when combined with precise information on the effects of water availability levels on plants [83]. The
application of a modified irrigation technique derived using compensated radiation integral, substrate moisture content and EC significantly increased paprika (Capsicum annuum) growth compared to conventional drip irrigation system [82]. Additionally, the development of an automated system for fertigation control in soilless tomato production based on transpiration estimates by the Penman-Monteith model and on leachate EC increased water and nutrient use efficiency as well as minimizing environmental problems related to effluent disposal of fertilizer [87].

CONCLUSION

Soilless culture systems, one of the most common production technique in today’s horticulture industry, can result in higher yields, even in areas with adverse growing conditions. Several organic and inorganic soilless substrates have been evaluated to improve growth and yield such as, peat-moss, perlite, cocopeat and volcanic tuff. The selection criteria of the soilless culture system is based on crop productivity, cost and longevity. Organic substrates had higher amount of microorganism communities compared to inorganic. Microorganisms enhance plant growth and productivity and reduce the impact of pathogens. In addition, microorganism inoculation (e.g. fungi) of inorganic substrate to increase their abundance is ineffective. The approaches used in manufacturing of inorganic substrates (e.g. rockwool and volcanic tuff) ensure that the substrate is chemically and biologically inert and free from pathogens and weed seeds. However, organic substrate last less than inorganic. In addition, peat-based growing substrate is not recommended by environmentalist because of its negative environmental impacts. Peat has a very slow regrowth rate and its harvest is causing loss of soil organic carbon and destruction of wetlands.

Soilless substrate physical and chemical properties play key roles in plant growth and productivity. Particle size of about 1 to 4 mm significantly improved growth, yield and flower quality. A very fine particle size substrate (less than 1 mm) has higher water holding capacity and less aeration when compared with well-aerated, coarse substrate (more than 5 mm). Low oxygen level through soilless substrate has an immediate negative impact on both water and nutrient uptake and the yield of the whole plant.

Agriculture demands for sustainable, environmental friendly soilless system highlights the benefits of using closed systems and the re-use of nutrient solution to avoid water and nutrient losses. This is because there must be at least 30% of leaching from the total volume of nutrient solution supplied to avoid salt accumulation in the substrate under open soilless systems. Interestingly, biological control of root rot pathogens is ideally suited to closed soilless systems due to the lower diversity of the microorganism community in closed structures. And this makes it easier for biocontrol agents to be effective. Moreover, using sand filtration in closed systems reduce pathogens propagules in the nutrient solution. However, substrate salinity is the main challenge in closed soilless systems as, The use of salt tolerance cultivars, large volume tank to stabilize EC development and minimal pH changes and subirrigation with low concentration nutrient solution are all strategies that can be used to manage the salinity level in the growing substrate.

Automated irrigation systems are wildly used in soilless culture systems to reduce, nutrient, water and labor costs. However, automatic irrigation design should be based on crop water requirements (not fixed watering schedules) to guarantee the high performance of the soilless system. Precision agriculture based on advanced sensing technologies such as smart irrigation systems and remote sensing are known to increase water use efficiency. Smart irrigation systems such as, wireless sensor networks for real-time sensing of substrate water, systems that use crop physiological factors to model water use and leachate EC status, and hyperspectral sensors that detect changes in spectral reflectance of soilless crops are holding promise as an effective approach for precision irrigation management of greenhouse soilless crops.

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FUTURE FOREST FIRE DANGER PROJECTIONS USING GLOBAL CIRCULATION MODELS (GCM) IN TURKEY

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ABSTRACT

Fire is one of the greatest threats to forests. Compared with other European countries, Turkey is in a relatively better situation according to the fire statistics. However, it is also known that the climate is rapidly changing in Turkey. Since the late 1990s, the scientists have been warning about summer temperatures and droughts. Although the annual precipitation undulates in general, 2013 and 2017 were also dry years like 2008. But, when considered in regional context, the warming and drought in the summer is stronger in southern and western regions where Calabrian pine (Pinus brutia Ten.) and maquis is common. After the drought in 2017, if 2018 is similarly going to experience a dry year, it is possible that it be a problematic season in terms of forest fires. In this study, the future weather conditions were projected using 3 different GCMs basing the current climate conditions in Turkey, and the case of fire risk for the years of 2050 and 2100 was tried to reveal according to the results obtained. Today, for a period of a 5-month fire season for Turkey, based on meteorological data by 114 stations, measurements were accordingly made by using Canada, Hadley and French circulation models. As a result of the study, possibility of fires in future is expecting increase. Dramatically the increases were stated in different FWI components and models at various levels, all the results showed the high fire risks.

KEYWORDS:
Forest fires, global climate models, The Canadian Wildland Fire Information System, fire danger

INTRODUCTION

Today, it is widely accepted that global climate warming will result in extreme heat and drought [1]. The urgent measures that need to be taken for the case were discussed in detail by all countries around the world at a recent climate change conference held in Paris about. It was decided on that a new agreement including the measures suggesting the decrease of global emission is to be put into practice soon after 2010 [2]. Forest fire regimes are influencing by climate and weather conditions as well as vegetation and socio-cultural factors [3, 4]. Research suggests that any decrease in the number of fires nor burning areas at high temperatures. On the other hand, extreme hot days are of a remarkable effect on large fires [5]. Today, a lot of research is carried out in order to attract the attention to the fire risk and climate change. Such predictions can be made through various GCMs. Global Circulation Models (GCMs) are numerical models that represent physical processes among atmosphere, oceans, cryosphere and land surface [6]. GCMs are used to make climate projections. GCMs come in various models and the measurements related to temperature, precipitation and other climate components can be made. The first climate model was developed in NOAA’s Geophysical Fluid Dynamics Laboratory in Princeton, New Jersey in the late 1960s [7]. In one research study where a GCM was used, Podur and Wotton [8] stated that an area twice as much in Ontario would have been damaged by 2040 because of the fires. In the study, as business-as-usual scenario suggests, there will be an 34% increase by 2040 and 92% by the end of 21st Century in escaped fires across Canada. In another study by Wotton et al. [9], a 140% rise in the number of fires by the end of 21st Century is predicted to occur according to the Hadley Centre GCM model. In the research conducted for the north hemisphere so far was stated an extension of fire season in future. This period of extension was defined as 20 days per year [10, 11]. Extreme summer temperatures, droughts have been observed in recent years. For this reason, numerous research studies have been carried out in order to specify climate change and probable climate conditions in future in the Mediterranean region of Europe, especially in the Eastern Mediterranean, the Middle East and around the Aegean Sea. In each study, the findings suggest increases in future temperatures and decrease in precipitation, especially during the summer, excluding beyond the north borders of the region [12, 13]. The Mediterranean region is seen to be one of the regions to be affected by a climate change all around the globe [13]. In all the Mediterranean region, most studies show that the utmost rise in temperatures take place in Iberia, the Balkan Peninsula and Turkey while the greatest decline in precipitation take place in the South Balkans, the South Aegean Sea, South Eastern Mediterranean, Southern Europe, Turkey and Arabian Gulf [12, 14, 15]. Apart from these regions where the
FWI components were measured for possible serious various levels in 3 models, extremely high levels of 37]. Although the research suggests the results at key [25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 17, Spain, Greece, Russia, Lebanon, Portugal and Tur- mining the fire risk by some research in the UK, Canada and it was found to be successful in deter- arranged according to pine fuel type, it is used across after the several additions. Although the system is Service [23, 24]. It has reached to the current status published in 1970 as a result of the research for a few ent forest layers [22]. Canadian FWI Index was first comprised of 6 components. Of these 6 components, three are used in determining the humidity of differ- forest layers [22]. Canadian FWI Index was first published in 1970 as a result of the research for a few years by some researchers from Canadian Forest Service [23, 24]. It has reached to the current status after the several additions. Although the system is arranged according to pine fuel type, it is used across Canada and it was found to be successful in deter- mining the fire risk by some research in the UK, Spain, Greece, Russia, Lebanon, Portugal and Tur- key [25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 17, 37]. Although the research suggests the results at various levels in 3 models, extremely high levels of FWI components were measured for possible serious climate changes in Turkey. A similar study was carried out using 3 GCMs in order to estimate fire risk for Russian and Canadian Boreal Forests by Stocks et al. [38]. As a consequence of this study, it was found out that serious changes will occur from the high to the extremely high fire risks under the conditions where CO$_2$ is twice as much for the both countries. The findings suggest a dramatic increase in fire activities and burning areas in these two countries. Moritz et al. [39] made global measurements for two periods is 2010-2039 and 2070-2099 based on 16 GCM and A2 scenarios. The study estimates an increase in the possibility of fire (62%) especially at medium-high altitudes and a global decrease (20%) mostly in tropical zones [39]. Of all the calculated FWI components, we presented FWI, FFMC, DC and DMC maps. These indices were given because they are a remarkable change. FWI was used to estimate the fire risk [23]. Two obvious examples are the strong relationship between man- caused fire occurrence and the Fine Fuel Moisture Code (FFMC) [40, 41, 42, 43, 44]. The Duff Moisture Code (DMC) is a numeric rating of the average moisture content of loosely compacted organic layers of moderate depth. This code gives an indication of fuel consumption in moderate duff layers and me- dium-size woody material. The Drought Code (DC) is a numeric rating of the average moisture content of deep, compact organic layers. This code is a use- ful indicator of seasonal drought effects on forest fuels and the amount of smoldering in deep duff lay- ers and large logs [45].

**MATERIALS AND METHODS**

The data obtained from 114 stations maintained by the General Directorate of Meteorology in Turkey was made of as the materials of this study. Current fire risk and the future climate conditions of Turkey were measured through the data continuously ob- tained from as many years as possible and daily data from the 5-month fire season of each year.

**Fire Danger and FWI System.** Canadian Fire Weather System (FWI) is a system which is calculated by maximum temperature, relative humidity, wind speed and 24-hour precipitation and is comprised of 6 components. Of these 6 components, three are used in determining the humidity of differ- forest layers [22]. Canadian FWI Index was first published in 1970 as a result of the research for a few years by some researchers from Canadian Forest Service [23, 24]. It has reached to the current status after the several additions. Although the system is arranged according to pine fuel type, it is used across Canada and it was found to be successful in deter- mining the fire risk by some research in the UK, Spain, Greece, Russia, Lebanon, Portugal and Tur- key [25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 17, 37]. Although the research suggests the results at various levels in 3 models, extremely high levels of FWI components were measured for possible serious climate changes in Turkey. A similar study was carried out using 3 GCMs in order to estimate fire risk for Russian and Canadian Boreal Forests by Stocks et al. [38]. As a consequence of this study, it was found out that serious changes will occur from the high to the extremely high fire risks under the conditions where CO$_2$ is twice as much for the both countries. The findings suggest a dramatic increase in fire activities and burning areas in these two countries. Moritz et al. [39] made global measurements for two periods is 2010-2039 and 2070-2099 based on 16 GCM and A2 scenarios. The study estimates an increase in the possibility of fire (62%) especially at medium-high altitudes and a global decrease (20%) mostly in tropical zones [39]. Of all the calculated FWI components, we presented FWI, FFMC, DC and DMC maps. These indices were given because they are a remarkable change. FWI was used to estimate the fire risk [23]. Two obvious examples are the strong relationship between man- caused fire occurrence and the Fine Fuel Moisture Code (FFMC) [40, 41, 42, 43, 44]. The Duff Moisture Code (DMC) is a numeric rating of the average moisture content of loosely compacted organic layers of moderate depth. This code gives an indication of fuel consumption in moderate duff layers and me- dium-size woody material. The Drought Code (DC) is a numeric rating of the average moisture content of deep, compact organic layers. This code is a use- ful indicator of seasonal drought effects on forest fuels and the amount of smoldering in deep duff lay- ers and large logs [45].

**Future Climate and GCMs.** With the carbon- induced climate change, Global Climate Models are used in order to estimate the future climate. General Circulation Models (GCMs) project the physical processes in atmosphere, ocean, cryosphere and earth surface [46]. GCMs have been the fundamental tool that is used for climatology since 1950s up to the date [47]. The Royal Swedish Air Force Weather Service in Stockholm is the first real time digital weather forecast in the world to be constantly used. The model was developed by the meteorologist Carl-Gustaf Rossby and the Institute of Meteorology at the University of Stockholm. Today, GCMs are the simulations of atmospheric movements of the earth and of the main equipments of climatology [48]. In our study, 3 contemporary GCMs were utilized to assume the future climate conditions and forest fire risks. These models are Canadian Climate Centre GCM [49], French GCM and the United Kingdom’s Hadley GCM. The fire risk for the years 2050 and 2100 is tried to be pointed out based on A1 and A2 scenarios by using these models.
RESULTS

As a result of our study, based on the rates of Canada, France and Hadley GCMs and A1 and A2 scenarios, it was put forward 220 Canada FWI components measured for the years of 2050 and 2100 and a map of percentage change. In the maps, the results were shown under 5 risk groups.

The representations for risk groups are “green as extremely low risk”, “yellow as low risk”, “orange as medium risk”, “red as high risk” and “dark red as extremely high risk”. Based on 2 different scenarios via 3 climate models used in the study, the maps belonging to the years of 2050 and 2100 according to various indices are explained separately in Figure 2 and Figure 3.

The situation of Today’s FWI indices calculated by the obtained meteorological data is given Figure 1. Although many indices were calculated, the map situations of FWI, FFMC, DC and DMC indices are shown. Today’s calculations of the given indices are given below in the same order.

In all 4 indices, the northern regions show milder results than the southern and southeastern ones do. Especially southeastern region show higher values in all 4 indices. Based on A1 and A2 scenarios, the situations in 2050 and 2100 are shown in Figure 2. and Figure 3. Different from the present day, they were shown with 24 maps for each (2050 and 2100) since 3 different GCMs were used according to these scenarios in the maps belonging to the forecasts. The maps for 2050 can be seen up (Figure 2).

When the maps are analyzed, it is seen that there are sometimes great similarities in different GCMs while the situations in the maps are sometimes quite different from each other. Among these 3 GCMs, it is seen that the French GCM is more optimistic while Canadian and Hadley GCMs make more pessimistic forecasts. The maps of 2100 forecasts are given below. When they are analyzed, firstly the drastic changes in the values, namely dark red ones compared to 2050 maps, stand out. All the maps belonging to FWI pointing out the fire severity are of low values, which means no green regions, while DC maps showing the impact of drought on the forest combustible materials bear high values in the extremely wide spaces (Figure 3).

The greatest changes in all the percentage changes for 2100 are foreseen in the north of Turkey while the fewest changes are foreseen to be in the west, south and the southeast parts of Anatolia. Relatively more moderate maps among percentage changes, the great change in the north is seen only in the Central and East Black sea; on the other hand, although great changes are seen in the Black Sea Region, the current smaller changes in the Southeastern, Southern and Western Regions respectively do not cause any changes in case the given 3 critical regions continue to be the regions being of the extremist values in Turkey.

DISCUSSION

In spite of the fact that forest fire regimes are shaped by climate-weather, fuels and human-induced factors, the fundamental factor is the climate itself [50]. These factors, today, are changing
because of the human-induced climate change. The impact of the probable climate changes on forests increasingly continues with the extreme events such as the high risk of forest fires, pests and diseases. The impacts of such events are seen in the South Europe in advance [51]. There is an increase in the number of inflammable materials and fire activities. The general tendency of drought as a result of early snow
melts and rise of temperature according to the pro-
jections will lead to the extension of fire seasons. As
a result of these changes in future, it is expected that
we will experience more severe fires, more burned
areas, more fires, more conducive conditions to fire
and longer fire seasons by warming climate
conditions [52; 53]. The areas expected to suffer
from severe fires are the Mediterranean region, Rus-
sia, Australia, Canada and the USA [54].

FIGURE 3
2100 FWI, FFMC, DC and DMC indices of Turkey with Canadian, French and Hadley GCMs according
to A1 and A2 scenarios.
The fire season for Turkey is the 5 months of May, June, July, August and September in the study. When the measured fire indices belonging to 2050 and 2100 are examined, the extremely high risk areas appear. According to these estimations, it is expected the extensions in fire seasons not only in the south and west coasts but also all across Turkey. The results obtained from 3 methods used in future climate modeling show that Hadley GCM is the one where the most pessimistic results are obtained and France GCM is the one where the most optimistic results are relatively obtained. In spite of this, the either scenario and 3 methods indicate that there will definitely be higher risks of fire compared to the actual situation for Turkey in future. Considering the risk conditions of regions, the South Eastern Anatolia is of the highest fire risk in the current conditions compared to other regions but of quite few forest lands. It is succeeded by the Mediterranean and Aegean regions. These three regions are stated to have the extremely high risk groups in all scenarios in future conditions. These regions are added respectively the Marmara, the Central Anatolia and sometimes the East Anatolia and The West Black Sea in some certain scenarios according to the risk groups.

When analyzed the percentage changes out of indices, the number of changes present adverse outcomes compared to those from indices. The most changes in percentage changes were assumed to be in the North East Anatolia and the East Black Sea while the least changes were assumed to be in the South East Anatolia region and the Mediterranean and Aegean regions subsequently. 2013 as the year where the highest number of fires took place and 1994, 2000 and 2008 as the years where the worst fires took place since 1990 never correspond to drought years excluding 1994. Thus, a significant relationship is between fire activity and drought in Turkey. The greatest fire ever in Turkey so far, damaging especially an area of 22,134 ha in Antalya in 2008, strengthens the possibility of it because it occurred in the year when Antalya had the lowest average annual precipitation belonging to Antalya since 1937 [55, 56].

The current forest protection systems are shaped according to the past climatic conditions [57]. The current firefighting organization, even if admitted either successful or not, is not able to prevent the high risk years from being those where the largest places burned. The firefighting organizations of many countries like Turkey fight against fires effectively and it is quite an achievement with a full capacity organization [58]. Forest fire risk status is measured to be in a high increase at a high level and the existent firefighting organizations would fall short. Today’s fire management policy focusing merely on extinguishment does not have to do with at all but only delays the inevitable outcome. Also, it cannot avoid more dangerous and damaging fires [54]. Therefore, in future conditions, prescribed burning, pruning and fuel management as a cost-effective method can be suggested [59]. Still, new and different methods must be found for effectiveness [1]. For this reason, reorganization is of high importance for firefighting organization in terms of getting prepared for the possible future conditions. So, a new fire management organization, especially aiming at decreasing fire risk, and making use of advanced and modern technological systems are necessary for a fire risk assumed for future.

ACKNOWLEDGEMENTS

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USING REED TO CLEAN STRONTIUM AND BARIUM CONTAMINATED SOLUTIONS

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ABSTRACT

Phragmites australis was cultured in Ba and Sr contaminated solutions for 2 weeks to investigate its potential to remove these metals. With the increase of time, the levels of metals in reeds increased. The more metals in solutions, the more metals entered the root system. After 2 weeks, 15.01±1.71 mg/g Ba and 3.93±0.05 mg/g Sr were sequestered in roots from solutions with 80 mg/L Ba and 20 mg/L Sr. Metals in shoots were slightly enhanced with the increase of metals in solutions. The translocation factor (TF) of Ba was not changed with time while the TF of Sr increased. The TF of Ba and Sr were less than 1 which further indicated that roots were the main organs for metal storage. Reed may be used to clean Ba and Sr contaminated water. Further in situ studies are needed to confirm the feasibility.

KEYWORDS:
Reed, Barium, Strontium, Phytoremediation, Flowback Liquid

INTRODUCTION

Hydraulic fracturing techniques have been in use in many countries to extract gas or oil. Hydro-fracturing may contaminate environment due to the “flowback” water which return to the surface when the hydraulic fracturing process is done [1]. High concentrations of heavy metals such as Barium (Ba) and Strontium (Sr) are reported in most flowback water [1, 2]. Once leaked or spilled, these heavy metals in “flowback” fluids can damage water quality and affect human health. High levels of Ba and Sr were reported in rivers near “flowback” fluids treatment outflows [3].

Phytoremediation technology can be used to remove metals from heavy metal contaminated water. Abundant research has been conducted to study the uptake and accumulation of metals in various plants. More than 500 plant species from 101 families have been investigated and reported [4], such as Pteris vitata (ladder brake), Brassica juncea (Indian mustard) and Thlaspi caerulescens (Alpine Pennygrass) and Helianthus annuus (dwarf sunflowers). Some wetland plants have also been studied for their potential to phytoremediation, such as Typha latifolia (cattail) and Phragmites australis (common reed). For instance, Phragmites australis have been applied to clean Fe, Al and Mn contaminated solutions [5]. It is also reported that Typha latifolia have successfully sequestered Zn, Cu, Cr, and Cd from municipal water [6]. Studies on phytoremediation of Ba and Sr contaminated water have not been widely reported. In this study, common reed (Phragmites australis) has been cultured in Ba and Sr contaminated solutions for 2 weeks to assess its potential to uptake these metals.

METHODS AND MATERIALS

The rhizomes of common reed Phragmites australis were purchased from Lorenz’s OK seeds, LLC (Okeene, Oklahoma). They were initially grown in commercial potting soil (Miracle-Gro lawn products, Inc) in pans (45 cm×25 cm×7.5 cm). Two centimeters of potting soil was placed in the bottom of each pan. This was followed by the rhizomes and another 2.5 cm of potting soil. The rhizomes were cultured in a greenhouse under natural light conditions. The average temperature of the greenhouse was 22°C and the humidity was 50%. Five hundred ml distilled (DI) water was sprayed into each pan every day to maintain the soil moisture. After 30 days of growth in the potting soil, seedlings with similar biomass were transferred into artificial solutions to initiate experiments. Prior to being transferred, the rhizomes of the reeds were rinsed with DI water to remove the attached potting soil.

Artificial hydraulic fracturing contaminated solutions were prepared by analytical grade metal salts: barium as BaCl2, strontium as SrCl2. Based on the typical concentration of hydrofracturing contaminated stream and the tolerant limits of plants, the metals concentration were (5 mg/L, 20 mg/L, 80 mg/L for Ba; 5 mg/L, 10 mg/L, 20 mg/L for Sr) [3]. The pH of the solution was adjusted to 7. In order to support the growth of reed, nutrients were added into
the solution. The main nutrients were N (620 mg N/L, NH₄NO₃) and P (94 mg P/L, KH₂PO₄) [5].

At day 1, the solution was prepared. Then plants were transferred to the solutions and cultured in greenhouse for 1 to 2 weeks. One reed was cultured in 1L solution which contained metals and nutrients. The experiments were conducted in triplicates. At the end of culturing period, reeds were harvested and then air dried. After drying, the tissues were separated into roots, rhizomes and shoots, then weighed and crushed with mortar and pestle. The milled tissues were then digested following the methods described in Guo and Cutright [5]. Finally, the solutions were filtered by 0.45 µm filters and analyzed by Atomic absorption spectroscopy 6300 (AAS).

Data on metal uptake in reeds were analyzed with one-way ANOVA using the Minitab statistical package (Minitab 16). Differences between specific metal levels were identified by Tukey's test at 5 % probability.

RESULTS AND DISCUSSIONS

Ba and Sr were found in reeds cultured in solutions for 1 and 2 weeks. It was not surprising that with the increase of time, the levels of metals in reeds increased. For instance, the roots of reeds grown in solutions with high level of metals (HM) accumulated 12.26±0.58 mg/g Ba after 1 week (Fig 1); while the roots uptake 15.01±1.71 mg/g Ba after 2 weeks (Fig 2). The amounts of Ba in shoots also increased with time. The shoots cultured in HM solutions sequestered 2.30±1.66 mg/g Ba and 3.92±0.53 mg/g Ba after 1 and 2 weeks, respectively (Fig 1 and 2). The similar trends were found for Sr. The roots of reeds grown in solutions with middle level of metals (MM) accumulated 2.17±0.20 mg/g Sr after 1 week (Fig 1); while the roots uptake 3.09±0.61 mg/g Sr after 2 weeks (Fig 2). The levels of Sr in shoots cultured in MM solutions increased from 0.15±0.12 mg/g Sr to 0.62±0.21 mg/g Sr after 2 weeks (Fig 2). The mechanism of metals uptake in plants from solutions was due to the metal diffuse through a membrane towards the bulk phase of the plant [7]. Thus with the time passing by and the growth of plants, more metals can enter the biomass of reeds.

Ba and Sr was mainly concentrated in the below ground tissues, especially roots of reeds. For example, after 2 weeks, 8.19±0.78 mg/g Ba and 2.23±0.35 mg/g Sr entered the roots cultured in solutions with low level of metals (LM) (Fig 2). It is not surprising as previous research also indicated that large amounts of heavy metals were preferentially accumulated in the roots and rhizomes of reeds [8]. Bonanno [9] also indicated that roots were the main organ to sequester Ba. High level of Sr was also found in roots other than shoots in reeds grown in an area affected by urbanization agriculture [9]. The levels of other metal were also much higher in the roots than that in the shoots of reeds [10]. For instance, Bonanno and Giudice [11] indicated that the subsurface organs of reeds were the primary areas of metal accumulation for Cd, Mn, Ni, and Zn. Lesage et al. [12] also reported that the Fe concentration in stems<leaves<belowground biomass of reed. To sequester most metals in roots, less metal can harm rhizomes which are the only persistent part of the plant [10]. Thus even less metals can transport to the aboveground tissue of reeds.

Error bar represented the standard deviation of triplicate samples. Different letters on the same plant organ indicated a significant difference at p<0.05. “Rhiz” represented “rhizome”. 

![FIGURE 1 (A)](image-url)
Error bar represented the standard deviation of triplicate samples. Different letters on the same plant organ indicated a significant difference at p<0.05. “Rhiz” represented “rhizome”.

Generally, with the increase of metals in solutions, the amounts of metals in the root system of reeds also increased. For example, after 2 weeks, the roots of reeds grown in solutions with MM accumulated 9.85±1.31 mg/g Ba and 3.09±0.61 mg/g Sr; while the roots in HM sequestered 15.01±1.71 mg/g Ba and 3.93±0.05 Sr (Fig 2). The same trend was found in rhizomes. The rhizomes of reeds grown in solutions with MM accumulated 3.60±0.40 mg/g Ba and 0.85±0.37 mg/g Sr after 2 week; while the rhizomes in HM sequestered 5.23±0.31 mg/g Ba and 1.39±0.25 mg/g Sr (Fig 2). It was in agreement with previous research that accumulation of metals in plant was correlated with concentrations in substrate [13]. Different from roots, the amounts of metals in the shoots of reeds were not enhanced with the increase of metals in solutions. For example, after 2 weeks, the shoots of reeds grown in solutions with LM accumulated (3.21±1.33 mg/g Ba and 0.57±0.20 mg/g Sr) (Fig 2). It was similar to those in the shoots in HM solutions (3.92±0.53 mg/g Ba and 0.84±0.06 mg/g Sr) (Fig 2). This may be related to the low mobility of Ba and Sr. As show in Table 1, the translocation factors (TF) of Ba and Sr which means the ratio of metal in plant shoots to that in roots was less than 1 [14]. The TF of Ba and Sr in reeds cultured in solutions
with different levels of metals was similar. It further proved that that most of these metals were stored in the roots and the translocation of metals into the shoots was limited. However, it was reported that the translocation of Sr in other plants such as sunflower was much higher [15]. It was understandable as the tolerant mechanisms and uptake models to metals were different for different plants [16]. The TF of Ba in reeds cultured 1 week was similar to those in reeds grown for 2 weeks. The value of Ba ranged from 0.19 to 0.39. It was similar to previous research which found that the TF of Ba was 0.19 and 0.31 [17, 18]. However, it was interesting to find that the TF value of Sr changed with time. It was around 0.07 in week 1 but increased to about 0.21-0.39 after 2 weeks (Table 1). It may be due to that the age of the plant can modify the translocation of Sr to shoot from roots [19]. With the growth of reeds, new shoots always emerge from the rhizomes of reeds, thus more metals can be transported to the above ground organs [20]. Taylor and Crowder [16] also indicated the growth of plants may increase the metal and nutrient transport. In addition, heavy metal transportation mechanisms differ with the type of metals [21]. Ba was easy to be precipitated which may inhibit its translocation in plants with time [9].

Previous research [22] even found that the TF of some metals (e.g. Cr) decreased with time. Besides, the translocation of metals in plants was also affected by other parameters such as the capacity of plant species, the availability/solubility of metals and the characteristics of environment [23, 24].

**CONCLUSIONS**

Hydraulic fracking leads to the boom of gas production but also consumes large amounts of water and produce flowback water. This project investigated the potential of phytoremediation technology (i.e. using *Phragmites australis*) to remove Ba and Sr from flowback liquids. The results indicated that it may be a promising treatment technology. With the increase of time, the levels of metals in reeds increased. The more metals in solutions, the more metals entered the root system of reeds. After 2 weeks, 15.01±1.71 mg/g Ba and 3.93±0.05 mg/g Sr were sequestered in roots from solutions that contained 80 mg/L Ba and 20 mg/L Sr. The amounts of metals in the shoots of reeds were not enhanced with the increase of metals in solutions. TF of Ba did not
change significantly with time, but the TF of Sr increased with time. The TF of Ba and Sr were still less than 1 after 2 weeks which further indicated that most metals were stored in the roots of reeds. Phragmites australis is a good candidate for phytoremediation, as it can survive almost everywhere all around the world and has high biomass. The limit of transferring heavy metals into shoot can sequester most metals in roots and avoid the passing of pollutants into food chain via herbivores. Further studies on the in situ remediation of flowback liquids contaminated environment are needed to confirm its potential for flowback liquids treatment in real word.

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DETERMINATION OF THE TOTAL AFLATOXIN LEVEL IN RED PEPPER MARKETED IN AFYONKARAHISAR, TURKEY

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ABSTRACT
Aflatoxins are toxic secondary metabolites of Aspergillus species (mainly A. flavus and parasiticus) that exhibit immunotoxic, hepatotoxic, carcinogenic, and teratogenic effects in human. Human exposure to aflatoxins may be occurred by consumption of risky foods such as cereals, milk, and various spices. The present study aimed to assess the total aflatoxin (B1, B2, G1, G2) contamination level of red pepper marketed in Afyonkarahisar province, Turkey. For this purpose, a total of 76 pepper samples including 48 red-scaled pepper, 28 red-powdered pepper were randomly collected from bazaars, herbal shops and supermarkets. The aflatoxin contamination was determined by ELISA. 49 of the 76 pepper samples (64.47%) contained aflatoxin in the range of 1.76-42.72 μg/kg. 5 (6.58%) samples exceeded the regulatory limits employed in Turkey and the European Union. Consequently, to protect public health from the adverse effects of aflatoxins, more precautions should be taken during every step of pepper production, transport and storage.

KEYWORDS:
Aflatoxin, red pepper, ELISA

INTRODUCTION
Fungus contamination is an important problem in agricultural products resulting in economics, food safety and human health concerns [1]. Their production is mainly affected by environmental conditions including humidity and high temperatures [2]. Several fungi genera such as Aspergillus, Fusarium, Penicillium, Claviceps produce mycotoxins as secondary metabolites. Also, aflatoxins are an important group of mycotoxins and they were mainly generated by Aspergillus flavus and parasiticus [3, 4]. Aflatoxin B1 (AFB1), B2, G1, and G2 are the most important mycotoxins in feeds and foods [1]. Aflatoxins were classified by the International Agency of Research on Cancer as group I carcinogens. Also, AFB1 is known as the most potent natural carcinogen and produced usually by toxigenic strains as major aflatoxin [3, 5]. Aflatoxins can be found in several types of foods such as milk [6], milk products [7, 8], cereals [9], and spices [10]. Humans may be exposed to these mycotoxins by means of risky foods including milk and milk products, spices, cereals, dried fruits, and coffee [5, 11]. Aflatoxins in foods can adversely affect the health of human and animal. They exhibit toxic, mutagenic, teratogenic, and carcinogenic effects [1, 12]. Also, chronic exposure to aflatoxins affect immunity and interferes with protein metabolism and multiple critical micronutrients affecting animal health [13].

Spices are employed in many cuisines for imparting aroma, colour, flavour and they also used for therapeutic purposes [12, 14]. Red pepper (Capsicum annuum L.) is the second most consumed spice in the world following the black pepper [1]. Also, Turkey ranks third in red pepper production after China and Mexico [15, 16]. Spices including red pepper can be easily contaminated with aflatoxins in the field, during the period of drying or storage and also in every processing stage [5, 17].

The present study aimed to determine total aflatoxin levels in red peppers (red-powdered and red-scaled pepper) marketed in Afyonkarahisar Province, Turkey. Moreover, this study may contribute to the risk assessment of red peppers regarding public health based on the obtained data.

MATERIALS AND METHODS
Material. During July-September 2018, a total of 76 red pepper samples including 28 red-powdered pepper and 48 red-scaled pepper samples were randomly collected from bazaars, herbal shops and supermarkets in Afyonkarahisar, Turkey. Also, 12 of the 28 red-powdered pepper samples were unpacked while the other 16 samples were packed. Moreover, 27 of 48 red-scaled pepper samples were unpacked and 21 samples were packed. Samples were transported to the laboratory under cold chain conditions.

Sample Preparation and Aflatoxin Analysis. Red pepper samples were prepared for ELISA analysis by using immunoaffinity column according to
the test kit manual (Rida Aflatoxin Column Art no.: R5001/5002, R-Biopharm, Darmstadt, Germany) [18]. 25mL of methanol (70%) was mixed with 5 g of red pepper samples. Then, the extraction of this solution was performed by means of gently mixing during at room temperature for 10 minutes. After the filtration of the extract by a paper filter (Whatman No.1), 5 ml of the extract was mixed with 15 ml distilled water. 0.25ml of Tween 20 was added to this mixture and stirred for 2 minutes. Following-ly, clean up procedure was performed for the obtained extract according to kit’s manual.

**Elisa Procedure.** Total aflatoxin concentration of the red-scaled and red-powdered pepper samples was determined by an enzyme immunoassay method (ELISA) according to manufacturer’s instruction (Ridascreen Aflatoxin Total, Art no.: 4701, R-Biopharm, Darmstadt, Germany) [19]. For this purpose, 50 μl of the prepared test sample or standard solutions were pipetted into the wells of a microtiter plate. Afterwards, enzyme conjugate (50 μl) and the antibody solution (50 μl) were pipetted into each well followed by an incubation period at room temperature (20±2°C) for 30 min. To remove the unbound conjugate, microtiter plate was washed three times with wash buffer. Then, substrate/chromogen solution (100 μl) was pipetted into each well and incubated at room temperature (20–25°C) for 15 min in the dark. Lastly, 1M H2SO4 as a stop solution (100 μl) was pipetted into each well and the absorbance was measured at 450nm in ELISA plate reader (Multiscan Go, Thermo Fisher Scientific Inc). The detection limit of the assay was 0.25 μg/kg. The obtained data were evaluated by Rida® Soft Win.net (Z9996) program.

**RESULTS**

Totally, 28 red-powdered pepper samples were analyzed for the presence of total aflatoxins (Table 1). It was determined that 10 (62.5%) of the packed samples were contaminated with different concentrations of aflatoxins whereas none of the samples was exceeded the maximum acceptable limit (10μg/kg). However, all of the unpacked powdered samples were contaminated with aflatoxins and 3 (25%) samples exceeded the maximum acceptable limit with the highest concentration of 38.30 μg/kg.

A total of 48 red-scaled pepper was analyzed to determine total aflatoxin contamination (Table 2). Different concentration of aflatoxins was found in 12 (57.14%) of 21 packed red-scaled pepper samples as well as in none of the packed samples, aflatoxin contamination level exceeded the 10μg/kg. Moreover, 15 (55.6%) of 27 unpacked red-scaled pepper was contaminated with aflatoxins and 2 (7.41%) samples exceeded the maximum acceptable limit with the highest concentration of 47.22 μg/kg.

A total of 76 red pepper samples (red-powdered pepper and red-scaled pepper) from which 39 unpacked and 37 packed was analyzed to determine the total aflatoxin contamination (Figure 1). It was determined that the total aflatoxin level in 30.77% unpacked and 40.54% of packed red pepper were lower than the detection limit (<0.25 μg/kg). Also, the aflatoxin level in 35.53% of the 76 red pepper samples was found to be under the detection limit. Moreover, 6.58% of all samples exceeded 10 μg/kg with the highest contamination level of 42.72 μg/kg while none of the packed red pepper samples exceeded maximum residue level (10 μg/kg).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>The total aflatoxin contamination of red-powdered pepper obtained from Afyonkarahisar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>n (%)</td>
</tr>
<tr>
<td>Unpacked</td>
<td>12</td>
</tr>
<tr>
<td>Packed</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
</tr>
</tbody>
</table>

Data were presented in number and percentage

<table>
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<tr>
<th>TABLE 2</th>
<th>The total aflatoxin contamination of red-scaled pepper obtained from Afyonkarahisar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>n (%)</td>
</tr>
<tr>
<td>Unpacked</td>
<td>27</td>
</tr>
<tr>
<td>Packed</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
</tr>
</tbody>
</table>

Data were presented in number and percentage
DISCUSSION

Red pepper is one of the most consumed spices in Turkey and the world. Also, red pepper is a suitable source for mould production. Therefore, aflatoxin contamination in red pepper can be a serious problem for public health [5,17,20]. There are several studies aiming to evaluate red peppers regarding the risk of aflatoxin contamination throughout the world. Romagnoli et al. [21] reported that aflatoxin contamination of hot pepper was determined as 45.5% (5/11) ranging from 0.57 to 30.7 μg/kg in Italy. In another study performed in Pakistan, aflatoxins contamination of 312 crushed chili, chili powder, chili sauce and whole chili samples were determined. According to the results of the study, 56.4% (176/312) of the samples were found to be positive for aflatoxins [22]. Jalili and Jinap [23] determined that 65.0% (52/80) of chili samples collected from Malaysia contains aflatoxins ranging from 0.2 to 79.7 μg/kg. Kursun and Mutlu [10] evaluated total aflatoxin level of 72 red pepper samples collected from a west part of Turkey and reported that all of the samples were contaminated with aflatoxins in the range from 3.55 to 9.55 μg/kg. In another study performed in Istanbul (Turkey), it was determined that 56.7% (17/30) of red-scaled pepper samples contained total aflatoxins ranging from 0.7 to 46.8 μg/kg while 36.7% (11/30) of red pepper samples contained total aflatoxins ranging from 0.8-15.4 μg/kg [11]. Moreover, Aydin et al. [20] assessed the aflatoxin B1 levels of 100 powdered red pepper samples collected from markets in Istanbul (Turkey). They determined that the level of aflatoxin B1 in 32% (32/100) of samples was lower than the detection limit of the immunoassay (0.025μg/kg) while 18% (18/100) samples exceeded the maximum residue levels (5 μg/kg). Also, Bircan [24] reported that 90% (27/30) of red ground pepper samples and all (15/15) of the chilli powder samples were contaminated with aflatoxins. The highest contamination level for red ground pepper samples was determined as 124.6 μg/kg while this level for the chilli powder samples was detected as 85.9 μg/kg. Besides, Ardic et al. [5] evaluated 75 red pepper samples obtained from bazaars and herbal shops in Sari province of Turkey regarding aflatoxin B1 contamination. They reported that 96% of the samples were contaminated with AFB1 in the range from 0.11 to 24.7 μg/kg and 14.7% samples exceeded European and Turkish regulatory limits. Set and Erkmen [25] evaluated 82 unpacked and 38 packed ground red pepper samples collected from Gaziantep province, Turkey and reported that 17.1% and 23.1% of unpacked ground red pepper samples exceeded legal limits, while only one packed sample contained aflatoxins higher than the legal limit. Gurbuz et al. [26] reported that 32% of 75 red ground pepper contained AFB1 residues at levels between 0.25 and 10 μg/kg. Ağaçoğlu [27] indicated that all of the 40 crushed red pepper samples collected from Van, Turkey was contaminated with aflatoxin B1 at different levels (1.10-44.00 ppb). Dokuzlu [28] determined that 46.66% of 30 red pepper samples obtained from Bursa were contaminated with aflatoxins in the range from 5 to 25 ppb.

To protect the health of consumers, maximum legal limits for total aflatoxins (10 μg/kg) and aflatoxin B1(5 μg/kg) in red pepper was established in both Turkey [29] and European Union [30].

In conclusion, this study evaluated total aflatoxin contamination in red pepper samples including red-powdered pepper and red-scaled pepper.
obtained from markets, herbal shops and bazaars in Afyonkarahisar, Turkey. It was determined the level of total aflatoxin in 93.42% (71/76) red pepper samples were found to be under the maximum residue level whereas 6.58% (5/76) of the samples exceeded these levels. The aflatoxin contamination in red peppers may pose a potential hazard for public health. Therefore, red peppers offered to sales should be regularly controlled regarding the presence of aflatoxins.

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LANDSCAPE INVENTORY AND CHARACTER ANALYSIS: A CASE STUDY FROM URLA, CESME, KARABURUN DISTRICT, TURKEY

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ABSTRACT

In this study, natural, socio-cultural and visual landscape characteristics of the study area were determined by using Geographical Information Systems (GIS) technologies in Izmir-Cesme and Karaburun district located in the north of Izmir-Cesme highway and landscape inventory and character analysis of the area were conducted. In this scope of the study, the natural and socio-cultural components of the landscape were examined in a planning approach that is sensitive to ecology, environment and natural disasters, in accordance with the conservation-use balance. In the first phase of the study, natural and socio-cultural indicators in the landscape inventory were compiled on the GIS base. In the second phase, the landscape function analysis, which reflects the natural functions of the study area for landscape character analysis, was produced through visual-aesthetic-perceptual landscape quality, water permeability, surface runoff potential, soil erosion risk and habitat value components. Finally, landscape character type maps specific to study area were produced using landuse/landcover, elevation, slope, forest stand map, great soil groups and rock permeability components. Cluster analysis was performed so that landscape character types can be reduced and interpreted numerically. Suggestions for landscape management strategies were developed by presenting the landscape inventory function and character types of Urla, Cesme and Karaburun districts in the direction of the findings obtained.

KEYWORDS:
Landscape inventory, landscape function analysis, Landscape Character Analysis (LCA), Geographic Information System (GIS)

INTRODUCTION

Landscape is the definition of an area as perceived by people, whose character is the result of the action and interaction of natural and human factors [1]. Landscape is a scene in which results of the interaction between nature and human [2]. This scene, where the dominant power is human, constantly changes. Change is a natural feature of a landscape. The combination of natural and socio-cultural influences and their complex relationships make one landscape different from another [3]. Changes seen in landscape can be large or small, fast or slow, distinct or indefinite, positive or negative. The increasing awareness on these changes has brought the landscape assessment studies, one of which is “Landscape Character Assessment” [4]. The determination of landscape character is an important tool for observing landscape changes.

Landscape is a combination formed by the following factors; geology, soil, topography, land use/landcover, hydrology, climatic components and cultural effect. This combination brings about landscape characters. Landscape Character (LC) is defined as a distinct, recognisable and consistent pattern of elements in the landscape that makes one landscape different from another, rather than better or worse [5, 6]. Landscape Character Assessment (LCA) is the process of identifying and describing combination in the character of the landscape [3], which is based on classification and mapping of different and separeative landscape. The purpose of LCA is to develop spatial planning strategies for specific landscape areas and types. Landscape areas and types are revealed through LCA that is the process of systematically analyzing landscape character areas by their natural and cultural landscape features [7].

LCA approach is seperated into two stages, identifying characters and judgement [8]. It is a function-character based landscape planning and management process that enables making judgement on conservation, development and management of landscapes [9]. The judgement stages of LCA is used with intent to develop landscape strategies, create landscape guides, determine the capacity and sensivity for the change that might happen [8]. Landscape character is analyzed through elements such as geology, soil, topography, climate, landcover, fauna and human action that a landscape
has [10], [11] stated that a landscape character is formed by (i) landscape structure, (ii) landscape function and (iii) landscape change.

The European Landscape Convention (ELC) encourages its member states to identify and describe the landscape covering of their entire territory, by making an inventory of the significant features that characterise them [1, 12]. Landscape Character Analysis has been a common concern for decision makers, stakeholders, planners and managers. In addition to contributing greatly to ELC’s implication [8], LCA facilitates the understanding of different characters and communicating about landscape leading to better environmental management and conservation [13]. Turkey had become a party of the ELC in 2003 and many studies on LCA conducted an in our country to make the obligations of this agreement and maintained. In this direction, there is a need in function-character based landscape planning and management process that enables making judgements on protection, development and management of country landscapes [9]. In this context, the structure, function and change of the landscape were presented on the basis of ecological features of Urla-Cesme-Karaburun Peninsula, for landscape inventory and character analysis of the study area. In this study, natural and social-cultural synthesis of landscape components was performed via landscape function analysis, in addition to assessments related to landscape disasters considering natural disaster sensitive planning approach.

STUDY AREA

The study was applied in Izmir, Karaburun district located in the western part of Turkey. The highway reaching Cesme from Izmir was determined as the border, with the purpose of increasing the spatial effect size of the study area. The districts of Cesme and Urla, located to the north of this highway, were also included in the study area. Thus, the study area was identified as the northern part of the Urla-Cesme-Karaburun peninsula (Figure 1).

Although study area is located in affected regions of Izmir having the third highest population in Turkey, it has maintained its original value until today [14]. This area has a distinctive identity with its topography, climate, flora and fauna, coasts, natural resources, historical sites and archaeological accumulation. The region covers an area of approximately 925 km² and its altitude ranges between 0 and 1200 m. Study area is typically characterized by Mediterranean climate with hot and dry summer and warm, rainy winter. The maximum temperature in the area is 31.2°C and the amount of rainfall is found at 34.9 mm to 43.6 mm [15]. The amount of rainfall is decreasing as it goes to the southern and coastal parts of Urla.
FIGURE 2
Visual-Aesthetic-Perceptual landscape quality inputs (landscape survey) [15]
TABLE 1
The base input information on the landscape character analysis

<table>
<thead>
<tr>
<th>Input data</th>
<th>Organization</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topography</td>
<td>General Command of Cartography</td>
<td>1/25000</td>
</tr>
<tr>
<td>Geology</td>
<td>General Directorate of Mineral Research and Exploration</td>
<td>1/25000</td>
</tr>
<tr>
<td>Soil</td>
<td>Ministry of Food, Agriculture and Livestock</td>
<td>1/25000</td>
</tr>
<tr>
<td>Corine (2006-2012)</td>
<td>Ministry of Forestry and Water Affairs</td>
<td>1/25000</td>
</tr>
<tr>
<td>Highways</td>
<td>Directorate General of Highways</td>
<td>1/25000</td>
</tr>
<tr>
<td>Stand</td>
<td>Ministry of Forestry and Water Affairs</td>
<td>1/25000</td>
</tr>
<tr>
<td>Stream network</td>
<td>Directorate General of State Hydraulic Work</td>
<td>1/25000</td>
</tr>
<tr>
<td>Climate</td>
<td>Directorate General of Meteorology</td>
<td>1/25000</td>
</tr>
</tbody>
</table>

DATASET

With digital and verbal data of natural landscape elements obtained in the study, creation of the database, digitization of the data, interpretation, associate, analysis and outputs of the data processes have been carried out by using ArcGIS version 10.3.1, a geographical information systems (GIS) software developed by ESRI. The key inputs used in the determination of landscape inventory, function and character types are given in Table 1.

In the study, socio-cultural landscape elements or factors are represented with visual, aesthetic and perceptual landscape quality values. In order to determine these quality values have been made landscape survey at 719 different point. The visual, aesthetic and perceptual landscape factors in the landscape survey were produced with the expert knowledge based on the scoring system applied by [9]. The socio-cultural landscape elements evaluated in three basic categories as visual, aesthetic and perceptual landscape factor are shown in Figure 2.
METHODOLOGY

The method of study is based on landscape character analysis which is applied previous studies by [6, 7, 9, 10, 12, 16-19].

Determination of landscape characters, identification of the landscapes of the study area and evaluation process consists of three basic stages as the following: (i) the creation of landscape inventory, (ii) landscape function analysis (habitat value, visual-aesthetic-perceptual landscape quality, water permeability, surface runoff potential, soil erosion risk and integrated function analysis) and (iii) production of landscape character types (Figure 3).

Landscape inventory. The landscape inventory stage is the part where the data collected from literature studies or government agency and database is created. In study area, natural and socio-cultural landscape inventories which are important parameter in determining landscape interactions were provided a aid for LCA. Natural landscape inventory are constituted with GIS based elevation, slope, aspect, Thornthwait climate classification, landuse/landcover, geologic structure, soil (great soil groups), forest stand map, hydrology and transportation maps. Socio-cultural landscape elements have been obtained from landscape survey studies.

Landscape function analysis. Landscape function and landscape character types will constitute a basis for landscape character analysis. Landscape function is a process that occurs with the mutual interaction of landscape components. Each landscape can be changed different fundamental and key process [9]. The keys of landscape functions in this study are defined as visual-aesthetic-perceptual landscape quality, habitat value, water permeability, surface runoff potential, soil erosion risk. This is the most important step in determining the landscape structure and ecosystem properties of the study area, and is the step in which the relationship between natural and socio-cultural processes is aligned with the natural environment. The landscape function analysis is second phase of this study and it is shown in the method (Figure 3).

(i) Visual - aesthetic - perceptual landscape quality. Landscape quality consists of the relationship between the characteristics of landscape and the influence of these characteristics on the users. Especially, landscape visual quality is a common product of the observer’s psychological (sight, perceptual, emotional and aesthetic) process which is in an interaction with apparent (visible) landscape characteristics [20]. Landscape assessment studies focus on evaluating the visual characteristics, locational installation, and social life of a place or a route on a perceptual basis (including all sense organs, especially the eyes) within a functional relationship. In order to understand the importance of environment, investigating how people react to different characteristics of the environment is required [21].

The land form, vegetation diversity, water existence, color, rarity and cultural change indicators were used to determine the visual quality value in this study. Six variables acquired from the landscape survey for visual quality; the land form, vegetation diversity, water existence, color, rarity and cultural change indicators, were scored as high (5), medium (3) and low (1) according to the expert knowledge. Six variables from 719 different points to reveal aesthetic quality value; aesthetics, enclosure, diversity, color, line and tranquility were scored within itself by taking expert knowledge. 5, 3, 1 for naturality and enclosure; 5.0 for diversity; 5, 2, 0 for color and line; and 2 and 5 for tranquility. Four variables, stimulus, contentment, safety and dominance, were used to determine the perceptual quality value, and they were scored by taking expert opinion within themselves. 5, 3, 0 for stimulus; 5, 3, -2 for contentment; 5, 0, -2 for safety; 0, 1, 2, 3, 4 and 5 for dominance were given.

For each of the 719 total points scored in ArcGIS software, Inverse Distance Weighting (IDW) method that is one of many spatial interpolation methods found in the literature was applied by adding visual, aesthetic and perceptual landscape quality assessment scores determined by expert knowledge. By this means, the visual landscape elements were mapped by generalizing from spatial point data. According to Wong (2017) [22], the basic objective of spatial interpolation is to estimate the value of a location where there are no data available using data that are available in other locations. By extension, if the method can estimate the value of a given location, it can estimate values for all locations in the study region [15].

(ii) Habitat value. The umbrella species which needs optimal spatial requirements were utilized while determining the habitat value of the study area. The umbrella species was designated as Eurasian Lynx (Lynx lynx L.) based on the study of Favilli (2013) [31]. When habitat requirements of the umbrella specie was determined, the landuse/landcover by 30%, topography by 10%, distance from facilities by human influence by 20%, elevation by 20% and distance from the road by 20% used. The determined habitat requirements were weighted overlay in the ArcGIS software and the habitat value analysis for the study area was estimated (Table 2).

(iii) Water permeability (Infiltration). Water permeability is an important factor for the development of ecological networks with the supply of underground water recharge. The two basic parameters of water permeability are the hydrological soil
properties and the geological structure of the area. In the the water permeability phase of study is based previous studies by [9, 18, 23-28].

The classification system developed by the USA Soil Conservation Service is used to identify hydrologic soil groups. According to this system, great soil groups obtained from soil map are evaluated in four basic categories. In this direction, the study area consisting of large soil groups were evaluated and reclassified as: (i) low surface runoff potential soil (A), (ii) medium low surface runoff potential soil (B), (iii) medium high surface runoff potential soil (C), and (iv) high surface runoff potential soil (D). In addition, another indicator used in water permeability which is for producing the rock permeability map, the attributes of the geological map were classified as permeable, semi-permeable and impermeable by the geologist's expert knowledge (Table 3).

The water permeability map was obtained according to Table 4 that is overlayed reclassifying rock permeability and reinterpretting of hydrologic soil groups.

### TABLE 2
Habitat suitability factors and weights of umbrella species [15]

<table>
<thead>
<tr>
<th>Landuse/Landcover (%)</th>
<th>Topo (%)</th>
<th>Dist from human (%)</th>
<th>Elevation (%)</th>
<th>Dist from road (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>%</td>
<td>Class</td>
<td>%</td>
<td>Class</td>
</tr>
<tr>
<td>Forest</td>
<td>100</td>
<td>0-30°</td>
<td>50</td>
<td>0-100 m</td>
</tr>
<tr>
<td>Pasture-Grassland</td>
<td>100</td>
<td>30°-60°</td>
<td>100</td>
<td>100-500 m</td>
</tr>
<tr>
<td>Bareground</td>
<td>50</td>
<td>60°-90°</td>
<td>20</td>
<td>500-1000 m</td>
</tr>
<tr>
<td>Urban</td>
<td>0</td>
<td>&gt;1000 m</td>
<td>100</td>
<td>1500-2000</td>
</tr>
<tr>
<td>Agriculture</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water bodies</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3
Rock Permeability reclassification [15]

<table>
<thead>
<tr>
<th>Geology structure</th>
<th>Rock Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alluvial fan</td>
<td>Permeable</td>
</tr>
<tr>
<td>Coglomerate-Sandstone-Mudstone</td>
<td></td>
</tr>
<tr>
<td>Sandstone-Mudstone</td>
<td></td>
</tr>
<tr>
<td>Sandstone-Mudstone-Limestone</td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td></td>
</tr>
<tr>
<td>Scree-Cone of Dejection</td>
<td></td>
</tr>
<tr>
<td>Coglomerate</td>
<td>Semi-permeable</td>
</tr>
<tr>
<td>Aglomera</td>
<td></td>
</tr>
<tr>
<td>Alluvial</td>
<td></td>
</tr>
<tr>
<td>Clay Limestone</td>
<td></td>
</tr>
<tr>
<td>Tufa</td>
<td></td>
</tr>
<tr>
<td>Tufa-Pyroclastic</td>
<td></td>
</tr>
<tr>
<td>Volcanic-Sediment</td>
<td></td>
</tr>
<tr>
<td>Andesite</td>
<td>Impermeable</td>
</tr>
<tr>
<td>Andesite-Bazalt</td>
<td></td>
</tr>
<tr>
<td>Bazalt</td>
<td></td>
</tr>
<tr>
<td>Granite-Granodiorite</td>
<td></td>
</tr>
<tr>
<td>Shale</td>
<td></td>
</tr>
<tr>
<td>Monzonite-Diorite</td>
<td></td>
</tr>
<tr>
<td>Ophiolite</td>
<td></td>
</tr>
<tr>
<td>Spilite</td>
<td></td>
</tr>
<tr>
<td>Trachyte</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 4
Rock and soil permeability overlay criteria [9, 15, 18]

<table>
<thead>
<tr>
<th>Rock permeability</th>
<th>Hydrological soil groups A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeable</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Semi-permeable</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Impermeable</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Water permeability degree: 5-very high, 4-high, 3-medium, 2-low, 1-very low
(iv) **Surface runoff potential.** Soil Conservation Service (SCS) Curve Number method developed by United States Department of Agriculture was used for surface runoff potential analysis. The SCS curve number method is a simple, widely used and efficient method for determining the approximate amount of runoff from a rainfall even in a particular area [29]. In this stage of study the runoff was obtained with using rainfall, hydrologic soil groups and landuse/landcover data. Landuse/landcover data was downloaded of CORINE 2012 dataset. The runoff formula shown below.

**Formula 1:**
\[ Q = \frac{(P - 0.2S)^2}{(P + 0.8S)} \]
\[ S = \frac{25400}{CN - 254} \]

In formula Q surface runoff value (mm); P rainfall (mm); S water retention potential (mm); CN surface runoff curve number were symbolised. Curve number value changes depending on landuse/landcover and hydrologic soil property. As curve number value increases, surface runoff increases and when it decreases, surface runoff decreases in the area.

(v) **Soil erosion risk.** The MAPA/ICONA [30] method developed by the Ministry of Agriculture, Nature Conservation and General Directorate of Agriculture in Spain was used to determine the risk of erosion in the study area. Soil erosion risk according to this method is demonstrated by the soil conservation level and the erodibility degree. The slope groups and the geological structure were reclassified (Table 5) according to the ICONA classification for the erodibility analysis. Slope degree are reclassified as (i) %0-5, (ii) %5-10, (iii) %10-15, (iv) %15-20 and (v) >%20.

Geologic structure of study area was categorized by means of MAPA/ICONA classification method. Erodibility degree was mapped in the five different violence degree as a result of Table 6 that ICONA rock reclass and slope groups are overlayed.

The another component of soil erosion risk is soil conservation level that produced with the aid of slope groups and landuse/landcover map. Slope groups was used same as erodibility analysis. Additionally, landuse/landcover category and index based on IFIE-Sección de Hidráulica Torrencial del Antiguo Instituto Forestal de Investigaciones y Experiencias (1968). This category is compiled i) shrub/maquis, (ii) bareground-at least vegetation, (iii) grassland, (iv) agriculture, (v) deciduous forest and (vi) coniferous forest. Soil conservation index was generated via landuse/landcover and slope groups overlaying (Table 7). In the finally phase for soil erosion risk analysis was mapped through overlay analysis with result of erodibility and soil conservation maps (Table 8).

<table>
<thead>
<tr>
<th>Geology structure</th>
<th>ICONA Rock Class</th>
<th>Geology structure</th>
<th>ICONA Rock Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglomerate</td>
<td>Limestone</td>
<td>Consolidated calcareous rock</td>
<td></td>
</tr>
<tr>
<td>Alluvial</td>
<td>Coglomerate</td>
<td>Compact siliceous rock</td>
<td></td>
</tr>
<tr>
<td>Andesite</td>
<td>Coglomerate-Sandstone-Mudstone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andesite-Basalt</td>
<td>Sandstone-Mudstone-Limestone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granite-Granodiorite</td>
<td>Sandstone-Mudstone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monzonite-Diorite</td>
<td>Clay limestone</td>
<td>Soft formation</td>
<td></td>
</tr>
<tr>
<td>Ophiolite</td>
<td>Shale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spilite</td>
<td>Massive rock</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trachyte</td>
<td>Alluvial fan</td>
<td>Clay, Sand and Quaternary deposit</td>
<td></td>
</tr>
<tr>
<td>Tufa</td>
<td>Scree-Cone of dejection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tufa-Pyroclastic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volcanic sediment</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 6**

<table>
<thead>
<tr>
<th>ICONA rock reclass and slope groups overlay criteria within the context of erodibility [9, 15]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICONA Rock Class</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>0-5</td>
</tr>
<tr>
<td>Massive rock</td>
</tr>
<tr>
<td>Consolidated calcareous rock</td>
</tr>
<tr>
<td>Compact siliceous rock</td>
</tr>
<tr>
<td>Soft formation</td>
</tr>
<tr>
<td>Clay, Sand and Quaternary deposit</td>
</tr>
</tbody>
</table>

Erodibility degree: 5-very high, 4-high, 3-medium, 2-low, 1-very low
TABLE 7
Soil conservation index table of landuse/landcover classification and slope groups [9, 15]

<table>
<thead>
<tr>
<th>Landuse/Landcover class</th>
<th>Slope groups (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5</td>
</tr>
<tr>
<td>Shrub / Maquis</td>
<td>1.0</td>
</tr>
<tr>
<td>Bareground/ at least vegetation</td>
<td>0.9</td>
</tr>
<tr>
<td>Agriculture</td>
<td>1.0</td>
</tr>
<tr>
<td>Deciduous forest</td>
<td>1.0</td>
</tr>
<tr>
<td>Coniferous forest</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Soil conservation index: 1.0-very high, 0.9-0.8-high, 0.7-0.6-medium, 0.5-0.3-low, 0.2-0.0-very low

TABLE 8
Erodibility and soil conservation overlay criteria within the context of potential erosion risk [9, 15]

<table>
<thead>
<tr>
<th>Erodibility</th>
<th>Soil conservation level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very low</td>
</tr>
<tr>
<td>Very low</td>
<td>4</td>
</tr>
<tr>
<td>Low</td>
<td>4</td>
</tr>
<tr>
<td>Medium</td>
<td>5</td>
</tr>
<tr>
<td>High</td>
<td>5</td>
</tr>
<tr>
<td>Very High</td>
<td>5</td>
</tr>
</tbody>
</table>

Potential erosion risk degree: 5-very high, 4-high, 3-medium, 2-low, 1-very low

TABLE 9
The dataset of natural landscape components determining the types of landscape characters [15]

<table>
<thead>
<tr>
<th>Landuse/Landcover</th>
<th>Code</th>
<th>Stand map</th>
<th>Code</th>
<th>Elevation</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban (High density)</td>
<td>1</td>
<td>Degraded high forest</td>
<td>1</td>
<td>685-1203</td>
<td>1</td>
</tr>
<tr>
<td>Urban (Low density)</td>
<td>2</td>
<td>High forest</td>
<td>2</td>
<td>345-685</td>
<td>2</td>
</tr>
<tr>
<td>Water surface</td>
<td>3</td>
<td>Normal degraded coppice</td>
<td>3</td>
<td>150-345</td>
<td>3</td>
</tr>
<tr>
<td>Bareground</td>
<td>4</td>
<td>Degraded coppice</td>
<td>4</td>
<td>0-150</td>
<td>4</td>
</tr>
<tr>
<td>Deciduous forest</td>
<td>5</td>
<td>Degraded high forest/ Degraded coppice</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grassland</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coniferous forest</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agriculture</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scrub/Maquis</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Great Soil Groups</th>
<th>Code</th>
<th>Rock permeability</th>
<th>Code</th>
<th>Slope</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chestnut soil</td>
<td>1</td>
<td>Semi-permeable</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Limeless brown forest soil</td>
<td>2</td>
<td>Impermeable</td>
<td>2</td>
<td>15-20</td>
<td>2</td>
</tr>
<tr>
<td>Brown forest soil</td>
<td>3</td>
<td>Permeable</td>
<td>3</td>
<td>10-15</td>
<td>3</td>
</tr>
<tr>
<td>Alluvial soil</td>
<td>4</td>
<td></td>
<td>4</td>
<td>5-10</td>
<td>4</td>
</tr>
<tr>
<td>Rendzina soil</td>
<td>5</td>
<td></td>
<td>5</td>
<td>0-5</td>
<td>5</td>
</tr>
<tr>
<td>Colluvial soil</td>
<td>6</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red brown mediterranean soil</td>
<td>7</td>
<td></td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red mediterranean soil</td>
<td>8</td>
<td></td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Integrated function analysis. The main objective of the integrated function analysis, the last stage of the functional analysis, is to conservation the landscape regardless of the risk or potential values of the landscape. When conducting integrated function analysis of Izmir, Urla, Cesme and Karaburun districts, the soil erosion risk, water permeability and habitat value components of the study area were utilized. Thus, conservation value of the landscapes of the study area was determined. The soil erosion risk and water permeability maps were reclassified to high, medium and low. Potential erosion risk, water permeability and habitat value maps were overlaid using GIS technologies and an integrated function analysis map was produced.

Landscape character analysis. At this stage of the study, natural landscape components that characterize the area and studies of [9, 16, 18], were taken as basis to determine landscape character types. Landuse/landcover, elevation, slope, forest stands, great soil groups and rock permeability components were used in the generation of landscape character types. An overlay analysis was performed using the six components shown in Table 9. For make easier of overlay and cluster analysis, each of the natural landscape components shown in Table 9 was grouped and re-coded.

Cluster analysis was performed to reduce the number of landscape character types generated and to group similar features as a result of the overlay analysis. The SPSS 24 statistical program was used and the clustering study was completed by the K-means method in cluster analysis process [15].
RESULT

In order to determine the structure, function and change of the landscape in the study area, to be able to identify possible landscape threats and to create a nature and natural disaster sensitive planning approach in this direction, based on the ecological characteristics of the area within the landscape inventory and character analysis study; landscape function analysis, integrated function analysis and landscape character types were specified.

Landscape function analysis. The most important step in determining the landscape and ecosystem of the study area is the landscape function analysis in which the relationship between natural and socio-cultural processes is revealed concert with the natural environment.

(i) Visual-aesthetic-perceptual landscape quality. For the visual-aesthetic-perceptual landscape quality components reflecting the socio-cultural landscape function, IDW analysis was applied and the results are given in Figure 4 that based on landscape survey and expert knowledge. According to result, the visual quality of the landscape along the Ildir district and its coast, on the north-western side of the study area, was determined at a high level. In addition to the highway section in the southern part of the area, was determined at a high level. In addition to the highway section in the southern part of the area, visual quality decreases in Urla and Cesme regions (Figure 4a).

Contrary to visual landscape quality, aesthetic and perceptual quality values are observed to increase towards urbanized landscapes instead of rural landscapes (Figure 4b). Aesthetic landscape quality has been obtained highly especially at Cesme and Urla districts, Karakoy, Germiyan, Nohutalan and Uzunkuyu villages. Perceptual landscape quality, on the other hand, did not show similarity with the aesthetic quality, but it showed an expansion in the Alacati district (Figure 4c). The lowest aesthetic and perceptual landscape quality was observed in the higher elevation sections of Karaburun district.

(ii) Habitat value. According to umbrella species habitat requirement, large part of the study area has highly suitable conditions. Besides, places with high habitat values are distributed intensively in Karaburun province, especially in settlements such as Akgazi and its surroundings, Kucukbahce, Parlak, Sarpincik, Yaylakoy, Yukariovacik and Asagiyovacik (Figure 5).

(iii) Water permeability. The production of the water permeability map contributes to the determination of the areas to be conserved due to their ecological characteristics in the study area. The two basic parameters of water permeability are the geological structure of the area with hydrological soil characteristics. Semi-permeable rocks are mostly found in the town of Cesme, permeable rocks in Karaburun province and impermeable rocks in Urla province dominantly. In the study area, low and medium water permeability is vastly dominant (Figure 5). Kucukbahce, Salman, Parlak, Sarpincik, Inecik, Kosedere, Eglenhoca settlements in Karaburun province; Urla settlements including the southern parts of Kadiovacik, Nohutani, Barbaros and Uzbek are low water permeability areas.

(iv) Surface runoff potential. According to the surface flow potential analysis carried out within the working area limits, there is a low surface runoff potential (Figure 5). Areas with a low surface runoff potential are; Saip, Ambarski, Kosedere, Eglenhoca, Mordogan, Asagiyovacik, Yukariovacik settlements in the coastal areas of Hasak, Karaburun; Ildir, Germiyan, Ovacik and Dalyan coastal areas of Cesme county; Nohutani, Barbaros, Uzbek, Kuscular, Kucukbahce, Guvendik and western parts of Mentes in Urla province. Areas with a high surface runoff potential are seen in settlement areas such as Kucukbahce, Salman, Parlak, Sarpincik, Hasaki, Parlak, Yaylakoy, Alacati, Ressere. At the same time, these areas with high surface runoff are areas for which significant surface runoff control is envisaged.
(v) Soil erosion risk. The erodibility degree and soil conservation levels are the determining factors in the production of the potential erosion risk. According to the erosive properties of rocks, there are five different rock erosion conditions in the study area, very low, low, medium, high and very high. Places where erodibility is high are generally areas of massive rocks, compacted siliceous rocks, soft formations and clay, sand and quaternary deposits as the geological structure where the degree of inclination is high. The least abundant erodibility in the area is the areas where rocks with very severe erosion extent are found. These areas are areas where the slope is low and the geological structure is formed by very hard rocks of massive rocks.

According to the result of soil conservation surface analysis which is another indicator of soil erosion risk, a high grade soil conservation surface is observed. When the level of soil conservation and soil erosion risk in Urla, Cesme and Karaburun provinces are associated; places where the level of soil conservation is low have a disadvantage of erosion, whereas those with high soil conservation level are interpreted as places where the risk of erosion is advantageous. According to the analysis of the soil erosion risk with the obtained findings; the erosion risk of the rocks is severe high and very high, having a very low soil conservation level, the risk of erosion is very high. In the study area, Akdag and its surroundings are areas where the soil erosion risk is severe high and very high due to the level of soil conservation, both in terms of slope degree. Areas with medium to high (severe) soil erosion risk appear to be less visible in the study area, and very low and low erosion dominates the Urla, Cesme and Karaburun districts (Figure 5).

Integrated function analysis. During the integrated function analysis of Urla, Cesme and Karaburun provinces soil erosion risk permeability and habitat value have been used as variables. In the study area, the places with the high function value are mostly found, and followed by medium function value. The study area places that has low function value in the area are the least found. The places with high function values are concentrated in settlements such as Kucukbahce, Parlak, Sarpincik, Yaylakoy, Yukariogacik, Asagiogacik, Ambarseki, Ildir and Ozbek (Figure 6).
Landscape character analysis. In the study, the six different components given in Table 9, the landuse/landcover, elevation, slope, forest stand type, great soil groups and rock permeability, which represents the characteristic features of the area, are grounded on while producing the landscape character type. The combination of the attributes of six different components resulted in 66368 number of character types output. The attributes are reduced by cluster analysis on the basis of the separating variables to reduce the combinations obtained at a very high level as a result of the combination. As a result of cluster analysis with k-mean techniques, the number of clusters was determined as eight. There are 126 landscape character types in the 1st cluster, 90 in the 2nd, 266 in the 3rd, 210 in the 4th, 232 in the 5th, 170 in the 6th, 80 in the 7th and 115 in the 8th. A total of 1289 different landscape character types have been obtained, which are reduced in number (Figure 7).
DISCUSSION AND CONCLUSION

Landscape character became a new paradigm, as well as time depth and landscape change trajectory or path (32). In presenting landscape inventory and character analysis, a planning approach should be adopted that is sensitive to ecology, environment and natural disasters, appropriate to the conservation and use balance of natural and socio-cultural components or elements that forms a landscape. In addition to some landscape pressure like urban growth, loss of agricultural area, increase of road network, demand for natural areas (33) disasters due to climate change causes alter landscape characters. There are few studies on the use of Landscape Character Assessment in the determination of landscape disasters and the development of measures (34, 35) However, it is an effective tool for producing solutions for landscape disasters.

Planning and monitoring of landscapes cannot be reduced to its outstanding features, but must take into account all its characteristics (36). In other words, landscape character analysis studies to be carried out throughout the country should be considered within the framework of an ecological-based understanding and approach to physical planning. Furthermore, the lack of ecological-based physical plans will lead to the formation of landscapes that have major problems within themselves and have a distorted cycle, thus making these physical plans will reveal more reliable results in character analysis studies. Herewith the fact that ecological-based physical planning approaches are pre-screened in landscape character analysis studies will allow a better understanding of the natural processes that make up the landscape.

In the light of increasing landscape changes, many national agencies have developed sophisticated Landscape Character Assessment tools that are scientifically sound, region-specific and stakeholder oriented – qualities that are considered as key issues for the future implementation of the EU policy and research agenda as well (37). This brings with a sensitive perspective on landscape scale and hierarchy in landscape character analysis studies to be done in our country. Because a hierarchical method is needed to solve problems that occurred or may occur on the scale being studied, and an ecological physical planning should be considered in order to provide solutions on the upper and lower scales and sustain natural resources. Many experts have emphasized the need to adopt a holistic approach in spatial planning studies (9, 38, 39). Although a number of countries have developed typologies for characterising landscapes, the examples illustrate that existing national approaches vary in terms of their methodologies and objectives. The national concepts should be ’nested’ with a hierarchical system of scales that build upon each other: regional, national, and European units should be part of one and the same methodological system (37).

This study includes a proposal model that can be used in landscape inventory and character analysis studies in our country. The landscape inventory of the study area is composed of natural, socio-cultural and visual elements, and the landscape character is composed of natural and socio-cultural elements. The interaction of these elements over time and the reflection of this interaction on the field were tried to be revealed. In addition, several landscape functions were analyzed. Functional analyzes, which are handled in two stages as regulatory and supportive, will help in the determination of the potential landscape disasters of the area. With similar studies could prevent the deterioration of ecosystems or landscape balances, at the same time, any precaution could be met in advance.

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STUDY ON A NEW PROCESS OF SYNTHESIS OF OMEPRAZOLE-LIKE AGENTS

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ABSTRACT

A new series of novel benzimidazole derivatives containing substituted pyrid-2-yl moiety and polyhydroxy sugar conjugated to the N-benzimidazole moiety has been synthesized and evaluated as orally bioavailable anti-inflammatory agents with anti-ulcerogenic activity. Effect of catalyst dosage, reaction temperature and reaction time on molar yield is investigated. Response surface methodology is applied to examine the relationship between each factor. The results show that reaction temperature is an important factor. Increasing catalyst dosage and reaction time can increase the molar yield. The optimal conditions are followed: temperature is 90°C, reaction time is 12 h, and catalyst dosage is 5 g.

KEYWORDS:
Omeprazole, 2-Methyl-1H-benzimidazole, Anti-ulcerogenic activity, catalyst; molar yield

INTRODUCTION

Gastrointestinal toxicity is the most common adverse effect of the currently available non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, indomethacin, and naproxen. Such adverse effects are manifested by dyspepsia, ulcers, or bleeding [1-3]. The gastrointestinal damage from NSAIDs is generally attributed to two factors; first, the local irritation by carboxylic acid group present in many NSAIDs (topical effect); second, the decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining gastrointestinal integrity and homeostasis [4]. The pharmacology of NSAIDs is linked to the inhibition of prostaglandin biosynthesis from arachidonic acid by inhibiting cyclooxygenases [5]. Therefore, patients treated with NSAIDs for long periods of time, may suffer from noticeable gastrointestinal toxicity. Consequently, synthetic approaches based on chemical mimicking NSAIDs have been taken with the aim of improving its safety profile. The concurrent use of NSAIDs with gastric proton pump enzyme (H+/K+-ATPase) inhibitors represents a major approach to minimize such adverse effects. Many pharmaceutical companies have spent considerable efforts in the identification of irreversible and reversible inhibitors of the H+/K+-ATPase. Substances belonging to the class of irreversible inhibitors are called proton pump inhibitors (PPIs) [6-8] such as omeprazole, lansoprazole, pantoprazole, rabeprazole and esomeprazole. Omeprazole is a member of the proton pump inhibitor family of drugs, extensively used in treatment of peptic ulcer, gastroesophageal reflux disease and Zollinger-Ellison syndrome. The fact that it is one of the drugs indexed in the World Health Organization's List of Essential Medicines points out the importance of enhancing the synthetic procedure used in the production of this pharmaceutical [9, 10].

Many of PPIs are benzimidazole derivatives [11, 12] which consist of two fragments of benzimidazole and pyridine. These PPIs act as prodrugs owing to protonation of the pyridine ring under the gastrointestinal acid environment, resulting in a chemical rearrangement which forms sulfenic acid then sulfonamide by dehydration. The active enzyme inhibitor is either the sulfenic acid or the sulfonamide which reacts with cysteine residues of the H+/K+-ATP enzyme (Fig. 1). [13, 14, 15].

The unique structural features and pharmaceutical activities of benzimidazoles have encouraged us to synthesize novel orally bioavailable 2-methyl-N-substituted benzimidazole sugar conjugates and study its anti-inflammatory and anti-ulcerogenic activities.

EXPERIMENTAL

All chemicals were purchased from common commercial suppliers and used without further purification. Melting points (mp) were determined on a Gallenkamp melting point apparatus and were uncorrected; IR spectra (KBr disks) were recorded on Bruker Vector 22 instrument. 1H and 13C NMR spectra were recorded on a Jeol ECX-spectrometer
FIGURE 1
Mechanism of acid transformation of 2-[(2-pyridylmethyl)sulfinyl]benzimidazole derivatives.
First, the gastric acidity causes protonation of the pyridine ring into intermediate, b. Then, a chemical rearrangement forms sulfenic acid c, which forms sulfonamide d by dehydration. The active enzyme inhibitor is either the sulfenic acid c or the sulfonamide d which reacts with cysteine of the H+/K+-ATP enzyme [13, 14, 15].

Effect of catalyst dosage and reaction time on molar yield is shown in Fig.2. Over the considered temperature range, molar yield increased. Molar yield increased when catalyst dosage increased.

The catalyst can change the chemical reaction rate (increasing or decreasing) of the reactant in the chemical reaction without changing the chemical equilibrium, and its quality and chemical properties are not changed before and after the chemical reaction. According to statistics, more than 90% of industrial processes use catalysts, such as chemical, petrochemical, biochemical, and environmental protection.

When the reaction temperature is raised, the reaction proceeds in the direction of reducing heat, that is, the exothermic reaction proceeds in the reverse direction, and the endothermic reaction proceeds in the forward direction; it is favorable for the formation of the reactant.
Effect of temperature and reaction time on molar yield is shown in Fig.3. The result shows that prolonging reaction time can increase molar yield. Molar yield increased when temperature increased.

Over the considered temperature range, the molar yield of 81.8%, 87.2%, 46.5% and 39.1% was observed for the temperature of 60, 80, 100, and 120°C, respectively.

Effect of catalyst dosage and reaction time on molar yield is shown in Fig.4. Effect of residence time is another important and crucial parameter on molar yield. The molar yield was studied by varying the synthesis process reaction time in the range of 3–15 h. It can be seen from Fig. 3, the reaction time has a strong effect on the molar yield. When reaction time increased from 3 to 15 h, the molar yield enhanced from 39.2% to 99.4%.
Objective function of Minitab’s response surface provides an intuitive tool for objective optimization and its unique response optimizer is a powerful tool for multi-objective problem to the solution encountered in the experimental design. And the target function is optimized by Minitab’s response optimizer. The result is shown in Fig. 5. It is calculated that the optimized conditions are present: temperature is 90°C, reaction time is 12 h, and catalyst dosage is 5 g.

CONCLUSIONS

A new series of novel benzimidazole derivatives containing substituted pyrid-2-yl moiety and polyhydroxy sugar conjugated to the N-benzimidazole moiety has been synthesized and evaluated as orally bioavailable anti-inflammatory agents with anti-ulcerogenic activity. Effect of catalyst dosage, reaction temperature and reaction time on molar yield is investigated. Response surface
The methodology is applied to examine the relationship between each factor. The results show that reaction temperature is an important factor. Increasing catalyst dosage and reaction time can increase the molar yield. Optimization result is calculated that the optimized conditions are present: temperature is 90°C, reaction time is 12 h, and catalyst dosage is 5 g.

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A NEW UNSTEADY PRODUCTIVITY PREDICTION METHOD OF FRACTURING HORIZONTAL WELLS IN LOW-PERMEABILITY GAS RESERVOIRS

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ABSTRACT

This study derived a new gas plane radial unsteady seepage formula, which is adaptable to even when the start-up pressure gradient introduced, which is unprecedented. In previous similar models, the scholars used unstable seepage formulas were derived under the traditional instantaneous point sink model where the planar radial flow is unsteady; but this formula would work is based on the assumption that the outer boundary conditions for the formation is infinite. However, this formula does not work when introducing the start-up pressure gradient. To solve this problem, we changed the boundary conditions, that is, outside the boundary condition is infinite formation changed to the circular constant pressure boundary. On this basis, the start-up pressure gradient is introduced into the unsteady seepage flow equation, which is adaptable to the reservoir environment. In the derivation, gas wells are not seen as a point sink. When \( r_w \rightarrow 0 \), the gas plane radial unsteady flow formula of instantaneous point sinks in circular formation is obtained, with taken the introduction of the start-up pressure gradient into consideration. Based on the new formula to deduce a new unsteady productivity prediction model. Furthermore, the high-speed non-Darcy effects and the stress sensitivity effect are added to the model.

KEYWORDS:
Low-permeability gas reservoir, fractured horizontal wells, productivity test, unstable seepage, start-up pressure gradient

INTRODUCTION

Many scholars have conducted research on theories explaining the production-forecasting model in fractured horizontal wells for gas reservoirs and other types of reservoirs [1-6]. In a steady-state productivity research based on the study of Fan Zifei, who derived a steady-state solution formula for rectangle reservoirs with vertical fractures, the steady-state solution with horizontal well is the deduced formula in a fractured reservoir [7]. Meanwhile, the work of Ning Zhengfu is based on the Lang Zhaoxin model, which considers the flow resistance and the pressure loss in the fracture re-derive, and corrects the fractured horizontal well steady-state production forecasting formulas [2, 8]. Sun Fujie and Han Shugang based their study on the steady-state solution of fractured horizontal wells in reservoirs, which considers the gas flow in the formation and horizontal wellbore pipe coupling to establish the corresponding model and solution [9, 10]. In studies on the steady-state productivity of fractured horizontal wells, Zhu Shiyian [11] considered the injection of the formation fluid directly into the horizontal wellbore, Zhang Yanming [12] considered inducing pressure drop through the loss of momentum and pipe wall from friction, Hu Yongquan [13, 14] considered the high-speed non-Darcy flow, and Jiang Kai considered fracture morphology.

However, no study has yet to consider using the start-up pressure gradient for a low-permeability gas reservoir; thus, high-speed non-Darcy flow effect and stress sensitivity of these factors are taken into account in the unstable production model in the current study. The unstable productivity prediction model for horizontal wells derived by previous scholars ruled out the start-up pressure gradient condition. The reason for not considering start-up pressure gradient might be that it leads to no solution by using the previous unstable seepage formula, after introducing the pressure gradient, and if the external boundary is not changed. We consider the work of Hao Fei to establish the start-up starting pressure gradient of an unstable seepage model derivation process in low-permeability reservoirs [15]. First, a new unstable production forecasting model of a fractured horizontal well in gas reservoir is deduced to build a plane radial unstable percolation model permeability gas reservoir, which considers the start-up pressure gradient. This gas
reservoir will further consider high-speed non-Darcy effect and stress sensitivity effect.

**EXPERIMENTAL**

**Start-up pressure gradient in the plane radial unsteady seepage equation.** Hypothesis: A circular reservoir is homogeneous and thick, and the upper and lower layers are closed with a radius of \( R_e \); the boundary is a constant pressure boundary; the gas center has a radius of \( r_w \) point sink, and is produced at a constant gas production \( Q \); formation pressure is equal before production; and \( p \) is the formation original pressure and is permeability. The seepage state can be classified as isothermal unsteady seepage. Here, the influence of gravity is not considered.

For the real gas, the equations is given by

\[
\frac{\partial^2 (p^2)}{\partial x^2} + \frac{\partial^2 (p^2)}{\partial y^2} + \frac{\partial^2 (p^2)}{\partial z^2} = \frac{\phi_0 C_i}{K} \frac{\partial (p^2)}{\partial t}. \tag{1}
\]

\( C_i \) is the isothermal compressibility of natural gas in the above equation. \( \eta \) is the real gas pressure coefficient, its expression is \( \eta = \frac{K}{\phi_0 C_i} \). In the polar coordinates, the formula (1) can be transformed into

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial p}{\partial r} \right) = \frac{\partial p}{\eta \partial t}. \tag{2}
\]

\[
\eta = \frac{K}{\phi_0 C_i}. \tag{3}
\]

After the introduction of pseudo pressure \( \Phi = 2\int \frac{p}{\mu Z} dp \), the above equations become

\[
\frac{\partial^2 \Phi}{\partial r^2} + \frac{1}{r} \frac{\partial \Phi}{\partial r} = \frac{1}{\eta} \frac{\partial \Phi}{\partial t}. \tag{4}
\]

Integrating the start-up pressure gradient in low-permeability reservoirs seepage equation results in the equation expressed as

\[
1 \left[ \frac{\partial}{\partial t} \left( \frac{1}{r} \frac{\partial p}{\partial r} - \lambda \right) \right] = \frac{1}{\eta} \frac{\partial p}{\partial t}. \tag{5}
\]

After finishing the above formula and combining it with the initial and boundary conditions, we arrive at

\[
\begin{align*}
\frac{\partial^2 p}{\partial r^2} + \frac{1}{r} \left( \frac{\partial p}{\partial r} - \lambda \right) = \frac{1}{\eta} \frac{\partial p}{\partial t} \\
p(r,0) = p_i \\
r \left( \frac{\partial p}{\partial r} - \lambda \right)_{r=r_w} = \frac{Q_w p_w T Z}{2\pi K h T} \\
p(R_e, t) = p_i
\end{align*}
\]

After the introduction of pseudo pressure \( \Phi = 2\int \frac{p}{\mu Z} dp \), the introduction of the start-up pressure gradient is \( \lambda_m = \frac{2p}{\mu Z} \), and then Formula (5) becomes

\[
\frac{1}{r} \left[ \frac{\partial}{\partial r} \left( \frac{\partial \Phi}{\partial r} - \lambda_m \right) \right] = \frac{1}{\eta} \frac{\partial \Phi}{\partial t}. \tag{7}
\]

Considering \( \psi = \Phi - \lambda_m (r - r_w) \), Formula (7) can be changed into

\[
\frac{\partial^2 \psi}{\partial r^2} + \frac{1}{r} \frac{\partial \psi}{\partial r} = \frac{1}{\eta} \frac{\partial \psi}{\partial t}. \tag{8}
\]

Equation (8) combines the initial condition, the inner boundary condition, and outer boundary conditions to obtain

\[
\begin{align*}
\frac{\partial^2 \psi}{\partial r^2} + \frac{1}{r} \frac{\partial \psi}{\partial r} &= \frac{1}{\eta} \frac{\partial \psi}{\partial t} \\
p(r,0) &= \Phi_i - \lambda_m (r - r_w) \\
r \left( \frac{\partial \psi}{\partial r} \right)_{r=r_w} &= \frac{Q_w p_w T}{\pi K h T} \\
\psi(R_e, t) &= \Phi_i - \lambda_m (R_e - r_w)
\end{align*}
\]

Introducing the transform form below

\[
u = \frac{r^2}{4\eta t}, \tag{10}
\]

the equation and the definite condition of Formula (9) are transformed into

\[
\begin{align*}
\frac{d^2 \psi}{du^2} + \frac{d \psi}{du} (1+u) &= 0 \\
2u \frac{d \psi}{du} \bigg|_{u=\frac{r^2}{4\eta t}} &= \frac{Q_w p_w T}{\pi K h T} \\
\psi(R_e, t) &= \Phi_i - \lambda_m (R_e - r_w)
\end{align*}
\]

Assuming that \( \psi' = \frac{d \psi}{du} \) has been established, the two order-differential equation of Formula (11) can be transformed into a one-order re-
duction given by
\[ \frac{d\psi'}{du} + \psi'(1 + u) = 0. \] (12)

After the separation of variables, we arrive at
\[ \frac{d\psi'}{\psi'} = \left( \frac{1}{u} + 1 \right) du. \] (13)

After including the integral available, the formula becomes
\[ \psi' = \frac{d\psi'}{du} = C_1 e^{-u} \] (14)

The boundary condition, \( 2u \frac{d\psi'}{du} \bigg|_{u=\frac{r_c^2}{4\eta}} = \frac{Q_{sc}p_{sc}T}{\pi K h T_c} \), can be used to fix constant \( C_1 \).

By integrating \( C_1 \) into Formula (14), we obtain
\[ \frac{d\psi}{du} = e^{-\frac{r_c^2}{4\eta}} \frac{Q_{sc}p_{sc}T}{2\pi K h T_c} \frac{e^{-u}}{u}. \] (15)

Again the following points are available:
\[ \psi(u, t) = e^{-\frac{r_c^2}{4\eta}} \frac{Q_{sc}p_{sc}T}{2\pi K h T_c} \int_u^{\infty} e^{-\frac{y}{y}} dy + C_2. \] (16)

in which the integration limit can be chosen arbitrarily. To easily determine the constant \( C_2 \), we use the lower limit of integration is \( \infty \), and then arrive at
\[ \psi(u, t) = -e^{-\frac{r_c^2}{4\eta}} \frac{Q_{sc}p_{sc}T}{2\pi K h T_c} \int_u^{\infty} e^{-\frac{y}{y}} dy + C_2. \] (17)

Depending on the outer boundary conditions, when \( r = r_c, \psi(r_c, t) = \Phi_i - \lambda_m (r_c - r_w) \).

\( u = \frac{R^2}{4\eta} \). By substituting them into Formula (17), we arrive at
\[ C_2 = \Phi_i - \lambda_m (r_c - r_w) + e^{-\frac{r_c^2}{4\eta}} \frac{Q_{sc}p_{sc}T}{2\pi K h T_c} \int_{\frac{r_c^2}{4\eta}}^{\infty} e^{-\frac{y}{y}} dy. \] (18)

Next, we calculate \( \lambda_m \approx \frac{2p_r}{\mu \lambda} \), and in a low-permeability gas reservoir, the introduction of start-up pressure gradient at any time and any place for circular formation potential distribution is expressed as
\[ \Phi(r, t) = \Phi_i + e^{-\frac{r_c^2}{4\eta}} \frac{Q_{sc}p_{sc}T}{2\pi K h T_c} \left[ E_i \left( \frac{r^2}{4\eta} \right) - E_i \left( \frac{R^2}{4\eta} \right) \right] - \frac{2p_r}{\mu \lambda} (r_c - r) \] (19)

where \( E_i(-u) = \int_u^{\infty} e^{-\frac{y}{y}} dy \).

In a rectangular coordinate system, if the point sink center is not in the origin, then the coordinates of the point sink \((x_0, y_0)\) and point sink radius \( r_w \) are smaller enough compared with \( R_c \). Therefore, \( r_w \rightarrow 0 \) and Formula (19) can be changed into
\[ \Phi(x, y, t) = \Phi_i + \frac{Q_{sc}p_{sc}T}{2\pi K h T_c} \left[ E_i \left( \frac{(x-x_0)^2 + (y-y_0)^2}{4\eta} \right) - E_i \left( \frac{R^2}{4\eta} \right) \right] - \frac{2p_r}{\mu \lambda} \sqrt{(x-x_0)^2 + (y-y_0)^2} \] (20)

where \( \eta \) is the pressure-transmitting coefficient, \( \eta = \frac{K}{\phi \mu C_g} \), with a unit of \( \mu m^2/MPa/(mPa.s) \), \( r \) is the radius with a unit of \( m \), \( \lambda \) is the start-up pressure gradient with a unit of \( MPa/m \), \( p \) is the pressure with a unit of \( MPa \), and \( t \) is time with a unit is of \( d \).

The gas reservoir fractured horizontal well productivity model. Physical model. In low-permeability gas reservoirs, based on the implementation of staged fracturing in horizontal well technology features, we assume that, after fracturing, horizontal wells produce \( N \) cracks that are perpendicular to the horizontal wellbore. Figure 1 is a schematic view of a physical model of fractured horizontal wells in a gas reservoir.

The assumptions are given below.

1. The circular formation is homogeneous with closed upper and lower bounds, and the outer boundary pressure is constant. The boundary and initial formation pressure is \( p_i \) without considering the influence of gravity.

2. Horizontal well lengths \( L \) form \( N \) vertical cracks after the implementation of staged fracturing, and symmetrically distribute on both sides of the horizontal wellbore. The fracture height equals the thickness of the gas reservoir \( h \).

3. The horizontal wellbore is located on the \( x \) axis, the toe of the horizontal well is located in the origin of coordinates, and the horizontal wellbore from toe to heel, which is divided into \( N \) segments, \( d = L/N \), is the length of each section. The midpoint of each section has a crack.

4. In formation, the gas-flow state is characterized as isothermal, single-phase and unstable seepage, thus corresponding to Darcy’s law.

5. The fluid along the wall-crack surfaces uniformly flow through the cracks in the horizontal wellbore, without considering the fluid directly flowing from the formation into the horizontal wellbore.
RESULTS AND DISCUSSION

Mathematical model. As shown in Figure 2, each crack of the left and right parts are divided into \( n \) equal parts. The crack of each equal part is treated as a point sink for research.

Calculation of the pseudo pressure at any point considering the mutual interference of the fractures in the formation. The coordinates of the center of the upper wing in the point sink \( j \) of crack \( i \) are expressed as

\[
(x_{ij}, y_{ij}) = \left( \frac{2i - 1}{2}, \frac{L}{N}, \frac{2n - 2j + 1}{2n} \right) X_{ji},
\]

\( j = 1, 2, \ldots, n \).

where \( X_{ji} \) is the crack half-length of crack \( i \) with a unit of m.

Similarly, the coordinates of the center of the under wing of the point sink \( j \) of crack \( i \) are expressed as

\[
(x_{bij}, y_{bij}) = \left( \frac{2i - 1}{2}, \frac{L}{N}, \frac{2j - 1}{2n} \right) X_{ji}.
\]

\( j = 1, 2, \ldots, n \).

Based on the start-up pressure gradient of the unsteady gas flow expressed in Formula (20), the production of point sink at time \( t \) is \( Q_{ij} \), and coordinates of \((x, y)\) to the formation of arbitrary point \((x, y)\) generated pseudo pressure are expressed as

\[
\Phi(x, y, t) = \ldots
\]
The principle of potential superposition can be known when the article crack \( i \) separates from the product at time \( t \) in the formation at an arbitrary point \((x, y)\), which is generated at the total pressure given by

\[
\Phi_i + \frac{q_{fisc} p_{fisc}}{2 \pi k H T_w} E_i \left\{ \frac{(x-x_0)^2 + (y-y_0)^2}{4\eta} \right\} - E_i \left( \frac{R_c^2}{4\eta} \right) - \frac{2 p_i}{\mu Z} \left[ R_c - \sqrt{(x-x_0)^2 + (y-y_0)^2} \right]
\]

(21)

where

\[
\Phi = 2 \int \frac{P_i}{\mu Z} \, dp
\]

\( P \) is the original formation pressure with a unit of MPa; \( P \) is the pressure at any point \((x, y)\) in the formation at time \( t \), with a unit of MPa; and \( P_{sc} \) is the pressure on the ground under the standard condition (0.101 MPa in this study). This study uses the following parameters:

- \( T \) is the formation temperature with a unit of K;
- \( T_{sc} \) is the ground temperature under standard conditions (293.15 K in this study);
- \( Z \) is the gas deviation factor;
- \( \mu \) is the gas viscosity with a unit of mPa.s;
- \( K \) is the formation permeability with a unit of \( \mu m^2 \);
- \( h \) is the gas reservoir thickness with a unit of m;
- \( t \) is seepage time with a unit of \( d \);
- \( \eta \) is the formation diffusivity coefficient;
- \( \eta = K/(\phi \mu C_v) \) with a unit of \( \mu m^2 \cdot MPa/(mPa \cdot s) \);
- \( \phi \) is the formation porosity;
- \( C_v \) is the total compressibility with a unit of MPa;
- \( q_{fisc} \) is the production of the point sink \( j \) of crack \( i \) in the horizontal well under the standard conditions with a unit of m\(^3\)/d;
- \( L \) is the length of the horizontal well bore with a unit of m;
- \( N \) is the number of fractures;
- \( \lambda \) is the start-up pressure gradient with a unit of MPa/m;
- \( R_c \) is the gas supply radius with a unit of m;
- \( X_{\beta} \) is the half-length crack \( i \) with a unit of m;

\[
 q_{fisc} = \frac{q_{fisc}}{2 n}, \text{ then } q_{fisc} \text{ is the production of crack } i.
\]

Horizontal wells have \( N \) cracks, and the \( N \) crack production at the same time in time \( t \) of the formation has an arbitrary point \((x, y)\). Thus, the total pseudo pressure is given by

\[
\phi(x, y, t) = \sum_{i=1}^{N} \frac{q_{fisc} p_{fisc}}{2 \pi k H T_w} E_i \left\{ \frac{(x-x_0)^2 + (y-y_0)^2}{4\eta} \right\} - \sum_{i=1}^{N} \frac{2 p_i}{\mu Z} \left[ R_c - \sqrt{(x-x_0)^2 + (y-y_0)^2} \right]
\]

(22)

\[
\Phi = 2 \int \frac{P_i}{\mu Z} \, dp
\]

(23)

Consideration of the high-speed non-Darcy effect in the fractures. Formation pressure is re-distributed because of the artificial cracks in the horizontal well bore. According to the previous hypothesis, the upper and lower wings of the crack vertical symmetry distribution on both sides of the horizontal well bore and the crack tip in the tow wings have equal pressures. With the lower wing crack tip pressure as the crack tip stress \( p_{t} \), the coordinate of lower wing crack tip of the crack \( i \) is given by

\[
(x_{bin}, y_{bin}) = \left( \frac{(2i-1)}{2} \frac{L}{N}, \frac{(2n-1)}{2n} X_{\beta} \right).
\]

At time \( t \), the pseudo pressure is at the lower wing crack tip of crack \( i \), which is expressed as
\[ \Phi_i = nN\Phi_f, \]

\[ R = \sqrt{2X_\beta/\pi}. \]

The gas flow process from the fracture to the wellbore can be expressed as

\[ \Phi_f - \Phi_{nf} = \frac{q_{flu}}{\pi K_f \delta T_{we}} \left( \ln \frac{\sqrt{2X_\beta h/\pi}}{r_w} + s \right), \]

where \( q_{flu} \) is the production of crack \( i \) under standard conditions with a unit of \( m^3/d \); \( K_f \) is the fracture permeability with a unit of \( m^2 \); \( \delta \) is the crack width with a unit of \( m \); \( r_w \) is the radius of the horizontal wellbore with a unit of \( m \); and \( s \) is the skin factor.

For high-production gas wells, formation and fracture make it likely that the high-speed non-Darcy effects would be produced; this effect is also called the “turbulence effect.” When the formation pressure is not too high and the production pressure is not too high, the formation of the high-speed non-Darcy effects can be ignored, as in the case when the high-speed non-Darcy effects occur only in the fracture. Therefore, the pressure drop of the high-speed non-Darcy effect mainly exists in the cracks. Similar to the treatment of skin effect, high-speed non-Darcy effect can be described using the skin factor \( D_{flu} \); hence, Formula (26) can be re-written as

\[ \Phi_f - \Phi_{nf} = \frac{q_{flu} p_{\nu}T}{\pi K_f \delta T_{we}} \left( \ln \frac{\sqrt{2X_\beta h/\pi}}{r_w} + s + D_{flu} \right), \]

where

\[ D = 2.191 \times 10^{18} \frac{By_s K_f}{\mu \omega_{we}}, \gamma_g \]

\[ \gamma_g = \frac{\rho_g}{\rho_{air}}, \text{ and } \beta \]

is the relative density of gas in the air, and \( \beta \) is a description of the turbulent influence coefficient of fracture with a unit of \( m^4 \).

The non-Darcy effect coefficient \( \beta \) has a variety of descriptions. To describe this coefficient, this study uses the Jones formula [16] given by

\[ \beta = 2.0127 \times 10^{11} / K_f^{1.55}. \]  

Given that the gas in the horizontal wellbore pressure loss is less than that in the matrix, it can be approximated that the pressures at the bottom of the crack and the proposed horizontal well pressure are equal, that is,

\[ \Phi_{nf} = \Phi_{nf}. \]

Formulas (27) and (24) can be obtained when considering the pressure gradient and high-speed non-Darcy effects, along with the fluid flow from the gas reservoir through the crack, which flows into the wellbore formula seepage flow process. This is expressed as

\[ nN\Phi_i - \Phi_{nf} = \]

\[ + \frac{q_{flu} p_{\nu}T}{\pi K_f \delta T_{we}} \left( \ln \frac{\sqrt{2X_\beta h/\pi}}{r_w} + s + 4.4096 \times 10^{-7} \gamma_{fl} K_s^{0.55} / \mu \omega_{we} q_{flu} \right), \]

where \( \Phi \) is a pseudo pressure at arbitrary point \((x, y)\) in the formation at time \( t \), and

\[ \Phi_i = 2 \int \frac{p}{\mu \omega} dp, \quad \Phi_{nf} = 2 \int \frac{p_{nf}}{\mu \omega} dp. \]

When \( \mu \omega \) is a constant,

\[ \Phi_{nf} = \frac{p_{nf}}{\mu \omega}; \]

hence, Formula (28) can be changed into

\[ nNp_i^2 - p_{nf}^2 = \]

\[ \frac{q_{flu} p_{\nu}T}{\pi K_f \delta T_{we}} \left( \ln \frac{\sqrt{2X_\beta h/\pi}}{r_w} + s + \right. \]
The sensitivity coefficient with a unit of MPa$^{-1}$ is known according to the given time.

When fluid from gas reservoir after the fracture, the high-speed (30) when the low-permeability gas reservoir considers the starting pressure gradient, the high-speed non-Darcy effect, and the near wellbore area of the non-Darcy effect.

Using MATLAB (Matrix laboratory), each element of all micro-cracks in the segment is a cumulative sum, whereas the same crack production coefficients are combined to produce the following: the coefficient matrix equations and the solutions of nonlinear equations using the Newton iteration method. The nonlinear equations contain $N$ unknowns and $N$ equations, and the iterative initial value is 0, thus the permissible error value is 1.0x10$^{-6}$. Crack production will finally obtain another sum, and produce yield $Q_{nc}$ for the fractured horizontal well.

Model validation. In this study, we examined a fractured horizontal well in the He 8 layer of Su 14 Well Area, which is located in the Sulige Gas Field. The basic parameters of the horizontal well are as follows:

- average thickness, 8 m;
- average reservoir permeability, 0.93x10$^{-3}$ mm$^2$;
- supply radius, 1500 m;
- average reservoir porosity, 8.2%;
- start-up pressure gradient, 0.00025 MPa/m;
- well bore radius, 0.076 m;
- horizontal well length, 900 m;
- formation temperature, 388.742 K;
- original formation pressure, 26.161 MPa;
- bottom-hole pressure, 12.918 MPa;
- compression coefficient, 0.03133 MPa$^{-1}$;
- relative gas density, 0.6056;
- gas deviation factor, 0.9847;
- gas viscosity, 0.02229 mPa.s;
- skin factor, 0.031;
- crack width, 0.009 m;
- production time, 30 d;
- fracture permeability, 60 mm$^2$; and
- actual output, 8.0618x10$^{4}$m$^3$/d.

In addition, the horizontal wells were formed after fracturing 6 cracks, with the crack length being 20 m. This productivity prediction method is used to calculate the capacity of the horizontal wells after fracturing and the fracture number from the toe end start number. The prediction method is also used to obtain the production histogram of the crack (Figure 3).

As shown in Figure 3, the end of the fracture horizontal wells (the first and the sixth cracks) has the highest production. The production on the second and the fifth cracks is lower than the production on the first and sixth cracks. Moreover,
the production on the third and the fourth crack is the lowest. Production is symmetric because the horizontal well center is in the center of symmetry, and both ends of the horizontal well fracture have a great discouraged area. Thus, we recommend that the fracturing should increase the crack length near the end, thus leading to a good yield-increasing effect.

The calculated gas production $Q_c$ is $8.1367 \times 10^4$ m$^3$/d. After comparison, the calculated value derived using the research method and the actual output of the horizontal well are both $8.0618 \times 10^4$ m$^3$/d with a relative error of only 3.8956%. This result meets the demands of engineering calculation; moreover, it indicates that the new unsteady model is reasonable and can be used in the fracturing of the horizontal well productivity calculation. In particular, the unstable model can calculate the outputs of different times compared with a stable model that conforms to the actual situation.

CONCLUSION

In this study, we obtained several conclusions listed below.

First, the author deduces the plane radial flow unstable-flow formula, which considers the start-up pressure gradient in the gas reservoir. Traditional models are based on the assumption that the outer boundary condition for the formation is infinite. However, the model doesn’t work when the start-up pressure gradient is introduced. In our study, we changed the boundary conditions, that is, outside the boundary condition is infinite formation changed to the circular constant pressure boundary. Thus, the model is solvable.

Second, based on the introduction of start-up pressure gradient in the radial unsteady seepage formula, the unstable productivity formula is established, while considering start-up pressure gradient, the high-speed non-Darcy effect, and stress-sensitive effect in a low-permeability gas reservoir. The formula can simulate the asymmetrical distributions of any crack length to simulate the output of every crack and horizontal well production. The formula for low-permeability gas reservoir also considers comprehensive factors and can calculate the production at different times.

Third, the model considers the start-up pressure gradient, high-speed non-Darcy effect, and stress-sensitivity effect of the case using the potential superposition principle, which considers the multiple interactions of cracks in close connection with the actual production. This model is true and accurate for predicting staged fracturing capacity of horizontal wells, identifying small errors, and implanting appropriate promotion and application. The calculation method of the model is simple and practical. The solution of the model is also stable and reliable.

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DETERMINATION OF CORRECT LOCATION OF CHEMICAL ATTRACTANT FOR TROPINOTA HIRTA (PODA, 1761) (COLEOPTERA: CETONIIDAE)

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ABSTRACT

The study was carried out in cherry orchard located in the boundaries of Atabey district in Isparta province. Totally ten chemical attractants approximately 2 m above from the ground were set up on branches of cherry trees. In the study conducted during the bloom period in which T. hirta was active on cherry trees, numbers of flowers and fruits were recorded daily to understand transformation of these flowers into fruits. As a result of the present study, fruit set was found out lower on branches with attractant than the branches without attractant 20.28 % and 30.86, respectively. This differences was found statistically significant. Therefore it is thought that the attractant hanging on the branches will increase the damage rate of Apple blossom beetle.

KEYWORDS:
Apple Blossom Beetle, Cherry, Epicometis hirta, Isparta, Turkey

INTRODUCTION

Tropinota hirta (Poda, 1761) (Coleoptera: Cetoniidae) damages the stamen and pistils of the flowers of several orchard trees and many ornamental plants [1-4]. Control methods of T. hirta are still being discussed at the present day. Unfortunately the one of the most common control method against T. hirta is the chemical control. The pesticide applications negative affected not only the target pest but also non-target organism as the honey bees which provides pollination [5-9]. Thus chemical control (using selective pesticides to pest) as well as other control methods should be considered for monitoring and controlling of mentioned pest. Recent scientific studies related with some biotechnical methods such as using color traps, and some attractants to Apple blossom Beetle have given valuable results and suggestions [6, 8, 10, 11].

For the last decades scientists have tried different attractants for monitoring of T. hirta [7, 12-20]. Tóth et al., (2003) [16] has found cinnamyl alcohol, transanethol and cinnamyl acetate as compounds attracting T. hirta. Accordingly Schmera et al., 2004 [13] affirmed that mixture of a bait of 1:1 combination of cinnamyl alcohol and transanethol and light blue color used in funnel traps were very effective trap for sampling of T. hirta. As for chemical attractive stimuli for apple blossom beetle, a binary floral attractant containing of (E)-cinnamyl alcohol and (E)-anethol has been improved [7, 15-17]. On the other hand there has been no scientific study about recommending for the correct position of the chemical attractant until nowadays. Therefore this study was investigated the correct location of chemical attractant (cinnamyl alcohol (3-phenyl-2-propen-1-ol) and transanethole [(1-methoxy-4-(1-propenyl) benzene)]) one of the most recommended methods with previous studies.

MATERIALS AND METHODS

The study was carried out in cherry orchard located in the boundaries of Atabey district in Isparta province between April 30 and May 14, 2018.

Totally ten chemical attractants were prepared with cinnamyl alcohol (3-phenyl-2-propen-1-ol) and transanethole [(1-methoxy-4-(1-propenyl) benzene)] by a 1:1 mixture according to the suggestions of Schmera et al., (2004) [13] and Tóth et al., (2004) [17] to sample adults of T. hirta. A total of 10 units of cottons prepared in equal length were impregnated with attractive (1:1 mixture-20 μl) and placed inside the perforated (20 pieces with a diameter of 0.3 mm) falcon tubes (50 ml). Falcon tubes (3x13 cm length) with lures fixed with upside part of the light blue funnels (22 cm diameters and 25 cm length). Plastic water containers (5 litres) which placed at the bottom of the funnels were used as traps to collect adults of apple blossom beetles. Totally 10 attractant traps approximately 2 m above from the ground were set up on branches of cherry trees.

In the study conducted during the blooming period in which T. hirta was active on cherry trees. Branches with attractant and without attractant were controlled daily for the numbers of flowers and fruits to understand transformation of these counted
flowers into fruits. Branches without attractant were randomly selected.

We analysed the differences of fruit sets between branches with attractant and branches without attractant with one way analyses of variance (ANOVA).

RESULTS

Totally 2890 flowers were counted in 10 branches where attractant had been hanged and it has been determined that 591 of them were turned into fruit. Transformation rate from flowers to fruit was found 20.28 % (Table 1). Bait were sampled entirely 87 adults of apple blossom beetle.

Totally 3141 flowers were determined in the randomly selected branches without attractant and it has been obtained that 961 of them were turned into fruit. Average of fruit set was found 30.86 % (Table 1).

Fully 87 adults of *T. hirta* were caught with attractants, *cinnamyl alcohol* and *transanethole* by a 1:1 mixture between April 30 and May 14, 2018.

Fruit set was found out lower on branches with attractant than the branches without attractant. This differences was found significant (P≤0.05) (F: 57.26; Sig.: 0.000). Therefore it is thought that the attractant hanging on the branches will increase the damage rate of Apple blossom beetle.

**TABLE 1**

<table>
<thead>
<tr>
<th>Branches with attractant</th>
<th>Number of flowers</th>
<th>Number of fruits</th>
<th>Proportion of fruit set</th>
<th>Branches without attractant</th>
<th>Number of flowers</th>
<th>Number of fruits</th>
<th>Proportion of fruit set</th>
</tr>
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<td>316</td>
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<td>241</td>
<td>68</td>
<td>28.22</td>
<td></td>
</tr>
</tbody>
</table>

Average: 289.1

**FIGURE 1**

Similarity dendrogram of number of flowers and fruits, and average of fruit set on branches with and without attractant hanging.

*FrSet_wAt: Average of fruit set on branches without attractant; FrSet_at: Average of fruit set on branches with attractant; Fr_wAt: Number of fruit on branches without attractant; Fr_At: Number of fruit on branches with attractant; Fl_wAt: Number of flowers on branches without attractant; Fl_At: Number of flowers on branches with attractant.
Percent similarity index showed that number of flowers on branches with attractant and without attractant hanging 85.76% similar to each others. Number of fruits and hence fruit set on branches with attractant and without attractant hanging were found 74.61% and 79.31%, respectively (Fig. 1).

DISCUSSION AND CONCLUSION

Attractant traps using for *T. hirta* have been set up with many different ways such as at the soil surface, in sunny places [13], on the ground surface or at a height of between 50 and 100 cm above [14], attached to poles or suspended from the vegetation at between 30 and 40 cm height above ground [7, 19, 21], hung among the branches of trees [10], etc. However there is no scientific study about comparing different positions of trap.

The result of the study showed that the location of chemical attractants for using *T. hirta* has very important role to defend flowers and the chemical attractants should not set up on the branch of the cherry trees. Because the attractants on the trees increased the damage rate of the cherry flowers. The apple blossom beetle could be more risky for the flowers when the chemical attractants were set up on branches. Therefore chemical attractants can be more suitable for hanging into the garden, but away from the trees, to reduce the rate of flower damage.

Recommended rate of fruit set is 30% however 40% of fruit set can be also tolerated if the orchards fertilization is applied according to recommendation. If the fruit set is over than 40% the fruits can be remained smaller than required calibre hence standardisation of fruits can be negatively affected. Under the 30% of fruit set, expected profit cannot be obtained due to the cost of fertilization, pesticides, harvesting, shipping, storage etc.

As a conclusion, more adults of *T. hirta* attracted by traps presents on branches with attractant than branches without attractant. Therefore it is thought that the attractant hanging on the branches will increase the damage rate of *T. hirta* and fruit set is affected negatively.

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EVALUATION OF SALINITY TOLERANCE IN RICE (ORYZA SATIVA L.) USING WATER POTENTIAL, BIOMASS, MEMBRAN DAMAGE AND OSMOPROTECTIVE COMPOUND

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ABSTRACT

We investigated the physiological (biomass, water relative content-RWC) and biochemical responses (osmoprotective compound-prolin, membran damage-MDA) of rice (Oryza sativa L.) varieties against stress factor at different concentrations (100, 200 and 300 mM NaCl). 2 local populations (Karacadağ, Hazro) and six rice varieties namely, Gala, Edirne, Şumnu, Neğiş, Tunca and Aromatik-1 were included in this study. As NaCl concentration increased, RWC decreased at different levels depending on the varieties, but the greatest decrease was detected in Gala (66%) and Karacadağ (74%). However, Tunca (85%) and Aromatik-1 (86%) were selected as the least affected varieties even at high NaCl concentration (300 mM). After 10 days of applications, the increase in proline (pro) content varied according to the varieties of rice and duration of application. When the malondialdehyde (MDA) contents of the varieties were examined, it was determined that Şumnu and Gala were highly affected even at 100 mM NaCl. Severe stress factor (300 mM NaCl) caused MDA increase in Tunca and Aromatik-1 but this increase were found higher in Karacadağ, Neğiş and Gala. As a result, when all parameters are evaluated Gala and Karacadağ was found a higher sensitivity while Tunca and Aromatik-1 had tolerant against salinity.

KEYWORDS:
Karacadağ, RWC, NaCl stress, proline, MDA

INTRODUCTION

The plants that are in constantly contact with the external environment, they are subject to stress due to lack of adaptation in adverse conditions in their environment. Therefore, plants give morphological, biochemical or molecular responses at different levels to protect themselves in adverse environmental conditions and called tolerant or sensitive to according to effect of the stress factor. The damage caused by stress factors can vary depending on the type of plant, its duration of development, tolerance and adaptability [1, 2]. As the population growth in the world causes the increase of demand for food, the rate of land used for agricultural activities is also increasing. The application of unconscious and incorrect irrigation techniques in these lands does not only destroy the structure of the soil but also causes the accumulation of salt in these soil. The excessive irrigation in arid and semi-arid regions is considered another condition that causes salt accumulation on agricultural land and the salinity problem is seen as a serious threat especially in arid and semi-arid regions.

Salinity which is one of the most important stress factors for plants, affects 23% of the agricultural areas worldwide and also threatened plant yield [3]. Similarly, in our country, soil salinity is considered as one of the most important environmental stress factors that limit agricultural productivity and it has been reported that about 1.5 million hectares of our soils (32.5% of irrigable areas) are facing salinity problems [4, 5]. In addition, together with the increase in agricultural land opened to irrigation every year, the use of dense chemical medicines and fertilizers lead to increase salt content in the soil [6]. Salinity, means "high concentrations of salt varieties in the soil". NaCl (sodium chloride), is one of the salt varieties that causes the most salinity in soil, and affects about 24% of irrigated land, about 10% of arable land [7].

It has been reported that lipid peroxidation, which occurs due to the negative effect of salinity in plants, is caused by oxidative stress, and this situation causes an increase in the amount of MDA, which is a marker of cell membrane damage [8, 9].

The increase in pro synthesis is considered as a common response to the stress factor in the majority of plants exposed to high salinity [10]. Many researchers reported that pro synthesis or accumulation continues in plants under stress conditions [11, 12, 13].

The effect of salinity on the growth and yield of high glycophyte plants such as rice (Oryza sativa L.) appears to be an important problem and which
limits rice production to a serious extent [14, 15]. Tolerance, which is an indication of durability against the salt stress factor, vary depending on plant type, development period and environmental conditions [16]. The rice plant is highly sensitive to the salinity factor in young seedling and flowering period but exhibits higher resistance during the tillering period [17].

The increase in plant populations and deterioration of land due to saltiness have led plant scientists to point out the idea of "salt-tolerant plant development" using genetic approaches [18]. There have been many studies on this subject but the mechanisms of salt tolerance in plants have not yet been adequately understood, the progress in developing salinity-tolerant varieties has been slow. Therefore, biochemical responses to salinity during the development stage of rice and studies to determine the effect of salt stress will provide important contributions to the development of salt tolerant rice varieties [19].

According to the results obtained from the literature reviews containing the negative effects of the salt stress factor, there was not enough study on rice varieties cultivated in our country. For this reason, we investigated the physiological and biochemical responses of some rice (Oryza sativa L.) varieties against the NaCl (100, 200 and 300 mM) stress factor. 2 local populations (Karacadag, Hazro) and six rice varieties namely, Gala, Edirne, Şümnu, Neğiş, Tunca and Aromatik-1 were included in this study. The sensitive and tolerant varieties were identified as a result of evaluating the responses of the varieties to the stress factors.

**MATERIALS AND METHODS**

**Plant material.** In this study, Aromatik-1, Edirne, Gala, Şümnu, Neğiş and Tunca varieties obtained from Trakya Agricultural Research Institute and Karacadag and Hazro rice varieties which in grown around Diyarbakir-Karacadag obtained from local producers were used as plant materials.

**Growth of plant material.** Seeds separated into groups were sterilized with 5% NaOCl (approximately 3 min) and then rinsed with distilled water for removal of NaOCl. Subsequently they were kept in distilled water for 1 night for the swelling of seeds. After this process, seeds planted into pots containing soil: perlite (3: 3: 1) and were allowed to develop in the plant growth chamber. The seeds were watered with ⅔ Hoagland nutrient solution during 4 weeks (28 days) based on field water capacity (65%). After 4 week development period, the plants were exposed to the salt stress factor with the NaCl. For this, all the pots were watered with ⅔ Hoagland nutrient solution prepared with 0, 100, 200 and 300 mM NaCl for 10 days. Plants in the control groups were watered at the same time and in the same manner as the NaCl-free ⅔ Hoagland nutrient solution.

**Growth parameters.** Following the 10th day, the plants were harvested and the aerial and roots parts were separated from each other. The fresh weights of leaves and roots were weighed, and root lengths were measured. The samples were then dried in etuve at 50 °C for 72 h, and the dry weights were determined. The other samples placed in the film boxes were labeled and stored at -80 °C until the time of analysis.

**Relative water content.** The plants were sampled and nodules were detached from the root. Sand was removed with the help of a soft brush and a light washing was given with distilled water and weighed immediately to take their fresh weight (FW) after removing excess of water by using filter papers.

The turgor weights (TW) were made by standing in di-H2O (de-ionized water) for 6 hours. After that the nodules were oven dried at 50 °C till a constant weight and the dry weight (DW) were made. The leaf RWC of the samples calculated as % relative to the Formula;

\[
\text{Leaf Relative Water Content} (\%) = \frac{(\text{TW} – \text{DW})}{(\text{FW} – \text{DW})} \times 100
\]

TW: Turgor weights; DW: Dry weight; FW: Fresh weight

**Determination of MDA amount.** The amount of MDA examined with thiobarbituric acid (TBA) test to determine the degree of damage to membranes in leaf tissues [20]. 0.1 g of leaf samples were homogenised with 2 mL of 5% TCA. The homogenate was centrifuged at 12000 rpm at 25 °C for 20 min. 0.1 µL of supernatant was precipitated by 20% TCA containing 0.5% TBA. The mixture was heated in a water bath shaker at 95 °C for 30 min and quickly cooled in an ice-bath. The absorbance was read at 532 nm after centrifugation at 1000 rpm for 10 min. MDA amount was determined using 1.1.3.3-Tetramethoxypropane standard curve.

**Determination of proline content.** Proline amounts were calculated according to the method of [21]. Samples (0.1 g) from each group were homogenized in 40% methanol and homogenate filtered through filter paper. 1 mL supernatant from each extract, 1 ml glacial acetic acid, 6 M orthophosphoric acid and 25 mg acid ninhydrin were put into a new eppendorf tube orderly. Before the incubation step, eppendorf tubes were mixed. Then, the tubes were incubated at 100°C for 60 min at heat block to hydrolyze proteins. Reaction was then stopped by using ice bath. The mixture was extracted with 5 ml toluene, and the absorbance of fraction
with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined using L-proline standard curve.

**Statistical analyses.** Data were statistically analyzed using SPSS 20.0 with One-Way Anova and Duncan tests. All of the results are represented as means ± SD of three independent replications (n=3). The significance refers to the statistical level at $P \leq 0.05$.  

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**FIGURE 1**

General view of rice varieties on the 10th of salt application:
(a) Gala (b) Edirne (c) Şumnu (d) Neşin (e) Tunca (f) Aromatik-1 (g) Karacadağ (h) Hazro
RESULTS

Under stress conditions formed with 0, 100, 200 and 300 mM NaCl was investigated responses physiological and biochemical of rice varieties in our research. 10 days after the salt applications, the treatment groups were compared with their own controls to determine morphological changes and at the high salt concentration (300 mM) it has been observed that the yellowing or wilting is significantly increased especially in Şunnu and Karacadag (Fig. 1).

Effect of salt stress on physiological parameters. When all treatment groups compared with control plants, only increase in FW values of Tunca and Neşri was detected from among the varieties. Although the FW values of Tunca increased at all three concentrations, the increase in Neşri was only observed at 200 and 300 mM application. Although there was a steady decrease in the FW values of Hazro and Aromatik-1 depending on the increase in NaCl, the values obtained was found not statistically significant (Fig. 2).

When leaf DW values of the varieties were examined, different values were obtained depending on the applied salt concentration. As shown in Fig. 3, a insignificant increase or decrease in the DW of Edirne, Hazro, Aromatik-1 and Gala leaves was noticed, in response to NaCl, although those changes were not found statistically significant.

In this study, a decrease in DW values of Hazro, Karacadag, Aromatik-1 and Edirne was detected among the varieties kept in high salt (300 mM) conditions. In contrast to the others, the value obtained from the Karacadag variety (20.5%) was found to be significantly lower compared to control group (Fig. 3).

![Figure 2](image)

**FIGURE 2**
Effect of NaCl stress factor on leaf FW.

Bars show SD, and data points marked with asterisks indicate that mean values are significantly different between treatments and control ($p \leq 0.05$).

![Figure 3](image)

**FIGURE 3**
Effect of NaCl stress factor on leaf DW.

Bars show SD, and data points marked with asterisks indicate that mean values are significantly different between treatments and control ($p \leq 0.05$).
RWC values of varieties were significantly affected by salt stress treatment and, it has been found that as salinity increases, RWC values decreases in all varieties with respect to control groups. When compared varieties exposed to 100 mM NaCl application, the highest RWC values were obtained in Negro, Tunca and Aromatik-1 (90%, 86% and 85%, respectively), on the contrast, the lowest RWC value (67%) was found at Gala.

With 300 mM NaCl application obtained the lowest RWC values from Gala and Karacağ (66%, 74% respectively) whereas, these rates were lower at 100 mM concentration were observed. However, the response given by Hazro and Negro for the same application was found different from the response of other varieties. As shown in Table 2, results varying according to the applied NaCl concentration were obtained. It was determined that when compared to the control group, the most affected varieties at 100 mM concentration were Şünmu and Gala. However, it was found that Tunca and Aromatik-1 were not affected much at high concentration (300 mM) whereas Karacağ, Negro and Gala varieties were more affected.

Pro is one of the most important organic osmolytes that plants accumulate in response to environmental stresses such as salinity, extreme temperature, drought and UV radiation [22]. For this reason, it has been used in many studies as an experimental parameter in determining the sensitive or tolerance level of plants. The differences in the pro contents of rice varieties exposed to 100, 200 or 300 mM NaCl stress application are shown in Table 3. In our research, pro accumulation in Gala, Şünmu, Aromatik-1, Karacağ and Hazro increased regularly with effect of salt stress. Pro accumulation was found lower in Tunca (6.480%) than Karacağ (53.70%) and Aromatik-1 (29.16%) in 100 mM NaCl application. In response to the same application, pro accumulation increased in other varieties, whereas decreased (-5.31%) in Negro. Statistical analysis shows that 300 mM NaCl application has almost the same effect on Karacağ (124.29%) and Negro (119.14%). However, it was observed that Tunca (26.321%) and Hazro (48.277%) were least affected by 300 mM NaCl compared to other varieties.

### TABLE 1

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Control</th>
<th>100 mM NaCl</th>
<th>200 mM NaCl</th>
<th>300 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gala</td>
<td>81.94±1.907 a</td>
<td>67.46±6.115 b</td>
<td>58.35±3.147 c</td>
<td>66.92±7.607 d</td>
</tr>
<tr>
<td>Edirne</td>
<td>88.82±3.351 a</td>
<td>83.89±4.132 b</td>
<td>81.78±3.355 b</td>
<td>81.81±3.054 a</td>
</tr>
<tr>
<td>Şünmu</td>
<td>83.91±1.011 a</td>
<td>80.81±1.231 c</td>
<td>80.53±0.898 c</td>
<td>80.33±3.974 a</td>
</tr>
<tr>
<td>Negro</td>
<td>95.64±0.786 a</td>
<td>90.08±1.114 a</td>
<td>86.02±1.050 a</td>
<td>84.65±4.053 a</td>
</tr>
<tr>
<td>Tunca</td>
<td>88.87±3.027 a</td>
<td>86.29±1.488 b</td>
<td>85.86±1.653 b</td>
<td>85.01±0.485 a</td>
</tr>
<tr>
<td>Aromatik-1</td>
<td>92.24±0.683 a</td>
<td>85.93±1.204 b</td>
<td>86.75±0.921 a</td>
<td>86.10±0.963 a</td>
</tr>
<tr>
<td>Karacağ</td>
<td>84.23±1.645 a</td>
<td>84.25±0.208 b</td>
<td>81.50±2.745 c</td>
<td>74.79±2.848 a</td>
</tr>
<tr>
<td>Hazro</td>
<td>95.53±0.872 a</td>
<td>84.10±1.297 b</td>
<td>83.91±1.034 a</td>
<td>83.73±3.838 a</td>
</tr>
</tbody>
</table>

Values represent mean ± SD of three replicates per treatment. Values of means within a column followed by the same letter(s) are not significantly different according to SPSS (p < 0.05).
**DISCUSSION**

Salt stress, which poses serious threats and causes loss of crops in plants, also causes economic damage in large quantities every year. Decrease in arable land due to increasing population density and when it is thought that food troubles may occur in the coming years, researches to reduce the product losses due to the stress factor have gained considerable importance for the whole scientific community. Therefore, researchers have been conducting a number of studies intended understand the defense mechanisms that provide resistance to stress factors in order to minimize loss of crops in important plant species. In this study, the effects of the NaCl stress applications on leaf fresh-dry weight, relative water content, pro biosynthesis, and lipid peroxidation levels in the plants were assessed.

Under saline conditions, plants that are exposed to stress use different physiological and biochemical mechanisms to deal with negative situa-

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Control</th>
<th>100 mM NaCl</th>
<th>200 mM NaCl</th>
<th>300 mM NaCl</th>
<th>Control-100 mM NaCl</th>
<th>Control-200 mM NaCl</th>
<th>Control-300 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gala</td>
<td>1.594±0.095</td>
<td>1.991±0.276</td>
<td>1.968±0.137</td>
<td>2.066±0.006</td>
<td>ba+24.924</td>
<td>ba+23.463</td>
<td>ba+29.611</td>
</tr>
<tr>
<td>Edirne</td>
<td>1.506±0.146</td>
<td>1.746±0.076</td>
<td>1.794±0.038</td>
<td>1.879±0.166</td>
<td>ba+15.936</td>
<td>ba+19.123</td>
<td>ba+24.787</td>
</tr>
<tr>
<td>Şümnu</td>
<td>1.268±0.012</td>
<td>1.705±0.286</td>
<td>1.301±0.403</td>
<td>1.612±0.070</td>
<td>ba+34.413</td>
<td>ba+5.269</td>
<td>ba+27.114</td>
</tr>
<tr>
<td>Neğiş</td>
<td>1.484±0.006</td>
<td>1.226±0.016</td>
<td>1.471±0.034</td>
<td>2.043±0.234</td>
<td>ba+17.377</td>
<td>ba+0.902</td>
<td>ba+37.623</td>
</tr>
<tr>
<td>Tunca</td>
<td>1.436±0.065</td>
<td>1.456±0.081</td>
<td>1.718±0.001</td>
<td>1.663±0.136</td>
<td>ba+1.343</td>
<td>ba+19.628</td>
<td>ba+15.772</td>
</tr>
<tr>
<td>Aromatik-1</td>
<td>1.859±0.102</td>
<td>1.892±0.161</td>
<td>1.958±0.036</td>
<td>1.948±0.099</td>
<td>ba+1.796</td>
<td>ba+5.308</td>
<td>ba+4.770</td>
</tr>
<tr>
<td>Karacadağ</td>
<td>1.656±0.329</td>
<td>1.739±0.206</td>
<td>1.940±0.062</td>
<td>2.328±0.144</td>
<td>ba+5.030</td>
<td>ba+17.149</td>
<td>ba+40.622</td>
</tr>
<tr>
<td>Hazro</td>
<td>1.702±0.070</td>
<td>1.616±0.059</td>
<td>1.977±0.148</td>
<td>2.170±0.141</td>
<td>ba+5.017</td>
<td>ba+16.175</td>
<td>ba+27.538</td>
</tr>
</tbody>
</table>

Values represent mean ± SD of three replicates per treatment.

**TABLE 3**

The changes in pro content of the varieties under stress conditions created with NaCl (mmol g⁻¹ T.A.)

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Control</th>
<th>100 mM NaCl</th>
<th>200 mM NaCl</th>
<th>300 mM NaCl</th>
<th>Control-100 mM NaCl</th>
<th>Control-200 mM NaCl</th>
<th>Control-300 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gala</td>
<td>5.007±0.050</td>
<td>5.573±0.168</td>
<td>6.600±0.005</td>
<td>7.570±0.087</td>
<td>ba+11.322</td>
<td>ba+31.831</td>
<td>ba+51.190</td>
</tr>
<tr>
<td>Edirne</td>
<td>3.532±0.001</td>
<td>4.395±0.290</td>
<td>3.820±0.058</td>
<td>5.627±0.522</td>
<td>ba+24.416</td>
<td>ba+8.138</td>
<td>ba+59.300</td>
</tr>
<tr>
<td>Şümnu</td>
<td>3.696±0.116</td>
<td>4.436±0.232</td>
<td>5.709±0.174</td>
<td>6.407±0.116</td>
<td>ba+19.998</td>
<td>ba+54.441</td>
<td>ba+73.330</td>
</tr>
<tr>
<td>Neğiş</td>
<td>3.861±0.000</td>
<td>3.655±0.174</td>
<td>6.654±0.348</td>
<td>8.461±0.116</td>
<td>ba-5.319</td>
<td>ba+72.339</td>
<td>ba+119.14</td>
</tr>
<tr>
<td>Tunca</td>
<td>4.119±0.063</td>
<td>4.386±0.046</td>
<td>4.185±0.052</td>
<td>5.204±0.040</td>
<td>ba+6.480</td>
<td>ba+1.594</td>
<td>ba+26.321</td>
</tr>
<tr>
<td>Aromatik-1</td>
<td>3.746±0.116</td>
<td>4.838±0.023</td>
<td>5.434±0.075</td>
<td>6.214±0.133</td>
<td>ba+29.167</td>
<td>ba+45.066</td>
<td>ba+65.899</td>
</tr>
<tr>
<td>Karacadağ</td>
<td>3.212±0.174</td>
<td>4.937±0.099</td>
<td>5.910±0.017</td>
<td>7.2046±0.116</td>
<td>ba+53.706</td>
<td>ba+84.013</td>
<td>ba+124.29</td>
</tr>
<tr>
<td>Hazro</td>
<td>3.573±0.174</td>
<td>3.943±0.116</td>
<td>4.805±0.406</td>
<td>5.298±0.290</td>
<td>ba+10.345</td>
<td>ba+34.484</td>
<td>ba+48.277</td>
</tr>
</tbody>
</table>

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Salt stress, which poses serious threats and causes loss of crops in plants, also causes economic damage in large quantities every year. Decrease in arable land due to increasing population density and when it is thought that food troubles may occur in the coming years, researches to reduce the product losses due to the stress factor have gained considerable importance for the whole scientific community. Therefore, researchers have been conducting a number of studies intended understand the defense mechanisms that provide resistance to stress factors in order to minimize loss of crops in important plant species. In this study, the effects of the NaCl stress applications on leaf fresh-dry weight, relative water content, pro biosynthesis, and lipid peroxidation levels in the plants were assessed.
ers have showed that the DW of the plant which is tolerant to salinity is higher than that of the sensitive varieties. According to our results, it was determined that in all varieties leaf FW were decreased compared to the controls, while in Tunca variety increased. A general decrease in FW or DW has been observed in all plant tissues subjected to salt stress. It has been reported that the loss of DW and FW in root and fresh green explants of pumpkin, wheat, melon, solanum, okra and pepper plants after stress application [32, 33, 34, 35, 36, 37, 38].

It is also known that salt stress affects the water status of the plant as well as leaf development. The osmotic effect resulting from soil salinity lead to the reduction of turgor pressure in cells, slow down the development indirectly and as a result cause defects water balance of the plants [39]. Khan and Panda [40] reported that the relative water content was negatively affected in Lunishree ve Beğenbıtechı rice roots exposed to different concentrations of NaCl, but that this decrease was greater in Lunishree. In the research presented, the varieties responded at different levels in terms of RWC values depending on the NaCl concentration. As the salt concentration increased, RWC decreased in all varieties compared to the control group, and responses to the stress differed generally in terms of statistical significance according to the varieties. When RWC data obtained from the NaCl applications evaluated, Tunca (85%) and Aromatik-1 (86%) was the least affected varieties from high salt concentration. Unlike it has been identified that Gala (66%) and Karacadag (74%) were the most affected varieties. Under stress conditions, the RWC parameter is accepted as an important and decisive criterion for selecting tolerant/sensitive varieties. Mekawy et al. [41] exposed Sakha 102 and Egyptian Yasmine Zea mays varieties to 50 mM NaCl for 14 days under salt stress conditions. While the RWC of Sakha 102 plants were 97.0% in control plants, it was 71.0% in application plants. However, the researchers reported that there was no significant difference between salinity-exposed plants (86.0%) and control group (88.0%) of the RWC of Egyptian Yasmme.

In case of stress, the amount of MDA in the cell increases in proportion to the increase in lipid peroxidation [42, 43]. Therefore, MDA level, a product of lipid peroxidation in plants, is frequently used as an indicator of oxidative damage in membranes in stress studies [44]. In our study, when control and application groups were compared, in generally the NaCl stress factor increased MDA content in all varieties. In support of our results, Khan and Panda [40] reported an increase in MDA levels in response to increased salinity. Similarly, when the responses of tolerant and sensitive varieties are investigated under salinity, many researchers reported that the increase in salt stress caused an increase in the amount of MDA in sensitive varieties [45, 46]. Lutts et al. [47] reported that the amount of MDA in salt-tolerant rice was low and that these values were higher in salt-sensitive rice varieties. Vaidyanathan et al. [48] investigated changes in lipid peroxidation in salt-sensitive Pusa Basmati-1 (PB-1) and salt-tolerant Pokkali (PK) rice at different concentrations of NaCl and they reported an increase in the lipid peroxidation values of sensitive/tolerant varieties depended to increased in concentration. Researchers reported that this increase was higher in salt-sensitive PB-1 at all concentrations. At the early stages of salt stress, Turan and Tripathy [49] reported that the level of lipid peroxidation was high in salt-sensitive PB-1 while the salt tolerant genotype (CSR10) showed resistance to stress as it reduced MDA accumulation. Lower levels of lipid peroxidation in CSR10 indicate that tolerant plants may be better protected from oxidative damage than sensitive plants, but sustained salt stress applications has blurred the distinction between tolerant and sensitive genotypes. According to the results from previous studies, the amount of MDA in tolerant varieties is less than in sensitive varieties. The low amount of MDA in tolerant varieties is considered to be indicative of less damage to the membranes. Similar to the results of the researchers, the low amount of MDA obtained suggests that Aromatik-1 (4.7%) is tolerant to salt stress. However, the higher amount of MDA in the Karacadag (40.6%) indicates that this variety is sensitive. It has been determined that the highest increase in MDA in Sunnu and Gala varieties at 100 mM NaCl applications. Similar increases were determined in Karacadag, Negis, Gala and Edirne varieties at 300 mM. In some studies in which the effect of salt stress was examined on the MDA content, it has been reported that the formation of oxidative stress resulted in lipid peroxidation. Researchers have reported that MDA is low in tolerant varieties that used higher levels of antioxidative enzymes [32, 35, 37, 38]. In response to increasing salinity in our applications, an increase in MDA levels in some species indicates that the plant is exposed to more damage. Compared to the control groups, the higher MDA content in Gala, Karacadag and Hazro indicates that these varieties are more sensitive and Tunca and Aromatik-1 varieties may be more tolerant. As Tunca and Aromatik-1 varieties have the lower level of MDA, we can conclude that these varieties better protect against oxidative damage caused by salt stress. As MDA-level that a product of lipid peroxidation, is a marker of oxidative damage in membranes [44]. Therefore we are of the opinion that the MDA results we have found in our study can be used as a parameter that leads to the correct conclusion about the tolerance of the varieties.

The presence of NaCI at high concentrations in environments where plants are found reduces water potential and it becomes harder for plants to
get water and this results in osmotic stress. Tang et al. [50] reported that one of the measures plants use to increase salt resistance is the accumulation of the compatible solutes. Plants tend to accumulate compatible solutes such as pro and glycine-betaine to cope with osmotic stress [51,52]. Pro accumulation is considered one of the most commonly adaptive responses of plants to salinity due to its antioxidant and osmo-compatible properties [53]. Chunthaburee et al. [31] compared the tolerance level of 12 rice cultivars with tolerant Pokkali and sensitive IR-29 and they reported that salt stress induced a significant increase in Pro accumulation in all varieties. Similarly, in our study, the pro content was increased in rice varieties depending on increase in salt concentration and all applications resulted in higher pro accumulation. Our results coincides with the expression "rapid accumulation of free pro is a typical response to salt stress" as Mansour [54] has pointed out. Lutts et al. [55] examined the effect of salt stress on osmotic regulation and pro accumulation in two rice varieties and they reported that 50 and 100 mM NaCl applications resulted in higher pro accumulation in sensitive rice varieties. As the researchers have noted, we can conclude that the varieties in which the amount of pro is higher can not adequately take water due to salinity and that osmotic stress is experienced in plants depending on the stress. In study of Bhattacharjee and Mukherjee [56], pro accumulation was increased in rice cultivars [salt-tolerant (Hamilton, SR26B) and sensitive (Ratna)] exposed to 100 mM NaCl and this accumulation was higher levels in salt tolerant varieties.

In conclusion, it is a known fact that a large part of the land in our country is faced with the problem of salinity due to both natural routes and misapplication. The great economic losses caused depending on decrease in yield and quality of the salinity occurred in the soil in plants are remarkable. For this reason, this study has made a significant contribution to the country's economy in terms of prevention of crop losses and the selection of salt-tolerant varieties in the areas / regions that have been faced with the problem of salinity. In the large-scale literature survey that done, was not observed detailed studies on the determination of salinity-tolerant and sensitive plants in 6 breeding rice cultivars and 2 local Karacadag rice populations cultivated and it is seen that there is a lack of important information on this subject. Findings obtained presented this study are very important in terms of removing this deficit in the literature. Physiological and biochemical results obtained in our study indicate that Tunca and Aromatik-1 rice varieties are tolerant and Gala and Karacadag are sensitive. It will be useful to conduct more advanced physiological and biochemical analyzes to determine the level of oxidative damage caused by salt stress in these varieties. In addition further studies to be made to elucidate the regulatory points which provide a large degree of salinity tolerance should be supported by molecular analyzes.

ACKNOWLEDGEMENTS

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INVESTIGATION OF EFFECTS OF VARIABLE BLANKHOLDER FORCE ON DEEP DRAWING PROCESS OF 6082 ALUMINUM ALLOY

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ABSTRACT

In this study, effects of various constant and variable blankholder force values on cup shaped cylindrical deep drawing of 6082-T6 aluminum alloy was investigated. Control of process parameters is extremely important for the effective completion of deep drawing process especially in materials in which vast amount of regional deformations occur due to high strength and low formability as in 6082-T6. It is aimed to complete deep drawing process having deep drawing ratio of 2 effectively under various constant and variable blankholder forces by considering analytically and empirically calculated blankholder forces in the literature. For this purpose, a finite element analysis (FEA) procedure which eliminates the negative environmental effects of the physical process caused by the huge amount of power consumption and waste oil generation was executed. In performed FEA studies whose accuracy was controlled via physical experiments; maximum deep drawing height, total deep drawing energy, maximum deep drawing force, average deep drawing force and thickness variation ratio values were improved by controlling the material flow without decrease in deep drawn product quality under various constant and variable blankholder forces.

KEYWORDS:
Deep drawing, Plastic deformation, Variable blankholder force, 6082 aluminum alloy, Finite element analysis.

INTRODUCTION

Deep drawing process is a widely used sheet metal forming method. Numerous products, particularly automobile, train, airplane and machine body panels; pressure vessels; white good bodies; kitchen sinks; cooking and medical cups; beverage cans and wheel rims are manufactured by deep drawing process [1]. Manufacturing of above mentioned products by deep drawing instead of welding of machined semi-products decreases manufacturing lead time as well as material losses. Thereby, negative effects of metal chips generated by machining process and waste gasses composed of welding process on environment can be eliminated considerably using deep drawing method.

Control of the effectiveness of the process is difficult due to the nonlinear plastic deformation characteristics and non-homogeneous material flow of deep drawing. Very-high press machine power consumptions are required in all plastic deformation processes as so in deep drawing applications. Appropriate determination of process parameters, especially decreasing the blankholder pressure, decreases the lubrication necessity and amount of waste oil as well as the power consumption considerably which has a major role in environmental pollution.

There are a number of studies on determination of deep drawing process parameters in the literature. Browne and Hillery experimentally investigated the effects of important parameters for process performance, namely punch and blankholder force, lubricant type and application region, deep drawing speed, punch and die edge rounding radius in their study in which they stated that it is critical to select deep drawing process parameters correctly, and determined conditions which make product's thickness distribution the most homogeneous [2]. Deep drawing ratio \( \beta = \frac{D}{d} \) decreases [3] and defects such as wrinkling, tearing, separation and earing occurs [4], if characterization is not made properly and process parameters are not selected correctly. Zheng et al. used macro-textured blankholder in deep drawing of 6082 aluminum alloy to prevent skirt wrinkling, and stated that increasing temperature and decreasing strain rate increases wrinkling resistance [5]. Numerous different methods such as variable blankholder force [3,4], vibrant blankholder [6], patterned blankholder [5], hydro-mechanical deep drawing [7], coating [8], hot deep drawing [5, 9], etc. are suggested in literature for the effective and defect-free completion of deep drawing process. Gavas and Izciler used an anti-lock brake system (ABS) in blankholder force control for improving formability and deep drawing ratio [6]. Vahdat et al. performed a numerical study
to minimize earing which is a critical defect seen in deep drawing, by decreasing anisotropy and friction effects. By performing the deep drawing process using a hydro-mechanical system [10], Gurun and Karaagac increased deep drawing ratio and decreased thickness variation of the product made of DC01, which is a widely used automotive material [7].Lim et al. coated metal sheets with PET and PVC to increase formability in deep drawing process, and developed a numerical formulation based on GTN (Gurson-Tvergaard-Needleman) damage model for predicting formability [8].

Generally, control of material flow is ensured with the pressure applied by an additional mold known as blankholder in deep drawing process (Fig. 1). If blankholder pressure is less than appropriate value, overmuch material enters to the gap between punch and die. This situation leads to wrinkling in the sheet metal. Thinning and tearing occurs in the sheet metal when blankholder pressure is more than appropriate value because of excessive material flow due to intervention of shearing on flanges of sheet. Determination of the appropriate blankholder pressure allows deep drawing of the work part to desired height properly. Although required blankholder pressure can be calculated approximately via various analytical and experimental formulas [1, 11, 12] depending on work part material’s yield strength and sheet/product geometry, a marked difference is seen between suggested formulas due to the significant influence of material properties, such as strain hardening and strain rate sensitivity exponent, on process. Various analytical and experimental formulas were suggested in the literature for the calculation of the appropriate value of blankholder force (Fbh) which procures pressure that has to be applied on sheet material for preventing wrinkling and tearing in deep drawing process Eqs. 1-4.

\[ F_{bh} = \frac{F_{dd}}{3} \]  
\[ F_{bh} = [(\beta - 1)^2 + \frac{D}{400r}] \sigma_{uts} A_{bh} \]  
\[ F_{bh} = [(\beta - 1)^2 + \frac{D}{500r}] \sigma_{uts} A_{bh} \]

Here; \( F_{dd} \) is deep drawing force [kN].

Calculated blankholder force values according to Eqs. 1-4 vary in a wide range between 30 and 104 kN as mentioned above. At the same time, chemical composition and plastic deformation behavior of the work part material together with deep drawing process parameters have significant influence on blankholder force. In addition, performed studies [13, 14] showed that application of constant blankholder force during deep drawing process generally prevents effective damage free completion of the process. A number of analytical [3, 13], experimental [2, 4, 5] and simulation based [13, 15] studies are found in the literature on the application of variable blankholder force in deep drawing process. Candra et al. analytically determined variable blankholder force approximately to prevent cracks occurring in deep drawing applied with constant blankholder force, and observed from finite element analyses results that formability and deep drawing height are increased considerably by applying variable blankholder force. In their numerical simulation based study [13], Kitayama et al. investigated the effects of variable and vibrant blankholder force in deep drawing process of difficult-to-draw materials to determine safe forming region of sheet material under constant blankholder force and, to optimize variable and vibrant force values [3]. Cheng-zhi et al. investigated the effects of variable blankholder force on formability of deep drawing of prismatic cups experimentally and numerically, and showed that formability is increased via controlling material flow by varying blankholder force regionally [4]. Yoshihara et al. showed that deep drawing ratio of magnesium alloy sheet which is a light material is increased with the application of variable blankholder force, especially at high temperatures [15].

Especially ever increasing environmental and economic issues increased the need to light and safe
structures. Thus; light metallic materials such as aluminum, titanium and magnesium have become irreplaceable for automotive and aerospace industries [16]. Usage of new generation aluminum alloy sheets in light structural applications become more popular each passing day under favor of their high specific strengths, economy and weight reduction capabilities. However; formability of these aluminum alloy sheets depends on numerous parameters such as punch speed, deep drawing ratio, holding time, heat treatment conditions, temperature, etc. and their formability is generally low in room temperature [14]. Especially, formability knowledge about 6, 7 and 8 series new aluminum alloy sheets is limited [17].

Effective deep drawing of newly developed 6 and higher series heat treated, high strength and low formability aluminum alloy sheets includes difficulties due to the occurrence of huge local plastic deformations during the process. For this reason, it is necessary to control the process precisely.

In this study, an experimentally verified FEA procedure for enhancing effectiveness of deep drawing process of 6082-T6 high strength low formability aluminum alloy was conducted to determine appropriate deep drawing conditions. For this purpose, various variable blankholder force conditions determined by considering wrinkling and tearing limit values under constant blankholder force conditions were examined.

**MATERIALS AND METHODS**

**Experimental study.** Experimental studies were conducted with a hydraulic deep drawing press having adjustable punch speed and blankholder pressure. Dimensions of punch, die and blankholder are given in Fig. 2. 6082-T6 aluminum alloy deep drawing sheet specimen having 135 mm diameter and 1 mm thickness was used in the experiments.

6082-T6 aluminum alloy is an artificially aged via solution treatment, aerospace industry material having high strength and low formability. Physical and mechanical properties of the work part material together with chemical composition are given in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Physical Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density [g/cm³]</td>
<td>2.71</td>
</tr>
<tr>
<td>Melting Temperature [°C]</td>
<td>575</td>
</tr>
<tr>
<td>Thermal Expansion Coefficient [10⁻⁶/oC]</td>
<td>23.1</td>
</tr>
<tr>
<td>Heat Capacity [W/m.K]</td>
<td>170</td>
</tr>
<tr>
<td>Electrical Conductivity (IACS) [%]</td>
<td>44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mechanical Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield Strength [MPa]</td>
<td>270</td>
</tr>
<tr>
<td>Ultimate Tensile Strength [MPa]</td>
<td>330</td>
</tr>
<tr>
<td>Elongation [%]</td>
<td>11</td>
</tr>
<tr>
<td>Elasticity Modulus [GPa]</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical Composition</th>
<th>Fe</th>
<th>Si</th>
<th>Cu</th>
<th>Mn</th>
<th>Mg</th>
<th>Zn</th>
<th>Cr</th>
<th>Others</th>
<th>Al</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>0.7</td>
<td>0.1</td>
<td>0.4</td>
<td>0.6</td>
<td>0.2</td>
<td>0.15</td>
<td>0.15</td>
<td>Remaining</td>
</tr>
</tbody>
</table>
A consistency more than 97% up to 99% was found between the results of experimental and simulation based studies performed in same conditions for determining the accuracy of finite element procedure as seen in Table 2.

**TABLE 2**
Comparison of experiment and analysis results under various blankholder pressures

<table>
<thead>
<tr>
<th>Blankholder Pressure [bar]</th>
<th>Maximum Deep Drawing Force [kN]</th>
<th>Consistency [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment</td>
<td>Simulation</td>
</tr>
<tr>
<td>0</td>
<td>67</td>
<td>66.20</td>
</tr>
<tr>
<td>40</td>
<td>98</td>
<td>96.96</td>
</tr>
<tr>
<td>80</td>
<td>137</td>
<td>132.29</td>
</tr>
<tr>
<td>120</td>
<td>180</td>
<td>184.20</td>
</tr>
</tbody>
</table>

In the experiments and simulations in which blankholder pressure was not applied, products having excessive wrinkles has obtained as seen in Fig. 4, and these wrinkles lead to tearing of the sheet metal due to stuck of work part between punch and die.

Deep drawing processes were completed effectively in the experimental studies under 40 and 80 bars blankholder pressures as seen in Fig. 5.

In the experiments and simulations under 120 bars blankholder force, tearing has occurred in the work part, because deep drawing force exceeded the critical value as seen in Fig. 6.

Variation of deep drawing force obtained from experimental and simulation based studies depending on punch stroke under 0, 40, 80 and 120 bars blankholder pressures is seen in Fig. 7.
Finite element analysis. FEA technique is a useful method for simulating physical phenomena in computer environment. Physical experiments can be performed in less time by using a validated and reliable FEA procedure. Performing deep drawing experiments via FEA accelerates experimental study period and eliminates the negative environmental effects of the physical process caused by the huge amount of power consumption and waste oil generation. “Simufact.forming” program, a finite element simulation software developed especially for metal forming processes, was used in this study. Experiment conditions were obtained by modeling deep drawing molds seen in Fig. 8 in “Simufact.forming” software and analyses were performed by applying experiment parameters. Analyses have been compared with experimental results for verifying simulation procedure.

Analyses were performed using elements having 2 mm side length and 3 layers in the thickness direction of the work part to obtain more reliable stress and deformation results in the sheet while punch, blankholder and die were defined as rigid body in the analyses performed in three dimensional conditions. Coulomb friction was chosen as friction type which is widely used for calculating friction conditions where contact stresses generally not exceeding the flow strength of friction couples.

Effectiveness of deep drawing process was evaluated by investigating maximum deep drawing height ($h_{\text{max}}$), total deep drawing energy ($E_t$), maximum deep drawing force ($F_{\text{max}}$), average deep drawing force ($F_{\text{avg}}$) and thickness variation ratio ($tvr$) values via performed analyses with various constant and variable blankholder forces using a verified FEA procedure. Deep drawing analyses were performed with 11 different blankholder force variation conditions in total. 3 of these were constant blankholder force conditions (A-C) and the other 8 were variable blankholder force conditions (D-K). Blankholder force variations of prominent conditions are specified in Fig. 9.

![FIGURE 8 Deep drawing analysis in Simufact.forming software](image)

![FIGURE 9 Variation of blankholder forces in simulation based studies](image)
Punch stroke also expresses obtained height of deep drawn product. Total energy ($E_t$), which is calculated as follows, expresses the work made during deep drawing process:

$$E_t = \int_0^{h_{\text{max}}} F_{\text{dd}} dh$$  \hspace{1cm} (5)

It is aimed to obtain maximum deep drawing height ($h_{\text{max}}$) with minimum total energy to increase the effectiveness of deep drawing process. Maximum deep drawing force ($F_{\text{max}}$) is the maximum value of force applied to the work part by the punch during deep drawing process. It is desired to make maximum deep drawing force as far as possible small by distributing applied force to the sheet metal homogeneously during the deep drawing process for preventing tearing in the sheet. Likewise, average deep drawing force ($F_{\text{avg}}$) is desired to be as far as possible small to realize deep drawing process as effectively with minimum energy and is calculated as follows since it is a standard magnitude expressing required energy per unit stroke:

$$F_{\text{avg}} = \frac{E_t}{h_{\text{max}}}$$  \hspace{1cm} (6)

Thickness variation of deep drawn product is also extremely important for the effectiveness of deep drawing process. In other words, quality of deep drawn product increases with decreasing thickness variation. Therefore, it is desired to obtain deep drawn product having more homogeneous thickness distribution. Thickness variation ratio ($tvr$) is calculated as follows by measuring thickness values of the product after deep drawing process from critical points shown in Fig. 10.

$$tvr = \frac{t_f - t}{t}$$  \hspace{1cm} (7)

In above equation, $t_f$ is the final thickness of the work part [mm] in critical points and $t$ is the original thickness of the work part [mm].

Thickness variation ratios in critical points under different blankholder force variation conditions determined as ‘B’ and ‘D’ are seen in Fig. 11. Thinning of the work part considerably decreases in blankholder force variation condition ‘D’ in which blankholder force was increased in the final stages of deep drawing process especially around critical point ‘3’ where edge rounding of punch contacts with work part which leads to tearing. Likewise, thickening of the deep drawn product decreases around critical point ‘5’ where edge rounding of die contacts with work part and more homogeneous thickness distribution was obtained. Lowest thickness variation ratio (17%) was obtained in deep drawing analysis ‘C’ performed with 10 kN constant blankholder force. However, deep drawing under 10 kN or lower blankholder force values resulted with wrinkling in the work part. On the other hand, although low thickness variation ratio values changing between 22% and 31% were obtained in the other analyses (E-K), maximum deep drawing height values were not increased considerably.
Thickness variation ratio, maximum deep drawing height and average deep drawing force variations depending on blankholder force variation conditions

Maximum deep drawing height ($h_{\text{max}}$), total deep drawing energy ($E_t$), maximum deep drawing force ($F_{\text{max}}$), average deep drawing force ($F_{\text{avg}}$) and thickness variation ratio ($tvr$) values obtained from analyses performed in 11 different blankholder force variation conditions having deep drawing ratio of 2 using 6082-T6 aluminum alloy sheet specimen having 150 mm diameter and 1 mm thickness are seen in Fig. 12.

Tearing occurred around critical point ‘2’ in blankholder force condition ‘A’ having 36 kN constant blankholder force value when deep drawing height reached to 34.25 mm with a maximum stress value of 355 MPa. In this analysis, thickness variation ratio was determined as 100%. Furthermore, maximum deep drawing height values were obtained in blankholder force conditions ‘B’ and ‘D’ in which maximum thickness variation ratios took place with 32%.

Minimum average deep drawing force occurred in blankholder force condition ‘C’ as seen in Fig. 13, since minimum limit value of blankholder force was applied in this condition. However, the lowest deep drawing height was obtained in this condition with wrinkling in the work part while analysis could be performed with low deep drawing force and energy requirement as seen in Fig.’s 13 and 14.
CONCLUSION

Maximum deep drawing height (h_{max}), total deep drawing energy (E_t), maximum deep drawing force (F_{max}), average deep drawing force (F_{avg}) and thickness variation ratio (tvr) values of high strength and low formability 6082-T6 aluminum alloy were investigated with an experimentally verified FEA procedure in this study for increasing effectiveness of deep drawing process and determining appropriate process conditions by performing analyses under various constant and variable blankholder force conditions.

It is observed from the results of experiments that deep drawing height increases with increasing blankholder force up to 36 kN, while tearing and cracking occurs in the work part when blankholder force exceeds 36 kN. On the other hand, it is observed that wrinkling occurs when blankholder force drops below 10 kN due to the decrease of deep drawing height. It is also observed from investigations that, increasing constant deep drawing force increases thickness variation ratio in the work part which leads to decrease in product quality.

Various increasing and decreasing variable blankholder forces were applied in this study during deep drawing process of 6082-T6 aluminum alloy sheet material for increasing deep drawing height and decreasing thickness variation ratio as well as consumptive energy.

Compared to analysis ‘B’ which was performed under 35 kN constant blankholder force, deep drawing height was increased with decrease in thickness variation ratio in measuring points ‘3’ and ‘5’ in analysis ‘D’ which was performed under variable blankholder force conditions.

Thickness variation ratio was lower in the successful variable blankholder force applications, namely E and G.

A considerable deep drawing height increase was not observed in unsuccessful variable blankholder force applications which were not included in the table. As a result of this study, it is observed that higher deep drawing height and more homogeneous thickness distribution can be obtained in deep drawing process of 6082-T6 aluminum alloy sheet by applying appropriate variable blankholder forces compared to constant blankholder force applications.

Declarations.

List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEA</td>
<td>Finite Element analysis</td>
</tr>
<tr>
<td>ABS</td>
<td>Anti-lock brake system</td>
</tr>
<tr>
<td>GTN</td>
<td>Gurson-Tvergaard-Needleman</td>
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</tbody>
</table>

Availability of data and materials. Some of the experimental data used in this study can be found in the Reference numbered 17. Simufactforming project files related with this manu-

script were zipped and attached to the submitted files.

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The bioactive compounds of detrital leaves of seaweed, *Zostera* spp. found as beach-cast were investigated by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Extracts of methanol, ethyl acetate, and n-hexane of detrital *Zostera* spp. leaves collected from Coasts of Aegean Sea, Izmir (Turkey) were used to determine the major bioactive constituents. Major bioactive elements in detrital leaves of *Zostera* spp. were elucidated as apigenin-7-O-glucoside, luteolin-7-O-glucoside, rosmarinic acid and diosmetin-7-sulfate. Minor amounts of apigenin-7-O-glucoside, luteolin-7-O-glucoside and rosmarinic acid were identified in the detrital leaves. Diosmetin-7-sulfate was the major compound in quantity. The antimicrobial activities of the extracts and individual bioactive compounds were tested for *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Candida albicans* and *Vibrio alginolyticus*. The antimicrobial activity of solvent extracts and the isolated compounds exhibited different sensitivities to the microorganisms tested. Antimicrobial activity was higher in the ethyl acetate extract in comparison to n-hexane and methanol extracts. Diosmetin-7-sulfate had no antimicrobial activity. Apigenin-7-O-glucoside, luteolin-7-O-glucoside and rosmarinic acid inhibited antifungal growth. Apigenin-7-O-glucoside and luteolin-7-O-glucoside showed antibacterial activity with different sensitivity towards bacteria. Dead biomass of *Zostera* spp. in beach-cast form can be used as a source for bioactive constituents. The bioactive potential of ‘detrital leaves’ of *Zostera* spp. may be of importance in projecting towards various biological applications.

**KEYWORDS:** Seaweed, *Zostera* spp., beach-cast, bioactive, antimicrobial
characterization and quantification of bioactive substances of beach-cast seaweed are essential for disclosing their potential application. The present study was undertaken to reveal the phytochemical structure of detrital leaves of Zostera spp. found as beach-cast at Aegean Sea Coasts, Izmir, Turkey and to evaluate the antimicrobial activities of different solvents and major bioactive compounds of detrital Zostera spp. leaves.

MATERIALS AND METHODS

Detrital Zostera spp. Samples. Detrital leaves of Zostera spp. were collected from the coasts of Guýbahce and Kalabak Coasts Izmir, Aegean Sea, Turkey at the coordinates of Lat. 09°17.417’N; Long. 07°08.558’E in June. Samples of detrital leaves were immediately transferred to the laboratory and washed thoroughly with tap water to detract from outer materials. Following to washing up the material was air-dried in shade until constant weight obtained and then placed in an electric mixer to get powder form. The powdered samples were kept in laboratory refrigerator until use.

Extraction and Isolation. The powdered Zostera spp. (2,270 g) samples were used to extract by maceration (20 L). The extract was filtered and densification under reduced pressure to pick up the crude methanol extract (638.82 g). The methanic extract was suspended in distilled water: methanol (1:1) mixture and fractionated by liquid-liquid extraction successively with n-hexane, and ethyl acetate, respectively. The reasons for the solvents selected are n-hexane allows to extract non-polar compounds (terpenoids, some aglycones, etc.), medium polar compounds (such as most of the aglycones (flavonoids, coumarins etc.); ethyl acetate to extract glucosides; methanol to extract polar compounds (polysaccharides, glycosides). Each fraction was concentrated to dryness under reduced pressure by evaporation to yield n-hexane fraction (23.30 g), ethylacetate fraction (8.88 g) and remaining methanol fraction (521 g). Each fraction was tested for their activities. Ethylacetate fraction was selected for further isolation procedure to determine active compounds. Ethylacetate fraction (6.2809 g) was subjected to column chromatography on sephadex LH-20 by eluting methanol to obtain 250 fraction of 10 mL. Fractions were combined to their phytochemical contents which controlled by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Z-4 (78.6 mg) and Z-2 (23.64 mg) compounds were obtained from fraction 125-250 (681.10 mg) and 73-89 (1434.50 mg), respectively by crystallization. Preparative TLC was also used to further purification of Z-1 (9.32 mg), Z-3 (23.4 mg) and Z-4 (78.60 mg) compounds from fractions 65-72 (475 mg); 73-89, 90-110 (239.2 mg), 111-124 (98 mg) and 111-124, 125-149, respectively. Ethyl acetate: methanol: water (80:13.5:10) mixture solvent system was used on silicagel 60 F254 (Merck 1.05744) plates for separation to obtain active components. Spectroscopic techniques such as MS, 1H-NMR and 13C-NMR were used for the structure elucidation of the isolated compounds.

HPLC Analysis. HPLC analyses were performed with an Agilent LC 1200 model chromatograph (Agilent Technologies, California, USA). The diode array detector (DAD) was adjusted at wavelength of 330 nm, and peak areas were integrated automatically with Agilent computer software. The chromatograms were plotted and processed by means of the software as expressed above. Separation was conducted using an ACE 5 C18 (250 mm×4.6 mm; 5 µm) column. The mobile phase was composed of 0.2 % phosphoric acid in water (A), acetonitrile (B) and in gradient elution: initial 0 min, A–B (90:10, v/v); then 0–20 min, linear change from A–B (90:10, v/v) to A–B (40:60); then 20.01–25 min, elution was changed as A–B (0:100) isocratic elution. The flow rate was 1.0 ml min⁻¹ and column temperature was kept at 40°C. The volume of the sample injection was 10 µL.

Preparation of Standard and Sample Solutions. Standard stock solutions were prepared for the relevant compounds. All compounds were weighed as 1 mg and dissolved in 1 mL of methanol: water solution (80:20). Different concentration levels (0.001 mg mL⁻¹, 0.005 mg mL⁻¹, 0.01 mg mL⁻¹, 0.05 mg mL⁻¹ and 0.1 mg mL⁻¹) were adjusted by adding the stock solution at different dilution rates. The injections (10 µL) were done for each standard solution in triplicate. The calibration curves were obtained by plotting the peak area of each solution against the concentration.

For HPLC analysis, dried and powdered Zostera spp. aerial parts were weighed (500 mg) and extracted with 10 mL of methanol: water (80:20) mixture in ultrasonic bath for 15 min x 4 times. Extract was filtered through a 0.45 µm membrane filter after adjusting to a final volume of 10 mL with the same solvent in volumetric flask. Triplicate 10 µL injections were performed for plant sample.

Determination of Antimicrobial Activity of Extracts and Isolated Compounds. In this study, n-hexane, ethyl acetate and methanol extracts of Zostera spp. were screened for their potential in vitro antibacterial activities against Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Bacillus subtilis ATCC 6633, Vibrio alginolyticus ATCC 17749 and antifungal activities against Candida albicans ATCC 10231. Extracts were dissolved in 20% DMSO at a concentration of 20.000 µg mL⁻¹.
Microbroth dilution method was used for determination of the minimum inhibitory concentrations (MIC) [27]. The cultures were obtained in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA). NaCl (2%) was added into Mueller Hinton Broth Medium for *Vibrio alginolyticus* ATCC 17749. Serial two-fold dilutions ranging from 10,000 to 78 μg mL<sup>-1</sup> were prepared in medium. A set of wells containing only inoculated broth and 10% DMSO were used as control. After incubation for 18-24 h at 37±1 °C for bacteria and 48 h for fungi, the last well with no microbial growth was recorded to represent MIC value (μg mL<sup>-1</sup>).

The isolated compounds (apigenin-7-O-glycoside, luteolin-7-O-glycoside, rosmarinic acid and diosmetin 7 sulphate) were dissolved in 20% DMSO at a concentration of 500 μg mL<sup>-1</sup>. The minimum inhibition concentrations for the isolated compounds were determined as explained above with the same microorganisms however, here, serial two-fold dilutions ranging from 2 to 250 μg mL<sup>-1</sup> were prepared in medium.

**RESULTS AND DISCUSSION**

In the current study, the isolated compounds from *Zostera* spp. were elucidated as apigenin-7-O-glucoside (Z-1), luteolin-7-O-glucoside (Z-2), rosmarinic acid (Z-3) and diosmetin-7-sulfate (Z-4) by their structures. H NMR and C NMR spectral data for bioactive compounds in detrital *Zostera* spp. extracts were presented in Table 1 and 2, respectively (in the tables Z3 was not included for the spectral data as the data of Z1, Z2 and Z4 were fair for the structure). Isolated compounds were quantitatively analysed in *Zostera* spp.

Structure elucidation of the compounds performed by spectroscopic techniques (MS, 1H-NMR and 13C-NMR) allowed for the identification four main metabolites; apigenin-7-O-glucoside (Z-1), luteolin-7-O-glucoside (Z-2), rosmarinic acid (Z-3) and diosmetin-7-sulfate (Z-4).

The results of this study were confirmed with data for the compounds which have been described in the literature [17–20, 25, 26]. The structure of the compounds was given in Fig 1.

Quantitative analysis of the isolated compounds has also been performed by HPLC. The contents of the plant, retention times and calibration curves were given in Table 3 for each compound. HPLC chromatogram of *Zostera* spp. was presented in Fig 2.

All the isolated compounds have obtained from detrital leaves and diosmetin-7-sulphate have been the major constituent with highest in quantity. The concentrations of four main bioactive compounds varied considerably and increased in the order of apigenin-7-O-glucoside< luteolin-7-O-glucoside< rosmarinic acid< diosmetin-7-sulfate. The major constituents detected in the detrital leaves of *Zostera* spp. in the present study comply with the bioactive compounds of *Zostera* species in the literature [7, 14, 17, 19, 20, 25, 26, 28]. However, the concentrations of the major constituents found in this study differed

<table>
<thead>
<tr>
<th>Position</th>
<th>Z-1</th>
<th>Z-2</th>
<th>Z-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6.84 (s)</td>
<td>6.72 (s)</td>
<td>6.82 (s)</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>6.80 (d, 2.0)</td>
<td>6.76 (d, 1.6)</td>
<td>6.52 (d,2.0)</td>
</tr>
<tr>
<td>7</td>
<td>6.42 (d,2.0)</td>
<td>6.42 (d, 1.6)</td>
<td>7.02 (d,2.0)</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1'</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>7.93 (d, 8.4)</td>
<td>7.39 (d, 2.4)</td>
<td>7.46 (d, 2.4)</td>
</tr>
<tr>
<td>3'</td>
<td>6.90 (d, 8.4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4'</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5'</td>
<td>6.90 (d, 8.4)</td>
<td>6.88 (d,8.0)</td>
<td>7.07 (d, 8.8)</td>
</tr>
<tr>
<td>6'</td>
<td>7.93 (d,8.4)</td>
<td>7.42 (dd, 2.0, 8.0)</td>
<td>7.56 (dd, 2.0, 8.4)</td>
</tr>
<tr>
<td>-OCH&lt;sub&gt;3&lt;/sub&gt; 1&quot; 2&quot;</td>
<td>5.04 (d, 7.2)</td>
<td>5.60 (d,6.8)</td>
<td>3.84 (s)</td>
</tr>
<tr>
<td>3&quot;</td>
<td>3.69-3.13</td>
<td>3.70-3.14</td>
<td></td>
</tr>
</tbody>
</table>

*Z-1: Apigenin-7-O-glucoside; Z-2: Luteolin-7-O-glucoside; Z-4: Diosmetin-7-sulphate
from the concentrations of the bioactive substances in previous studies. Diosmetin-7-sulfate is the most available major component of beach-cast Zostera spp. with the concentration of 1.413 mg g⁻¹ dry weight in Gulbahce and Kalabak samples (Izmir) in this study. The dominance of diosmetin-7-sulfate was in agreement with the previous study on the flavonoid content of Z. nolitii samples from Arcachon [14]. The diosmetin-7-sulfate quantity was within the range of 0.18 and 5.69 mg/g dry weight in the samples from the Mediterranean and Atlantic region including the sampling site, Dikili (Izmir) which is in the same province of our sampling area [20]. Here, apigenin-7-O-glucoside concentration was 0.031 mg g⁻¹ dry weight in the detrital leaves of Zostera spp., which was lower than that of the results reported by Grignon-Dubois et al. [14]. The concentrations of apigenin-7-O-glucoside were 98 ± 2 μg g⁻¹ dry weight in October and 69 ± 2 μg g⁻¹ dry weight in June in the aqueous crude extracts of the Z. nolitii samples from Cadiz. Luteolin-7-O-glucoside was found to be at the concentration of 0.046 mg g⁻¹ ry
weight in our samples. This value of luteolin-7-O-glucoside is within the range of 0.04 and 1.42 mg g⁻¹ dw reported for Z. noltei across the Atlantic and Mediterranean region [20]. Rosmarinic acid concentration in this study was found to be 0.431 mg g⁻¹. Guan et al. [19] reported that the concentrations of rosmarinic acid in the range of 15.6 and 106.8 μg mL⁻¹ on the surface of Z. marina as well as 2.683 μg gdw⁻¹ in the tissue of the leaves. The quantities of rosmarinic acid in fresh Z. nolitii leaves in Cadiz Bay (11.254 μg g⁻¹ dry weight) and Alfacs Bay (823 μg g⁻¹ dry weight) were reported by Grignon-Dubois et al. [14]. The level of phytochemicals in fresh leaves can be higher in comparison to dead leaves. Therefore, rosmarinic acid concentration was compared with the detrital leaves of Z. nolitii and Z. marina. It is reported that the concentrations of rosmarinic acid ranged from 2.2 to 18.0 mg g⁻¹ for detrital leaves of Z. nolitii and 1.3 to 11.2 mg g⁻¹ dry weight for detrital leaves of Z. marina collected from Arcachon lagoon (France) by Achamlale et al. [25]. Wang et al. [7] assessed the mean value of 3.13 mg g⁻¹ rosmarinic acid in dry eelgrass tissue. Rosmarinic acid concentration (0.431 mg g⁻¹ dry weight) in our samples can be considered low, in general.

The variations in the concentrations of bioactive constituents can be explained by the fact that the phytochemical composition of the marine plant varies with species, physiological condition, environmental characteristics such as climate, location, salinity, temperature, collection period [16]. Thus, the lower amounts of the bioactive substances in Zostera spp. in the present study may be related to the physiological state of the detrital leaves. Schulz et al. [29] reported that the metabolite profile of the various substrates such as algae or decaying matter regardless of their origins may have similar profile however, differ in quantitatively. In contrast, geographic source for Z. noltei had influence on chemotypes, as indicated in characterization of the flavonoids in Z. noltei [20]. The quantitative dissimilarity of the detrital leaves in our samples may indicate the physiological state of the leaves and biogeographical variations. Geographic genetic variability may have an effect on the phenolics content in Zostera species, however, differentiation by the geography is still unclear and needs further investigation [20].

TABLE 3
Spectral (HPLC) data and the quantification of four compounds isolated from the detrital leaves of Zostera spp.

<table>
<thead>
<tr>
<th>Compound*</th>
<th>Calibration curves</th>
<th>mg/g ± SD</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-1</td>
<td>y = 26556.5816x – 4.6118</td>
<td>0.031±0.0001</td>
<td>10.970</td>
</tr>
<tr>
<td>Z-2</td>
<td>y = 29398.6760x – 15.4027</td>
<td>0.046±0.0001</td>
<td>9.739</td>
</tr>
<tr>
<td>Z-3</td>
<td>y = 25613x – 2.346</td>
<td>0.431±0.0007</td>
<td>11.570</td>
</tr>
<tr>
<td>Z-4</td>
<td>y = 37182.5359x – 24.1002</td>
<td>1.413±0.0018</td>
<td>13.117</td>
</tr>
</tbody>
</table>

*Z-1: Apigenin-7-O-glucoside; Z-2: Luteolin-7-O-glucoside; Z-3: Rosmarinic acid; Z-4: Diosmetin-7-sulphate

FIGURE 2
HPLC profile of detrital Zostera spp. extract collected from Izmir Coast, Turkey
Lee et al. [32] tested methanol extracts of 17 seaweeds for their antimicrobial activity and of the seaweeds screened the MIC of Neorhodomela aculeata methanol extract ranged from 8 to 32 mg mL⁻¹ against the three human skin pathogens, showing similarity to the MICs of methanol extracts in the present study. However, methanol extracts showed activity only for E. aerogenes and marine pathogen, V. alginolyticus in our samples. The inhibitory effect of n-butanol fraction of Z. marina for S. aureus was reported as MIC of 20 mg mL⁻¹ by Zheng et al. [33], showing higher value than our overall MIC values. The overall range of the MIC values of detrital Zostera extracts were detected in compliance with the range of MICs for various seaweeds [34]. Hence, our MICs ranged from 0.625 to 10 mg mL⁻¹ for all fractions in our study general, showing similarities and differences from the results in various studies. The antimicrobial activities and bacterial inhibition capacity of green alga Ulva rigida extracts to the given bacteria differed considerably among the solvents used in the extraction phase [35]. In the tests with different solvents it was found that the solvents have an impact on the antimicrobial activity. The inhibitory activity by the solvent should be considered in evaluating the MIC values of the extracts. Furthermore, the physiological state of the leaf material may have an impact in terms of antimicrobial capacity. Thus, Harrison and Chan [30] reported that the extracts from the aged leaves of Z. marina and long-term drying in the field may lead to loss of anti-bacterial activity.
The isolated compounds; apigenin-7-O-glycoside, luteolin-7-O-glycoside, diosmetin-7-sulfate and rosmarinic acid exhibited different activities to the microorganisms tested (Table 5). Apigenin-7-O-glycoside was effective for *E. coli*, *E. faecalis*, *V. alginolyticus*, *C. albicans* Luteolin-7-O-glycoside was effective for *E. faecalis*, *V. alginolyticus* and *C. albicans*. Diosmetin-7-sulfate did not exhibit any antimicrobial activity to the microorganisms tested. Rosmarinic acid was only effective for *C. albicans*.

In terms of isolated compounds, the strongest antifungal effect was assessed in apigenin-7-O-glycoside with the MIC of 0.0625 mg mL⁻¹ to *C. albicans*. Luteolin-7-O-gluco-side and rosmarinic acid also exhibited antifungal effect with MICs of 0.125 mg mL⁻¹ and 0.250 mg mL⁻¹, respectively. Rosmarinic acid did not show any antibacterial effect in our MIC tests. In contrast, Wang et al. [7] reported the antibacterial effects of rosmarinic acid at the concentration of 1 mg mL⁻¹ against some bacteria strains: *Klebsiella* sp., *Stenotrophomonas maltophilia*, *Streptomyces* sp.and *Pantoea* agglomerans. On the other hand, Guan et al. [19] reported that rosmarinic acid and diosmetin-7-sulfate did not inhibit bacterial growth. Our results for rosmarinic acid and diosmetin-7-sulfate are in agreement with the findings of Guan et al. [19] as rosmarinic acid and diosmetin-7-sulfate had no antibacterial activity in our test. Strong antimicrobial effect of luteolin was reported by Xu and Lee [36]; Yamamoto and Ogawa [37], confirming our results for luteolin-7-O-glucoside with antimicrobial effects to *E. faecalis*, *C. albicans* and *V. alginolyticus*. The bioactivities of the compounds exhibited different antimicrobial effects by the microorganisms tested. This can be explained by that microorganisms show different sensitivities towards phenolics, as reported by Puupponen-Pimia [38]. Inhibition of bacterial growth may be linked to the interruption of cell-to-cell communication due to the bioactive compounds, manifesting failure in quorum sensing (QS) mechanism. Production of QS inhibitory compound by *macroalgae* was observed by Carvalho et al. [39] however, QS inhibition showed variations by the algae species, indicating specific activity of the metabolites found in the *macroalgae* to the bacteria. Furano crystals isolated from benthic marine macroalgae, *Delisea pulchra*, acting as intercellular signal antagonist for cell-cell communication were reported for *Serrata liquefaciens* [40]. Antagonistic effect of benthic marine macroalgae, *D. pulchra* on intercellular quorum sensing mechanism of *Vibrio harveyi* was detected in prawn infections [41]. Marine pathogen, *V. alginolyticus* inhibited by apigenin-7-O-glucoside and luteolin-7-O-glucoside in our tests, indicating their probable antagonist effect. Further investigation is required to determine the mechanism of QS for apigenin-7-O-glucoside and luteolin-7-O-glucoside isolated from detrital *Zostera* spp.

The economic value of beach-cast sea grasses due to their phenolic acid contents was reported for *Cymodocea nodosa* by Grignon-Dubois and Rezzonico [15]. In particular, the sulfated flavonoids are of high economic value because they are not commercially available [19]. Considering the results of this study, the beach-cast seaweeds in waste condition can be utilized for their content of phenolics, particularly sulfated flavonoids. Nonetheless, it should be emphasized that over-exploitation of the natural resources including the beach-cast for industrial purposes may be challenging for the next years. Here, we underline the significance of waste seaweed utilization for extracting the bioactive compounds instead of fresh plant or deposited on the beach.

In conclusion, the studies on the bioactive compounds of beach-cast of *Zostera* spp. are limited. Detrital *Zostera* spp. leaves found as beach-cast could be considered as renewable source of bioactive substances. In the present study, bioactive compounds were identified as diosmetin-7-sulfate, apigenin-7-O-glucoside, luteolin-7-O-glucoside and rosmarinic acid. Antimicrobial activities of the detrital Zostera spp extract and individual bioactive compounds were detected, showing the variation with the characteristic of the microorganisms and the metabolites as well as solvents used in extraction phase. Of the individually isolated compounds in detrital leaves of *Zostera* spp., apigenin-7-O-glucoside and luteolin-7-O-glucoside exhibited antibacterial activity despite having different sensitivity towards bacteria. Rosmarinic acid inhibits fungal growth. Diosmetin-7-sulfate did not exhibit any antimicrobial activity for the microorganisms tested. Basing on the results of this investigation the beach-cast *Zostera* spp. could be considered as bioactive compounds source for pharmaceuticals and food additives.

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ANTIBACTERIAL EFFECTS, FATTY ACID PROFILES, AND \( \gamma \)-TOCOPHEROL CONTENTS OF PISTACHIO (PISTACIA VERA L.) AND WALNUT (JUGLANS REGIA L.)

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ABSTRACT

Pistachio (Pistacia vera L.) and walnut (Juglans regia L.) are frequently consumed in daily life owing to their taste and health benefits. This study aimed to analyze the pistachio and walnut oils for antibacterial effects against certain bacteria and to record their fatty acid profiles and \( \gamma \)-tocopherol contents. Antibacterial activities were evaluated by agar well diffusion and broth microdilution methods, fatty acid profiles were recorded by GC/MS, and \( \gamma \)-tocopherol contents were quantified by HPLC. The results revealed that, pistachio and walnut exhibited weak antibacterial effect against the test bacteria. It was also observed that the most abundant fatty acid in pistachio was cis-9-oleic acid (61.72%) while that in walnut was linoleic acid (59.65%). The \( \gamma \)-tocopherol content was observed to be 17.98 mg/kg and 10.55 mg/kg in pistachio and walnut, respectively. Consequently, extracts of walnut and pistachio displayed the weak antibacterial effect but kernels of them possessed the essential fatty acids and \( \gamma \)-tocopherol contents which were supported the human health.

KEYWORDS:
Antibacterial effect, fatty acid, \( \gamma \)-tocopherol, Pistacia vera L., Juglans regia L.

INTRODUCTION

Pistachio (Pistacia vera L.), belongings to the family Anacardiaceae [1], is widely consumed around the world because of its taste, nutritional value, and health-related benefits [2]. Although pistachio is native to Central and Western Asia, it is also being cultivated in the Mediterranean basin in recent times [3].

Walnut (Juglans regia L.) belongs to the Juglandaceae family [4] and is also consumed by people around the world. The nuts or the kernels are the edible parts of both pistachio and walnut. Because of their taste and health benefits, these nuts or kernels are frequently consumed as snacks and are used in making several desserts in Turkey. These nuts or the kernels contain unsaturated fatty acids, dietary fibers, and plant proteins in specific quantities [5]. The consumption of these nuts in the daily diet is beneficial for human health. Pistachio has been endorsed to be a good source of fiber for humans, containing as much as 9.9 g of dietary fiber per 100g [6]. It has also been reported that pistachios are the only nuts that contain anthocyanin pigments, which give them their characteristic color [2].

Natural products for the medicinal purposes have been used around the world for centuries. Because the increasing antibiotic resistance has become a global problem, several researchers have focused on the investigation of the antimicrobial effects of plants and natural products in recent years. The natural matrices are also widely described as “prototypes of new antimicrobial agents” [7]. Hence, this study aimed at investigating the antibacterial properties, fatty acid profiles, and \( \gamma \)-tocopherol contents of the pistachio and walnut.

MATERIALS AND METHODS

Sample Collection. The walnut samples were collected from Fistikozu Village (Sanliurfa, Turkey) and the pistachio samples were collected from Yesilozen Village (Sanliurfa, Turkey) during the 2017 harvest. Kernels of the air-dried pistachio and walnut samples were separated manually and were separately ground using a kitchen-type grinder.

Preparation of Extracts. The extraction process was performed following the method published by Dulger and Gouuz [8], with some modifications. The ground kernels of pistachio and walnut (20 g each) were extracted using 300 mL of ethanol for 24 h in a Soxhlet apparatus. The solvent was then evaporated from the extracts at 55 °C using a SciLogex RE100-Pro Rotary Evaporator. The filter-sterilized and concentrated extracts were stored at -20 °C until use and used for antibacterial activity and minimal inhibitory concentrations (MIC) analysis.

Assay for Evaluation of Antibacterial Activity. The antibacterial activity was evaluated using
the agar well diffusion method [9]. The extracts of pistachio and walnut were tested against the following bacteria: Klebsiella pneumoniae ATCC 33495, Bacillus subtilis subsp. spizizenii ATCC 6633, Staphylococcus aureus ATCC 6538, and Enterobacter aerogenes ATCC 13048. Mueller Hinton Agar was used as a culture medium for the growth of the test bacteria. Using a sterilized cork borer (11 mm diameter), wells were made in the agar medium. The extracts were dissolved in 10% aqueous dimethyl sulfoxide (DMSO), filter-sterilized, and 100 μL of each of the extracts was added to the wells. All the Petri plates were incubated at 37 °C for 48 h. Erythromycin was used as the positive control, while 10% aqueous DMSO was used as the negative control. The inhibition zones were measured by a digital caliper. All the tests were performed in triplicate.

Minimal Inhibitory Concentrations (MIC). The MIC values of pistachio and walnut extracts against Klebsiella pneumoniae ATCC 33495, Bacillus subtilis subsp. spizizenii ATCC 6633, Staphylococcus aureus ATCC 6538, and Enterobacter aerogenes ATCC 13048 were determined using the broth microdilution method [10, 11]. Aliquots of 100 μL of the Mueller Hinton Broth were placed into each well of 96-well microplates. Then, 100 μL of each of the extracts was added to the first row of the microplates, and dilutions of the compounds (100-0.39 mg/mL) were made. To these wells, 100 μL of each of the 16-hour-old bacterial cultures were added after adjusting to the 0.5 McFarland Standard, and the microplates were incubated at 37 °C for 18 h. After the incubation period, 20 μL of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC) solution was added to the wells and the plates were incubated again for 30 min. Wells in which the color change was not observed indicated corresponding MICs.

Determination of The Fatty Acid Profile. A BUCHI B-811 extraction system was used to extract the oils from pistachio and walnut samples. Hexane was used as the solvent, which was removed from the extracts using an evaporator after extraction of the oil. 10 mL of hexane were added to 0.1 g of oils and mixed. To this mixture, 0.5 mL of 2N methanolic KOH solution was added and mixed. The mixture was kept in dark for 1-2 h till a clear supernatant was obtained. These supernatants were then used for determination of the fatty acid profiles. The fatty acid compositions of pistachio and walnut were determined using gas chromatography coupled with a mass spectrometer (GC/MS) (Shimadzu 2010 QP Ultra), equipped with a Flame-ionization detector (FID). A Restek Rtx-2330 capillary column (60 m x 0.25 mm ID, 0.10 μm) was used to separate the fatty acid methyl esters from 1 μL of the injected sample. Helium, at a flow rate of 30 mL/min and split rate of 100:1, was used as the carrier gas. The injection temperature was adjusted at 250 °C. The initial temperature was fixed at 90 °C for 4 min, while the final temperature was set at 230 °C for 9 min. The temperature of the FID was 255 °C.

Determination of the γ-Tocopherol Content. 10 g each of the pistachio and walnut samples were extracted using 50 mL of hexane, which was removed by an evaporator after completion of the extraction process. To 100 μL of the oils obtained from pistachio and walnut, 900 μL of hexane was added and vortexed. Then, 200 μL of the resulting mixtures was mixed with 800 μL of methanol and centrifuged at 15000 rpm for 10 min. The supernatants were filtered through a filter of pore size 0.45 μm, and were then used for γ-tocopherol analysis [12]. The γ-tocopherol contents of pistachio and walnut were determined using High Performance Liquid Chromatography (HPLC) (Thermo Scientific Ultimate 3000 HPLC) in conjunction with a diode-array detector (DAD). The chromatographic separation was achieved using a 5 μm Zorbax C18 column (250 mm x 4.6 mm) with solvent A (water) and solvent B (methanol) as the mobile phase under the 95% B and 5% A gradient condition. The injection volume was 20 μL, the flow rate was 1 mL/min, and the temperature of the column thermostat was 30 °C. The absorbance was measured at 300 nm.

Statistical Analysis. The results obtained from triplicate measurements of antibacterial activities were presented as “mean ± standard deviation” and analyzed using one-sample t-test, with p values ≤ 0.05 considered to be significant. The statistical analyses were made using the statistical package SPSS (version 20).

RESULTS AND DISCUSSION

The antibacterial activities of pistachio and walnut against the test bacteria were determined by two methods and the results are presented in Table 1. The results indicated that pistachio did not produce inhibition zone against the test bacteria, while walnut exhibited (13.78 mm) against only Klebsiella pneumoniae ATCC 33495. This inhibition zone was significantly greater (p ≤ 0.05) than that shown by erythromycin with 12.50 mm. Pistachio was observed to have MIC value of 100 mg/mL against all the test bacteria. In case of walnut, the MIC values were 50 mg/mL against Klebsiella pneumoniae ATCC 33495, Staphylococcus aureus ATCC 6538, and Enterobacter aerogenes ATCC 13048, and 100 mg/mL against Bacillus subtilis subsp. spizizenii ATCC 6633.
Enterobacter aerogenes terebinthus

Another study tested the extracts from 12 (Escherichia coli, negative bacteria (and essential oil obtained from the gum of bacteria such as 

mg/mL inhibited the growth of Gram-positive test 

[15]. Pereira et al. [16] reported that the extract of 

effects of the lipophilic extracts of 

researchers reported the antimicrobial activity of 

Shelled pistachios have a greater antimicrobial effect than roasted-salted pistachios. In other studies, researchers reported the antimicrobial activity of essential oil obtained from the gum of Pistachio vera L. [14], and the antibacterial and antifungal effects of the lipophilic extracts of Pistachio vera [15]. Pereira et al. [16] reported that the extract of fresh walnut leaves at a concentration of 100 mg/mL inhibited the growth of Gram-positive test bacteria such as Bacillus cereus, Bacillus subtilis, and Staphylococcus aureus, but not the Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumonia) and fungi (Candida albicans, and Cryptococcus neoformans). Another study tested the extracts from 12 Pistachio terebinthus (terebinth) fruits against Staphylococcus aureus, Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella enterica subsp. enterica serovar typhimurium and reported that Listeria monocytogenes and Salmonella typhimurium were more susceptible to the terebinth extracts than other bacteria [17]. The results reported in previous studies are consistent with the findings of our study.

The fatty acid compositions of pistachio and walnut were evaluated using GC/MS and results are presented in Tables 2 and 3, respectively. It was observed that fatty acid concentrations in the pistachio ranged between 0.10% and 61.72%. In addition, cis-9-oleic acid was the most abundant fatty acid in pistachio, followed by linoleic acid and palmitic acid (61.72%, 25.51%, and 8.29%, respectively).

Fatty acid concentrations in the walnut ranged between 0.10% and 59.65%. In the walnut, linoleic acid was the most abundant fatty acid, followed by cis-9-oleic acid and γ-linolenic acid (59.65%, 17.84%, and 9.79%, respectively). It was also observed that both pistachio, as well as walnut, possessed palmitic acid, palmitoleic acid, stearic acid, cis-9-oleic acid, γ-linolenic acid, α-linolenic acid, and cis-11,14-eicosadienoic acid, at varying concentrations.

The health-related properties of various nuts could be attributed to the high content of fatty acids and other micronutrients such as vitamin B, carotenoids, and tocopherols [18, 19]. Plant and animal foods contain dietary fats that can facilitate the absorption of fat-soluble vitamin such as vitamin A, D, E, and K. Certain plant and animal foods are also good sources of essential fatty acids such as linoleic acid and α-linolenic acid. The α-linolenic acid is a n-3 fatty acid, while linoleic acid is an n-6 fatty acid. These two fatty acids cannot be synthesized by the human body [6]. According to our findings, the pistachio and walnut contained α-linolenic acid (0.60% and 0.16%, respectively).

A previous study reported that oleic acid was the most abundant fatty acid in Pistacia terebinthus L., with concentrations ranging between 42.13% and 55.16% [17]. We observed that the most abundant fatty acid in pistachio was cis-9-oleic acid (61.72%), while that in walnut was linoleic acid (59.65%). These results suggested that the kernels of the pistachio and walnut possessed health-related properties owing to their high linoleic acid and cis-9-oleic acid contents. Moreover, it has been reported that oleic acid has a lowering effect on LDL [20]. In addition, polyunsaturated fatty acids are beneficial for the human cardiovascular system [21], because they regulate the lipid levels in the body [22].

The γ-tocopherol contents of pistachio and walnut were evaluated using HPLC (Figure 1) and results are summarized in Table 4. It was observed that the γ-tocopherol content of the pistachio was higher than that of the walnut (17.98 mg/kg and 10.55 mg/kg, respectively).

TABLE 1
Antibacterial activities of pistachio and walnut extracts

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Pistachio (mm)</th>
<th>Walnut (mm)</th>
<th>Ery (mm)</th>
<th>10% aqueous DMS (mm)</th>
<th>MICs of pistachio (mg/mL)</th>
<th>MICs of walnut (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13.78 ± 0.10a</td>
<td>-</td>
<td>12.50 ± 1.65a</td>
<td>-</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>ATCC 33495</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis subsp.</td>
<td>32.46 ± 1.42</td>
<td>-</td>
<td>24.45 ± 1.39</td>
<td>-</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>spizizenii ATCC 6633</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>12.20 ± 0.40</td>
<td>-</td>
<td>-</td>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>ATCC 6538</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 13048</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ery: Erythromycin, *mean ± standard deviation, *Superscript letter indicates significant differences (p ≤ 0.05) between walnut and erythromycin in Klebsiella pneumoniae ATCC 33495.
γ-tocopherol is an important form of vitamin E [23]. Humans and animals generally obtain vitamin E from plants, because they cannot synthesize it indigenously. γ-tocopherol is often found in several plant seeds [24]. Because of this, people obtain γ-tocopherol by consumption of seeds such as pistachio, walnut, almond, and hazelnut. It was reported that γ-tocopherol possesses anti-inflammatory properties and reduces the risk of cardiovascular disease and prostate cancer [23]. Lux et al. [25] reported the γ-tocopherol contents of pistachio and walnut to be 7.87 mg/100 g and 7.60 mg/100 g, respectively, while Stuetz et al. [26] reported values of 30.6 mg/100 g and 32.3 mg/100 g in raw pistachio and walnut, respectively.

**CONCLUSION**

In the present study, the antibacterial effects, γ-tocopherol contents, and fatty acid profiles of pistachio (*Pistacia vera* L.) and walnut (*Juglans regia* L.) were investigated. Both pistachio and walnut demonstrated weak antibacterial effect against the test bacteria; however, they can be beneficial for human health because of their γ-tocopherol and fatty acid contents.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Fatty acid compositions of the pistachio extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>Fatty acid</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>Myristic acid</td>
</tr>
<tr>
<td>C16:0</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>C18:0</td>
<td>Stearic acid</td>
</tr>
<tr>
<td></td>
<td>Unsaturated fatty acids</td>
</tr>
<tr>
<td>C18:3 n3</td>
<td>α-linolenic acid (ω-3)</td>
</tr>
<tr>
<td>C18:3 n6</td>
<td>γ-linolenic acid (ω-6)</td>
</tr>
<tr>
<td>C18:2 n6c</td>
<td>Linoleic acid (ω-6)</td>
</tr>
<tr>
<td>C16:1</td>
<td>Palmitoleic acid (ω-7)</td>
</tr>
<tr>
<td>C20:1</td>
<td>Cis-11-eicosanoic acid (ω-9)</td>
</tr>
<tr>
<td>C18:1 n9c</td>
<td>Cis-9-oleic acid (ω-9)</td>
</tr>
<tr>
<td>C20:2</td>
<td>Cis-11,14-eicosadienoic acid</td>
</tr>
<tr>
<td>C20:3 n3</td>
<td>Cis-11,14,17-eicosenioic acid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Fatty acid compositions of the walnut extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>Fatty acid</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>C11:0</td>
<td>Undecanoic acid</td>
</tr>
<tr>
<td>C16:0</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>C18:0</td>
<td>Stearic acid</td>
</tr>
<tr>
<td></td>
<td>Unsaturated fatty acids</td>
</tr>
<tr>
<td>C18:3 n3</td>
<td>α-linolenic acid (ω-3)</td>
</tr>
<tr>
<td>C18:2 n6c</td>
<td>Linoleic acid (ω-6)</td>
</tr>
<tr>
<td>C18:3 n6</td>
<td>γ-linolenic acid (ω-6)</td>
</tr>
<tr>
<td>C16:1</td>
<td>Palmitoleic acid (ω-7)</td>
</tr>
<tr>
<td>C18:1 n9c</td>
<td>Cis-9-oleic acid (ω-9)</td>
</tr>
<tr>
<td>C20:1</td>
<td>Cis-11-eicosanoic acid (ω-9)</td>
</tr>
<tr>
<td>C20:2</td>
<td>Cis-11,14-eicosadienoic acid</td>
</tr>
<tr>
<td>C22:6 n3</td>
<td>Cis-4,7,10,13,16,19-docosahexaenoic acid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4</th>
<th>The γ-tocopherol content of pistachio and walnut samples (R²: 0.9981)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>γ-tocopherol value (mg/kg)</td>
</tr>
<tr>
<td>Pistachio extract</td>
<td>17.98 ± 0.36^</td>
</tr>
<tr>
<td>Walnut extract</td>
<td>10.55 ± 0.23</td>
</tr>
</tbody>
</table>

^ mean ± standard deviation
REFERENCES


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**ABSTRACT**

Coniferous and broad-leaf mixed forest is an important vegetation formation and has a large distribution area in Huanglong Mountain Nature Reserve, Shaanxi Province, China. The spatial patterns of dominant woody species give important clues about the underlying processes driving regeneration and succession of the forest. In this paper, the trees of a forest were mapped, characterized and the spatial distribution patterns and spatial associations of dominant woody species (*Pinus tabulaeformis* Carr., *Quercus wutaishanica* Mayr., *Acer ginnala* Maxim. and *Populus davidiana* Dode) were analyzed using O-ring univariate O(r) and bivariate O12(r) statistics. We found that: (1) there are 28 species of trees which DBH is greater than 1 cm, the species are rich and the density of trees is larger. The stand diameter distribution appears an inverted "J" curve and the tree height distribution is similar to normal distribution. (2) *P. tabulaeformis*, *A. ginnala* and *P. davidiana* population show aggregative distribution on a small scale of 0-5m, and the aggregation intensity decreased with scale increase, and behaves randomly at other scales. The spatial patterns of *Q. wutaishanica* population are aggregative distribution from 0 to 44 m. Meanwhile the distribution patterns get more and more aggregative as its density increased, and the degree of aggregation decreases with the increasing of scales. (3) *P. tabulaeformis* and *Q. wutaishanica*, *P. tabulaeformis* and *P. davidiana*, *Q. wutaishanica* and *P. davidiana* show positive correlation at medium and small scales, while *A. ginnala* shows negative correlation with other species at some scales.

**KEYWORDS:**
Point pattern analysis, Inter-specific association, Species coexistence, O-ring Statistic

**INTRODUCTION**

The spatial distribution pattern and population structure of trees are the direct results of comprehensive interactions among species characteristics, environmental factors, and intraspecific and interspecific plant-plant interactions over a long period [1]. Studying and clarifying the pattern of population distribution patterns are helpful to deeply understand community structure. It is of great significance to correctly describe forest spatial distribution pattern judging the forest distribution regular, grasping the process evolution and predicting the trend of the changes [2]. In recent years, point pattern analysis has been widely used in ecology. As an effective tool for studying spatial patterns on different spatial scales, it overcomes the shortcomings that the traditional methods can only study the spatial distribution and connection of species on a single scale.

Loess Plateau is the largest region with serious land degradation caused by disturbance and is known for problems from drought and soil erosion, as well as for its poorly developed economy [3, 4]. Huanglong Mountain Forest is located on the Loess Plateau of Shaanxi. Coniferous and broad-leaf mixed forest is one of the typical zonal vegetation in Huanglong Mountain Forest. In this area, forest vegetation has been destroyed by human, therefore, restoration of these disturbed lands is urgent for regional development. The most effective way to improve the ecological conditions is the restoration of vegetation [3, 5]. Many scholars have studied the growth pattern, structural characteristics and dynamic changes of different tree species in the forest community [6-9]. Most researchers use the spatial point pattern method which applied in different growth conditions and different tree species populations to study the spatial distribution and spatial associations. However, there are few reports on forest spatial pattern in the Loess Plateau of Shaanxi Province. In this study, the spatial distribution and associations of the dominant tree species in coniferous and broad-leaved mixed forests of Huanglong Mountain are analyzed in detail with point pattern analysis. We attempt to answer the following...
questions: How does the spatial distribution and associations of the dominant tree species change at different spatial scales? What are the possible mechanisms that affect spatial distribution and associations?

**MATERIALS AND METHODS**

**Research Area Overview.** The study area was located in the Huanglong Mountain natural forest protection reserve, Shaanxi Province, China (35°29'–35°41'N, 109°42'–110°00'E), it is located at a staggered belt in the gully region of the Loess Plateau in Huanglong County. With an area of 194,174 hm², which forest land occupies 99.8% of the total land area. The region is a warm temperate zone with sub humid continent climate, annual average temperature ranges 8.6 °C, precipitation ranges 611. 8mm, frost-free time adds up to 2370 hours, and elevation ranges 1100-1300 m. Trees in the forest area are mainly composed by natural recovered forests that had been destroyed before. The main components of natural forest are P. tabulaeformis and Q. wutaishanica, secondly are P. davidiana and Betula platyphylla Suk, and most soil is cinnamon soil. Due to differences site conditions and other reasons, the whole forest is non-uniform distribution.

**Methods of Investigation.** According to the spatial pattern of the study sample plot requirements, the sample must have a certain scale in order to ensure that the various species can occur [10], at the same time the rectangular or square sample is better [11]. In August 2014, according to the technical requirements and research objectives, a 100 m × 100 m permanent plot with typical features was set up in the farm with TOPCON total station through a comprehensive survey of the Guanzhuang Forest Farm. By using the adjacent grid method, the plots were divided into 25 plots of 20 m × 20 m, and the trees with a diameter at breast height (DBH) ≥ 1 cm was recorded, together with their DBH (cm), tree height(m), crown breadth(m), and growth status. At the same time, the spatial location and coordinates of each tree species and environmental factors such as soil and litter were surveyed and recorded. The distribution of trees in the plot is shown in Figure 1.

**Data Analysis. Species important value and other parameters of the calculation.** The importance value (IV) is an important quantitative index of community and is often used to measure the importance of a certain species in a community [12]. The formula IV = (relative density + relative frequency + relative significance) / 3. The relative density (relative abundance) = individual number/ all tree species number × 100%; the relative frequency = the frequency of a specie / the frequency of all species × 100%; the relative significance = the thoracic area of a tree / the thoracic area of all tree species × 100%.

**Tree Diameter and Height Distribution.** Stand diameter structure is the most basic stand structure. It is a basis for measuring and studying stand diameter, area, volume and the growth of these factors, and is the basis of many forest management techniques [13]. In this study, the DBH of trees was taken as the index to measure the age of trees [14]. The tree was divided according to the DBH size and the tree structure was replaced by the age structure analysis community within the population dynamics, under normal climatic conditions, the diameter is thicker, the age greater, and vice versa. In this paper, P. tabulaeformis, Q. wutaishanica, A. ginnala and P. davidiana were set as the main tree species. The other species in the tree layer of the community were all identified as other tree species. The diameter at breast height was analyzed with 2 cm as diameter class, and 2cm < DBH ≤ 4cm is diameter class one, 4cm < DBH ≤ 6cm is diameter class two… and so on. The distribution of forest trees was discussed.

Tree height is closely related to diameter and volume, and tree height growth was less affected by stand density, largely determined by the merits of site conditions. Therefore, in forest management, the relationship between height and stand age (even-aged forest) or stand diameter (uneven-aged forest) is often used as the basis to evaluate site quality. In this study, 1 m was used as height class to analysis tree height distribution, and 1.3m < H ≤ 2m is height class one, 2m < H ≤ 3m is height class two… and so on.

**Spatial Pattern Analysis.** Point pattern analysis base on plant species in the spatial coordinates as the basic data, and use its spatial distribution point picture to do pattern analysis [15]. The method is widely used to study multi-scale spatial distribution patterns of plant populations and multi-scale correlation between two species. The O-ring function based on the Ripley K (d) and incorporates a new point pattern analysis method of the mark correlation function [16]. O-ring is a ring-scale analysis method, using the annular band width of 1 m instead of circular scale analysis, eliminating the cumulative effect of the size scale caused by the Ripley K (d) function on a scale of 1m steps [17]. Therefore, the univariate O-ring statistic, O(r) was employed to discuss the spatial distribution patterns of the main tree species at different scales, and the bivariate O-ring statistic, O12(r) was chosen to depict the spatial correlation among tree species.

The generalized O-ring equation is:

\[ O(r) = \lambda g(r) \]  

(1)


\[ g(r) = \frac{dk(r)}{dr} / 2\pi r \]  

(2)

For the CSR, O(r) is equal to \( \lambda \). If O(r) > \( \lambda \), the individuals obey aggregated distribution at distance r; if O(r) < \( \lambda \), the individuals obey regular distribution. To remove edge effect, the study area was divided into many small cells. Then, the O-ring statistic is estimated as:

\[ O^w(r) = \frac{\sum_{i=1}^{n} \text{Point}[R^w_i(r)]}{\sum_{i=1}^{n} \text{Area}[R^w_i(r)]} \]  

(3)

\[ O^w_{12}(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \frac{1}{\text{Area}[R^w_{i1}(r)]} \]  

(4)

Equations (3) and (4) are respectively the formulas for the univariate and bivariate O-ring statistics. In Equation (4), \( n_1 \) is the number of points of object 1; \( R^w_i(r) \) is the ring with radius \( r \) and width \( w \) centering at the \( i \)-th point of object 1.

Points \( [R^w_i(r)] = \sum_{\text{all} x} \sum_{\text{all} y} S(x, y)P_2(x, y)I^w_i(x, y, y) \)  

(5)

Area \( [R^w_i(r)] = z^2 \sum_{\text{all} x} \sum_{\text{all} y} S(x, y)I^w_i(x, y, y) \)  

(6)

\[ I^w_i(x, y, y) = \begin{cases} 1 & \frac{w}{2} \leq \sqrt{(x-x_i)^2 + (y-y_i)^2} \leq r + \frac{w}{2} \\ 0 & \text{otherwise} \end{cases} \]  

(7)

In Equation 5, points \([X]\) is the number of points of object 2 in region X, the area of which is determined by operator Area[X] in Equation 7. Where \( (x_i, y_i) \) are the coordinates of the \( i \)-th point of object 1; \( I^w_i \) is the ring radius \( r \) and width \( w \) centering at the \( i \)-th point with coordinates \( (x_i, y_i) \); S(x, y) is a variable that equals 1 if \( (x, y) \) are inside the study area and equals 0 if otherwise; \( P_2(x, y) \) is the number of points of object 2 in each cell; \( I^w_i \) is a variable that varies in the circle with radius \( r \) centering at the \( i \)-th point of object 1; \( z^2 \) is the area of one cell. The univariate statistic calculated under the condition that object 2 equals to object 1.

Data Processing. Using SigmaPlot12.0 software package plot tree distribution map, and using ecological software Programita (2006) completed O-ring statistical analysis [18]. The spatial scale varied from 0 to 50 m at step widths of 1m. Then, the Monte-Carlo simulation was performed 99 times. From which a 95% confidence interval was derived. When O(r) located over the upper envelope, it presented aggregate distribution, and the interspecies relationship is a positive correlation; when O(r) located between the upper and lower envelope, the distribution is random and the relationship between species is irrelevant; When O(r) locate below lower envelope, the distribution is uniform, the relationship between species is negative correlation.

RESULTS AND DISCUSSION

Stand Structure. The species composition of the study site is shown in Table 1. It can be seen from Table 1 that there are 28 species of tree species in the plot with a density of 1866 plants per hectare and an average diameter of 8.54 cm. The first four important species are P. tabulaeformis, Q. wutaishanica, A. ginnala and P. davidiana. The sum of the significant values is 72.48%, the sum of relative densities is 81.68%, and the sum of relative saliency reaches 94.46%. P. tabulaeformis is the unique coniferous trees among the 28 species, the relative density is 33.23%, the rest of broadleaf trees is 66.77%, the ratio of needle to broadleaf trees is 3: 7, and the ratio of area of breast height is 4: 1.

Forest Diameter and Tree Height Distribution. Figure 1 shows the diameter (DBH) and height (H) profiles of all trees in the sample plot. As can be seen from Figure 2, the diameter distribution of the stands ranges from 2 to 42 cm, which is generally inverted J-shaped distribution. Among them, the number of trees with 1 diameter class (2.0 ~ 3.9cm) is the largest, accounting for 20.53% of the total number of trees. Then, with the increase of diameter, the number of tree individuals decreased sharply. The tree height ranged from 1 m to 23 m, which is similar to the normal distribution, with the highest number of individuals ranging from 3 to 4 m, accounting for 28.36% of the total. The above results show that the main stands are young and middle-aged trees, and there are few over-mature trees. The diameter and height of main tree species distribution are similar to other species.

The spatial distribution patterns of the main tree species. Figure 2 displays the spatial distribution of all trees in the plot, but we can not research the relationship between the distribution and spatial scales. As can be seen from Figure 3, P. tabulaeformis, A. ginnala and P. davidiana population show aggregated distribution on a small scale of 0-5 m, the aggregation intensity decreased with the scale increased; and show random distribution on other scales. The population of Q. wutaishanica was aggregated in most scales, and the aggregation degree decreased with the increase of the scale. P. tabulaeformis population is aggregated distribution at scales < 7 m, and the degree of aggregation weakens with the increase of scale, and then with the scale
increases, the whole species exhibited random distribution. *Q. wutaishanica* present aggregated distribution on all scales from 0-44 m, especially significant at the scale of < 4 m, and show random distribution at the large scale > 44 m. *A. ginnala* populations show random distribution over most of the scales and aggregated distribution at the 0-2 m, 4-5 m, 7-11 m and 13-16 m scales, while at 11-12 m, 22 m, 24-25 m and 29 m scales show uniform distribution. *P. davidiana* population show aggregated distribution at the 0-14 m, 40 m and 43-45 m scales, but random distribution at other scales.

### TABLE 1
Composition of tree species in the sample plot in the coniferous and broad-leaf mixed forest in Huanglong Mountain.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number/hm2</th>
<th>DBH/cm Average</th>
<th>DBH/cm SD</th>
<th>Basal area at breast height/m²</th>
<th>Relative Density/%</th>
<th>Relative Frequency/%</th>
<th>Relative Significant degree/%</th>
<th>Important Value/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinus tabuliformis Carr.</td>
<td>620</td>
<td>16.6 9.0</td>
<td></td>
<td>1746.00</td>
<td>10.87</td>
<td>80.40</td>
<td>41.50</td>
<td></td>
</tr>
<tr>
<td>Quercus wutaishanica Mayr</td>
<td>581</td>
<td>4.0 4.2</td>
<td></td>
<td>152.09</td>
<td>31.14</td>
<td>10.87</td>
<td>7.00</td>
<td>16.34</td>
</tr>
<tr>
<td>Acer ginnala Maxim.</td>
<td>170</td>
<td>7.0 5.3</td>
<td></td>
<td>101.42</td>
<td>9.11</td>
<td>10.00</td>
<td>4.67</td>
<td>7.93</td>
</tr>
<tr>
<td>Populus davidiana Dode</td>
<td>153</td>
<td>4.6 4.7</td>
<td></td>
<td>51.88</td>
<td>8.20</td>
<td>9.57</td>
<td>2.39</td>
<td>6.72</td>
</tr>
<tr>
<td>Swida macrophylla (Wall.) Sojak</td>
<td>81</td>
<td>3.9 3.8</td>
<td></td>
<td>19.04</td>
<td>4.34</td>
<td>7.39</td>
<td>0.88</td>
<td>4.20</td>
</tr>
<tr>
<td>Betula albo-sinensis Burk</td>
<td>54</td>
<td>2.1 0.9</td>
<td></td>
<td>2.22</td>
<td>2.89</td>
<td>6.52</td>
<td>0.10</td>
<td>3.17</td>
</tr>
<tr>
<td>Toxicodendron vernicifluum (Stokes) Barkl</td>
<td>34</td>
<td>3.4 5.9</td>
<td></td>
<td>12.31</td>
<td>1.82</td>
<td>6.96</td>
<td>0.57</td>
<td>3.12</td>
</tr>
<tr>
<td>Betula platyphylla Suk</td>
<td>30</td>
<td>10.4 7.8</td>
<td></td>
<td>39.23</td>
<td>1.61</td>
<td>6.96</td>
<td>1.81</td>
<td>3.46</td>
</tr>
<tr>
<td>Cornus officinalis Sieb</td>
<td>30</td>
<td>2.8 2.9</td>
<td></td>
<td>3.91</td>
<td>1.61</td>
<td>5.65</td>
<td>0.18</td>
<td>2.48</td>
</tr>
<tr>
<td>Ulmus macrocarpa Hance</td>
<td>27</td>
<td>4.4 3.5</td>
<td></td>
<td>6.47</td>
<td>1.45</td>
<td>5.65</td>
<td>0.30</td>
<td>2.47</td>
</tr>
<tr>
<td>Cotonoeaster submutilflorus Popov</td>
<td>24</td>
<td>1.9 0.6</td>
<td></td>
<td>0.78</td>
<td>1.29</td>
<td>0.87</td>
<td>0.04</td>
<td>0.73</td>
</tr>
<tr>
<td>Populus simonii Carr</td>
<td>12</td>
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<td>0.00</td>
<td>0.34</td>
</tr>
<tr>
<td>Pinus koraiensis Sieb. et Zucc</td>
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**FIGURE 1**
Distribution of diameter and height of all trees in the sample plot in the coniferous and broad-leaf mixed forest in Huanglong Mountain. "The dominant species" include *P. tabuliformis, Q. wutaishanica, A. ginnala* and *P. davidiana*, while the other is "Other species".
The solid line is the O(r) value. Dashed lines correspond to the confidence intervals generated from 99 Monte Carlo simulations under a null hypothesis of complete spatial randomness.

The dominant type of spatial relationship among the main tree species was that of an independent distribution (Figure 4). \textit{P. tabulaeformis} and \textit{Q. wutaishanica} show positive correlation at most scales, and with no correlation at the few large scale of 32-35 m and 37-40 m. \textit{P. tabulaeformis} and \textit{A. ginnala} show significantly positive correlation at the scales of 28-37 m, but negative correlation at the scales of 9-10 m and 14-15 m, no correlation in other scales.

\textbf{Spatial relationships among the main tree species.} The dominant type of spatial relationship among the main tree species was that of an independent distribution (Figure 4). \textit{P. tabulaeformis} and \textit{Q. wutaishanica} show positive correlation at most scales, and with no correlation at the few large scale of 32-35 m and 37-40 m. \textit{P. tabulaeformis} and \textit{A. ginnala} show significantly positive correlation at the scales of 28-37 m, but negative correlation at the scales of 9-10 m and 14-15 m, no correlation in other scales.

\textbf{FIGURE 2}
Spatial distribution of all trees in the sample plot in the coniferous and broad-leaf mixed forest in Huanglong Mountain.

\textbf{FIGURE 3}
Spatial distribution patterns of the main tree species.
The solid line is the O(r) value. Dashed lines correspond to the confidence intervals generated from 99 Monte Carlo simulations under a null hypothesis of complete spatial randomness.
The solid line is the O(r) value. Dashed lines correspond to the confidence intervals generated from 99 Monte Carlo simulations under a null hypothesis of complete spatial randomness.

(a) P. tabulaeformis - Q. wutaishanica (b) P. tabulaeformis - A. ginnala (c) P. tabulaeformis - P. davidiana (d) Q. wutaishanica - A. ginnala (e) Q. wutaishanica - P. davidiana (f) A. ginnala and P. davidiana

study scale. P. tabulaeformis and P. davidiana show a significant positive correlation at 19-27 m, 39-43 m and 46-48 m scales, negative correlation at 12 m scale and no correlation at other scales. Q. wutaishanica and A. ginnala show a significant positive correlation at the scales of 23-26 m and 29-45 m, negative correlation at the scales of 3 m, 6-7 m and 10 m, and no correlation with other research scale. Q. wutaishanica and P. davidiana show a significant positive correlation with 6-11 m, 16-25 m, 28-31 m, 33-40 m and 43-50 m scales. A. ginnala and P. davidiana show negative correlation at the scales of 3-13 m, 32-38 m and 42-43 m. A. ginnala and other species show different degrees of negative correlations, indicate that there is a strong competition between A. ginnala and other species.

The study area is a secondary forest with rich tree species and complicated stand structure (Table 1). P. tabulaeformis, Q. wutaishanica and P. davidiana play an important role in maintaining structure, function and stability of the coniferousbroad-leaved forest. Although the mixed coniferous and broad-leaved forest is a typical zonal community in Huanglong Mountain, the average DBH of all tree species in the whole plot is small, indicating that the sample is in the initial stage of community succession [19]. The diameter distribution of stands is inverted "J", that is, the number of trees with small diameter class is large, indicating that the seedlings are abundant and the population is in good regeneration. With the increase of diameter, the number of trees begins to decrease gradually, which accords with the basic rules of diameter structure of natural mixed forest, and the ecosystem has strong stability [20]. The tree height is similar to the normal distribution, showing that the mixed coniferous and broad-leaved forest is in good condition overall.

The spatial distribution pattern of plant populations has a close relationship with spatial scale. Zhang et al. [3] considered that at a smaller scale, a
species’ spatial pattern and spatial correlation may be influenced by intraspecific competition and the method of seed dispersal, whereas at a larger scale, they may be determined by heterogeneity or patchiness of the species distribution and different environmental conditions. The results of this study show that *P. tabuliformis*, *A. ginnala* and *P. davidiana* are aggregated distribution at a small scale of 0-5m, while *Q. wutaishanica* population indicates aggregative distribution at the most scales. The results of previous studies prove that most natural populations follow a cluster pattern that includes conifer species [21]. *P. tabuliformis* population indicates from aggregated distribution to random distribution with the increase of spatial scale, mainly due to its seed dispersal mechanism. The cones are big and heavy and can not be spread by wind or gravity. Its distribution is mostly dependent on the activities of animals. The limited distribution characteristic of *P. tabuliformis* is the reason why *P. tabuliformis* is aggregated distribution on the small scale. At the same time, habitat heterogeneity is an important driving force for population aggregated distribution. Around the adult tree is usually habitat suitable for population growth, contributing to the survival of the renewal body, resulting in aggregation of different population individuals on a smaller scale [22]. The role of canopy gaps in creating aggregated spatial patterns of trees was emphasised by Stewart [23]. In this study, *A. ginnala* and *P. davidiana* as concomitant species grow in the gaps of *P. tabuliformis* and *Q. wutaishanica* and form aggregated patches, so the gathering radius of these two species is smaller than that of *P. tabuliformis* and *Q. wutaishanica* population. At the same time, the individual number of *A. ginnala* and *P. davidiana* population is small, and the time for the two species to begin their breeding in the forest is not long enough to reach the level of making full use of space resources. The stronger aggregation intensity at the smaller aggregation radius makes the two associated species some interspecies competitive advantage. The main tree species in the forest change from aggregated distribution to random distribution, and there is a trend of gradual homogenization. This is one of the mechanisms for population self-regulation, which is conducive to making better use of environmental resources and is also related to population self-thinning [24].

Spatial association of different populations varies with the spatial scale. There is significant trends towards negative or positive association between different tree species suggests that there are direct influences which are essential in shaping relationships among tree species at small spatial scales. The results show that *P. tabuliformis* and *Q. wutaishanica*, *P. tabuliformis* and *P. davidiana*, *Q. wutaishanica* and *P. davidiana* (Figure 4 (a), (c), (e)) show a positive correlation at the middle and small scales, indicating that these three species have similar biological characteristics, and have similar ecological adaptability and overlapping ecological niche to the habitat. They are less competitive and have certain spatial dependency. Meanwhile, it is known as transitivity in interspecific association [25]. Negative association between the *A. ginnala* and other species (Figure 4 (b), (d), (f)) at the small scales, especially, the interspecies competition of *A. ginnala* and *P. davidiana* population is fierce. The results indicate that *A. ginnala* and other tree species have different biological characteristics, with different ecological adaptability and ecological niche separated from each other. *P. davidiana* inhibits the growth of *A. ginnala*. Wang et al. [26] found that the seedlings of *A. ginnala* are very abundant and produce a large number of seedlings. When the upper canopy density is high, as a species fond of light, *A. ginnala* population will induce intense interspecific competition for the light or other resources. Above results indicate that, under the same growth environment and competition condition, the tree species with the same or similar habitat requirements show significantly positive correlations in habitats suitable for their growth, and the differences in habitat requirements and resource utilization will inevitably lead to interspecific competition for the use of resources, the tree species tend to exhibit significant negative correlations, which has been verified in previous studies [27].

CONCLUSIONS

Exploring tree population distribution patterns and interspecific spatial associations are helpful in elucidating the mechanisms underlying species coexistence in forest communities. If a species has a competitive advantage, it will monopolize those resources to avoid coexistence with other tree species. However, if species sufficiently partition the biotic or abiotic environment, or if there are tradeoffs in resource allocation, then different species can coexist where conditions are spatially or temporally heterogeneous. In this paper, *P. tabuliformis* and *Q. wutaishanica* are two important native tree species in Huanglong Mountain Forest, they have a strong competitive advantage. At the same time, *A. ginnala* population are moderate thinning, it is helpful to guide the forward succession of the mixed coniferous and broad-leaved forest.

ACKNOWLEDGEMENTS

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SOIL CHARACTERISTICS AND WATER HOLDING CAPACITY OF THREE TYPICAL SHRUBS IN THE SOUTHERN SLOPE OF QILIAN MOUNTAINS

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ABSTRACT

In order to grasp the situation of soil properties and water storage capacity of three typical shrubs in the southern slope of Qilian Mountains, NW China, soil characteristics and water holding capacity under three typical shrubs (Potentilla fruticosa, Caragana jubata and mixture of these two) in the Southern Slope of Qilian Mountains were investigated in this project. Soil bulk density, porosity, moisture content and water holding capacity were studied based on both field survey and laboratory experiments. Results showed that: (1) with the increase of the depth, bulk density increases while the total porosity decreases in the three typical shrubs. (2) Soil bulk densities follow the high to low order that 0 to 50 cm soil layer is mixture shrub (1.19 g/cm³), Caragana jubata (1.08 g/cm³) and Potentilla fruticosa (0.95 g/cm³). The order of the total porosity is Potentilla fruticosa (62.53 %), Caragana jubata (58.35 %) and mixture shrub (54.73 %). (3) In the soil layer of 0 to 50 cm, the soil saturation water storage capacity under Potentilla fruticosa, Caragana jubata and mixture shrub is 3126.74 t/hm², 2917.54 t/hm², 2736.71 t/hm², respectively. (4) The soil non-capillary water holding capacities of the three shrubs are 1788.08 t/hm², 1446.13 t/hm², 1164.12 t/hm², respectively. As a result, we suggest that in the region of the southern slope of Qilian Mountains, the soil under the Potentilla fruticosa has a better soil structure, followed by Caragana jubata, while mixture shrub had the poorest characteristics. Similarly, the soil under the Potentilla fruticosa has the better water holding capacity, which is followed by Caragana jubata in order, while mixture shrub has the weakest capacity.

KEYWORDS:
Soil properties, bulk density, water holding capacity, shrub, Qilian Mountains

INTRODUCTION

Soil physical properties, including soil thickness, bulk density, total porosity, capillary porosity and non-capillary porosity and other indices, boast the comprehensive embodiment of soil’s structure, water storage capacity, water retain capacity and infiltration capacity. Moreover, Soil properties are significant on the soil’s water conservation, soil and water conservation and reduce surface runoff [1, 2]. As the main body of conservation of precipitation, soil provides storage for the soil moisture. Soil moisture is not only the limiting factor for the development of eco-system in arid and semi-arid areas, but also an important index to evaluate the capacity of water conservation capacity [3]. The shrub community is an important component of water conservation forests and plays an important role in the protection of regional ecological environment [4-6]. However, there are differences in the capacity of water storage and water conservation because of different species composition, litter volume and degree of decomposition, soil physical and chemical characters [7].

In the Qilian Mountains of NW China, shrubs cover an area of 266,000 hectares. There is an important part of forest which accounts for 67.32% of the area of forest land in the Qilian Mountains. Shrubs is the main forest vegetation type in this region [8, 9]. Zhang Ping [4], Liu Xiande [9] and Zhao Jinmei [23] et al. all did some researches on the soil characters and water source conservation functions under shrubs in the northern slope of Qilian Mountains; Sun Yanhong et al. researched the soil characters and water conservation of different forest types in Jinyun Mountain [15]; Wang Heizilai et al. researched the water conservation function of forest in ecological valley in Daxing’ anling [16]; Pan Chunxiang et al. did some researches on the soil water holding capacity under four typical ecosystems in Wuyunjie nature reserve of Hunan province [27]. But the researches on the soil characters and water source conservation functions under shrubs in the southern slope of Qilian Mountains are still deficient. The southern slope of Qilian Mountains is an important water sources conservation area in arid
area of northwestern China. Its strength of function of water conservation is directly related to the ecological environment and the production and living water of Qinghai and Gansu province. Thus, it is significant to research the water sources conservation functions in the southern slope of Qilian Mountains.

**RESEARCH AREA**

The research area is located in the northeast of Qinghai province and borders Gansu province, NW China. Geographic coordinate is 98°08′13″-102°38′16″ E, 37°03′17″-39°05′56″ N and the altitude is 2257~5235 m (Figure 1). With the typical plateau continental climate, the temperature changes a lot in a day, and the mean annual precipitation is 400 mm [10]. In the region, the main arbor vegetation type includes Picea crassifolia, Populus simonii Carr, Betula platyphylla Suk, Pinus and Sabina przewalskii. Shrubs have Potentilla fruticosa, Caragana jubata, Potentilla glabra Lodd, Salix oritrepha Schwein, Rhododendron parvifolium Adams. Herbs are mainly Oxytropis falcata Bge, Polygonum viviparum L, Elymus nutans Griseb., Rheum palmatum L, Saussurea stella and Kobresia tibetica et al. Soil types include Castanozems, Chernozems, Brown pedocals, Meadow soils and Litho soils [11].

**METHODS**

**Sampling.** In the summer of 2015, representative shrubs such as Potentilla fruticosa, Caragana jubata and mixture shrub were chosen, and a standard sample size was set at the size of 20 m×20 m. Three quadrats were chosen within each sample. There are 11 samples and 30 quadrats of Caragana jubata, 19 samples and 58 quadrats of Potentilla fruticosa, 8 samples and 18 quadrats of mixture. Soil samples were taken by a soil auger. The distance between the layers is 10 cm and sampling depth is 50 cm. The samples are placed in the bag after the roots and leaves litter were removed, then weighed the fresh samples timely. Soil physical properties such as soil bulk density and moisture content of samples were measured by conventional methods in Qinghai Province Key Laboratory of Physical Geography and Environmental Process.

**Measurements and Calculating Formula.** The soil moisture content was measured by the drying method. Soil bulk density was measured by a steel ring tool [12]. The formulas of the various indices are as follows [13-16]:

**Soil bulk density:**

\[
B_d = \frac{M_1 - M_2}{V}
\]

Where \( B_d \): Soil bulk density (g/cm³); \( M_1 \): Sum of drying soil weight and ring knife weight (g); \( M_2 \): Ring knife weight (g); \( V \): Ring knife bulk (g).

**Soil moisture:**

\[
W = \left( \frac{M_2 - M_4}{M_2} \right) \times 100\%
\]

Where \( W \): Soil moisture (%); \( M_4 \): Wet soil weight (g); \( M_1 \): Drying soil weight (g).

**Total porosity, Capillary porosity, Non-capillary porosity:**

\[
T_p = 93.947 - 32.995B_d
\]

\[
T_c = W \times B_d
\]

\[
T_n = T_p - T_c
\]

Where \( T_p \): Total porosity (%); \( T_c \): Capillary porosity (%); \( T_n \): Non-capillary porosity (%); \( W \): Soil moisture (%); \( B_d \): Soil bulk density (g/cm³).
### Table 1

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</tr>
<tr>
<td>30-40</td>
<td>27.05</td>
<td>32.32</td>
<td>21.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-50</td>
<td>24.60</td>
<td>31.88</td>
<td>19.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>28.92</td>
<td>35.76</td>
<td>23.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Saturation water storage capacity, capillary water storage capacity, Non-capillary water storage capacity:

\[
W_p = 10^4 (m^2) \times T_p \times H \times 1.0 (g/cm^3) \tag{6}
\]

\[
W_c = 10^4 (m^3) \times T_c \times H \times 1.0 (g/cm^3) \tag{7}
\]

\[
W_n = 10^4 (m^3) \times T_n \times H \times 1.0 (g/cm^3) \tag{8}
\]

Where \( W_p \): Saturation water storage capacity (t/hm²), \( W_c \): Capillary water storage capacity (t/hm²); \( W_n \): Non-capillary water storage capacity (t/hm²); \( T_p \): Total porosity (%); \( T_c \): Capillary porosity (%); \( T_n \): Non-capillary porosity (%); \( H \): Soil depth (m).

**RESULTS**

The changing properties of soil bulk density at different shrub types. Soil bulk densities under three types of shrubs all increase with the increase of soil depth (Table 1). That is, the surface soil is relatively loose and the underlying soil is relatively tight. The reasons are that the content of organic matters is higher, the activities of the root system and soil organic matter is stronger. The underlying is contrary to the top [17, 18]. As far as the soil profile, the variation range of soil bulk density under the Caragana jubata, Potentilla fruticosa and the mixture is 0.84 g/cm³ to 1.24 g/cm³, 0.78 g/cm³ to 1.06 g/cm³ and 0.79 g/cm³ to 1.55 g/cm³, respectively. In the 0 to 50 cm of soil layer, the average of soil bulk density under the mixture is the maximum (1.19 g/cm³), followed by the Caragana jubata (1.08 g/cm³), and Potentilla fruticosa is the minimum (0.95 g/cm³). This illustrates that soil structure of Potentilla fruticosa is superior to the other two.

The changing properties of the soil porosity. Soil total porosity under three kinds of shrubs all decrease with the increase of soil depth (Table 1), and this is contrast to the change of soil bulk density. The variation range of total porosity of the Caragana jubata, Potentilla fruticosa and they mixture is 53.13 % to 66.20%, 58.91% to 68.06% and 42.69% to 67.86%, respectively. Table 1 illustrates that the different layers of the total porosity of soil is greater than or close to 50%, which indicated that the soil’s permeability and sponginess is better and the hydrological regulation ability is quite strong [18, 19]. In the 0 to 50 cm of soil layer, the average of total soil porosity under the Potentilla fruticosa is the mixture (62.53%), then is Caragana jubata (58.35%), the mixture is the minimum (54.73%). Soil capillary porosity and non-capillary porosity under three kind of shrubs all reduce generally with the increase of soil depth. The averages of non-capillary porosity under Caragana jubata, Potentilla fruticosa and mixture are 28.92%, 35.76% and 23.28%, respectively, and the average capillary porosities of them are 29.43%, 26.77% and 31.45%, respectively.
The changing properties of soil water storage capacity. Except for the soil capillary water storage of Potentilla fruticosa, soil water content, retention and conservation under three types of shrubs all decrease with the increase of soil depth (Figure 2; Table 4). The capillary water storage of soil of Potentilla fruticosa increases first and then decreases, thereby illustrating the soil moisture under Potentilla fruticosa has obvious irregularity. Among different types of shrubs (Table 2), the average of soil water content changes is small in 0 to 50 cm profile and ranged from 32.45 % to 33.19 %. In the 0 to 50 cm profile the sum of soil saturation water storage capacity, capillary water holding capacity and the non-capillary water storage capacity is rather changeable. The sum of soil saturation water storage capacity under Caragana jubata, Potentilla fruticosa and mixture is 2917.54 t/hm², 3126.74 t/hm² and 2736.71 t/hm², respectively; the sum of soil non-capillary water storage capacity is 1446.13 t/hm², 1788.08 t/hm² and 1164.12 t/hm², respectively; the sum of soil capillary water storage capacity is 1471.40 t/hm², 1338.67 t/hm² and 1572.59 t/hm², respectively.

DISCUSSIONS

Soil properties. The results show that soil bulk densities increase with the increase of soil depth under three types of shrubs, while soil total porosity decrease together with the increase of soil depth in the Southern Slope of Qilian Mountains. The reasons are that there are lots of vegetation roots and plant litters in the soil surface which can make soil loosening, prevent soil hardening, and improve the structure of soil. In the three types shrubs, soil bulk density is the maximum while the total porosity is the least under mixture shrub, however, the increase or decrease is
more evident in 20 to 50 cm soil layer. We suggest that the brush roots system are mainly distributed in 0 to 20 cm deep surface soil, there are plenty of heat, moisture and mineral nutrition provided for the shrub roots to assimilate [20]; meanwhile, interspecific competition of mixture shrub may be fierce which leads to the most roots of mixture shrub being concentrated in 0 to 20 cm soil depth and making the best of this resource for survival advantage. But the soil layer below 20 cm, hydrothermal and permeability are poor and roots decrease, the soil bulk density increases and total porosity decreases.

The soil bulk density and total porosity under different vegetation types were listed in the table 3. It is not hard to find that the soil bulk densities under three types of shrubs all are less than the soil bulk densities of non-forest land and degraded alpine meadow, while the soil total porosities are all greater than them. The soil total porosity under three types of shrubs are totally greater than the soil bulk density of Picea crassifolia forestry land, Rhododendron simsii shrub land and mixture plantation of Picea crassifolia-Larix gmelinii, the soil total porosities are less. That is to say, the soil structure, loosen level, air permeability, water storage capacity, etc. is worse than Picea crassifolia forestry land, Rhododendron simsii shrub land and mixture plantation of Picea crassifolia-Larix gmelinii, is better than non-forest land and degraded alpine meadow. Result is on the contrary to previous researches, because the different research area, species composition, the active level of the roots, litter stocks and gravel contents in the soil can lead to the different results.

### Soil water storage capacity

Except for the soil capillary water storage of Potentilla fruticose, which increases first and then decreases with the increase of soil depth, the others decrease with the increase of soil depth. These trends are not different from those of the soil bulk density. Both soil water content and storage capacity have a good linear relation and the variation range of correlation coefficient is 0.56 to 0.99. The possible reason is that the soil is loosen and the porosity is big when the soil bulk density is less which leads to a better soil water storage capacity; the soil is hardening, the porosity reduce and the space of store water will reduce when the soil bulk density is bigger, which can drop the Soil water storage capacity [26]. It is easy to find that both the soil water content and soil bulk density have a good logarithmic function under three kind of shrubs from figure 3. The equation of correlation is $y = -54.42ln(x) + 36.799, R^2 = 0.9901, y = -31.84ln(x) + 30.716, R^2 = 0.9876, y = -51.56ln(x) + 39.957, R^2 = 0.999$. That is to say, the soil water content will decrease with the increase of the soil bulk density, and this result is similar to that from Li Zhuo [26].

<table>
<thead>
<tr>
<th>Site</th>
<th>Bulk Density (g/cm³)</th>
<th>Total Porosity (%)</th>
<th>Data Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xishui Site, Qilian Mountains</td>
<td>1.25</td>
<td>47.25</td>
<td>Non-forest [21]</td>
</tr>
<tr>
<td>Pai Lugou valley, Qilian Mountains</td>
<td>0.49</td>
<td>73.85</td>
<td>Picea crassifolia forestry [22]</td>
</tr>
<tr>
<td>Jingjiang river valley, Qilian Mountains</td>
<td>0.77</td>
<td>65.11</td>
<td>Rhododendron simsii shrub [23]</td>
</tr>
<tr>
<td>E38°20′~38°30′, N101°20′~101°30′</td>
<td>0.86</td>
<td>67.56</td>
<td>Picea crassifolia+ Larix gmelinii [24]</td>
</tr>
<tr>
<td>Beilu, Source region of the Yangtze river</td>
<td>1.307</td>
<td>40.82</td>
<td>Degraded alpine meadow [25]</td>
</tr>
<tr>
<td>This thesis</td>
<td>0.92~1.10</td>
<td>57.74~63.44</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4

**Relationship between Water Content and Soil Depth**

<table>
<thead>
<tr>
<th>Water Content</th>
<th>Equation of Correlation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caragana jubata</td>
<td>$y = -1.7264x + 87.299$</td>
<td>0.8802</td>
</tr>
<tr>
<td>Potentilla fruticosa</td>
<td>$y = -3.9724x + 159.21$</td>
<td>0.9654</td>
</tr>
<tr>
<td>Mixture</td>
<td>$y = -1.1406x + 67.014$</td>
<td>0.973</td>
</tr>
<tr>
<td>Saturation Water Storage Capacity</td>
<td>$y = -0.289x + 198.61$</td>
<td>0.9492</td>
</tr>
<tr>
<td>Caragana jubata</td>
<td>$y = -0.4201x + 292.68$</td>
<td>0.9424</td>
</tr>
<tr>
<td>Potentilla fruticosa</td>
<td>$y = -0.1601x + 117.62$</td>
<td>0.9964</td>
</tr>
<tr>
<td>Mixture</td>
<td>$y = -0.5568x + 193.86$</td>
<td>0.6751</td>
</tr>
<tr>
<td>Soil Capillary Water Storage Capacity</td>
<td>$y = 1.2703x - 310.11$</td>
<td>0.5648</td>
</tr>
<tr>
<td>Caragana jubata</td>
<td>$y = -0.2411x + 105.84$</td>
<td>0.9365</td>
</tr>
<tr>
<td>Potentilla fruticosa</td>
<td>$y = -0.4511x + 160.46$</td>
<td>0.9347</td>
</tr>
<tr>
<td>Mixture</td>
<td>$y = -0.3368x + 150.46$</td>
<td>0.9055</td>
</tr>
<tr>
<td>Non-capillary Water Storage Capacity</td>
<td>$y = -0.3198x + 104.46$</td>
<td>0.7487</td>
</tr>
</tbody>
</table>
At present, most scholars take the soil non-capillary water storage capacity that MA Xuehua presented as the index to evaluate the soil water storage capacity [27, 28]. In the study area, the soil bulk density is the least and the total porosity, saturation water storage capacity, non-capillary water storage capacity are the maximum under the Potentilla fruticosa shrub, but the soil capillary water storage capacity is the minimum. The soil bulk density is the maximum and the total porosity, saturation water storage capacity, non-capillary water storage capacity are the minimum under the mixture shrub, but the soil capillary water storage capacity is the maximum. Therefore, this thesis considers that the soil water storage capacity under Potentilla fruticosa shrub is the better, then is the Caragana jubata shrub, the soil water storage capacity under mixture shrub is the weakest. Although the soil water storage capacity under Caragana jubata shrub is weaker than Potentilla fruticosa shrub, Caragana jubata shrub has an advantage that Caragana jubata shrub has root nodule. Root module can enhance the soil fertility [29], which can make the quantity of microorganism increase in the soil thus can improve the soil’s structure and enhance the soil water storage capacity.

Table 5 is soil bulk density, total porosity and water storage capacity under different arbor. It is easy to find that soil bulk density under Picea crassifolia, Pinus tabulaeformis and Platycladus orientalis are bigger than these in this thesis. Soil saturation water storage capacity, capillary water storage capacity and non-capillary water storage capacity are less than these in this thesis. This result of contrast is contrary to what mentioned above. So in practice, it should be grasped on what kind of forest for water source conservation is good in water storage capacity and protect them to prevent them destroyed by human activity.

**CONCLUSIONS**

The study aimed at the soil characteristics under three typical shrubs in the research area of the Southern Slope of Qilian Mountains and discovered: Soil bulk density increased with the increase of soil depth while total porosity decrease with the increase of soil depth. Capillary porosity and non-capillary porosity are generally decreased with the increase of soil depth. Within the range of 0 to 50 cm, the order
of soil bulk density under three kinds of shrub is mixture (1.19 g/cm³) > Caragana jubata (1.08 g/cm³) > Potentilla fruticosa (0.95 g/cm³); The order of total porosity is Potentilla fruticosa (62.53 %) > Caragana jubata (58.35 %) > mixture (54.73 %). Soil permeability and porosity under three kinds of shrub are good and the soil’s structure of Potentilla fruticosa is the best.

Except for the soil capillary water storage of Potentilla fruticosa which increases first and then decreases with the increase of soil depth and the others totally decrease with the increase of soil depth. Both soil water content and storage capacity have a good linear relation and the variation range of correlation coefficient is 0.56 to 0.99. Both the soil water content with soil bulk density have a good logarithmic function under three kind of shrubs and correlation coefficient close to 1. The order of saturation water storage capacity is Potentilla fruticosa (3126.74 t/hm²) > Caragana jubata (2917.54 t/hm²) > mixture (2736.71 t/hm²); The order of capillary water storage capacity is mixture (1471.40 t/hm²) > Caragana jubata (1338.67 t/hm²) > Potentilla fruticosa (1572.59 t/hm²); The order of non-capillary water storage capacity is Potentilla fruticosa (1788.08 t/hm²) > Caragana jubata (1446.13 t/hm²) > mixture (1164.12 t/hm²). Soil water storage capacity under Potentilla fruticosa is better, then is Caragana jubata, and mixture shrub is the weakest.

ACKNOWLEDGEMENTS

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DEVELOPMENT TREND AND POROUS TEXTURE CHARACTERIZATION OF TIGHT RESERVOIRS IN CHINA

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ABSTRACT

Tight reservoir is characterized by small pore throat size, complex pore structure, strong heterogeneity and large difficulty in characterization. Through systematically summarizing the development situation of current characterization technology of Tight reservoir, some notable problems in the characterization of pore structure of tight reservoir were pointed out. This paper provides a comprehensive literature review on the simulation techniques being developed in recent years for describing unique flow behaviors in tight gas reservoirs. The future development of pore structure characterization technologies of tight reservoir was put forward.

KEYWORDS:
Reservoir simulation, Adsorption and desorption, Tight reservoir, Characterization of pore structure

INTRODUCTION

Unconventional gas reservoirs, such as shale and tight gas reservoirs, are rich resources of natural gas. In unconventional formations, huge amount of gas is trapped in the location where it is generated, without the primary migration process. Featured by extremely low permeability, shale and tight gas reservoirs are different from each other, even with similar porosity, pore pressure, and in-situ stress conditions. As shown in Fig. 1 [1], permeability values of organic-rich shales are in the range of sub-nanodarcy to tens of microdarcy, and permeability of deep organic-lean mudrocks can be even less. Some correlations can also be observed between the permeability values and the porosity of shales. The diameter of pore throat in tight gas formations is usually between 0.01 μm and 1μm [2], while the pore throat diameter of shale formation varies from tens of nanometers to as low as a few nanometers [3]. Recent studies even revealed sub-nanometer pores inside shale formations that are beyond the scope of scanning electron microscope (SEM) based on CO2 and N2 adsorption experiments [4] and atom-scale reconstruction technique. Due to these extremely small pores, the hydrocarbon transport mechanisms in unconventional reservoirs turn out to be very different from those in conventional reservoirs.

Fluid flow behavior in shale and tight gas reservoirs is featured by multiscale single-phase (gas) and/or multiphase fluid (gas, gas condensate and/or brine) flow and transport in ultra-low permeability, highly heterogeneous porous/fractured, and stress-sensitive rocks. Compared with flow in conventional reservoirs, fluid flows in unconventional reservoirs with extremely low permeability are highly non-linear and complex processes, including non-linear adsorption/desorption, non-Darcy flow in the entire range from high flow rate to low flow rate, strong rock-fluid interaction, and rock/organic matter deformation within nano-pores or micro-fractures. Therefore, quantifying flow in unconventional gas reservoirs has become a challenging issue for conventional REV (Representative Elementary Volume)-based Darcy’s law approaches, which may not be suitable and thus must be improved in order to handle these realistic problems in shale and tight gas reservoirs.

MAIN TECHNOLOGY

Small-angle scattering (SAS). Characterizing nanoporous materials is particularly challenging because the structural information is generally available in a variety of different and often indirect forms. Direct imaging of the structure via microscopy is sometimes possible but this is limited to materials that can withstand the required vacuum and electron dose. Moreover, the amount of material that is sampled by electron microscopy seldom exceeds a few femtograms [5], which always raises the question of data robustness. For that reason, workers in the field also rely on indirect data that provides nanometer-scale information based on macroscopic sampling; this is notably the case of nitrogen adsorption, mercury intrusion, and small-angle scattering (SAS), among others. In principle, small-angle scattering is particularly well suited for mesoporous materials because the resolution of typical SAS setups (from about 1 to 100 nm) encompasses the IUPAC
FIGURE 1
The relationships of porosity and permeability for shale gas reservoirs (FWB-Fort Worth Basin, WV #6 well-Marcellus Shale well in West Virginia) in North America obtained from core analysis

Due to the complexity of the pore structure of tight reservoirs, the existing mature interpretation models are not applicable, and the diffraction intensity and data model are not explained in the published literature. In addition, for the small-angle scattering analysis of tight reservoirs, a unified analysis process has not been formed, and there are differences between sample preparation and blank extraction. Therefore, it is recommended to carefully select the small-angle scattering technique in the evaluation of pore structure of unconventional tight reservoirs [9, 10].

Transmission electron microscope (TEM). Various materials characterization techniques (for instance, X-ray diffraction, neutron diffraction, X-ray absorption spectroscopy, nuclear magnetic resonance and etc.) have been widely employed in characterization of LrLOs, and most of them can just collect spatially averaged information. However, further understandings of the current issues, as mentioned before, relies heavily on the recognitions of specific nanodomains, such as local defects, interfaces, and boundaries. Based on the viewpoint, the capability of transmission electron microscope (TEM) to precisely probe the structural/chemical evolutions at an ultrahigh spatial resolution provides us a powerful analytical tool beyond other macroscopic characterization methods [11].

In early days, the original STEM was intended for biological research, the samples were used more as resolution tests than for materials studies. Adoption of the STEM technique was slow owing to difficulties in sample preparation of bulk materials and a lack of widespread instrument availability as a result of the vacuum and electronics requirements.
With the development of a commercially manufactured STEM and better specimen preparation tools such as ion-milling, there was a renewed interest in the instrument as an analytical tool for metallurgy and in the semiconductor industry. At present, new generations of commercial field emission TEMs with scanning attachments made STEM techniques widely available to the materials research community and industry [12].

However, the ability of TEM to characterize the pore structure of the sample surface is not as good as that of field emission scanning electron microscopy. The main reason is that TEM uses the electron beam to penetrate the sample and form a two-dimensional image that not only contains information on the surface of the sample, but also includes. The information inside the sample is similar to the supervised display of viscer a and bone in the X-ray film. Therefore, TEM has a short plate in the pore structure characterization. The previous research results confirm this view. Since TEM requires electron beam to penetrate the sample for imaging, considering the absorption of the electron beam by the sample and the scattering of the electron beam, the sample of the transmission electron microscope is extremely thin, generally less than 100 nm, and needs to be prepared by focusing ion beam scanning electron microscopy. Difficult to use, and the field of view is small, the representation of the chosen field of view is often questioned. Therefore, it is recommended that the pore structure be characterized without using transmission electron microscopy, and the internal structure and lattice characteristics of organic matter can be studied to determine the composition of organic matter and its relationship with pore development [13-18].

**Low-temperature CO\textsubscript{2} adsorption technology.** Low-temperature CO\textsubscript{2} adsorption technology [19-25] mainly studies pore structure less than 2 nm, and has the characteristics of high analysis temperature (273.1 K), strong energy and fast balance. In recent years, with the successful commercial development of shale gas. Many scholars use low-temperature CO\textsubscript{2} adsorption technology to study the pore distribution in shale.

Mastalerz M et al. [26] use a new non-destructive atomic force microscopy technique to map the elastic modulus of organic and inorganic components at the nanometer scale in shales containing Type II kerogen from three different levels of thermal maturation. They found that when vitrinite reflectance increases from 0.40 to 0.82, the average Young modulus of kerogen increases from 6.1 GPa to 16.0 GPa. A high degree of variance is registered in the elastic moduli, particularly at higher levels of thermal maturation, probably reflecting the inherent heterogeneity in the depositional organic matter present in the shales. The mean modulus for bitumen in the same samples - identified as void filling organic matter that was present only at intermediate and higher levels of maturation - was relatively constant with mean values of 7.5 GPa and 8.5 GPa, respectively. In the samples that experienced catagenesis, the modulus maps reveal that individual kerogen macerals possess soft regions - interpreted as exuded bitumen - which act to soften the overall structure of the kerogen. As well as providing high resolution mechanical data, this technique could be used to track the way bitumen and other compounds are generated from kerogen during catagenesis.

Tian Hua et al. [27] choose the gas adsorption of carbon dioxide at °C (D-R), capable of the access to the porosity as fine as 0.35 nm, to determine micropores (<2 nm). Mesopores (2~50 nm) were determined using the nitrogen adsorption (at -196°C) method (BET theory) while macropores (>50 nm) measured with the high-pressure mercury injection method. Therefore, a combination of mercury-injection data with low-pressure nitrogen and carbon dioxide adsorption analyses can provide a more comprehensive assessment of different pore-size distributions. Characteristics of different pores in typical marine and lacustrine shales in China and their influencing factors were recognized by means of the above-mentioned approach as follows: (1) pores are well developed in marine shales of the Sichuan Basin and the highest porosity is found in the Niutitang and Wufeng Formation shale; (2) macropores in shales develop mostly related to minerals, while micro and mesopores develop mainly related to organic matter; (3) with the increase of thermal maturity, organic pores in shales becomes gradually larger.

Li Teng-fei et al. [28] focused on the pore structures of Upper Ordovician-Lower Silurian black shales sampled from well YC7 in southeastern Chongqing area. Nitrogen and carbon dioxide gas adsorption were conducted at 77.4 K and 273.15 K, respectively, and the pore structures were characterized by modified BET, BJH, DFT and Stockecki methods. The results show that the micropore size distribution curves from the Stockecki method using CO\textsubscript{2} adsorption at 273.15 K can be well correlated with that calculated by DFT method using nitrogen adsorption at 77.4 K. This indicates that a continuous pore size distribution could be achieved for micropores, mesopores and part of macropores in shales by combing together the N\textsubscript{2} and CO\textsubscript{2} adsorption data.

Zhu Yanming et al. [29] investigated shale in the upper Yangtze area using a field-emission scanning electron microscope, high pressure mercury intrusion, low-temperature nitrogen adsorption and carbon dioxide adsorption. The results indicate that the combination of the high pressure mercury intrusion, low-temperature nitrogen adsorption and carbon dioxide adsorption curves enabled the description of the pore size distributions for micro-, micro-, and macroporosity. The pore size distributions arc
In the actual application process, there are some problems in the low temperature CO$_2$ adsorption technology: (1) The current pretreatment and key experimental parameters of the experimental samples have not been unified, and most scholars did not mention the pretreatment process of the samples in the research. Only some scholars mentioned the sample quality, but the key parameters affecting the experimental results such as the particle size and the pressure increase rate of the sample are not mentioned; (2) The theoretical model of micropore distribution interpretation is diverse, and the results obtained by different models are quite different [30-32].

**High pressure Mercury intrusion techniques.** Tight gas sandstone reservoirs in China are suggested to be reservoirs with porosity less than 10%, in situ permeability less than 1 mD, pore throat diameter less than 1 μm and gas saturation less than 60% [33]. These sandstones are all characteristic by typically poor reservoir quality and showing strong heterogeneities [34, 35]. Pore structure is one of the important factors affecting the properties of rocks, and processes of fluid transport through underground reservoirs are closely related with the microscopic pore structure [36]. However, the complexity and irregularity of pore structure make it difficult to quantitatively characterize pore structure by Euclidean geometry and other traditional experimental methods [37]. Since the concept of fractal was firstly proposed by Mandelbrot [38], fractal geometry has been successfully used to characterize the spatial heterogeneities of different patterns over a wide range of pore spaces in sedimentary rocks. Fractal theory is an effective method for investigating pore structure of rocks, which builds a bridge between microscopic pore structure and macro performance (porosity, permeability) [39]. Many scholars have developed the fractal theory to study the fractal characteristics of pore structure in rocks [40-42]. They confirmed that the pore distribution in rocks is statistically self-similar, i.e., identically independent of the scale of magnification, such fractal objects are characterized by the fractal dimension Df [43]. Fractal dimensions have been extensively used in quantifying the complexity of pore structure and physical properties of rocks.

High pressure Mercury intrusion techniques are widely used for the determination of total pore volumes and pore size distributions for reservoirs rocks [44]. Fractal dimensions calculated from capillary pressure curves are frequently used to evaluate the microscopic heterogeneities of reservoir rocks.

Jin Lai and Guiwen Wang [45] performed routine rock properties measurements and high-pressure mercury intrusion tests (HPMI) on a suite of the Bashijiqike tight gas sandstone samples to delineate the pore network characteristics of these reservoir rocks. Thin section epifluorescence and scanning electron microscopy (SEM) analyses were used to gain insight into pore geometry and pore size distribution of these sandstones. The results show that the pore system of the sandstones mainly consists of intergranular macropores and intragranular micropores. The HPMI analysis shows that these reservoir rocks have complex, heterogeneous microscopic pore structure. There are clear inflection points on the fractal curves of log $(S,H_g)$ versus log$(P_c)$, i.e., the fractal curves break into two segments at the capillary pressures corresponding to the apex of the Pittman’s hyperbola (plot of the ratio of mercury saturation over capillary pressure against mercury saturation).

Gautier Njiekak et al. [46] provided in the paper a number of examples where micro-CT was used in addition to standard petrophysical measurements (helium and mercury intrusion porosimetry) to get estimates of porosity in dolomites and limestones from the Weyburn oilfield in southwestern Saskatchewan, Canada. Optical microscopy and SEM were used to have an overview of the pore shapes and sizes and, whenever needed, to condition the micro-CT image segmentation parameters.

On about half of the studied twelve samples, the micro-CT porosity agrees within ±2% with the mercury and helium porosities and is lower (up to 16%) on the rest of the samples. On an oolitic limestone, we show how combining micro-CT and mercury porosimetry can allow quantitative estimation of connected and non-connected pores.

According to porosity measured by high-pressure mercury intrusion experiments and helium, and analysis of oil saturation data of 30 samples of tight formations of Member 4 of Cretaceous Quantou Formation in Rangzijing slope zone, southern Songliao Basin, Yanjie GONG et al. [47] determined the lower limit of flowing porosity of tight oil and its controlling factors of the samples. By conversion between capillary pressure in reservoir conditions and capillary pressure from high-pressure mercury intrusion experiments, flowing porosity in various injection pressures in reservoir condition can be calculated. By calculating the minimum flowing porosity of oil-bearing samples and the maximum flowing porosity of the samples without oil, it is confirmed that 3.2% is the lower limit of flowing porosity in the oil-bearing samples in the study area; and the corresponding injection pressure in reservoir conditions is 0.35 MPa. If the injection pressure is higher than 0.35 MPa, tight oil can effectively flow and accumulate. Based on the tight oil lower limit of flowing porosity and injection pressure in reservoir conditions, the discriminant chart of effective accumulation of tight oil was set up.
FIGURE 2
3-D views illustrating the common porosity characteristics in samples L7, L3 and D2.
The 2-D raw micro-CT image at the top of the stacked images is shown in (a), (d) and (g) for samples L7, L3 and D2, respectively. The 3-D rock solid matrix is shown in (b), (e) and (h) and only the pore space is presented in (c), (f) and (i). In (c), the intercrystalline pores in sample L7 are shown in light gray and the intracrystalline pores in dark gray. Note the high connectivity between the intercrystalline pores. In (f), the pores are subdivided into three groups in sample L3: large and medium-sized pores (dark and light gray colors, respectively) will cut through the micritic matrix where small pores (black color) are common. In (i), the porosity (dark gray color) captured by the micro-CT (5 μm resolution) in sample D2 is less than 1%. A large part of the rock porosity is not resolved at this resolution (see text for further explanation) [46].

For shale reservoirs, some scholars have tried to analyze the pore size distribution by high pressure mercury intrusion [48, 49], which we believe should be treated with caution. Compared with tight sandstone, the shale clay has high clay mineral content and smaller pore throat size. If traditional plunger samples are used, the sample preparation is difficult and cracks are easy to occur, and the high pressure on the shale seams is obvious during the experiment. It reduces the accuracy of the experimental results. If high-pressure mercury intrusion experiments are carried out using irregular samples, the results are not satisfactory.

FUTURE DIRECTION
Unconventional oil and gas is gradually becoming an important exploration and development field in the petroleum industry. In recent years, China’s unconventional oil and gas industry has developed rapidly. Therefore, the core-tight reservoir evaluation of unconventional oil and gas exploration has become the focus of academic and industrial research. Because dense reservoirs have the characteristics of poor physical properties and strong heterogeneity compared with conventional reservoirs, the fine study of pore structure has become a key content in the evaluation of tight reservoirs. At present, the
study of pore structure is mostly based on laboratory fine characterization. We believe that the future research of pore structure will develop from static to dynamic, from laboratory to field application, and improve the existing evaluation by combining with macroscopic geological parameters and engineering parameters. The practicability of the parameters, provide important technical support for tight reservoir evaluation and favorable area prediction.

Based on the summary analysis of previous studies, we believe that in the study of pore characterizing tight reservoirs, the lithologic facies analysis should be carried out in a single well, and representative samples should be selected for different facies. Micron to nanoscale of these samples should be carried out. The pore structure test is used to obtain the corresponding pore structure parameters. At the same time, the correlation between the pore structure parameters and the logging response characteristics of the corresponding lithofacies is established. Finally, the digital core technology is applied to establish a digital model, and finally from the sample to the well to the entire three-dimensional space pore structure characterization.

CONCLUSIONS

(1) The application of microscopic pore structure characterization methods for various reservoirs shows that various characterization techniques have their own characteristics.

(2) Several issues to be noted in the characterization of pore structure of tight reservoirs:

(a) Emphasis on improving the resolution while strengthening the sample representative scale study;
(b) At the same time as the pursuit of new technologies/methods, it is necessary to strengthen the study of the scope of effective application of technology;
(c) Emphasis on quantitative characterization of data splicing, while strengthening multi-scale qualitative and quantitative data fusion to improve characterization accuracy.

(3) Defining the pore structure of tight reservoirs The future development direction: Different levels of pore structure analysis of dense reservoirs according to different levels, based on the test simulation of sampling points, space for the entire tight reservoir structure by digital core technology construction.

ACKNOWLEDGEMENTS

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EVALUATION OF HEAVY METAL LEVELS IN IZMIT BAY COASTAL WATERS BY USING ULVA LACTUCA L., ENTEROMORPHA SP., AS BIOLOGICAL INDICATORS IN TURKEY

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ABSTRACT

In order to study the heavy metal levels of Izmit bay four stations were selected. The study was carried out seasonally between December 2015 and September 2016. This research was conducted to determine heavy metal levels (Al, Cd, Cr, Cu, Fe, Ni, Mn, Pb, and Zn) in seawater, Ulva lactuca L. and Enteromorpha sp. were identified by using ICP-OES. According to the results of this study, heavy metal levels were arranged in the following sequence: Fe > Al > Zn > Cr > Mn > Cu > Pb > Ni > Cd for Ulva lactuca, Fe > Al > Mn > Zn > Cu > Cr > Pb > Ni > Cd for Enteromorpha sp. It was determined that Fe was the highest metal in the Ulva lactuca, Enteromorpha sp.. Heavy metal concentrations at the sampling stations may be used for background levels for interspecific comparison with the Izmit Bay. Besides, physical and chemical parameters of sea water were determined. Water temperature, pH and conductivity varied from 10.11-24.22 °C, 7.45-8.25, dissolved oxygen values as 8.10-12.70 mg L⁻¹, respectively.

KEYWORDS:
Ulva lactuca, Enteromorpha sp., Heavy metals, Izmit Bay.

INTRODUCTION

The rapid development in technology and industrialization are bringing both advantages and disadvantages. Domestic and industrial pollutants affect the environment negatively. The sources of anthropogenic contamination or pollution of the environment by heavy metals include different sources of industry such as the power industry, transport, municipal waste management, waste dumping sites, fertilisers and waste used to fertilise soil. The heavy metals from these sources are scattered in the environment and contaminate soil ecosystem, water and air ecosystems [1]. Many contaminants participates the marine environment, however, they have the ability to move into different solid components of the ecosystem [2]. Environmental pollution by heavy metals is considered to be a significant problem due to its toxicity and ability to accumulate in the biota [3]. Heavy metals, such as lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), and nickel (Ni), are among the most common pollutants found in both industrial and domestic wastes. In low concentrations (Cu, Ni, and Mn) are essential trace elements for photosynthetic organisms; however at higher concentrations, it is important to determine the cause of crucial toxic influence of these metals [4]. Marine microalgae which are important for the primary production are generally used as indicators for metal pollution in sea water. However, it is very important in macro algae species for aquatic ecosystem. For this purpose, the macroalgae species of Fucus sp., Enteromorpha sp., Laminaria sp. and Ulva sp. generally the most used indicator types in determining heavy metal levels in sea. In the world and Turkey is also a lot of work carried out on this issue [1-19]. Different organisms, both animal and vegetal species, it is used as biomonitors. For example, bivalve molluscs, mussels in particular, algae species and other organisms. In this research, it is aimed to some heavy metal levels in Ulva lactuca, and Enteromorpha sp. which have sustained dispersion in the littoral region at Izmit Bay and its spatial distribution. Moreover, the correlations between metal concentrations in seawater and Ulva lactuca, Enteromorpha sp. were statistically examined.

MATERIALS AND METHODS

Apparatus. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used. This is an efficient technique to simultaneously measure almost all metals, including refractory metals and some non-metals. ICP-OES systems have now become the analytical method of choice for a wide range of applications. For this reason, it is preferred by the most research centres.
Study area. Izmit Bay is 50 km long and 1.8-9 km wide and has a surface area of 261 km². The eastern section, the smallest component of the entire system, is about 15 km in length and relatively shallow with a maximum depth of about 35 m. Central section of the Izmit Bay, being the largest component of the system, is about 20 km long and the bottom topography varies considerably in the northsoutherly direction; its northern part is relatively shallow with an average depth of about 60 m, increasing approximately 180 m towards the southern section [12]. Station 1, was located at the Tavşancıl coast of the bay. This station was surrounded by public beach and by houses used in the summer. The coordinates 40° 40' 03'' N between 029° 34' 12'' E. Station 2, was located at Derince coast. There is a recreational park area around. The coordinates 40° 45' 01'' N between 029° 48 '43'' E. Station 3, was located at Naile cafe’s coast of Izmit Bay. The coordinates 40° 44° 11''N between 029° 56' 30'' E. Station 4, was located at Kavaklı coast. The coordinates 40° 43° 32''N between 029° 50' 17'' E (Fig. 1).

Sample collection and analytical procedure. In this study, samples were collected from December 2015 to September 2016 (December, May, August, and September). A total of 16 collections were conducted and samples taken every seasonally at 4 sampling stations. Macroalgae samples were collected in littoral region (up to 2.5 m) and brought to Barem Environment Laboratory in labelled bags. *Ulva lactuca* L. and *Enteromorpha sp*. samples were dried as 1 gr wet at 105 °C for 24 hours in drying kiln in heat-resisting glass tubes after they have been cleaned up from living and non-living materials by means of distilled water. The dried samples stayed at ambient temperature for 24 hours after they have been added by 3 mL nitric acid. They put on the heat table in low temperature until their coloured steams disappeared completely in order to make them absolutely mineralized and then they were completely dissolved after they had been added with 1 mL sulphuric acid (H₂SO₄). After these procedures, the samples were diluted in 50 mL and got ready to be read after they have been filtered through blue band filter papers [13]. Heavy metal levels were indicated as μg/g wet weight. Water temperature, dissolved oxygen and pH, were measured in the field with portable instruments (IQ scientific instrument). The difference of the metal levels among stations was examined by One-Way ANOVA test. However, correlation analysis was performed to determine the relationship of metal levels with each other and physico-chemical parameters. Statistical analyzes were performed in SPSS v.11.5 package program and p <0.05 value was evaluated significantly [20].

RESULT

In this study, heavy metal accumulation levels of the *Ulva lactuca* and *Enteromorpha sp.* were determined. At the same time, it was determined in the relationship between the metal concentrations in
macro-algae samples and the physico-chemical parameters in sea water. Water temperature, pH and conductivity varied from 10.11 - 24.22 °C, 7.45 - 8.25, dissolved oxygen values as 8.10 - 12.70 mg L⁻¹, respectively. The averages and standard deviations of heavy metal concentrations of *Ulva lactuca* and *Enteromorpha* sp. species were given in Table 1 and Table 2, and some physicochemical parameters of sea water was given in Table 3.

It was determined that the Fe ratio for *Ulva lactuca* and *Enteromorpha* sp. were high in all stations. The highest value was determined at the third station. Heavy metal levels of *Ulva lactuca* was determined as Fe> Al> Zn> Cr> Mn> Cu> Pb> Ni> Cd (Figure 2).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Metal concentrations determined in <em>Ulva lactuca</em> (µg/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy Metal</td>
<td>Station 1</td>
</tr>
<tr>
<td>Al</td>
<td>24.50-26.76</td>
</tr>
<tr>
<td></td>
<td>25.24±1.03</td>
</tr>
<tr>
<td>Cd</td>
<td>0.73-1.96</td>
</tr>
<tr>
<td></td>
<td>1.37±0.50</td>
</tr>
<tr>
<td>Cr</td>
<td>14.91-17.35</td>
</tr>
<tr>
<td></td>
<td>16.02±1.03</td>
</tr>
<tr>
<td>Cu</td>
<td>10.71-16.77</td>
</tr>
<tr>
<td></td>
<td>14.49±2.66</td>
</tr>
<tr>
<td>Fe</td>
<td>138.25-161.50</td>
</tr>
<tr>
<td>Ni</td>
<td>1.40-1.50</td>
</tr>
<tr>
<td>Mn</td>
<td>1.47±0.05</td>
</tr>
<tr>
<td>Zn</td>
<td>15.34-11.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Metal concentrations determined in <em>Enteromorpha</em> sp. (µg/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy Metals</td>
<td>Location</td>
</tr>
<tr>
<td>Al</td>
<td>24.42-43.52</td>
</tr>
<tr>
<td></td>
<td>36.96±12.10</td>
</tr>
<tr>
<td>Cd</td>
<td>1.30-1.40</td>
</tr>
<tr>
<td></td>
<td>1.37±0.05</td>
</tr>
<tr>
<td>Cr</td>
<td>2.97-8.11</td>
</tr>
<tr>
<td></td>
<td>6.21±2.26</td>
</tr>
<tr>
<td>Cu</td>
<td>4.31-8.23</td>
</tr>
<tr>
<td></td>
<td>6.11±1.69</td>
</tr>
<tr>
<td>Fe</td>
<td>104.00-204.60</td>
</tr>
<tr>
<td>Ni</td>
<td>2.15-4.47</td>
</tr>
<tr>
<td>Mn</td>
<td>2.87±1.07</td>
</tr>
<tr>
<td>Zn</td>
<td>12.5±2.33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Physico-chemical parameters in sea water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Location</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>10.11 - 22.00</td>
</tr>
<tr>
<td>pH</td>
<td>7.45-7.72</td>
</tr>
<tr>
<td></td>
<td>7.59±0.13</td>
</tr>
<tr>
<td>Dissolved oxygen (mg L⁻¹)</td>
<td>8.50-12.70</td>
</tr>
</tbody>
</table>

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Heavy metal levels of *Enteromorpha* sp. were determined as Fe> Al> Mn> Zn> Cu> Cr> Pb> Ni> Cd (Figure 3).

The Spearman correlation coefficients of the metal concentrations were determined for *Ulva lactuca* that indicate a positive correlation between Cr-Cd, Mn-Al, Mn-Cd, Zn-Al, Zn-Cd, Zn-Mn (p<0.05) (Table 4).

### TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Mn</th>
<th>Pb</th>
<th>Zn</th>
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<tbody>
<tr>
<td>Al</td>
<td>1.000</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.042</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>-0.299</td>
<td>0.611</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.004</td>
<td>0.243</td>
<td>-0.096</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.200</td>
<td>-0.266</td>
<td>-0.284</td>
<td>-0.312</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.374</td>
<td>-0.356</td>
<td>-0.373</td>
<td>0.057</td>
<td>-0.083</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.636</td>
<td>0.662</td>
<td>0.234</td>
<td>0.400</td>
<td>-0.240</td>
<td>0.150</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>-0.059</td>
<td>0.304</td>
<td>0.052</td>
<td>-0.039</td>
<td>0.452</td>
<td>-0.196</td>
<td>-0.009</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.583</td>
<td>0.568</td>
<td>0.015</td>
<td>0.382</td>
<td>0.056</td>
<td>0.177</td>
<td>0.798</td>
<td>0.125</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* Bold values indicate that there is strong statistical relationship (p<0.05)
TABLE 5
Spearman correlation coefficients between the measured metal concentrations for Enteromorpha sp.

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Mn</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.094</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.573</td>
<td>-0.220</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.844</td>
<td>0.330</td>
<td>0.263</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.679</td>
<td>-0.094</td>
<td>0.645</td>
<td>0.553</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ni</td>
<td>0.469</td>
<td>-0.380</td>
<td>0.708</td>
<td>0.186</td>
<td>0.551</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.808</td>
<td>0.282</td>
<td>0.337</td>
<td>0.718</td>
<td>0.458</td>
<td>0.388</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.367</td>
<td>0.157</td>
<td>0.589</td>
<td>0.227</td>
<td>0.378</td>
<td>0.615</td>
<td>0.458</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.793</td>
<td>0.472</td>
<td>0.255</td>
<td>0.907</td>
<td>0.453</td>
<td>0.174</td>
<td>0.388</td>
<td>0.344</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* Bold values indicate that there is strong statistical relationship (p<0.05)

TABLE 6
Spearman correlation coefficients between physico-chemical parameters and heavy metal concentrations

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>pH</th>
<th>Dissolved Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>0.352</td>
<td>0.419</td>
<td>0.182</td>
</tr>
<tr>
<td>Cd</td>
<td>0.649</td>
<td>0.194</td>
<td>0.271</td>
</tr>
<tr>
<td>Cr</td>
<td>0.235</td>
<td>-0.079</td>
<td>0.103</td>
</tr>
<tr>
<td>Cu</td>
<td>0.315</td>
<td>0.034</td>
<td>0.447</td>
</tr>
<tr>
<td>Fe</td>
<td>-0.409</td>
<td>-0.368</td>
<td>0.030</td>
</tr>
<tr>
<td>Ni</td>
<td>-0.027</td>
<td>0.265</td>
<td>0.143</td>
</tr>
<tr>
<td>Mn</td>
<td>0.770</td>
<td>0.528</td>
<td>0.531</td>
</tr>
<tr>
<td>Pb</td>
<td>-0.233</td>
<td>0.020</td>
<td>0.080</td>
</tr>
<tr>
<td>Zn</td>
<td>0.706</td>
<td>0.253</td>
<td>0.689</td>
</tr>
</tbody>
</table>

* Bold values indicate that there is strong statistical relationship (p<0.05)

Spearman correlation coefficients of metal concentrations for Enteromorpha sp. were determined as: Cr-Al, Cu-Al, Fe-Al, Fe-Cr, Fe-Cu, Ni-Cr, Ni-Fe, Mn-Al, Mn-Cu, Pb-Cr, Pb-Ni, Zn-Al, Zn-Cd, Zn-Cu. This is a positive correlation between the metals was found (p<0.05) (Table 5).

According to the correlation between physico-chemical parameters and heavy metals were determined in research stations. It was determined that there was a positive correlation between sea water temperature, Cd, Mn, Zn, pH to Mn, and oxygen value to Zn (p<0.05) (Table 6).

Differences between stations and seasons of heavy metal levels were analyzed by One-Way ANOVA test. The results of the statistical analysis showed that the values of Ulva lactuca, Al, Cu (p<0.05) were significant for the 1st and 2nd stations. However the Cr value for the same stations were not significant in terms of p < 0.05, but, it was found that there is a value to be considered. However, it was determined that Cd values were significant in May, September and August (p<0.05). As a result of the statistical analysis for Enteromorpha sp., heavy metal ratios are not significant compared to the all stations, but, Al, Cd, Cu, Ni, Zn values were found to be significant in May, September and August (p<0.05). However, there is a significant relationship between seawater temperature and heavy metal levels (p <0.05).

DISCUSSION AND CONCLUSIONS

The amount of heavy metals in terrestrial and aquatic ecosystems varies depending on industrial and domestic activities. However, the geological structure of the region is very effective. Macro algae are in the groups that are frequently used indicating heavy metal pollution levels. The heavy metals absorbed by macro algae indicates variations subjected to many factors such as pH, salinity, oxygen, light, organic substance, food elements, levels of the metals in the aquatic system [13, 21, 22]. It was reported that Fe > Zn > Cu sequence is one of the elements required for the biological activities of macroalgae and their high accumulation levels are good nutrients for metabolic activities [13, 23]. In the research, heavy metal levels were determined Fe> Al> Zn> Cr> Mn> Cu> Pb> Ni> Cd in Ulva lactuca, and Fe> Al> Mn> Zn> Cu> Cr> Pb> Ni> Cd in Enteromorpha sp. In the study, it was determined that the values were suitable for the order of Fe > Zn > Cu for the metabolic activities of algae. Pb and Cd levels, which have toxic effects in aquatic systems, were found to be lower in research. The high iron content in both Ulva lactuca and Enteromorpha sp. may be due to the absorption of contaminants from industrial activities by algae, except for their biological activities. In the study, the highest concentration of iron was measured Ulva lactuca 587.60 μg/g and Enteromorpha sp. 207.61 μg/g. Many studies have been carried out in
Egypt, Brazil, Spain, Greece and Turkey seawater. In these studies, the highest iron accumulation ratio was also reported in Enteromorpha sp., Enteromorpha sp., Ulva lactuca and Enteromorpha sp. in the comparison of other metal ions [13]. In the study, the iron level of both species were determined at the highest rate compared to other metals levels. In the research, the highest average value after iron was measured for Al. High level of detection of this metal after iron, intensive industries in the vicinity of research stations, ports, maritime transport and may be due to allochthonous factors. Pb> Ni> Cd levels was found similar and low in Ulva lactuca and Enteromorpha sp. On the other hand, detection of the highest heavy metal accumulation levels seen in August when the sea water temperature is highest in month. This bears a resemblance to the data of relation between heavy metal and warm water that was given in literature [18]. This similar results has been observed in many research in Turkey's seas. The differences in heavy metal accumulation levels of algae were caused by the many factors such as the physico-chemical structure, geological formations and environmental impacts [5-16]. The similar differences were also determined in the global aquatic systems due to the geographic factors [2-4, 18, 23].

In recent years, local governments and Ministry are trying to reduce the pressure on the marine area. As a result, it is very important to regularly monitor the accumulation of metal in the sea and other living things, to implement the necessary legal sanctions and to take environmental measures in order to exploit the marine ecosystem.

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THE EFFECT OF THE MUSIC ON THE GROWTH AND DEVELOPMENT OF BEAN PLANT IN DIFFERENT MEDIUM

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ABSTRACT

The experiment was carried out in the Department of Horticulture of Cukurova University in 2014 using the bean genotype in two different climate controlled plant breeding chambers and by growing the plants with hydroponics and vermiculite. It was performed until the young plant stage in hydroponics and vermiculite medium. In the study, classical music (Yann Tiersen-comtine D’un Autre Ete) was played for beans. In addition, every day between 11.00 - 15.00 hours music was played to the plants. Photosynthesis rate CO2, leaf stomatal conductance, respiration rate, leaf temperature, plant height, leaf number, stem diameter, shoot fresh and dry weight, root fresh and dry weight, root length were investigated. Plant height, leaf number, and stem diameter were determined every four days and by 5 separate measurements for each day. The rate of photosynthesis CO2, leaf stoma conductivity, respiration rate and leaf temperature in bean plant was measured after 22 days than sowing. The root length, root fresh, the fresh and dry weight of the green component and dry weight of the plants were measured after finished testing the bean plant. In this study, according to their own controls, differences were observed of the effect of music in the hydroponics and vermiculite tests. In addition, the effect of music was also different between the two trials (hydroponics and vermiculite) in different environments.

KEYWORDS:
Music, Yann tiersen-comtine D’un AutreEte, bean, Photosynthesis, Hydroponics, Stomatal

INTRODUCTION

The influence of music on creatures has been a spoken topic for many years. Dr. T. C Singh showed the interaction between music and plants is harmonic sound waves which affect the development, flowering, fruit and seed yield of plants. He indicated that the most suitable music for the plants was classical music [1]. Later studies showed that plants were not indifferent to music and gave positive responses [2, 3, 4, 5]. From this point, we decided to examine the effect of the classical music on plants. However, we have done this work using soilless agriculture, which is an alternative way of agriculture that has emerged in recent years as a result of erosion-ineffective soils, drought and diminishing agricultural areas [6]. It has been found that playing the appropriate melodies promotes the synthesis of the favour able protein of the plant [7].

Soilless agriculture is an hydroponics technique used mostly in the greenhouses. It is bifurcated as hydroponics and substrate culture. We studied the effect of music on plants for a bean plant growing in a dead water culture which is a kind of hydroponics, and for a bean plant growing in vermiculite which is a kind of substrate culture. The reason of choosing the bean plant is that the bean is a fast growing plant, besides that it is the traditional food of the Turks (dry bean) and the production of 514,000 tons of beans annually in our country. With this production, our country is in 2nd place in the production of beans in the world ranking after China [8].

MATERIALS AND METHODS

The experiment was carried out in two separate 9 square meter climate controlled plant breeding chambers at Cukurova University Faculty of Agriculture Department of horticulture and by cultivating plants hydroponics and vermiculite technique. Have bean genotypes used name is spir and origin Erzurum province, Turkey. Music was played in one room, and control application without music was performed in the other room. Characteristics of climate chambers: 16 hours light 8 hours dark, Temperature was 25 °C ± 2 in the daytime and 18 °C ± 2 at night, Humidity: 60-70 % RH. First, the bean seeds were planted into the violes and after the beans germinated the transfer process was performed. After this process, Nutrient Solution containing nutrients that plants need was given in hydroponics and vermiculite tests. Leaf temperatures of bean plants were measured using a Minisight brand infrared thermometer. Photosynthesis rate, respiratory rate and in bean plants were measured by (LI-6400XT system brand) and leaf stoma conductivity by (Delta T Devices marka AP4) devices. Root fresh weight fresh green parts of plants
were weighed by analytical balance (Metler Toledo 0.01 grams sensitive), and the length and the root diameter of the plants were also measured. The number of leaves was counted in bean plants.

The randomized block trial pattern was carried out in 4 replicates. The data obtained at the end of the experiment were subjected to JMP package program and statistical analysis, with respect to the control % change and correlation analysis was performed, and the averages were compared according to the LSD test. In this way, the Nutrient solution was made ready (Table 1).

### TABLE 1
The nutrient solution used to grow the beanplant Consolidation of elements

<table>
<thead>
<tr>
<th>Nutrient Elements</th>
<th>Concentration (mgL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>130</td>
</tr>
<tr>
<td>P</td>
<td>35</td>
</tr>
<tr>
<td>K</td>
<td>220</td>
</tr>
<tr>
<td>Mg</td>
<td>45</td>
</tr>
<tr>
<td>Ca</td>
<td>150</td>
</tr>
<tr>
<td>S</td>
<td>70</td>
</tr>
<tr>
<td>Fe</td>
<td>1.50</td>
</tr>
<tr>
<td>Mn</td>
<td>0.80</td>
</tr>
<tr>
<td>B</td>
<td>0.50</td>
</tr>
<tr>
<td>Zn</td>
<td>0.15</td>
</tr>
<tr>
<td>Cu</td>
<td>0.10</td>
</tr>
<tr>
<td>Mo</td>
<td>0.10</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

Bean plants growing in 2 different environments since seeding that were 22 days old and listening to music for 14 days were compared with control plants. The morphological measurement values of the bean plant showed that the growth rates, leaf counts and plant lengths of the bean plants that were listened the classical music were higher than those of the control group. This result suggests that classical music has positive effects on plant growth. 5 measurements were taken at intervals of every 7 days, for the plant size, body diameter, and leaf number. In the hydroponics environment, there was a difference in plant height between control and music experiment. The bean genotype under the music effect was increased by 12.40 % and 14.80 % in the fourth and fifth measurements, respectively, in terms of % change according to the control (Table 2). It is known that sound affects the growth of plants [9]. Studies have also shown that sound vibration can be used to stimulate a seed or plant [10]. Previous studies have shown that musical sound has a significant impact on the number of germinated seeds compared to noise and untreated control, and that sound vibrations directly affect living biological systems [9].

In the vermiculite substrate environment, a difference in plant height and music was observed. In comparison with their own control, 8.41 %, 26.19 %, and 15.16 % increase were observed in the second, third and fifth measures, respectively of the bean genotype under the music effect. A difference in plant height control and music experiment was noted in the Vermiculite substrate environment showed. 8.41 %, 26.19 %, and 15.16 % increase were observed in the second, third and fifth measures, respectively in the bean genotype under music influence, according to their control. However, at the last measurement in hydroponics and vermiculite media, plant size was measured the average 22.41 cm and 32.12 cm respectively. According to this, the effect of the music on the plant height was higher in the vermiculite medium than in the aqua culture medium (Table 3).

### TABLE 2
Effect of music application on plant height (cm plant⁻¹) in bean plant at different time intervals in hydroponics medium and % change relative to control.

<table>
<thead>
<tr>
<th>Hydroponics environment</th>
<th>Plant height 1measurement (cm plant⁻¹)</th>
<th>Plant height 2measurement (cm plant⁻¹)</th>
<th>Plant height 3measurement (cm plant⁻¹)</th>
<th>Plant height 4measurement (cm plant⁻¹)</th>
<th>Plant height 5measurement (cm plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control 6.3</td>
<td>Control 7.31</td>
<td>Control 12.4</td>
<td>Control 13.5a</td>
<td>Music 22.41a</td>
</tr>
<tr>
<td>Music</td>
<td>Music 6.7</td>
<td>Music 7.09</td>
<td>Music 12.09</td>
<td>Music 15.5 b</td>
<td>Control 19.52b</td>
</tr>
<tr>
<td>Change compared to control (%)</td>
<td>6.34</td>
<td>-3.01</td>
<td>-2.5</td>
<td>12.40</td>
<td>14.80</td>
</tr>
<tr>
<td>LSDns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.37</td>
<td>1.94</td>
</tr>
</tbody>
</table>

ns: not significant

### TABLE 3
Effect of music application on plant height (cm plant⁻¹) in bean plant at different time intervals in vermiculite medium and % change relative to control.

<table>
<thead>
<tr>
<th>Vermiculite environment</th>
<th>Plant height 1measurement (cm plant⁻¹)</th>
<th>Plant height 2measurement (cm plant⁻¹)</th>
<th>Plant height 3measurement (cm plant⁻¹)</th>
<th>Plant height 4measurement (cm plant⁻¹)</th>
<th>Plant height 5measurement (cm plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Music 10.31</td>
<td>Music 11.21a</td>
<td>Music 19.32a</td>
<td>Music 22.78</td>
<td>Music 32.12a</td>
</tr>
<tr>
<td>Music</td>
<td>Control 9.70</td>
<td>Control 10.34b</td>
<td>Control 15.31b</td>
<td>Control 21.70</td>
<td>Control 27.89b</td>
</tr>
<tr>
<td>change compared to control (%)</td>
<td>6.28</td>
<td>8.41</td>
<td>26.19</td>
<td>4.47</td>
<td>15.16</td>
</tr>
<tr>
<td>LSDns</td>
<td>ns</td>
<td>0.73</td>
<td>0.60</td>
<td>ns</td>
<td>0.51</td>
</tr>
</tbody>
</table>

ns: not significant
In the hydroponics medium, a difference was observed in the number of leaves both in music and control trials. % Change values for the bean genotype under the music effect was higher as 33.33 %, 37.5 %, and 41.93 % in the third, fourth and fifth measures, respectively than in the control (Table 4). This suggests that the plants enjoy music and respond to different music genres and wavelengths. Optimal plant growth occurs when the plant is exposed to pure tones according to wave tones [7].

There was the difference in leaf count values between control and music experiments in the vermiculite substrate medium. According to the control of the bean genotype under the influence of music, there was a slight increase in 16.21 %, 1.25 % and 10.74 %, respectively, and a decrease of 1.38 % in the fourth. On the other hand, the trunk diameter of the bean genotype under the influence of music was measured as 4.81 (mm plant⁻¹) and 5.67 (mm plant⁻¹) respectively, in hydroponics and vermiculite media. According to this, the effect of music on the trunk diameter was greater in the vermiculite medium than in the hydroponics medium (Table 5).

There was no difference in body size for control and music experiment in the hydroponics medium. A decrease by 3.87 % and 2.07 % in the fourth and fifth measurements, respectively, and by 8 % in the third measurement was observed in the bean genotype under music in comparison with control (Table 6). In the vermiculite substrate environment, little difference was observed in case of body diameter measurements in control and music experiment. In the second, third, and fifth measures of percent change according to its own control of the bean genotype under music influence, there was no change in body size for control and music experiment in the vermiculite substrate medium. A decrease by 1.38 % in the fourth. On the other hand, the trunk diameter of the bean genotype under the influence of music was measured as 4.81 (mm plant⁻¹) and 5.67 (mm plant⁻¹) respectively, in hydroponics and vermiculite media. According to this, the effect of music on the trunk diameter was greater in the vermiculite medium than in the hydroponics medium (Table 7). Corn plants exposed to music sprout faster, are greener
and are thicker and harder than corn plants in silence [11].

In the experiments for photosynthesis rate CO₂, leaf stoma conductance, and respiration rate, statistically different results were obtained between the control experiment and experiment with music in the hydroponics environment. According to its own control, the increases in % change rates of bean genotype, which was under the effect of music, by 71.20 %, 75 %, and 26.66 % were observed in photosynthesis rate CO₂, leaf stoma conductance, respiratory rate, respectively (Table 8). Statistically, different results were noted for Photosynthesis Rate CO₂, leaf stoma conductance, respiratory rate of the control and music trials in vermiculite substrate medium. With respect to the % change in the bean genotype under the music influence, as against the control group, the percentages of photosynthesis rate CO₂, leaf stoma conductance, respiration rate, and temperature were increased by 30.79 %, 16.17 %, 16.59 %, and 5.46 %, respectively. However, the photosynthetic rate CO₂ of the music-affected bean genotype was 9.69 (mmol m⁻² s⁻¹) and 9.43 (mmol m⁻² s⁻¹), respectively; and the leaf stoma conductance was 189 (mmol m⁻² s⁻¹), respectively; respiration rates were as 3.23 (mmol m⁻² s⁻¹) and 2.60 (mmol m⁻² s⁻¹) respectively and the temperature was 23.27 (°C) and 23.35 (°C) respectively. According to these results, the effect of music on the photosynthetic rate CO₂, leaf stoma conductance, and respiratory rate was found to be higher in hydroponics medium compared to vermiculite medium (Table 9). The effects of acoustic frequencies of "Hezuo 918", a kind of tomato species, on growth characteristics, photosynthetic and chlorophyll fluorescence parameters were investigated. The results showed that acoustic frequency treatment not only increased the number of infusions, number of fruits, body diameter, and leaf chlorophyll content of tomatoes, but also improved photosynthesis and fluorescence parameters, particularly increasing the content of intracellular CO₂ concentration. It has been deduced that acoustic frequency processes play an important role in supporting the growth and photosynthesis of tomatoes [12]. Various studies have been conducted to investigate and understand the effect of sound and music on plant and plant growth. Under optimal stimulation conditions (100dB and 800Hz) it was reported that the sound field could increase the growth of Chrysanthemum callus and moderate stress stimulation could increase the assimilation of tissues or cells, increase physiological activities, and accelerate the growth of plants [13].

In the experiments for root fresh and dry weight, root length, the fresh and dry weight of plant green compost statistically different results were obtained between the control experiment and experiment with music in the hydroponics environment. According to its own control, the increases in % change rates of bean genotype, which was under the effect of music, by % 62.83, % 21.84, % 42.01 and % 84 were observed in root fresh weight, the fresh weight, root dry weight and the dry weight, respectively and there was a 3.21% decrease in root length (Table 10). Other studies show that growth of chrysanthemum roots can be accelerated under appropriate sound stimulation and that sound stimulation can increase the metabolism of roots and growth [14]. The rate of water from the leaves is also affected by sound waves [15].

### TABLE 7

<table>
<thead>
<tr>
<th>Vermiculite environment</th>
<th>Stem diameter 1measurement (mm plant⁻¹)</th>
<th>Stem diameter 2measurement (mm plant⁻¹)</th>
<th>Stem diameter 3measurement (mm plant⁻¹)</th>
<th>Stem diameter 4measurement (mm plant⁻¹)</th>
<th>Stem diameter 5measurement (mm plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Music</td>
<td>3.64</td>
<td>Control 3.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Control 3.70b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change compared to control (%)</td>
<td>4.29</td>
<td>16.21</td>
<td>1.25</td>
<td>-1.38</td>
<td>10.74</td>
</tr>
<tr>
<td>LSDa,b</td>
<td>ns</td>
<td>0.35</td>
<td>ns</td>
<td>ns</td>
<td>0.46</td>
</tr>
</tbody>
</table>

ns: not significant

### TABLE 8

<table>
<thead>
<tr>
<th>Hydroporics environment</th>
<th>Photosynthesis rate CO₂ (mmol m⁻² s⁻¹)</th>
<th>leaf stomatal conductance (mmol m⁻² s⁻¹)</th>
<th>respiration rate (mmol m⁻² s⁻¹)</th>
<th>leaf temperature (°C)</th>
<th>% change relative to control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Music</td>
<td>9.69 a</td>
<td>189 a</td>
<td>3.23 a</td>
<td>23.27 a</td>
<td>71.20 a</td>
</tr>
<tr>
<td>Control</td>
<td>5.66 b</td>
<td>108 b</td>
<td>2.55 b</td>
<td>22.34 a</td>
<td>75</td>
</tr>
<tr>
<td>Change compared to control (%)</td>
<td>71.20</td>
<td>75</td>
<td>26.66</td>
<td>4.16</td>
<td>0.99</td>
</tr>
<tr>
<td>LSDa,b</td>
<td>ns</td>
<td>20.86</td>
<td>0.33</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns: not significant
### TABLE 9
Effect of music application on Photosynthesis rate CO2 (mmol m⁻² s⁻¹), leaf stomatal conductance (mmol m⁻² s⁻¹), respiration rate (mmolm⁻²s⁻¹), leaf temperature (°C) in Vermiculite medium and % change relative to control.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Photosynthesis rate CO2 (mmol m⁻² s⁻¹)</th>
<th>Leaf stomatal conductance (mmol m⁻² s⁻¹)</th>
<th>Respiration rate (mmolm⁻²s⁻¹)</th>
<th>Leaf temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermiculite</td>
<td>Music 9.43 a</td>
<td>Music 158 a</td>
<td>Music 2.60 a</td>
<td>Music 23.35 a</td>
</tr>
<tr>
<td>Change compared</td>
<td>Control 7.21 b</td>
<td>Control 136 b</td>
<td>Control 2.23 b</td>
<td>Control 22.14 b</td>
</tr>
<tr>
<td>to control (%)</td>
<td></td>
<td></td>
<td>30.79</td>
<td>15.62</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td></td>
<td></td>
<td>0.21</td>
<td>0.66</td>
</tr>
</tbody>
</table>

ns: not significant

### TABLE 10
Effect of music application on root height (cm Plant⁻¹), root fresh weight (g Plant⁻¹), shoot fresh weight (g plant⁻¹), root dry weight (g Plant⁻¹), shoot dry weight (g plant⁻¹) in bean plant in hydroponics medium and % change relative to control.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Root height (cm Plant⁻¹)</th>
<th>Root fresh weight (g Plant⁻¹)</th>
<th>Shoot fresh weight (g Plant⁻¹)</th>
<th>Root dry weight (g Plant⁻¹)</th>
<th>Shoot dry weight (g Plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroponics</td>
<td>Control 44.78a</td>
<td>Music 13.19a</td>
<td>Music 27.00a</td>
<td>Music 0.55a</td>
<td>Music 2.76a</td>
</tr>
<tr>
<td>Change compared</td>
<td>Music 43.34b</td>
<td>Control 8.10b</td>
<td>Control22.16b</td>
<td>Control 0.39b</td>
<td>Control 1.50b</td>
</tr>
<tr>
<td>to control (%)</td>
<td>-3.21</td>
<td>62.83</td>
<td>21.84</td>
<td>41.02</td>
<td>84</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td>0.59</td>
<td>1.55</td>
<td>0.62</td>
<td>0.09</td>
<td>0.10</td>
</tr>
</tbody>
</table>

### TABLE 11
Effect of music application on root height (cm Plant⁻¹), root fresh weight (g plant⁻¹), shoot fresh weight (g plant⁻¹), root dry weight (g Plant⁻¹), shoot dry weight (g plant⁻¹) in bean plant in Vermiculite medium and % change relative to control.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Root height (cm Plant⁻¹)</th>
<th>Root fresh weight (g Plant⁻¹)</th>
<th>Shoot fresh weight (g Plant⁻¹)</th>
<th>Root dry weight (g Plant⁻¹)</th>
<th>Shoot dry weight (g Plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermiculite</td>
<td>Music 41.61 a</td>
<td>Music 17.89a</td>
<td>Music 26.72a</td>
<td>Music 0.96a</td>
<td>Music 2.83a</td>
</tr>
<tr>
<td>Change compared</td>
<td>Control 28.78 b</td>
<td>Control 13.12b</td>
<td>Control22.31b</td>
<td>Control 0.67 b</td>
<td>Control 2.24b</td>
</tr>
<tr>
<td>to control (%)</td>
<td>44.57</td>
<td>36.35</td>
<td>19.76</td>
<td>43.28</td>
<td>26.33</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td>1.77</td>
<td>0.66</td>
<td>0.48</td>
<td>0.10</td>
<td>0.19</td>
</tr>
</tbody>
</table>

In the experiments for Root height, Shoot fresh weight, Root fresh weight, Root dry weight and Shoot dry weight statistically different results were obtained between the control experiment and experiment with music in the Vermiculite environment. According to its own control, the increases in % change rates of bean genotype, which was under the effect of music, by 44.57 %, 36.35 %, 19.76 %, 43.28 % and 26.33 % were observed in Root height, Root fresh weight, Shoot fresh weight, Root dry weight, Shoot dry weight, respectively.

However, the Root height of the music-affected bean genotype was 43.34 (cm Plant⁻¹) and 41.61 (cm Plant⁻¹), respectively; and the Root fresh weight was 13.19 (g Plant⁻¹) and 17.89 (g Plant⁻¹), respectively; and the Shoot fresh weight was 27.00 (g Plant⁻¹) and 26.72 (g Plant⁻¹), respectively; and the Root dry weight was 0.55 (g Plant⁻¹) and 0.96 (g Plant⁻¹), and the Shoot dry weight was 2.76 (g Plant⁻¹) and 2.83 (g Plant⁻¹) respectively. According to these results, the effect of music on the Root fresh weight, Root dry weight, and Shoot dry weight was found to be higher in vermiculite medium compared to hydroponics medium. And the effect of music on the Root height, and Shoot fresh weight was found to be higher in hydroponics medium compared to vermiculite medium (Table 10,11).

### CORRELATION
As a result of the study performed with the bean genotype under the musical influence, differences were observed in the findings of the correlation between physiological parameters and morphological parameters. A positive correlation between plant height and fresh green flesh weight was found. A positive correlation between plant height and fresh green flesh weight (r = 0.5006* P=0.05), dry green flesh weight (r = 0.5135* P=0.05), Photosynthesis rate CO2 (r=0.8046** P=0.01), leaf stoma conductivity (r = 0.8466** P=0.01), leaf temperature (r = 0.6994* P=0.05) and leaf number (r = 0.9580** P=0.01) was found. A positive correlation between respiration rate and Photosynthesis rate CO2 (r = 0.9636** P=0.01), leaf stoma conductivity...
(r= 0.9764** P=0.01) was found. A positive correlation between leaf number and Photosynthesis rate CO2 (r= 0.9138** P=0.01), leaf stoma conductivity (r= 0.9633** P=0.05), respiration rate (r= 0.9887** P=0.01) and leaf temperature (r= 0.5166* P=0.05) was found.

CONCLUSION

As can be seen from the graphs based on the morphological measurements, the growth rates and the number of the leaf of the plants that were listened the classical music are higher than those of the plants that do not listen to music, both in hydroponics and vermiculite environments. This result shows that classical music has a positive effect on plant growth. In both media (hydroponics and vermiculite), the effect of music on the photosynthetic rate CO2, leaf stoma conductance, and respiratory rate was found to be statistically different from, according to their own controls (Tables 10 and 11).

In both media, vermiculite and hydroponics, although the same amount of nutrient solution was used growth and increase in leaf number increased. As a result of this increase, iron deficiency was primarily observed in the group listened to the music compared to the control (Table 2, 3, 4, 5), (Figure 1). In the experiment presented here, it is seen that the data obtained by using landless cultivation methods in a very short time is quite hopeful. These results guide to the modern greenhouses using soilless hydroponics systems in the greenhouse how they can use music to improve yield and product quality during vegetable growing. Experiments to be carried out after that, farming practices can be planned with music reaching crop yield in the greenhouses. We believe that these studies have the potential to include data that the greenhouse owners can use in practice. We also believe that the classical music to bee activities that pollination and fertilize in the greenhouse and the people working in it (Figure 2).

![Figure 1](image1.png)

**FIGURE 1**
Changes in plants affected by classical music in 2 different media (vermiculite, hydroponics) compare to the control group.

![Figure 2](image2.png)

**FIGURE 2**
Changes in plants affected by classical music in 2 different media (vermiculite, hydroponics).
REFERENCES


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DETERMINATION OF BOTANICAL COMPOSITION, YIELD AND PASTURE QUALITY RATINGS OF INFERTILE PASTURES IN KOZLUK DISTRICT OF BATMAN PROVINCE OF TURKEY

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ABSTRACT

This study was carried out to determine the yield, quality, capacity and species diversity in Kozluk pastures of Batman province of Turkey. Studies were carried out in pastures of five villages (Yenicaglar, Karpuzlu, Kavakdibi, Kahveci and Yaprakli). Vegetation measurement was conducted by wheeled loop method. Total average plant coverage rate of relevant pastures was 84.7% where the ratio of graminea in the botanical composition was 25.4%, the ratio of legumes was 36.8% and the rate of other family plants was 37.8%. The ratio of the decreasers was 1.8%, the ratio of increasers were 9.5% and the rate of the invaders was 88.8%. The average green grass yield of the pastures was 1.2 t / ha and the hay yield was 0.3 t / ha. The average pasture quality grade of the pastures was 3.89. As a result of vegetation surveys, it was concluded that three of five pastures were at medium condition and two were in need of improvement treatments.

KEYWORDS:
Pasture, yield, botanical composition, decreaser, increaser, invasive

INTRODUCTION

Forage crops, pastures and meadows are main sources of animal feed. Meadows and pastures are the cheapest and main feed resources for livestock in Turkey. 65-70% of total inputs in average livestock enterprises are feed expenses [1].

Botanical composition, yield capacity, grazing capacity and pasture condition must be known for the appropriate utilisation of the pastures. It is possible to determine the management rules of a pasture through land survey studies to understand the management requirement of these areas. In this study, it is aimed to determine the existing condition of pastures of Kozluk district of Batman province to orient future pasture improvement practices.

MATERIALS AND METHODS

Field studies were carried out in the pastures of Yenicaglar, Karpuzlu, Kavakdibi, Kahveci and Yaprakli villages of Kozluk district of Batman province between 15 April and 15 June 2015. Topographical information of the study area is given in Table 1.

According to the meteorological data recorded between 1963-2017 which were obtained from General Directorate of Meteorology, the average temperature in Batman province is 16.4 °C, the average highest temperature is 23.6 °C, the average lowest temperature is 8.9 °C and the total rainfall is 487.5 mm. The hottest months are July and August, and the maximum precipitation receiving months are March and April [2]. The vegetation measurements of the pastures were determined by the modified wheeled loop method adopted from Koc and Cakal, [3]. The vegetation survey was carried out on four lines at each stop on a total of 400 points, oriented to east, west, north and south directions at flowering time of the dominant plants in pastures. Diagnosis of plant species encountered during the study was conducted according to Davis, [4]. Plant coverage ratio and participation rates of the plants in the botanical composition were calculated by method of Gokkus et al. [5]. In this method, plant coverage ratio was determined by the proportioning the "number of points including a plant" to "total number of points". The determined number of each plant species in the botanical composition were proportioned to the total number of plants to calculate "species rate in botanical composition". The diagnosed plant species were divided into three groups: legumes, graminea and other family plants and the proportions of these groups in the botanical composition were determined. To determine quality degree status of the pastures, below equations were used which were adopted from Gokkus et al. [5].

\[
PQD: \frac{(\Sigma RxQS)}{100}
\]

PQD: Pasture Quality Degree,
R: Rate of species in the botanical composition
QS: Quality Score
TABLE 1  
Topographical information of the studied pastures

<table>
<thead>
<tr>
<th>Village</th>
<th>Surface Area (m²)</th>
<th>Altitude (m)</th>
<th>Slope (%)</th>
<th>Soil Depth</th>
<th>Grazing Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yenicaglar</td>
<td>9686</td>
<td>666</td>
<td>2-5</td>
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<td>Intensive</td>
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<tr>
<td>Karpuzlu</td>
<td>900</td>
<td>800</td>
<td>6-11</td>
<td>Shallow</td>
<td>Intensive</td>
</tr>
<tr>
<td>Kavakdibi</td>
<td>1935</td>
<td>730</td>
<td>6-11</td>
<td>Shallow</td>
<td>Medium</td>
</tr>
<tr>
<td>Kahveci</td>
<td>3370</td>
<td>652</td>
<td>2-5</td>
<td>Shallow</td>
<td>Medium</td>
</tr>
<tr>
<td>Yapakli</td>
<td>1265</td>
<td>532</td>
<td>6-11</td>
<td>Shallow</td>
<td>Medium</td>
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</table>

TABLE 2  
Pasture Status Scale

<table>
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<tr>
<th>Pasture Quality Grade</th>
<th>Pasture Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>Very weak</td>
</tr>
<tr>
<td>2-4</td>
<td>Weak</td>
</tr>
<tr>
<td>4-6</td>
<td>Medium</td>
</tr>
<tr>
<td>6-8</td>
<td>Good</td>
</tr>
<tr>
<td>8-10</td>
<td>Very good</td>
</tr>
</tbody>
</table>

Quality score lists of Bakir [6] and Gokkus et al. [5] were used to determine the quality scores of plant species encountered in pastures. Moreover, for determining the quality scores of plant species which are not included in the lists, the characteristics of the species efficiency, grazing convenience and palatability were taken into consideration, as described by the authors. The scale showing the pasture status is given in Table 2.

33x33 cm frame was placed on the 20th m of each line in each pasture parcel and the grass in the frame were cut from the soil level. The grass were weighed and the green grass yield per hectare was calculated. The samples used for the calculation of green grass yield were dried at 70 °C for 48 hours in a drying cabinet to calculate dry grass yield.

The grazing capacities of the investigated pastures were calculated using the following equation in accordance with Erkun, [7], Yilmaz, [8] and Tukel, [9].

\[
\text{Grazing capacity} = \frac{\text{Pasture Area (ha)} \times \text{Pasture Yield (kg/ha)} \times \text{Utilizable Forage Rate}}{\text{Forage consumption of one animal per day (kg) \times Number of Grazing Days (days)}}
\]

Since pastures are located in dry zone, benefici-cable feed rate was considered 50%, which is recommended for arid pastures by Tukel and Hatipoglu, [10]. The average capacity of pastures were calculated as AU (animal unit). Therefore, in the above equation, a daily feed (dry grass) requirement of an animal was taken as 12.5 kg / day, considering that a cattle weighing 500 kg could consume 2.5% of the dry weight of the live weight. Pasture grazing period was covered 150 days.

The data obtained from the study were analyzed by JUMP statistical package program and the variance analysis was performed according to the randomized block design.

RESULTS AND DISCUSSION

Detected plant species and related informations in five village pastures of Kozluk district of Batman province in Turkey are given in Table 3.

A total of 23 plant species including 3 gramineae, 4 leguminous and 16 other family plants were determined in Yenicaglar village pasture. 10 of the identified species were found perennial, 13 were annual, 2 were decreaser, 4 were increaser and 17 were invasive. Plant coverage rate of Yenicaglar village pasture was calculated as 83.0% and the pasture quality grade was 4.18. In Karpuzlu village pasture, a total of 20 plant species including 4 gramineae, 3 leguminous and 13 other family plants were identified. It was determined that 5 species were perennial, 15 were annual, 1 was decreasing, 2 were increaser and 17 were invasive. Plant coverage rate of Karpuzlu village pasture was 84.5% and the pasture quality grade was 3.36. A total of 26 plant species including 6 gramineae, 11 legume and 9 other family plants were identified in this pasture. It was determined that 5 species were perennial, 21 were annual, 1 was decreaser, 2 were increaser and 23 were invasive. Plant coverage rate of Kavakdibi village pasture was 91.5% and the pasture quality grade was 4.39. A total of 24 plant species including 24 gramineae, 10 leguminous and 3 other family plant species were identified in Kahveci village pasture. Six of the identified species were perennial, 18 were annual, 2 were decreaser, 3 were increaser and 19 were invasive. Plant coverage rate of Kahveci village was 85.5% and the pasture quality grade of the pasture was 4.06. In Yapakli village pasture, 1 graminea, 4 legumes and 14 other family plants were determined. It was observed that 9 of the identified species were perennial, 10 were annual, 1 was increaser and 18 were invasive. The plant coverage ratio were 79.0% and the quality of the pasture was 3.48.
### TABLE 3
Species determined in the pastures, plant families, life span, its effects, plant coverage rate (PC), botanical composition (BC) and participation rate in composition (PRC)

<table>
<thead>
<tr>
<th>Yenicaglar Village</th>
<th>Family</th>
<th>Species</th>
<th>Life Span</th>
<th>Effect</th>
<th>PC</th>
<th>BC (%)</th>
<th>PRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poaceae</td>
<td>Bromus tectorum</td>
<td>Annual</td>
<td>Invasive</td>
<td>3.25</td>
<td>3.92</td>
<td>0.04</td>
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<tr>
<td>Poaceae</td>
<td>Lolium perenne</td>
<td>Perennial</td>
<td>Decreaser</td>
<td>2.50</td>
<td>3.01</td>
<td>0.24</td>
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</tr>
<tr>
<td>Poaceae</td>
<td>Poa bulbosa</td>
<td>Perennial</td>
<td>Increaser</td>
<td>4.50</td>
<td>5.42</td>
<td>0.22</td>
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<tr>
<td>Fabaceae</td>
<td>Coronilla scorpionides</td>
<td>Annual</td>
<td>Increaser</td>
<td>1.50</td>
<td>1.81</td>
<td>0.11</td>
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<tr>
<td>Fabaceae</td>
<td>Hibiscus rosa-sinensis</td>
<td>Annual</td>
<td>Invasive</td>
<td>2.50</td>
<td>3.01</td>
<td>0.03</td>
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<tr>
<td>Fabaceae</td>
<td>Medicago rigidula</td>
<td>Annual</td>
<td>Invasive</td>
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<td>3.61</td>
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<td>1.81</td>
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<tr>
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<td>Invasive</td>
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<td>5.72</td>
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<td>9.64</td>
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<td>0.90</td>
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<td>0.01</td>
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<td>0.90</td>
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<td>Increaser</td>
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<td>Invasive</td>
<td>3.25</td>
<td>3.92</td>
<td>0.04</td>
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<td>Invasive</td>
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<td>6.33</td>
<td>0.06</td>
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<td>0.90</td>
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<th>Life Span</th>
<th>Effect</th>
<th>PC</th>
<th>BC (%)</th>
<th>PRC</th>
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<td>1.18</td>
<td>0.04</td>
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<td>2.66</td>
<td>0.05</td>
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<td>0.07</td>
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<th>Species</th>
<th>Life Span</th>
<th>Effect</th>
<th>PC</th>
<th>BC (%)</th>
<th>PRC</th>
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<th>Effect</th>
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<th>BC (%)</th>
<th>PRC</th>
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**TABLE 4**

Number of plant species and families determined in the pastures and the rates of increaser, decreaser and invasive plant species in botanical composition

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<th>Rate of Invasives</th>
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<td><strong>Average</strong></td>
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The characteristics of the plant species identified in the village pastures are given in Table 4. *Lolium perenne*, *Sanguisorba minor*, *Chrysopogon gryllus* were decreaser species and *Poa bulbosa*, *Coronilla scorpioides*, *Teucrium polium*, *Plantago atrata*, *Plantago lanceolata* and *Hordeum bulbosum* were increaser species encountered in the studied pastures. Other species are included in the invasive group. As seen in Table 4, no decreaser plant species was encountered in Yaprakli, village pasture.

The ratio of the decreasers and increasers in similar studies carried out in Turkey were respectively as %10.88 and %30.6 [11]; %18.07 and %28.41 [12]; %14.72 and %24.80 [13], %12.62 and %19.98, [14]; %28.86 and %28.06 [16].

It was observed that the rate of participation of decreasers and increasers in botanical composition were generally lower than previous studies. It is estimated that the source of this change is related to different grazing pressures in different regions.

The ratio of the plant coverage rates and the ratio of graminea, legumes and other family plants determined in the pastures surveyed in the study is given in Table 5.

The plant coverage ratio of the pastures were ranged between 79.0-91.5% where the average was 84.70%. There was no statistically significant difference between the village pastures in terms of plant coverage ratio.

It was observed that the difference in terms of the rate of the graminea, legumes and other family plants in the vegetation were statistically significant. Highest graminea coverage rate was observed in Kahveci village.

Plant coverage rates of pastures in similar studies in Turkey were between %71.9-99.0 [16]; [12]; [17]; [11]; [18]. Proportion of gramineas in vegetation in similar studies in Turkey were between %19.3-57.0 [16]; [12]; [17]; [11]; [18]. Proportion of legumes in vegetation in similar studies in Turkey were between %1.3-31.0 [16]; [12]; [17]; [11]; [18]. Proportion of other family species in vegetation in similar studies in Turkey were between %25.4-64.5 [16]; [12]; [17]; [11]; [18]. The results obtained from this study and the results of previous studies were generally similar.

Green grass and hay yields determined in the studied pastures are given in Table 6.

The difference between green grass and hay yields was statistically significant (1%). The highest yield of green grass (1.600 kg / ha) was obtained from the village of Kahveci, and the lowest green grass yield 800 kg / ha was obtained from the Yaprakli village pasture. The highest hay yields were obtained from Kahveci (450 kg / ha) and Kavakdibi (400 kg / ha) villages.

Previous studies in Turkey showed that green grass yields ranged between 1.781-6.072 kg / ha [19]; [20]; [21] and hay yields ranged between 465-2.208 kg / ha [22]; [16]; [19]; [11]; [21]. Green grass and hay yields were likely to be lower than previous studies due to the differences in grazing activities in pasture areas and ecological and topographical factors of these pastures.

The pasture quality grades, pasture conditions and grazing capacities determined in the surveyed pastures are given in Table 7.

### Table 5

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<th>Plant Coverage Rates</th>
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<th>Rates of Legumes</th>
<th>Rates of Other Plant Family</th>
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<td>Rates (%)</td>
<td>Rates (%)</td>
<td>Rates (%)</td>
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<td>50.37 a**</td>
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<td>19.98 c</td>
<td>35.12 ab</td>
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<td>CV: %26.60</td>
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<td>CV: %21.80</td>
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* LSD P≤0.05; ** LSD P≤0.01

### Table 6

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<th>Green Yields</th>
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<td>Green Yields (kg / ha)</td>
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<td>CV: %14.09</td>
<td>CV: %19.83</td>
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*P≤0.05 is significant according to LSD test
TABLE 7
Pasture quality grades, pasture conditions and grazing capacities of Kozluk pastures

<table>
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<th>Grade</th>
<th>Pasture Condition</th>
<th>Grazing Capacity</th>
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<tr>
<td>Karpuzlu</td>
<td>3.36</td>
<td>Weak</td>
<td>4.80</td>
</tr>
<tr>
<td>Kavakdibi</td>
<td>4.39</td>
<td>Medium</td>
<td>20.60</td>
</tr>
<tr>
<td>Yaprakli</td>
<td>3.48</td>
<td>Weak</td>
<td>6.10</td>
</tr>
<tr>
<td>Yenicaglar</td>
<td>4.18</td>
<td>Medium</td>
<td>67.20</td>
</tr>
<tr>
<td>Average</td>
<td>3.89</td>
<td></td>
<td>27.80</td>
</tr>
</tbody>
</table>

The pasture qualities were ranged between 3.36 to 4.39, with an average of 3.89. The main reason for high grazing capacity of Yenicaglar village pasture and Kahveci village pasture compared to other pastures was high surface area of these pastures (Yenicaglar village pasture was 969 ha and Kahveci village pasture was 337 ha). In similar studies in our country, pasture quality grade were found weak or very weak where these values were between 1.86-4.53 in studies of [16]; [19]; [11]; [23]; [20]. Results obtained from the study are similar to the previous studies.

CONCLUSION

In this study, pastures of five villages of Kozluk district of Batman province were surveyed and observed that the ratio of the areas covered by the plant is high, number of plant species in the decreaser and increaser group was found low and the invasive plant species in the pasture areas were dominant. In general, the number of legumes and graminea family species were at the desired level. Karpuzlu and Yaprakli pastures were low scored in terms of plant coverage rate, participation of legumes and gramineas in botanical composition and green and dry grass yields. This situation was also found reflected to pasture quality and pasture status observations. It was concluded that suitable grazing systems should be applied in the village pastures of Kahveci, Kavakdibi and Yenicaglar, and improvement of pasture status should be made with appropriate method in pastures of Karpuzlu and Yaprakli.

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STUDY ON ECOLOGICAL CARRYING CAPACITY OF CHARACTERISTIC TOWN

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ABSTRACT

China is experiencing rapid progress in industrialization and urbanization. Characteristic towns (Tese Xiaozhen) are one of the important drivers for China’s urbanization, industrialization and agricultural modernization in the 21st century. This paper first explores the designation of a brand-new type of new towns in China, i.e. “characteristic towns”, to meet the need of the current urbanization in China. Eco-environment carrying capacity of a characteristic town was studied, the results showed that the recent ecological footprint of the characteristic town was 674.72 hm², effective ecological carrying capacity was 841.15 hm², and ecological surplus was 166.43 hm². In terms of current and short-term planning of the characteristic town, tourism activities will not bring serious damages to the ecological environment, and ecological footprint of the study area can be further developed. But from the perspective of ecological carrying capacity, the characteristic town needs a proper long-term planning. Targeted suggestions and construction emphasis on characteristic town in the future have been put forward according to the influence.

KEYWORDS:
Characteristic towns, Urbanization, China, Eco-environment, Ecological footprint, Ecological carrying capacity

INTRODUCTION

Characteristic towns (Tese Xiaozhen) are one of the important drivers for China’s urbanization, industrialization and agricultural modernization in the 21st century. In 2016, China’s urbanization level reached a record high of 57.53%, with per capita GDP at 53,980 RMB (about 7800 US dollars) [1, 2]. Meanwhile, only 41.2% of the population are registered urban residents. That means 223 million migrant workers and their families are unqualified urban residents. The Hukou registration system in China makes these migrants unable to have equal access to public services and welfare in urban, such as housing, education, medical care, and social insurance. The rapid urbanization has further made China the world factory with numerous industrial parks, resulting in excess production capacity in raw materials like crude steel. Characteristic towns in China is an innovative platform integrating characteristic industries, culture, tourism and community functions. Unlike the rural-urban fringe zones, which are often located in the suburban areas [3], characteristic towns are located in a variety of places.

With more than a decade of spectacular growth, some had cast doubt on the sustainability of the “Chinese economic model”. By the fourth quarter of 2016, China’s GDP growth rate was 6.7%, the lowest growth rate since the global financial crisis in 2008. It is evident that the traditional engines of economic growth, such as manufacturing and real estate, have been weakened. China needs a new growth engine that can transform these ever-prosperous areas into ones that feature growth quality instead of quantity, agglomeration instead of simple concentration, livability instead of congestion, sustainability instead of pollution, and innovation instead of imitation. Meanwhile, China’s rapid urbanization has created an enabling environment for the rapid economic development with abundant labor, low-cost land and good infrastructure. According to National New Urbanization Plan (2014–2020) released in 2014, China’s urbanization level in 2020 will reach the benchmark of 60%. In other words, another 100 million rural population will become new urban residents. Meanwhile, the worsening pollution, urban sprawl and congestion make megacities and large cities no longer feasible to absorb surplus rural population with urbanization advancing. The growing urban population has brought housing tension and urban environmental pollution [4]. On the other hand, the lagging development of infrastructure has been the bottleneck of China’s rural economic development [5].

Given the existing regional imbalance in China, Prime Minister Li Keqiang has revealed that further urbanization is not only critical to China’s economic success, but also a fundamental way to reduce regional disparities. In this regard, the development of characteristic towns aims to address issues associated with the rapid urbanization, such as the imbalance between urban and rural areas, urban amenity and economic transitions mentioned above. Since
economic reforms started from 1978, industrial clusters and regions with characteristic industries have prospered in China, especially in the eastern coast, alongside the needs of urbanization.

The cultivation of characteristic towns affirmed by the central government has become one of the national initiatives. China's traditional economic model is resource and labor oriented, which is incompatible with the rising trend for sustainable growth. To increase the competitiveness in the wave of globalization, it is critical to upgrade the industry, integrating big data, artificial intelligence and other high-end technologies. The development of characteristic towns aims to enhance the regional competitiveness, while help to alleviate the congestion and pollution brought by the traditional mode. In 2016, the Ministry of Housing decided that 127 towns in 32 provinces and cities were the piloting characteristic towns, with a goal to cultivate about 1000 (accounting for about 5% of the total number of towns) distinctive, vibrant characteristic towns by the end of 2020. China's New Urbanization Plan (2014–2020) further revealed the strategic objectives of the new urbanization “to speed up the cultivation of small cities and small towns” by accelerating urban development transformation.

At present, the economic growth continues at the same time the environment is worsening, the ecological damage is becoming more and more serious. Comprehensive evaluation of the environmental economy has been widespread concern in academia [6, 7]. Scholars have studied from the perspective of coordinated development on social economy and ecological environment, ecological security warning, economic growth and environmental pollution, ecological economy [8, 9]. But on the whole, there were few studies on qualify the relationship between social economic development with ecological quality and environmental quality at home and abroad, and lacked of macroeconomic research on the development stage and path of regional environmental economy. Some developed countries mainly focus on the study of micro-scale, such as atmospheric environment [10], water environment [11] and other aspects of pollution characteristics, pollution on biological and human health and macro-scale focuses on pollution emissions and economic growth, cross-border pollution and regional environmental quality [12-14] and so on.

Single study cannot guide the overall regional environmental governance; other issues had increasingly arisen in the process of solving some problems [15]. At present, there are several methods used in regional environmental assessment, such as ecological footprint, coordination degree, matter element analysis [16] and ecological health risk analysis.

The concept and research method of ecological environment carrying capacity. The carrying capacity of the ecological environment is to determine the maximum ability of the ecosystem to human activities. The so-called maximum capacity for human activities refers to the intensity of human activities that the ecosystem can withstand without destroying the function of ecosystem services. The ecological environment carrying capacity is based on the study of the various service functions of the ecosystem, combined with the conditions of the operation of the social economic system, including the support of the ecosystem to the socio-economic system and the pressure of the socio-economic system on the ecosystem, which can be described the relationship between ecosystems and human socioeconomic activities [17]. Relevant research on the carrying capacity of the ecological environment can rationalize the relationship between human and ecological environment and realize the coordinated and sustainable development between man and nature [18].

Ecological environment carrying capacity, the key indicator to measure the sustainable development of regional economy, society, and ecology, has become a research hot spot [19]. This concept was born under the background of ecological disruption, resource shortages, and environmental pollution with the evolution of several conceptions such as the population carrying capacity, grazing capacity, land carrying capacity, and the environmental carrying capacity. Ecological environment carrying capacity was once the first ecological discipline and one of the most important notions of bioscience. Currently, it is becoming more and more mature, from researches of biotic population growth law to practical problem studies serving for human economic development, from carrying capacity restricted by a single factor like food, water, or land to systematic capacity involving multi-factor constraints. With sustainable development pursued by nations all over the world, ecological environment carrying capacity is widely employed in urban planning, resource and environmental management, regional development, and other macro-social economic activities. Mountain urban construction or low-slope hilly land exploitation acts as a typical social practice for which ecological carrying capacity theories can potentially provide huge guidance.

**EXPERIMENTAL**

Research method. The assessment of the ecological footprint includes the following six different types of natural use: cultivated land, woodland, pasture, construction land, fossil energy land and waters. The calculation of the ecological footprint of a known population includes the production of resources consumed by these populations and the total area of bio-productive land required to consume the waste generated. The ecological carrying capacity of
a region refers to the sum of the areas of bio-productive land that this region can provide to humans. The ecological footprint reveals how many natural resources humans have utilized, and the ecological carrying capacity reflects the product and service capabilities that nature can provide. The difference between the two is an ecological deficit or an ecological surplus, which can be used to evaluate the sustainable development of status and trends for the research object.

The ecological footprint can be calculated separately from the perspective of consumption and production. The per capita ecological occupancy calculated from the perspective of personnel consumption can be compared with the per capita ecological occupancy of the region; the ecological occupancy calculated from the production perspective (especially the production using or occupying ecological resources) is used to analyze the characteristics. The ecological impact and ecological profit and loss of human activities in the town. From a production perspective, all products, including for their own consumption and for trade, occupy (consume) bio-productive land, which is a bio-productive land within the study area. Therefore, we mainly calculate the production-type ecological footprint of the study area.

Ecological footprint calculation. The calculation of the ecological footprint is based on the following two facts: (a) Humans can estimate most of the resources, energy and waste generated by themselves; (b) These resources and waste can be converted into organisms that produce and absorb these resources and wastes. Productive area. Wackernagel divides bio-productive land into arable land, woodland, pasture, construction land, fossil energy land and waters. Due to the large difference in production capacity per unit area of these six bio-productive land, in order to make the results comparable, usually an equalization factor is multiplied before each type of bio-productive land area. The ecological footprint calculation formula is as follows:

$$\text{EF} = \text{Ne} \times \sum r_i a_i$$  \hspace{1cm} (1)

$$a = C_i \times Y_i (i = 1, 2, 3, \ldots, 6)$$  \hspace{1cm} (2)

Where \(i\) is the type of consumption; \(EF\) is the total ecological footprint; \(N\) is the total population of the region; \(ef\) is the regional per capita ecological footprint; \(r_i\) is the equilibrium factor for \(i\)-type land; \(a_i\) is the per capita bio-productive land area converted by \(i\) consumption type \(C_i\) is the per capita consumption of \(i\) consumption type; \(Y_i\) is the average production capacity of \(i\) consumption type.

Ecological carrying capacity calculation. The ecological footprint method defines the sum of all bio-productive land areas (including waters) actually provided to humans in a region as the ecological capacity of the region, and realizes the biophysical indicators of bio-productive land area to indicate and evaluate the ecological footprint. And ecological carrying capacity makes the ecological carrying capacity and ecological footprint comparable. The average yield of a certain type of bio-productive area varies from country to country, so the yield factor is introduced. Calculated as follows:

$$\text{REC} = (1 - 12\%) \times \text{EC}$$  \hspace{1cm} (3)

$$\text{EC} = \text{N} \times \text{ec}$$  \hspace{1cm} (4)

$$\text{ec} = \sum \text{ec}_i$$  \hspace{1cm} (5)

$$\text{ec} = \text{a}_i \times \text{C}_i \times \text{Y}_i$$  \hspace{1cm} (6)

In the formula, \(REC\) is the effective ecological carrying capacity; \(EC\) is the regional total ecological carrying capacity; \(N\) is the regional population; \(ec\) is the per capita ecological carrying capacity of \(i\)-type land; \(a_i\) is the per capita bio-productivity of \(i\)-type land Area; \(r_i\) is the equilibrium factor; \(y_i\) is the production factor (productivity coefficient).

RESULTS AND DISCUSSION

Ecological footprint. Conceptually, ecological footprint can be described as the pressure of human activities on nature. A key aspect of the EF methodology is that it encapsulates a wide range of environmental data into a single indicator. Also, it is incorporated as an indicator of environmental performance by many governmental and intergovernmental organizations like European Energy Agency, European Union, European Commission and United Nations since it provides a basis for setting goals, identifying options for action, and tracking progress toward stated goals. Therefore, understanding stochastic behaviors of the EF is important, especially in terms of policy implementation.

The main types of consumption in the characteristic towns are biological resources and energy. Among them, biological resources include food, edible vegetable oil, fresh vegetables, pork, beef and mutton, fresh eggs, poultry, aquatic products, sugar, fresh fruits, fresh milk, and wood; energy includes gasoline, liquefied petroleum gas, natural gas, and electricity. According to the calculation formula of per capita bio-productive land area, the calculation results of per capita bio-productive land area of biological resources and energy consumption are shown in Table 1 and 2, respectively.

The calculation results of the per capita ecological footprint in the characteristic towns are shown in Table 3. Since different types of land have different production capacities, the equilibrium factor \(n\) is introduced, that is, when the world average 1 hm\(^2\) bio-productive land-output unit is 1 unit, 1 hm\(^2\) of arable land can produce 2.8 units; 1 hm\(^2\) grassland Can produce 0.5 units; 1 hm\(^2\) forest and fossil energy land can produce 1.1 units; 1 hm\(^2\) water area can produce 0.2 units; construction land is generally better cultivated land, so its equilibrium factor and The
TABLE 1
Bio-productive land area per capita consumption of biological resources in characteristic town

<table>
<thead>
<tr>
<th>Biological resources</th>
<th>Global average production (kg/hm²)</th>
<th>Per capita consumption in characteristic town (kg)</th>
<th>Per capita bioproductive land area (hm²)</th>
<th>Bioproductive land type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>2744</td>
<td>53.330</td>
<td>0.0195</td>
<td>Arable land</td>
</tr>
<tr>
<td>Edible vegetable oil</td>
<td>431</td>
<td>7.009</td>
<td>0.0164</td>
<td>Arable land</td>
</tr>
<tr>
<td>Vegetables</td>
<td>18000</td>
<td>33.505</td>
<td>0.0018</td>
<td>Arable land</td>
</tr>
<tr>
<td>Pork</td>
<td>74</td>
<td>15.616</td>
<td>0.2111</td>
<td>Arable land</td>
</tr>
<tr>
<td>Beef and mutton</td>
<td>33</td>
<td>2.802</td>
<td>0.0848</td>
<td>Grassland</td>
</tr>
<tr>
<td>Fresh eggs</td>
<td>400</td>
<td>9.298</td>
<td>0.0233</td>
<td>Arable land</td>
</tr>
<tr>
<td>Birds</td>
<td>764</td>
<td>10.216</td>
<td>0.0135</td>
<td>Grassland</td>
</tr>
<tr>
<td>Aquatic products</td>
<td>29</td>
<td>9.932</td>
<td>0.3426</td>
<td>Territorial waters</td>
</tr>
<tr>
<td>Sugar</td>
<td>4997</td>
<td>1.941</td>
<td>0.0005</td>
<td>Arable land</td>
</tr>
<tr>
<td>Fresh melon</td>
<td>18000</td>
<td>18.528</td>
<td>0.0011</td>
<td>Arable land</td>
</tr>
<tr>
<td>Fresh milk</td>
<td>502</td>
<td>10.323</td>
<td>0.0207</td>
<td>Grassland</td>
</tr>
<tr>
<td>Wood</td>
<td>1.99</td>
<td>0.027</td>
<td>0.0132</td>
<td>Woodland</td>
</tr>
</tbody>
</table>

TABLE 2
Per capita bio-productive land area for energy consumption in characteristic town

<table>
<thead>
<tr>
<th>Energy</th>
<th>Per capita consumption (t)</th>
<th>Conversion factor (GJ/t)</th>
<th>Per capita consumption (GJ/person)</th>
<th>Global average energy footprint (GJ/hm²)</th>
<th>Per capita biological productive land area (hm²)</th>
<th>Bioproductive land type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gasoline</td>
<td>0.0138</td>
<td>43.125</td>
<td>0.592</td>
<td>93</td>
<td>0.0065</td>
<td>Fossil energy land</td>
</tr>
<tr>
<td>Liquefied petroleum gas</td>
<td>0.0225</td>
<td>50.201</td>
<td>1.131</td>
<td>71</td>
<td>0.0158</td>
<td>Fossil energy land</td>
</tr>
<tr>
<td>Natural gas</td>
<td>0.0003</td>
<td>840.001</td>
<td>0.169</td>
<td>93</td>
<td>0.0019</td>
<td>Fossil energy land</td>
</tr>
<tr>
<td>Electric power</td>
<td>280.7001 (kWh)</td>
<td>0.009</td>
<td>2.331</td>
<td>1000</td>
<td>0.0024</td>
<td>Building land</td>
</tr>
</tbody>
</table>

TABLE 3
Per capita ecological footprint

<table>
<thead>
<tr>
<th>Land type</th>
<th>Per capita area (hm²)</th>
<th>Equilibrium factor</th>
<th>Per capita ecological footprint (hm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arable land</td>
<td>0.274</td>
<td>2.8</td>
<td>0.7672</td>
</tr>
<tr>
<td>Woodland</td>
<td>0.014</td>
<td>1.1</td>
<td>0.0154</td>
</tr>
<tr>
<td>Grassland</td>
<td>0.118</td>
<td>0.5</td>
<td>0.0590</td>
</tr>
<tr>
<td>Building land</td>
<td>0.003</td>
<td>2.8</td>
<td>0.0084</td>
</tr>
<tr>
<td>Territorial waters</td>
<td>0.344</td>
<td>0.2</td>
<td>0.0688</td>
</tr>
<tr>
<td>Fossil energy land</td>
<td>0.027</td>
<td>1.1</td>
<td>0.0297</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0.9485</td>
</tr>
</tbody>
</table>

TABLE 4
Ecological carrying capacity of characteristic town

<table>
<thead>
<tr>
<th>Land type</th>
<th>Land area (hm²)</th>
<th>Equilibrium factor</th>
<th>Yield factor</th>
<th>Ecological carrying capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arable land</td>
<td>36.93</td>
<td>2.8</td>
<td>1.86</td>
<td>192.29</td>
</tr>
<tr>
<td>Woodland</td>
<td>672.71</td>
<td>1.1</td>
<td>0.93</td>
<td>688.18</td>
</tr>
<tr>
<td>Grassland</td>
<td>57.19</td>
<td>0.5</td>
<td>0.31</td>
<td>8.87</td>
</tr>
<tr>
<td>Building land</td>
<td>12.15</td>
<td>2.8</td>
<td>1.73</td>
<td>58.82</td>
</tr>
<tr>
<td>Territorial waters</td>
<td>38.56</td>
<td>0.2</td>
<td>1.00</td>
<td>7.72</td>
</tr>
<tr>
<td>Fossil energy land</td>
<td>0</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total ecological carrying capacity</td>
<td>955.88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REC</td>
<td>841.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cultivated land is the same. Therefore, the total ecological footprint is: EF = Nef = 674.72 hm².

Ecological carrying capacity. The calculation results of total ecological carrying capacity (EC) and effective ecological carrying capacity (REC) are shown in Table 4.

Among them, the production factor is obtained by comparing the ecological productivity per unit...
area of the characteristic town with the world average productivity. Subtracting 12% of the area used to protect biodiversity, the available per capita ecological carrying capacity (effective ecological carrying capacity) is obtained.

Comparison of ecological footprint and ecological carrying capacity. The comparison between the effective ecological carrying capacity and the actual production ecological footprint in the characteristic town reflects the ecological profit and loss of the characteristic town. It can be seen from the above calculation results that the ecological footprint of the characteristic towns in the near future is 674.72 hm², the effective ecological carrying capacity is 841.15 hm², and the ecological surplus is 166.43 hm². Therefore, for current and near-term planning, tourism activities will not have a major impact on the ecological environment of the characteristic towns. The ecological footprint of the characteristic town only takes up 80% of the effective ecological carrying capacity, and there is room for further development.

In addition, the per capita ecological footprint of the characteristic towns is 0.941 hm², which is lower than the national per capita ecological footprint (1.6 hm²) in 2012. In 2012, a total of 142,000 tourists were received, and the growth rate was 20% per year. On the basis of the improvement of the living standards of the residents in the characteristic towns, the future will obviously have an adverse impact on the ecological environment of the characteristic towns. Therefore, in terms of ecological carrying capacity, special towns should pay special attention to long-term planning.

CONCLUSION

China is experiencing rapid progress in industrialization and urbanization. Characteristic towns (Tese Xiaozhen) are important drivers for China’s urbanization, industrialization and agricultural modernization in the 21st century, serving as a strategic choice of regional innovation and development. On the one hand, characteristic towns help to gather entrepreneurial innovation elements, optimize supply system, and accelerate economic transformation and upgrading. On the other hand, the coordinated development of large cities and small towns makes the characteristic town an innovative administration reform in China.

The research shows that the recent ecological footprint is 674.72 hm², the effective ecological carrying capacity is 841.15 hm², and the ecological surplus is 166.43 hm². The current and recent tourism will not have a big impact on the ecology, but from the perspective of ecological carrying capacity, attention should be paid to the long-term planning. At the same time, the protection of ecological resources such as soil, vegetation, forests and birds should be strengthened to maintain a good state of the ecological environment.

Promote the sustainable development of ecological environment carrying capacity:
(1) Control the number of visitors to prevent over-saturation of the ecological footprint.
(2) Adjust the energy structure. Reduce the use of energy-based firewood, fully develop and utilize renewable energy, and reduce the ecological footprint of fossil energy.

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BLEEDING STATUS, HANDLING TIME AND INITIAL MORTALITY OF RECREATIONAL FISHERIES IN CHUB (SQUALIUS CEPHALUS) (LINNAEUS 1758)

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ABSTRACT

The aim of this study is to determine the bleeding status, handling time, and initial mortality caused by the anatomical status of fish hooks in a fish’s oral mouth. Observations were conducted during the course of a total of 30 amateur recreational fishing expeditions to catch Squalius cephalus between July, 2013, and December, 2015. Anatomical conditions of the hooks as lodged in a fish’s jaws (lower, upper, external, upper and lower jaws), hook removal times, and overall length of the captured fish were compared on the basis of the type of lure and hook used (i.e., spinner, spoon). Only four (of the 111 fish caught in total) were observed to have died within one hour of capture. All of these deaths occurred in fish caught by spinner. It was observed that the bleeding rates proportionally increased with the increase in the amount of time required to remove the hook. The difference between the anatomical status and the time of removal from the hook was statistically found to be significant (ANOVA, F = 8.842 p <0.01). In the fish that were hooked on both jaws, the time to remove the hook was longer. Therefore, bleeding (and mortality) was more prevalent in these fish. To create more sustainable recreational fisheries, it can be concluded that the awareness of fishermen (and state authorities) should be focused on greater selectivity in the types of hooks that are used; fish hooks that cause less damage to the fish lead to an increase in overall fish welfare, and ultimately facilitate a more sustainable fishing industry.

KEYWORDS:
Catch and Release, Mortality Rate, Squalius cephalus, Spinner, Spoon

INTRODUCTION

The global importance of high intensity commercial fishing is decreasing in favour of fish-stock farming and sport fishing, activities that have become increasingly significant today as a result of responsible stewardship and protection of remaining fish stocks. There are very few studies on the social and economic characteristics of the fisheries that demonstrate this sector as having much great commercial potential in Turkey. In this study, the aim was to evaluate both the spinner and spoon-type fishing lure and hook systems via catch-and-release (C&R) of fish living in the Keban Dam Lake; this dam system is on the Euphrates River, located in the Kemaliye fishing region of Elazığ Province, southeastern Turkey. Both commercial and recreational fisheries are in great demand in the Keban Dam water system of the Firat River watershed [1, 2].

Recreational fishing is an important social and economic activity in the exploitation of fish stocks in many developed countries of the world [3, 4, 5, 6]. Participation rates in recreational fisheries is increasing in proportion to global population growth. It is estimated that 10% ±6.9 of the world’s population is currently engaged in recreational fishing activities. Catch-and-release practices in developed countries in the world have led to significant improvements in fisheries management and sport-related angling [7].

There has been much debate in recreational fisheries management about the practice of releasing captured fish [5, 8, 9, 10]. According to some researchers, the captured fish subsequently died after they were returned to the water [10]. Researchers also reported that there were decreases in the life activities of those fish that initially survived after they were returned to the water [11, 12]. In recreational fishing, it is thus important to develop new fishing equipment and methods that will cause the least amount of permanent damage to the fish; moreover, upon returning captured fish to the water, this new equipment (and methods) must consider the causation of various unwanted side-effects in the fish including physiologic effects, elapsed time before returning to the water, the effect of hook size, bleeding condition, and so forth. Recently, it has been reported that fishing tools used in amateur fishing have been evaluated according to the fish species, type of feed, selectivity and size of hook.

Catch-and-release (C&R) angling is a well-known and popular practice in recreational fishing [13, 14, 15, 16]. Nonetheless, C&R applications require further investigation (in terms of economic, social, and fish welfare issues) due to reports of harm and injury to the fish due to excessive bleeding and
other physiological events. In recreational fishing, it is estimated that more than 60% of fish caught each year are returned to the water whether voluntarily or in compliance with government regulations [17, 18, 19]. Despite significant variability due to limited acute effects (e.g., bleeding status, type of fish hook, living environment), fish released into their natural environment are generally expected to survive [12, 18]. Many of these studies provide important information regarding which elements of angling have the greatest influence on fisheries management and how C&R has particularly contributed to discovering potential solutions and changes to implement best practices in fisheries management (and influence fishermen’s behaviour).

In recent years, C&R policies and practices in recreational fisheries have become an important tool used by management agencies to revive depleted stocks of marine and freshwater fish species by reducing fish mortality rates from hooks [10, 20, 21]. However, the success of this approach is estimated to be the minimum mortality rate caused by the C&R process. It is estimated that more than 60% of the roughly 47 billion fish caught by recreational fishermen are released each year, whether voluntarily or to comply with government regulations [22, 23].

Catch-and-release angling can sometimes be a firm legal requirement in certain regulatory regimes, yet is also often a voluntary practice by many anglers [12, 24]. Regardless of the reason for any given release practice, a great deal of international effort is nonetheless underway to reduce unwanted damaging effects to fish due to C&R (e.g., bleeding, hook release time, hook type, etc.). For more effective fishery management, it is necessary to bring to the forefront certain issues such as fishing gear, bait type, hook type, and other specific fish welfare concerns suitable for the targeted fish species [12, 13]. For example, appropriate fishing gear choices, and bait type (and size), might reduce injury, and enable more selective exploitation of the target species, thereby contributing to more effective fisheries management [25] and improved fish welfare.

Recreational fishing studies in Turkey have, thus far, been generally focused on the socio-economics of fishermen [26, 27, 28, 29] and hook selectivity [30, 31]. However, this study is one of the first research specifically concerning catch-and-release applications in this country’s inland waters.

Squalius cephalus (also called Chub) is categorized as a game fish in FishBase, a global species database. The Squalius genus is a large group represented by 45 species in the Cyprinidae family. Squalius cephalus (Linnaeus 1758) is a species that is widely spread in European fresh waters, the Black Sea, the Caspian Sea, and Anatolia [32]. Different populations of this species have already been studied in terms of growth, nutrition, and quality of food [33, 34]. However, Squalius cephalus has not been studied in terms of catch efficiency, mortality rate, and C&R effects, particularly in recreational fisheries applications. Both commercial and sport-fishing landing size was determined to be 20 cm.

**MATERIALS AND METHODS**

This study was carried out in the Keinan Dam Lake fishing region through a total of 30 fishing operations conducted on weekends between July, 2013, and December, 2015. In total, 111 Squalius cephalus (Chub) were caught and released during the study. For this purpose, four anglers were selected, all with the same level of experience in recreational fishing. All fishing gear that was used were similar in design and structure. Captured fish, still on the hook, were landed and segregated by individual fisherman in their own designated one-by-one meter net cages. After one hour from removal of the fish from the hook, various parameters were recorded, including bleeding status, hooking location, and hook removal times.

Each angler was supplied with considerable training before the fishing activity began, and was provided with both measuring equipment and a diary (in which to record specific details). At the time of fish capture, the following data points were recorded: fish total length (size), the hook type (spinner or spoon), hooking location (upper jaw, lower jaw, both upper and lower jaw), bleeding (none, little, or very), and weight (in grams). If a fish lost equilibrium, it was observed for one hour to determine initial mortality. If fish were still alive after the initial one-hour observation period, they were kept in the water although it is already known that initial hooking mortality rates underestimate total hooking mortality [20, 35]. After landing, the location of the hook penetration (lower, upper, external, upper and lower jaw), and presence (or absence) of bleeding were recorded. Bleeding status was recorded only after the hook was removed. During data sampling, bleeding status was categorized in a range from 0 to 3 (0 = no bleeding, 1 = little bleeding, 2 = very bleeding) [41]. However, unexpectedly, hooking depth was shallow in all of the captured Chub, and thus bleeding was observed to be minor overall (and occurred only at the hook’s wound site). Therefore, for recording purposes, the previously detailed categorization was collapsed into either bleeding absent or bleeding present. No chronic bleeding was observed in our experiment. Additionally, the fish total length was recorded. All measurements were conducted by the same person for all fish that were caught.

One-way ANOVA, and the differences among groups, were statistically analysed using Tukey’s HSD Test. The changes in the fish’s anatomic status, hook removal times, hooking location (lower, upper, external, upper and lower jaw), total length (cm), and hook type (spinner or spoon) were statistically assessed using these methods.
During the study, 111 Chub were caught with both spoon and spinner types of hooks. There were no observed differences across the dataset by type of hook (spinner or spoon) versus total fish body length (cm). Fish ranged between 31.2 cm and 53.6 cm in total length. Average (±SE) total lengths of fish were 40.70 ± 0.796 cm and 38.22±0.587 cm, captured by spoon and by spinner, respectively. Average weights of fish were 786.48±18.62 g and 670.17± 15.71 g, captured by spoon and by spinner, respectively.

The difference between the anatomical status and the hook removal times (s) were statistically significant (ANOVA, F=8.842, p<0.01); that is, versus the maximum time to when the fish hook was removed from the upper and lower jaws.

The difference between total fish length (cm), and capture by spinner or spoon-type hooks, according to the anatomical state of the fish, was not statistically significant (p>0.05) (ANOVA, F=1.537).

Of all 111 Chub caught, only four died within one hour after capture. The mortality rate from the spoon-type hook is 6.89%. No deaths were observed in the fish captured by spinner. Initial mortality from hook-type was not related to either spinner or spoon fishing methods. Mortality rates varied only depending on bleed status, hook removal times, and anatomical status of the fish.
FIGURE 3
The removing time from hook of the captured according to the anatomical status of the fish

FIGURE 4
Change of bleeding status in the captured fish according to status of the hook.

FIGURE 5
Hook-related mortality rates in the captured fish according to bleeding conditions.
TABLE 1

The comparison of spinner and spoon according to the hook type, hook removal times and anatomical status of captured fish

<table>
<thead>
<tr>
<th>Bleeding Status</th>
<th>Hook Types</th>
<th>Hook Removal Times (s)</th>
<th>Anatomic Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spoon</td>
<td>Spinner</td>
<td>0-19</td>
</tr>
<tr>
<td>No</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Little</td>
<td>37</td>
<td>75.5</td>
<td>0</td>
</tr>
<tr>
<td>Very</td>
<td>12</td>
<td>24.5</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>100</td>
<td>12</td>
</tr>
</tbody>
</table>

Despite the fact that bleeding occurred in 36.9% of the captured fish, their mortality rate from the hook was only 6.89%. However, there was no statistically significant difference between bleeding rate and mortality rate. When it took more than 40 seconds to remove the hook, both bleeding rates and mortality rates increased.

Bleeding was not observed in the 75.5% of the fish caught with the spoon and in 50.0% of the fish caught with the spinner. While 6.44% of the fish caught with spinners had high bleeding, there was no similarly high amounts of bleeding observed in the fish caught with the spoon.

Significant differences were found between the hook removal time and the bleeding status. Although the bleeding was higher in the fish that were removed from the hook after more than 40 seconds, there was not a large amount of bleeding in the fish that were removed in under 40 seconds. According to anatomical conditions, the rate of bleeding was higher in fish caught on an external surface and in both jaws. The mortality rates in the fish with no, little, and very bleeding status were 0%, 5.13%, and 50%, respectively.

DISCUSSION AND CONCLUSION

This study is important because it is the first known study conducted on catch-and-release activity in recreational fishing in Turkey. In recent years, recreational fishing has become popular not only in Turkey, but also in most developed countries. Previous studies on fishing in Turkey mostly focused on the socio-economic aspects of fishermen, and the selectivity of fishing equipment [26, 27, 28]. However, formal studies have been limited on specific topics like the increasing prevalence of C&R fishing activity in the waters of the subject country, fish mortality rates due to hooks, bleeding conditions, anatomic impacts, and the time to removal of fish from hooks. Catch-and-release activity, especially in light of increased interest in fish welfare and fishery sustainability, has attracted the notice of both amateur anglers and scientists alike. This increased attention has been extended to the type of hooks used by fishermen, bait applications, water temperature, and fish species. Additionally, the number of fishing professionals and amateur enthusiasts who now choose, on a daily basis, to leave the small fish (and captured fish) in the water is greatly contributing to an ethos of sustainable fishing. However, because of the physiological effects (bleeding, anatomic condition, removal time from the hook, etc.) of C&R on the fish, to improve survival, it is becoming apparent that they be thoroughly checked before being returned to the water. One study on Northern Pike found that only 10 out of 415 fish (2.40%) died from hook-related causes [35]. In this study, the Chub death rate was found to be only 4 of 111 fish (3.60%). Moreover, the mortality rates vary according to the type of hook, fish species and the fishing area.

In various studies, significant differences were found in the initial mortality rates from parameters such as hook types, feed types, water temperature, and size of fish; one study (mentioned earlier) discussed the effects of hooks on C&R mortality in Northern Pike [26]. Some researchers found very low mortality rates of 1.7% [27] and 4.8% respectively [28]. However, other studies reported higher mortality in the ranges of 5.3 to 10.5% [38] and 4.5 to 11.3% [39].

In C&R-type studies, it was found that hook localization and bleeding status, relative to specific fish species, are the most important factors influencing mortality rates [13, 40]. Therefore, it is important to develop fishing tools and methods which cause the least damage to the fish, when they are returned to the water after capture, by taking into consideration the undesirable effects of hook size, bleeding, and so forth [35].

In addition, comparisons were made according to governmental regulation regarding legally allowable fish lengths at time of capture. The minimum legal fishing length for Chub is 20 cm according to Turkish fish-size catch regulations. In the present study, all of the fish caught were above the 20 cm minimum. Accordingly, no further evaluation was made based on the legal fishing length. Our results are thus important only in terms of sustainability of recreational fishing.

There was an observed increase in fish mortality rates with an increase in bleeding at the hook site [35, 37]. In this study, four out of 62 fish captured...
with spinners (6.45%) exhibited high amounts of bleeding. They were kept in water for one hour and then died. However, there was no bleeding in the fish captured with the spoon-type lure, and their hook-related mortality rates were, consequently, zero.

REFERENCES


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MECHANISM STUDY OF SULFUR REMOVAL AGENT FOR DRILLING FLUID

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2Zhong yuan Petroleum Engineering Company Drilling Company III, Lankao 473000, China
3Sinopec Hua dong Petroleum Engineering Company Jiangsu Drilling Company, Nanjing 210000, China

ABSTRACT

When drilling in sour gas reservoirs, chemical desulfurizer is added to drilling fluids to react with hydrogen sulfide to reduce the harmful concentration of hydrogen sulfide at home and abroad, however, the research on the conditions and effect of sulfur removal reagents in drilling fluid is not thorough enough. In this paper, the physical and chemical properties of two kinds of new type of sulfur removal material and common sulfur removal material are studied, and the effect of sulfur removal agent on the performance of drilling fluid is evaluated by experimenting. The effect of sulfur removal agent on desulfurization is also evaluated by conducting experiments at China Environmental Protection Product Quality Supervision and Inspection Center. Experiments have shown that the optimum drilling fluid pH values of sponge iron, basic zinc carbonate, composite sulfur removal agent and ferrous urate saccharate are 9, 11, 10 and 12 respectively. Sponge iron has the worst effect of removing hydrogen sulfide, while the compound sulfur removal agent and ferrous gluconate have a good effect. In the Zhongu well 6, the compound sulfur removal agent was used for on-site test, and the sulfur removal effect was obvious, which was consistent with the conclusion drawn in the laboratory.

KEYWORDS:
Drilling fluid, hydrogen sulfide, sulfur removal agent, action mechanism

INTRODUCTION

When drilling in sulfur-bearing formations, the sulfide immersed in wellbore is mainly removed by sulfur removal agent, which can reduce the harmful concentration of hydrogen sulfide and slow down the corrosion of dissolved hydrogen sulfide to downhole drilling tools [1-3]. Sponge iron and basic zinc carbonate are often used to remove hydrogen sulfide gas immersed in wellbore during drilling, but their application conditions are limited to a certain extent. In this paper, besides studying the above two substances, physical and chemical properties of compound desulfurizer, ferrous gluconate and other desulfurizers are also studied, and laboratory experiments are carried out. The experiment evaluates the influence on drilling fluid performance, judges the effect of removing hydrogen sulfide gas, and determines the optimum pH environment. The conclusion provides a theoretical basis and technical reference for the exploitation of high sulfur oil and gas reservoirs.

EXPERIMENTS

Sulfur removal agent. Sponge iron. Sponge iron is a kind of synthetic iron oxide with high porosity and nonmagnetic properties, which is spongy porous. It is obtained by chemical reaction of high active pig iron powder through special process. The surface area of the reaction is large, with the active surface about 10m²/g, the density being 415-416 g/cm³ in dry state, and the particle size is 1.5-50 micron, almost 90% of the reaction in 2-20 microns [4, 5]. Sponge iron is a solid material, and its interaction with sulfide depends on the effective surface area. The reaction needs to penetrate into the internal solid. Slow diffusion of sulfur ions will limit the overall reaction speed and degree [6-9].

Basic zinc carbonate. The molecular formula of basic zinc carbonate is ZnCO₃·2Zn(OH)₂·H₂O, which is formed by the reaction of alkali metal carbonate and zinc carbonate. It is white powder, tasteless, odorless, slightly soluble in water, and its density is about 4.4 g/cm³. After dissolution, the dissociated Zn²⁺ rapidly reacts with S²⁻ to precipitate.

Compound sulfur removal agent. Compound sulfur removal agent is prepared by double decomposition of zinc powder, zinc-based compounds and polymer materials. It is characterized by high purity, optimized monomer ratio, high molecular weight and high zinc content of effective agent. It mainly contains calcite, kaolinite and zinc sulfide, and it contains a small amount of quartz and illite. The saturated Na₂S solution was used to react with inorganic compound sulfur removal agent, and the
experimental environment was alkaline. Diffraction results show that the material mainly contains calcite, calcium hydroxide, quartz, smithsonite, kaolinite and sodium montmorillonite. Its main reaction formula with sulfide is \( \text{CaCO}_3 + \text{H}_2\text{S} \rightarrow \text{CaS} + \text{H}_2\text{O} + \text{CO}_2 \) or \( \text{ZnCO}_3 + \text{H}_2\text{S} \rightarrow \text{ZnS} + \text{H}_2\text{O} + \text{CO}_2 \).

**Ferrous gluconate.** Ferrous gluconate is gray or yellowish green powder or granule after drying, slightly caramel odor, and of high solubility. 1 g sample can be dissolved in about 10mL water under slightly heated conditions, almost insoluble in alcohol [10, 11]. The saturated \( \text{Na}_2\text{S} \) solution was used to react with inorganic compound sulfur removal agent, and the experimental environment was alkaline [12, 13]. As shown in Figure 2.

When ferrous gluconate is added into the aqueous solution, it dissolves immediately, and the solution is light green. When a small amount of \( \text{Na}_2\text{S} \) solution is dripped into the solution, the dissolved iron ions react with sulfur ions rapidly and form black precipitation immediately, which is faster than sponge iron and hydrogen sulphide [11-15]. The color of the solution turns black instantly. The main reaction patterns of sulfide and sulfide are as follows: \( \text{Fe}^{2+} + \text{S}^{2-} \rightarrow \text{FeS} \downarrow \).

In this performance test, various sulfur removal agents were added by 1%. Drilling fluid formula is below: 1% bentonite + 0.1% caustic soda + 0.3% CX-215 + 0.3% DRISPAC + 1.2% JMP + 1.0% PFPRD + 1.75% YX-1 + 1.25% YX-2 + limestone powder.
TABLE 1
Effect of sulphur removal agent on drilling fluid performance indicators
(sulfur removal agent added after aging)

<table>
<thead>
<tr>
<th></th>
<th>Φ_{600}</th>
<th>Φ_{300}</th>
<th>Φ_{100}</th>
<th>Φ_{3}</th>
<th>AV/</th>
<th>PV/</th>
<th>YP/</th>
<th>γ</th>
<th>pH</th>
<th>API</th>
<th>FL/</th>
<th>HTH</th>
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</thead>
<tbody>
<tr>
<td>Base bulp</td>
<td>192</td>
<td>144</td>
<td>22</td>
<td>26</td>
<td>96</td>
<td>48</td>
<td>491</td>
<td>1.042</td>
<td>11</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After aging</td>
<td>65</td>
<td>47</td>
<td>1</td>
<td>3</td>
<td>32.5</td>
<td>18</td>
<td>148</td>
<td>1.04</td>
<td>9</td>
<td>6.6</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Adding sponge iron</td>
<td>86</td>
<td>52</td>
<td>1</td>
<td>2</td>
<td>43</td>
<td>34</td>
<td>92</td>
<td>1.05</td>
<td>9</td>
<td>6.8</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>Adding Basic zinc carbonate</td>
<td>84</td>
<td>56</td>
<td>2</td>
<td>3</td>
<td>42</td>
<td>28</td>
<td>143</td>
<td>1.05</td>
<td>9</td>
<td>6.5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Adding Compound sulfur removal agent</td>
<td>76</td>
<td>47</td>
<td>1</td>
<td>3</td>
<td>38</td>
<td>29</td>
<td>92</td>
<td>1.044</td>
<td>10</td>
<td>6.5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Adding Ferrous gluconate</td>
<td>40</td>
<td>22</td>
<td>1</td>
<td>3</td>
<td>20</td>
<td>18</td>
<td>21</td>
<td>1.042</td>
<td>7</td>
<td>7</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

Notes: pH=11; Aging temperature: 140°C

The experiment of removing hydrogen sulfide by sulfur remover was carried out at China Environ-
mental Protection Products Quality Supervision and Inspection Center.

Experimental drugs. Sponge iron, basic zinc carbonate, compound sulfur removal agent-A, com-
 pound sulfur removal agent-B and organic sulfur removal agent

FIGURE 3
Physical drawing of sulfur removal tester at room temperature and atmospheric pressure

Experimental apparatus. Experimental methods. The test environment is at room tempera-
ture and standard atmospheric pressure (100.6 KPa). The inlet concentrations (standard gas concentration, 10^{-6} L/L) are 998 ppm and 1998 ppm, flow rate being 120 L/H. The outlet hydrogen sulfide concentration is detected by adding a certain amount of sulfur removal agent reaction. In this experiment, the desul-
furization effect of 0.5% & 1.0% as well as 2 concentra-
tions of sulfur remover were detected accord-
ing to the actual conditions, to screen and evaluate several kinds of sulfur remover reagents suitable for field use.

The sulfur removal agents experimented for contrast studies include sponge iron, basic zinc carbonate, compound sulfur removal agent and fer-
rous gluconate, among which ferrous gluconate is a new product. The sulfur removal agent sample was added to the low solid drilling fluid to form a propor-
tionally dehydrosulfide drilling fluid system.

The drilling fluid formula is below: 1% bentonite + 0.1% caustic soda + 0.3% CX-215 + 0.3%
DRISPAC + 1.2% JMP + 1.0% PF-PRD + 1.75%
YX-1 + 1.25% YX-2 + limestone powder

Experimental procedures.
1) Connect the glassware and apparatus as shown in Figure 4 and place them in the ventilation cabinet to maximize the air volume

2) The hydrogen sulfide standard gas cylinder is placed outside the ventilation cabinet and connected to the test instrument

3) Remove the glass sieve plate from the ab-
sorption bottle and pour the 50 mL sample into the absorption bottle [6-7].

4) Insert the glass tube with glass sieve plate and another glass tube into the rubber plug, then in-
sert it into the absorption bottle, and close the rubber bottle plug so that the sieve plate is 3 mm from the bottom of the absorption bottle. Open the H_{2}S standard gas cylinder, adjust the pressure relief valve, and adjust the flow rate of H_{2}S gas to 120 L/H. Start the stopwatch at the same time [4]

5) The concentration of H_{2}S gas, i.e. C2 released from the outlet of the bottle with glass sieve plate was measured by PGM250Q detector every mi-
nute, until C2 reached or approached the upper limit of the measurement range of the PGM250Q detector.

6) Calculate the removal efficiency of H_{2}S gas by the following formula:

\[ G = \frac{(C1-C2)}{C1} \times 100\% \]

In the formula, G is the removal efficiency of hydrogen sulfide gas as sample, % (V / V). C1 and C2 are the concentration of hydrogen sulfide gas of inlet and outlet, respectively, 1 \times 10^{-6} L/L.
RESULTS AND DISCUSSION

1) In order to determine the optimal desulfurization pH environment of various sulfur removal agents, the experiments were carried out under the conditions of the same concentration of inlet H₂S, the same concentration of sulfur remover and different pH values of drilling fluid. The test data of sulfur removing is shown in tables 3 ~ 14, respectively. The data in the table reflect the relationship between inlet concentration, outlet concentration and time.

(1) When inlet concentration of H₂S is 1998 ppm in the drilling fluid, sponge iron added by 1%, the outlet concentration is tested under the conditions of pH being 7, 9, 11, and the results are drawn in the same coordinate system as shown in Figure 4.

It can be seen from the diagram that when the pH value of drilling fluid is 7 and 11, the desulfurization effect of sponge iron is not as good as that of pH value of 9. Because when the pH of drilling fluid is high, the flocculating solubility of hydroxide Fe(OH)₃ of the generated trivalent iron is relatively small, even in the environment of high pH value drilling fluid. Some iron groups react with hydroxyl radical to form more stable hydroxide flocculating precipitates, which leads to insufficient iron base substance to react with hydrogen sulfide, so the sulfur removal effect becomes worse.

(2) When inlet concentration of H₂S is 1998ppm in the drilling fluid, basic zinc carbonate added by 0.5%, the outlet concentration is tested under the conditions of pH being 8, 10, 11, and the results are drawn in the same coordinate system as shown in Figure 5.

It can be seen from the diagram that the outlet concentration of hydrogen sulfide reached 169 ppm in the first minute, when pH of drilling fluid is 8 that the desulfurization effect of basic zinc carbonate is very poor under this condition. After 5 min of hydrogen sulfide injection, the desulfurization of drilling fluid of pH being 11 is better than that of pH being 10. At the 6th minute, the outlet concentration of hydrogen sulfide in drilling fluid of pH being 11 is 23ppm, and that of H₂S in drilling fluid of pH being 10 is 100 ppm. Therefore, the basic zinc carbonate has the best desulfurization effect when the pH value of drilling fluid is 11.

(3) Here are the test results of the compound sulfur removal agents under the same inlet concentration of H₂S, the same concentration of sulfur removal agents and different pH values of drilling fluid. When inlet concentration of H₂S is 1998 ppm in the drilling fluid, compound removal agent added by 1%, the outlet concentration is tested under the conditions of pH being 8, 10, 11, and the results are drawn in the same coordinate system as shown in Figure 6.

It can be seen from the diagram that when the pH value of drilling fluid is 8, the desulphurization effect is very poor, and the outlet concentration of hydrogen sulfide reaches 136ppm in the 3rd minute, and the slope of the experimental curve with pH value of 8 is very large. With the extension of time, the removal efficiency of hydrogen sulfide became worse and worse. When the drilling fluid pH being 11, the concentration of hydrogen sulfide outlet reached 61 ppm at the time of experiment to the 10th minutes, and the slope of the experimental curve was increasing, indicating that the sulfur removal agent was gradually exhausted. However, in the case of drilling fluid pH being 10, the removal of hydrogen sulfide in the first 10 min is very good, and the outlet concentration of hydrogen sulfide is within 12 ppm.
Therefore, for the development of oil and gas fields with high hydrogen sulfide content, in order to remove most of the hydrogen sulfide flowing into the drilling fluid in a short time, the pH value of the drilling fluid should be controlled at 10.

(4) The test results of ferrous gluconate under the same inlet concentration of H$_2$S, the same concentration of desulphurizer and different pH values of drilling fluid are below.

In Table 2-4, with the same hydrogen sulfide inlet concentration and the same amount of hydrogen sulfide (1%), but under different pH conditions, the sulfur removal data of ferrous gluconate are drawn in the same coordinate system, as shown in Figure 7.

### TABLE 2
1% ferrous gluconate H$_2$S inlet concentration 998 ppm pH=9 120L/H

<table>
<thead>
<tr>
<th>test time(min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td>outlet concen.</td>
<td>79</td>
<td>110</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10^-6/L/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3
1% ferrous gluconate H$_2$S inlet concentration 998 ppm pH=11 120L/H

<table>
<thead>
<tr>
<th>test time(min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>outlet concen.</td>
<td>1</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>16</td>
<td>19</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>(10^-6/L/L)</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>test time(min)</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
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<tbody>
<tr>
<td>outlet concen.</td>
<td>36</td>
<td>45</td>
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<td>61</td>
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<td>66</td>
<td>79</td>
<td>96</td>
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### TABLE 4
1% ferrous gluconate H$_2$S inlet concentration 998 ppm pH=12 120L/H

<table>
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<tr>
<th>test time(min)</th>
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<td>0</td>
<td>0</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>(10^-6/L/L)</td>
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<td>4</td>
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<td>15</td>
<td>16</td>
<td>18</td>
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<tr>
<td>(10^-6/L/L)</td>
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<th>35</th>
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<th>37</th>
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<tr>
<td>outlet concen.</td>
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<td>23</td>
<td>27</td>
<td>29</td>
<td>31</td>
<td>34</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>(10^-6/L/L)</td>
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<th>46</th>
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<th>48</th>
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<tbody>
<tr>
<td>outlet concen.</td>
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<td>47</td>
<td>52</td>
<td>57</td>
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<td>68</td>
<td>75</td>
<td>83</td>
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<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>outlet concen.</td>
<td>91</td>
<td>100</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
It can be seen from the diagram that when pH is 9, the outlet concentration of hydrogen sulfide reaches 79 ppm at the first minute, and the effect of sulfur removal is better when the pH value of the drilling fluid is 11, but after 15 min, the desulfurization efficiency becomes worse obviously. Only when the pH value of the drilling fluid is 12, the effect is obvious, and the maximum absorption efficiency is achieved in the first 25 min, the outlet concentration of hydrogen sulfide being 0 ppm. The outlet concentration is only 12 ppm at the 40th minute, and even the outlet concentration of hydrogen sulfide is only 85 ppm after 1 h. Therefore, the optimal pH value of ferrous gluconate is 12.

2) In order to compare the desulphurization effect of various kinds of sulfur removal agents, the experiment was conducted on the conditions that all the sulfur removal agents’ pH value are the optimal and density are the same, and H₂S inlet concentration are the same.

The test data of sulfur removal are shown in Table 5 - 8, respectively. The data in the table reflect the relationship between inlet concentration, outlet concentration and time.

### TABLE 5

1% ferrous gluconate H₂S inlet concentration 1998 ppm pH=12 120L/H

<table>
<thead>
<tr>
<th>test time(min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
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<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>test time(min)</td>
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<td>18</td>
<td>19</td>
<td>20</td>
<td>21</td>
<td>22</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
<td>19</td>
<td>21</td>
<td>23</td>
<td>26</td>
<td>31</td>
<td>35</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>test time(min)</td>
<td>25</td>
<td>26</td>
<td>27</td>
<td>28</td>
<td>29</td>
<td>30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
<td>51</td>
<td>59</td>
<td>68</td>
<td>77</td>
<td>88</td>
<td>98</td>
<td>—</td>
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</tr>
</tbody>
</table>

### TABLE 6

1% sponge iron H₂S inlet concentration 1998 ppm pH=9 120L/H

<table>
<thead>
<tr>
<th>test time(min)</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
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<td>1</td>
<td>3</td>
<td>7</td>
<td>12</td>
<td>18</td>
<td>26</td>
<td>36</td>
</tr>
<tr>
<td>test time(min)</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
<td>47</td>
<td>61</td>
<td>76</td>
<td>94</td>
<td>115</td>
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</table>

### TABLE 7

1% basic zinc carbonate H₂S inlet concentration 1998 ppm pH=11 120L/H

<table>
<thead>
<tr>
<th>test time(min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
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<td>8</td>
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<td>test time(min)</td>
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<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
<td>26</td>
<td>34</td>
<td>42</td>
<td>53</td>
<td>68</td>
<td>85</td>
<td>105</td>
<td>—</td>
</tr>
</tbody>
</table>

### TABLE 8

1% compound desulfurizer H₂S inlet concentration 1998 ppm pH=10 120L/H

<table>
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<tr>
<th>test time(min)</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>test time(min)</td>
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<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>outlet concentration (10⁻⁶L/L)</td>
<td>12</td>
<td>11</td>
<td>23</td>
<td>28</td>
<td>36</td>
<td>46</td>
<td>96</td>
<td>—</td>
</tr>
</tbody>
</table>
A comparison of desulphurization effects under optimal pH values of various sulfur removal agents

The data in Table 5-8 are drawn in the same coordinate graph, and the effects of various sulfur removal agents are compared and observed as shown in Figure 8.

The results showed that sponge iron had the worst desulphurization effect, while ferrous gluconate had the best sulfur removal effect, and compound sulfur removal agent was the second. Sponge iron (Fe(OH)₃) has the worst desulphurization effect and its main principles are as follows: the effect of sponge iron (Fe₂O₃) on sulfur removal was the biggest difference from that of organic desulphurizer. Because spongy iron is a solid, and the potential for the reaction is largely dependent on the effective surface area. Any reaction requires a reaction between the permeable surface and the internal solid, so, the slow diffusion of sulfur ions to Fe₂O₃ will limit the overall reaction speed and degree. Usually, about 30% of iron reacts, and the best reaction efficiency is when the pH value is no less than 9, on the contrary the high pH value has a negative effect on it. However, when the pH value is less than or equal to 7, the trivalent iron precipitates to form a flocculent metal hydroxide colloid, and the solubility product of Fe(OH)₃ is very small and harder to dissolve, which makes it easier for some trivalent iron ions to form a ferric hydroxide colloid. The effect of sulfur removal is very poor because of its influence on the combination of sulfur ions. However, organic sulfur removers are easily dissolved in drilling fluids, mainly in the form of divalent iron ions. At the beginning, the following reactions can be observed in sulfur-containing drilling fluids as below:

\[
\begin{align*}
\text{Fe}^{2+} + 2\text{OH}^- & \rightarrow \text{Fe(OH)}_2 \\
\text{Fe}^{3+} + \text{S}^2- & \rightarrow \text{FeS} \\
\text{Fe(OH)}_2 + \text{H}_2\text{S} & \rightarrow \text{FeS} + 2\text{H}_2\text{O}
\end{align*}
\]

The solubility product constant of Fe(OH)₂ is \(8 \times 10^{-16}\), bigger than that of FeS \(6 \times 10^{-18}\). FeS is more stable and much more difficult to dissolve [14-16]. More Fe²⁺ reacts with S²⁻ to form FeS precipitation, achieving better sulfur removal effect. So the sulfur removal effect of ferrous gluconate is better than that of sponge iron.

The compound sulfur removal agents contains sodium montmorillonite, which expands in water and produces ion exchange. Metal ions in solution such as Zn²⁺, Mg²⁺, Fe²⁺ replace Al³⁺ in octahedral wafers, and Al³⁺ replaces Si⁴⁺ in tetrahedron wafers. As a result, Zn²⁺, Mg²⁺, Fe²⁺ plasma can not be exposed to sulfur ions in the first time, which affects the sulfur removal effect [14-16].

Basic zinc carbonate is a single substance, which contains only zinc reacting with sulfur ion to form precipitate, so its sulfur removal effect is not as good as that of complex desulfurization agent containing many metal ions.

Field test study on sulfur removal agent. Field Test of Tazhong Oilfield in Tarim. The content of H₂S in Tazhong area is high, mainly in Ordovician formation. During drilling, the highest content of hydrogen sulfide is 27000 ppm, and the compound desulfurization agent is selected to carry out field test when drilling for sulfur-bearing layer of Zhonggu well 6. The formula of drilling fluid for Zhonggu well 6 is as follows: 1% bentonite + 0.1% caustic soda + 0.3% CX-215 + 1%CX-508 + 1% CMF +1%JMP-1 + 2% PEG + 2% TYRF-1 + 1.0% PF-PRD + 3% KCL + 1% YX-1 + 1% YX-2 + lime-stone powder.
TABLE 9
Performance table of drilling fluid plus 0.8% compound desulphurizer

<table>
<thead>
<tr>
<th>density (g/cm³)</th>
<th>Viscosity s</th>
<th>AVmpa·s</th>
<th>YP pa</th>
<th>Gel pa</th>
<th>API FL/K ml</th>
<th>HTHP FL/K ml</th>
<th>KF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.14</td>
<td>50</td>
<td>8</td>
<td>5.5</td>
<td>2.0/6.0</td>
<td>4.8/0.2</td>
<td>9.6/1</td>
<td></td>
</tr>
<tr>
<td>Sand Content (%)</td>
<td></td>
<td></td>
<td></td>
<td>Solid content (%)</td>
<td>Oil content (%)</td>
<td>Water content (%)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>11</td>
<td>2468</td>
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<td>12</td>
<td>0.5</td>
<td>87.5</td>
<td>13.44</td>
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</tbody>
</table>

TABLE 10
Performance table of drilling fluid with 0.8% compound desulphurizing agent in overflow well

<table>
<thead>
<tr>
<th>density (g/cm³)</th>
<th>Viscosity s</th>
<th>AVmpa·s</th>
<th>YP pa</th>
<th>Gel pa</th>
<th>API FL/K ml</th>
<th>HTHP FL/K ml</th>
<th>KF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>50</td>
<td>9</td>
<td>4.5</td>
<td>1.5/6</td>
<td>4.8/0.3</td>
<td>9.6/1</td>
<td></td>
</tr>
<tr>
<td>Sand content (%)</td>
<td></td>
<td></td>
<td></td>
<td>Solid content (%)</td>
<td>Oil content (%)</td>
<td>Water content (%)</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>11</td>
<td>2171</td>
<td>80</td>
<td>16</td>
<td>0.5</td>
<td>83.5</td>
<td>13.3</td>
</tr>
</tbody>
</table>

According to the monitoring concentration of H₂S, 0.5 ~ 2% compound sulfur remover was added to drilling fluid in Zhonggu well 6. The designed quantity of four-cut drilling fluid is 250 m³, and the density of water-based drilling fluid was raised to 1.14 g/cm³, with 2 tons of compound desulphurizer added accounting for 0.8% of the amount of drilling fluid. After preparation, the performance of drilling fluid reached the designed requirements of four-hole drilling fluid. Downhole condition is normal during drilling.

When drilling to the depth of 6172.73 m of the Ordovician, the speed is quickened, and the overflow occurs during circulation, being 0.3 m³. Immediately the well was shut off. The casing pressure is closed from 0 MPa to 4.5 MPa, the vertical pressure being 0 MPa. There is a float valve inside the drilling tool. The surface slurry density is raised from 1.14 g/cm³ to 1.18 g/cm³. Throttling well killing rate is 8L / S, riser pressure increased from 10.5 MPa to 15 MPa, casing pressure raised from 4.5 MPa to 6MPa then to 0MPa, and the density of returning drilling fluid is raised to 1.21 g/cm³ (adding 1.1 tons of sulfur remover to the extraction density). Then the 1.18 g/cm³-1.21 g/cm³ drilling fluid was pumped into by 105 m³. The separator outlet was ignited and the flame was 2.5 meters high. Half an hour later, it was found that the highest concentration of H₂S was 800 ppm at the well head, and the H₂S contaminated drilling fluid was gray in color, smelling of rotten eggs and strong in taste. 2 hours later, the H₂S concentration in the wellhead was 0 ppm, and the fire-works were self-extinguishing.

Finally, the drilling was wound, 3³/₂ drill pipe and drill collar without damage, which shows that the compound sulfur removal agent has achieved the expected sulfur removal effect, which is in agreement with the results of laboratory test.

CONCLUSION

The compound sulfur remover and ferrous gluconate have a good effect on sulfur removal, while sponge iron has the worst effect on hydrogen sulfide removal. Evaluated from the experiment, it is concluded that the optimum pH values of sponge iron, basic zinc carbonate, compound sulfur removal agent and ferrous gluconate were 9, 11, 10 and 12 respectively.

ACKNOWLEDGEMENTS

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REFERENCES


EFFECT OF HARVEST STAGE AND ENSILING PERIOD ON SILAGE QUALITY OF GRASS PEA (*LATHYRUS SATIVUS* L.)

Erdem Gulmuser*  
Bilecik Şeyh Edebali University, Faculty of Agriculture and Natural Science, Department of Field Crops, Bilecik, Turkey

**ABSTRACT**

The aim of current study was to determine the effect of different harvest stages and ensiling periods on silage quality of grass pea (*Lathyrus sativus* L.) grown in the experimental field of Agricultural Application and Research Centre of Bozok University during 2016-2017 vegetation periods. The plants were harvested at the flowering and seed filling stage, chopped and ensiled in 2 kg special jars. Then, they were stored at 25±2 °C under laboratory condition for 15, 30 and 45 days. Physical properties such as colour, structure, odour are observed, and chemical contents are determined as follows: dry matter ratio, crude protein ratio, crude ash ratio, lactic acid, acetic acid, butyric acid, phosphorus (P), calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), sulphur (S), boron (B), chromium (Cr), cobalt (Co), molybdenum (Mo) and ODAP. All silage samples determined in the study were in quality class of silage. The highest lactic acid was determined as 4.05%, while the lowest was 0.85%. Acetic and butyric acid ranged between 0.93-1.82% and 0.10-0.40%, respectively. The highest ODAP content was determined as 2.48 mg/g, while the lowest was 0.66 mg/g. Generally, mineral nutrients were high and within the livestock requirement limits.

As a result, harvesting at the flowering stage and ensiling for 45 days was found out to be the best treatment for the successful grass pea silage.

**KEYWORDS:**  
Grass pea, silage quality, harvest time, ensiling period.

**INTRODUCTION**

The good dry matter ratio (35%) and availability of fermentable substrates and energy density in plants represents quality silage [1, 2]. When the plant matures, the fermentation activity of bacteria decreases depending on the water-soluble carbohydrates [3]. Harvest stage damages the energy density and affects the optimum moisture level required for good silage preservation [2]. Therefore, the successful silage and high nutrient content depend on the maturity stages of the plants.

Silage fermentation is mostly completed within 30-45 days regarding the harvest stage and climatic condition [4]. Kung and DerBedrosian [5] reported that 3-4 week is enough for silage fermentation, while Ward and Ondarza [6] indicated that it requires minimum 4 months. On the other hand, Jaster [7] stated that lactic acid bacteria fermentation can be completed in 3 weeks.

Scientists who study on global climate change scenario claim that impact of the drought and high summer temperatures will increasingly be felt more in the future, and they claim that agriculture will be affected from this change [8, 9, and 10]. Therefore, the importance of plant cultivars which are resistant to extreme ecological conditions (i.e. drought or flood) increase. For instance, Grass pea (*Lathyrus sativus* L) which belongs to the Fabaceae is extremely tolerant to the high temperature, drought and marginal areas with an acceptable yield even under adverse climatic and soil conditions [11, 12, 13]. Grass pea improves soil as it provides organic nitrogen and this improves the efficiency and quality of the subsequent product. Researchers indicated that grass pea has the potential to fix 108-125 kg N ha⁻¹. Besides, grass pea has a natural insecticide characteristic which makes it nature-friendly [14].

This plant is also an important source of mineral contents which is used as a forage crop for livestock in Mediterranean type environments [15, 16, and 17]. Basaran et al. [18] reported that there is no significant difference in the yield and quality of grass pea compared with other legumes. However, it should be noted that over-consumption of grass pea for long-term can result motor neuron disorders called lathyrism disease. This disease causes spastic paralysis and weakness of the legs in animals. Lathyrism is caused by the ODAP content of grass pea and is recommended it should be less than 2.0 mg / g to be consumed safely by livestock [19, 20].

The grass pea is cultivated for different purposes such as a second and low-input crop, human consumption and forage crop. The literature shows that the plant as a forage crop is used both as roughage and grazing. However, studies on the silage quality of grass pea is limited. For this reason, the current experiment aimed to investigate the changes of the silage quality regarding the harvesting time and ensiling period of grass pea silage.
In this study, the effect of different harvest (flowering and seed filling) and ensiling period (15, 30 and 45 days) on silage quality of grass pea (Lathyrus sativus L.) was determined in the Research Field of the Faculty of Agriculture at Yozgat Bozok University located in Yozgat-Turkey. The experiment was carried out in 2016-2017 vegetation period. Plant material consisted of a Gurbuz-2001 variety of grass pea. Plant materials harvested at flowering and seed filling stages were ensiled in 2 kg special jars and stored at 25±2 °C under laboratory conditions for 15, 30 and 40 days, separately. After 15, 30 and 45 days of ensiling period, the jars were opened and silage samples were taken for physical analysis. Silage samples were analysed physically (colour, smell and structure) as described by Yalcinkaya et al. [21]. Accordingly, the 1-14 scale was used to identify the colour, the 1-2 scale was used to identify the smell, the 1-4 scale was used to identify the structure. Flieg score (Flieg Score = 220 + (2 x Dry Matter% - 15) - 40 x pH) was calculated by a pH and dry matter ratio [22]. The Flieg score between 81-100 was considered as very good, between 61-80 was considered as good, between 41-60 was considered as medium, between 21-40 was considered as poor, and between 0-20 was considered as poor silage quality. Besides, the quality and average nutrient loss values were given in Table 2. Total point of silage samples was determined and it was evaluated according to the 0-20 scale.

Mineral element analyses. The determination of phosphorus (P), calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), sulphur (S), boron (B), chromium (Cr), cobalt (Co) and molybdenum (Mo) in grass pea silage were performed by inductively coupled plasma mass spectrometry (ICP-MS) using a Thermo Scientific- iCAPQc (Bremen, Germany).

Dry matter ratio. The fresh weights of three plant samples taken from each jars were determined and they were dried in a hot-air oven at 105 °C for 72 hours; consequently, dry matter ratio (%) was calculated.

Crude protein and crude ash ratio. Silage samples were dried at 65 °C until constant. Then, samples were ground through hammer mill particle size for about 0.5 to 1 ml. Nitrogen (N) contents of samples were determined using Kjeldahl apparatus (FOSS 984.13) and then, CP content was calculated by multiplying the N concentration by a factor of 6.25. Crude ash ratio was also determined by burning the silage samples at 550°C for 6 h [23].

ODAP content analyse. Quantitative value of ODAP was estimated by the modified method of Rao [24]. Regent; O-phthalaldehyde (OPT) reagent was prepared by mixing OPT ethanol (95%) with borate buffer (152.7 g potassium tetraborate and 500 ml distilled water). Diaminopropionic acid (DAP) was used as a standard. All the chemicals are analytical regent. 20 mg finely powdered silage sample was taken to a test tube and 2 ml of distilled water was mixed. The tubes were kept in boiling water and cooled to room temperature and then centrifuged. Supernatant + 0.2 ml 3 N KOH were placed in the tube and kept in boiling water for 30 minutes. After hydrolysis, 0.7 ml of water and 2 ml OPT regent was added to the tube. Finally, the yellow colour was measured by a spectrophotometer (Perkinelmer Lambda 25) at 425 nm.
TABLE 3
Physical traits and silage quality of grass pea

<table>
<thead>
<tr>
<th>Traits</th>
<th>Flowering time</th>
<th>Seed filling time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-day</td>
<td>30-day</td>
</tr>
<tr>
<td>Smile (0-14)</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Structure (0-4)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Colour (0-2)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total point</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Quality class</td>
<td>VG</td>
<td>VG</td>
</tr>
<tr>
<td>Average nutrient loss (%)</td>
<td>10-15</td>
<td>10-15</td>
</tr>
</tbody>
</table>

G: Good; VG: Very good.

TABLE 4
Fleig score and silage quality of grass pea

<table>
<thead>
<tr>
<th>Traits</th>
<th>Flowering time</th>
<th>Seed filling time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-day</td>
<td>30-day</td>
</tr>
<tr>
<td>pH</td>
<td>4.90</td>
<td>4.93</td>
</tr>
<tr>
<td>Dry matter (%)**</td>
<td>29.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fleig score**</td>
<td>68.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.80&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quality class</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

**: p< 0.01, There is no difference between the same letters in each column (p<0.05); G: Good; VG: Very good.

TABLE 5
Lactic, acetic, butyric, crude protein and crude ash ratio of grass pea silage

<table>
<thead>
<tr>
<th>Traits</th>
<th>Flowering time</th>
<th>Seed filling time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-day</td>
<td>30-day</td>
</tr>
<tr>
<td>Lactic acid (%)**</td>
<td>2.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetic acid (%)**</td>
<td>1.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.63&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butyric acid (%)**</td>
<td>0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude protein ratio**</td>
<td>24.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude ash ratio (%)**</td>
<td>9.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**: p< 0.01, There is no difference between the same letters in each column (p<0.05)

Statistical analyses. All data were statistically analysed by repeated measure analysis in SPSS version 18.0 and means were separated by Duncan’s Multiple Range test.

RESULT AND DISCUSSION

Physical traits and Fleig score of grass pea silage were given in Table 3 and 4. The silage total points were ranged 16-20. As a result, all silage samples were very good and good quality class. This shows that silages were obtained correctly and were air proofed. While 45FE silage sample average nutrient loss ranged 15-20%, other silage samples were ranged 10-15% (Table 3).

As seen in Table 4, the dry matter ratio and Fleig score were significantly affected (p<0.01) by harvest time and ensiling period, while pH was insignificant. Kung and Shaver [25] aimed that pH values of quality silage should range between 3.7 and 4.2. However, in the current study, pH values (4.90 – 4.94) were higher than the values suggested by Kung and Shaver [25]. This may be due to the silage samples used in the current study were legumes. Because, legumes have high protein and pH values. The highest dry matter ratio was determined 39.00% in 30SE, while the lowest was 27.50% in 30FE and 45FE. According to Panyasak and Tumwasorn [26], good quality silage should contain 60-75% moisture. If the silage contains more than 75% moisture, its palatability lowers [27, 28]. Our study, silage samples exhibited an average of 67.67% moisture content. Although Fleig scores of silages samples vary, all treatments determined in the study were very good and good quality class of silage (Table 4).

Organic acids (lactic, acetic, butyric) contents, and crude protein and crude ash ratios of grass pea were given in Table 5. All traits were significantly affected (p<0.01) by harvest time and ensiling period. The highest lactic acid was determined as 4.05% (45FE), while the lowest was 0.85% (45SE) and 1.37% (30SE). Acetic and butyric acid ranged from 0.79-1.82% and 0.10-0.40%, respectively. Alcicek and Ozkan [29] and Kilic [30] stated that the lactic acid in silage should be more than 2.0% and acetic acid should be less than 0.8%. In addition, butyric acid is undesirable in the silage, but its presence between 0.1% to 0.6% would not affect the silage quality [31]. It is determined that harvest at flowering stage and ensiling for 45 days were suitable for grass pea silage with these criteria (Table 5). Crude protein ratio ranged 20.18% (45SE) – 24.84% (15FE). Basaran et al. [13] found that CP
contents of grass pea ranged between 20.95-26.31%. The highest crude ash ratio was determined as 9.66%, while the lowest was 7.08%.

The mineral element content of grass pea silage were between 2.42 – 2.93%, 0.23 – 0.27%, 0.77 – 0.96% and 0.32 – 0.35% for K, P, Ca and Mg, respectively (Table 6). NRC [32] and Tekeli and Ates [33] reported that the requirements for lactating beef are 0.6-0.8% for K, 0.18 – 0.39% for P, 0.18-0.44% for Ca and 0.04-0.1 % for Mg. Additionally, Kidambi et al. [34] indicated that forage should contain 0.21% P, 0.30% Ca and 0.10% Mg for the ruminant. Within this respect, in this study, while ratios of Ca, Mg and K were very high, P was similar with the suggested ratios in grass pea silages. The highest Na content was determined as 0.14% (15FE) while the lowest was 0.09% (30SE) and they were more than the value (0.07%) suggested by NRC [32].

NRC [32] indicated that the requirements for growing and finishing cattle are 50 ppm for Fe, 43-55 ppm for Zn, 12-15 ppm for Mn, <10 ppm for Co, 10-30 ppm for Cu, and 0.20 ppm for Se. In the current study, ratios of Zn, Mn, Cu Se and Co were very low compared to the values suggested by NRC [32]. Besides, the Fe content of grass pea obtained at the 15FE, 45FE, and 30SE was higher than the values suggested by NRC [32], while other silages were low. The Mo, Cr and S contents of grass pea silage ranged from 0.146-0.198 ppm, 1.70-3.01 ppm, and 246.58-286.06 ppm, respectively (Table 6).

ODAP contents of grass pea silage samples were given Figure 1. The highest ODAP content was determined as 2.48 mg/g in 30FE, while the lowest was 0.66 mg/g in 30SE. ODAP content in the present silage samples other than 30FE are relatively low compared to 2 mg/g content reported as critical value by Abd El-Moneim et al. [35].

**TABLE 6**

<table>
<thead>
<tr>
<th>Mineral element</th>
<th>Flowering time</th>
<th>Seed filling time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-day</td>
<td>30-day</td>
</tr>
<tr>
<td>Potassium content (K) (%)*</td>
<td>2.64(^a)</td>
<td>2.93(^b)</td>
</tr>
<tr>
<td>Phosphorus content (P) (%)**</td>
<td>0.26(^a)</td>
<td>0.27(^a)</td>
</tr>
<tr>
<td>Calcium content (Ca) (%)</td>
<td>0.89</td>
<td>0.96</td>
</tr>
<tr>
<td>Magnesium content (Mg) (%)</td>
<td>0.35</td>
<td>0.32</td>
</tr>
<tr>
<td>Sodium (Na) (%)</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>Iron content (Fe) (ppm)</td>
<td>69.18</td>
<td>40.11</td>
</tr>
<tr>
<td>Zinc (Zn) (ppm)</td>
<td>3.54</td>
<td>3.28</td>
</tr>
<tr>
<td>Mangan (Mn) (ppm)*</td>
<td>7.14(^b)</td>
<td>7.30(^a)</td>
</tr>
<tr>
<td>Cobalt (Co) (ppm)</td>
<td>0.037</td>
<td>0.033</td>
</tr>
<tr>
<td>Copper (Cu) (ppm)</td>
<td>1.13</td>
<td>1.29</td>
</tr>
<tr>
<td>Selenium (Se) (ppm)</td>
<td>0.004</td>
<td>0.003</td>
</tr>
<tr>
<td>Molybdenum (Mo) (ppm)</td>
<td>0.149</td>
<td>0.146</td>
</tr>
<tr>
<td>Chrome (Cr) (ppm)</td>
<td>2.89</td>
<td>2.36</td>
</tr>
<tr>
<td>Sulphur (S) (ppm)</td>
<td>266.19</td>
<td>273.67</td>
</tr>
</tbody>
</table>

*: p < 0.05, **: p < 0.01, There is no difference between the same letters in each column (p<0.05)
CONCLUSION

The shortage of good quality fodder is the major constraint for livestock production. Especially, during summer and winter, this shortage poses a great challenge for livestock farmers to feed their animals. Therefore, silage is a viable solution to supply quality forage in production periods. Grass pea has high protein content and it adapts best to extreme conditions. It is also resistant to diseases and has low input requirement for its cultivation. Hence, it is used for different fields, especially forage crops. On the other hand, the plant is affected by toxic ODAP causing irreversible spastic paralysis of the legs known as neurolathyrism. For this reason, ODAP content of grass pea must be less than 2 mg/g.

As a result, harvesting at the flowering stage and ensiling for 45 days was found out to be the best treatment for the successful grass pea silage.

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LEAF PHYSIOLOGICAL AND ROOT MORPHOLOGICAL PARAMETERS OF GRAFTED TOMATO PLANTS UNDER DROUGHT STRESS CONDITIONS

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²Institute of Biological Production Systems, Vegetable Systems Modelling Section, Leibniz University of Hannover, Hannover, Germany

ABSTRACT

Grafting of tomatoes becomes more and more popular particularly under adversarial soil conditions such as low and high soil temperature, salinity, alkalinity and drought. This study focused on the interactions between tomato rootstock and scion varieties regarding growth, development and yield under different water supply levels to identify the morphological and physiological plant traits related to drought tolerance of a tomato crop. Different tomato scion (S) varieties (Pannovy and Treasury) were grafted on different tomato rootstocks (R) (Brigeor and Maxifort) varieties and were grown under well watered (WW) and drought stress (DS) conditions. As control plants the S varieties were grafted on themselves. To evaluate growth and morphological effects, various plant parameters such as leaf area, fruit fresh matter, fruit diameter, main stem length, total plant dry matter, root fresh and dry matter, fruit water use efficiency (WUE), shoot WUE, total root length, root length density and root diameter were investigated at final harvest. To determine physiological plant traits, leaf water potential, leaf relative water content and leaf osmotic potential were assessed. Morphological parameters such as fruit fresh matter, fruit diameter, main stem length, total plant dry matter, fruit WUE, leaf area, root dry matter, total root length, and root length density were higher under WW than under DS conditions. As well, in terms of physiological plant parameters such as leaf relative water content (LRWC) was higher under WW than under DS conditions. Furthermore, drought stress can affect photosynthesis directly, by affecting various biochemical processes involved in photosynthesis, and indirectly, by reducing the intake of CO₂ through the stomata of the leaves [3, 4]. Due to stomata closure, a decline in net assimilation rate, leaf area and chloroplast activity occurs [5, 6, 7] which hastens the senescence of leaves [8].

Tomato (Lycopersicon esculentum Mill.) is an important vegetable crop in many parts of the world particularly in Europe, America and Asia. Since the tomato plants are sensitive to drought stress and so usually show a high correlation between evapotranspiration and crop yield [9]. The study by Giardini et al. [10] indicated that low or deficit irrigation rates significantly decreased the yield and fruit size of the tomatoes under greenhouse conditions, since translocation of assimilates to the roots may be increased. In dry soils mechanical impedance and low soil water potential are the dominant stress factors on root growth [11]. Root development is usually decreased or stopped under drought stress and roots tend to become suberized to their tips, reducing their capacity to absorb water [12]. However, drought stress can greatly modify the differential sensitivity of roots and shoots which leads to large increases in the root/shoot ratio [13]. Tomato plants tend to grow a denser root system at soil water potentials which are slightly less than field capacity [14]. Several physiological, morphological, biochemical, and genetic studies were carried

KEYWORDS:
Grafting, drought tolerance, tomato, stomatal conductance, rootstock

INTRODUCTION

In many countries, as a consequence of global climate changes and environmental pollution, water use for agriculture production was considerably reduced, whereas a great emphasis was shown on the crop physiology and crop management studies in dry conditions aimed to develop and use plants which can efficiently use the available water [1]. Generally, the plants are adversely affected when more than 40% of the total available soil water has been depleted [2]. Furthermore, drought stress can affect photosynthesis directly, by affecting various biochemical processes involved in photosynthesis, and indirectly, by reducing the intake of CO₂ through the stomata of the leaves [3, 4]. Due to stomata closure, a decline in net assimilation rate, leaf area and chloroplast activity occurs [5, 6, 7] which hastens the senescence of leaves [8].

Tomato (Lycopersicon esculentum Mill.) is an important vegetable crop in many parts of the world particularly in Europe, America and Asia. Since the tomato plants are sensitive to drought stress and so usually show a high correlation between evapotranspiration and crop yield [9]. The study by Giardini et al. [10] indicated that low or deficit irrigation rates significantly decreased the yield and fruit size of the tomatoes under greenhouse conditions, since translocation of assimilates to the roots may be increased. In dry soils mechanical impedance and low soil water potential are the dominant stress factors on root growth [11]. Root development is usually decreased or stopped under drought stress and roots tend to become suberized to their tips, reducing their capacity to absorb water [12]. However, drought stress can greatly modify the differential sensitivity of roots and shoots which leads to large increases in the root/shoot ratio [13]. Tomato plants tend to grow a denser root system at soil water potentials which are slightly less than field capacity [14]. Several physiological, morphological, biochemical, and genetic studies were carried
out on the tomato crop because of its valuable traits such as easy cultivation, short life cycle and the amenability for various horticultural manipulations, including grafting, or cutting [15, 16]. As an alternative, to diminish losses in production and to improve water use efficiency under limited water supply conditions in high-yielding genotypes could be grafted onto rootstocks competent of decreasing the effect of water stress on the shoot [17, 18].

In the past, first vegetable grafting was performed in Korea and Japan in the late 1920’s by grafting watermelon onto gourd rootstocks [19, 20]. It is an innovative technique for the suitable cultivation of fruit-bearing vegetables (tomatoes, bean, eggplant, cucumber, melon, and watermelon) in Japan, Korea, the Mediterranean basin, and several European countries [21], where land use is very intensive and continuous cropping is a common practice [22]. Later on, it has been a widely used technique in vegetable seedling production in order to get the advantages of the rootstock such as resistance to pests and diseases [23, 24, 25, 26], robust growth, resistance against low [27] and high [28, 29] temperatures, enhancement in fruit yield and quality [30], to increase the absorption of nutrients and the mineral content in the aerial portion of the plant [31], enhance vegetable tolerance to salinity [32, 33], flooding [34], under limited water supply [35, 36] and to improve plant vigour and the post-harvest lifetime of the fruits [37]. Several studies have been conducted to improve drought tolerance by grafting with different tomato scion and rootstock varieties [38, 19, 20, 39, 34]. For instance, the study of Passam et al. [36] clearly demonstrated that the fruit size and the fruit number of eggplant was significantly increased when grafted onto tomato rootstock compared to non-grafted controls under greenhouse conditions. Similar positive grafting effects on fruit yield were obtained when the watermelon plant was grafted onto squash rootstocks [28]. Cuartero et al. [40] reported that when the tomato scion variety Jaguar was grafted with the tomato rootstock variety of Radja and the tomato rootstock variety of Volgogradskij, fruit yield increased more than 60% in both graft combinations as compared to self-grafted Jaguar tomato plants under drought stress conditions.

In drought tolerance, the morphological and physiological characteristics of both shoot and root systems play a crucial role and may change the response of the plant. Therefore, a vigorous rootstock under deficit water supply may increases the water absorption from soil due to deep, wide-spreadening and a much branched root system and maintains water supply to the shoot [41]. Differences in xylem diameter in the roots can also influence hydraulic conductivity and hence the water status of the shoot [42]. For instance, the study by Weng [7] showed under water deficit leaves of grafted tomato with Solanum mammosum rootstock maintained higher leaf water potential and leaf conductance as compared to standard tomato roots. This might be due to amount and distribution of the root system which can influence the amount of abscisic acid produced in root tips as the soil dries and may affect leaf stomatal conductance rapidly [43].

As mentioned above, up to now the effects of grafting on tomato plants were carried out by many authors. Nevertheless, limited information is available on the mechanisms of the grafting effects on the morphological plant traits related to drought tolerance in tomato crop. More comprehensive studies on tomato plants have to be conducted to better understand whether grafting could improve drought tolerance. Therefore, this study focused on the effects of grafting between tomato rootstock and scion varieties regarding growth, development and yield under different water supply levels to identify the morphological plant traits related to drought tolerance of a tomato crop. Thus, different tomato scion (S) varieties were grafted with different rootstocks (R) varieties. As control plants the S varieties were grafted on themselves.

MATERIALS AND METHODS

Location, plant material and growth conditions. Seeds of the tomato scion and rootstock varieties were germinated for 7-14 days in a moist peat substrate and then the appropriate seedlings were selected for the grafting process. When the plants started to initiate their fourth true leaf, the tomato scion varieties of Pannovy and Treasury were grafted onto the different rootstock varieties of Brigeor and Maxifort using the tube grafting method. As control plants the scion varieties were grafted on themselves. After completion of grafting process, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the climate chamber for one week [44]. In order to prevent grafted plants from wilting by the excessive transpiration and to enhance healing, the tunnel was closed for the first three or four days of healing and acclimatization period. For the next three or four days, the opening and closing of the tunnel were done depending on the conditions of grafted plants and growth room. This was done for the acclimatization of grafted plants to environmental conditions outside tunnel. After the end of healing and acclimatization, grafted plants were transplanted into a double 2 x 30 L pot and grown in the greenhouse at 20°C/16°C day and night temperature regulated ventilation. The experiment was conducted in an automatically controlled greenhouse of the Institute of Biological Production Systems, Faculty of Natural Sciences, Gottfried Wilhelm Leibniz University Hannover, Germany.
The tomato plants were grown in a mixture of sandy soil with 8.3 g Osmote Start-Controlled Release Fertilizer (12:11:17+2 MgO). The homogenized sandy soil was dried before using for each experiment and sub-samples were taken to determine the initial soil water content. Soil water holding capacity was measured by subtracting the weight of pots filled with dry sand and the weight after saturating soil with water and allowing the exceeding water to drain until there is no change in the weight. For this calculation, water saturated soil samples were taken with aluminum cylinders and dried in an oven at 105 °C for 48 h.

The 2 x 30 L pots were placed on moveable carts in the greenhouse. To measure the necessary irrigation water, a weighing system with 2 rollers and a scale was used. Transferring the heavy pots to the weighing system by rolling was easy and safety for the plants. To avoid evaporation, each pot was covered with 6 kg gravel to construct a nearly 5 cm height surface layer. To calculate the evaporation water, one of the pots was left without plant.

Soil water holding capacity (SWHC) was maintained at a value of 85% for the well watered and additionally at 45% for the drought stress treatments. Therefore, the pots were weighed daily in the morning to irrigate to the target value for well watered and drought stress treatments. To give the plants of the drought stress treatments time to adopt, in the beginning only 50% of the transpired water of the previous day was replaced in order to increase the stress level. This was stopped when the pots reached 45% of water holding capacity. Treatments were defined by a factorial combination of two irrigation levels and two grafting treatments. The treatments were arranged in a randomized complete block design with three replicates per treatment.

**Measurements and analysis.** In the experiment, plants were harvested by separating them into shoot and roots. For the fresh matter, determination shoot was fractioned into the leaf, stem and fruits. After measuring the fresh weights of each shoot fraction, samples were dried in a ventilated oven at 74 °C for 72 hours. Starting from first florescence up to last one on the tomato plant, each single fruit were destructively collected for fresh weight and on the same fruit for the diameter measurements by using a caliper device. Main stem length (cm) was measured by using a ruler. The root length of tomato plants was measured by using the special software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada). After root length measurement, root samples were dried in ventilated oven at 74 °C for 72 hours.

Leaf water potential was determined using a Scholander type pressure chamber (Plant Moisture Systems, Santa Barbara, CA, USA) during daytime between 11:00 and 14:00 h, because Fischer and Sanchez [45] reported that leaf water potential was reasonable stable during noon. It was measured in the youngest fully expanded leaf every 48 h for well watered and drought stressed plants during the water stress period with three replicates per treatment. Leaves were excised with a smooth, clean cut using a sharp razor blade and leaf water potential was measured immediately afterwards, thus minimizing errors due to transpirational losses. Increase of pressure within the chamber was done very slowly and the end point (i.e. the point at which the first water bubbles appeared on the cut surface) was observed carefully with a hand lens.

Relative water content measurements were done on the youngest fully expanded leaves between 11:00 and 15:00 h every 48 h using three replicates per treatment. For measurement of relative water content 3 cm leaf disks were excised from young fully expanded leaves using a cork borer. Immediately after cutting the leaf disks, they were quickly transferred to the laboratory and afterwards fresh weights were determined. After the fresh weight was obtained, the leaf disks were floated for 24 h on distilled water in covered Petri dishes kept at low light intensities and in a constant temperature room (20 °C), until they became fully turgid. After soaking, leaves were quickly and carefully blotted dry with tissue paper in preparation for determining turgid weight. Thereafter the leaf disks were stored in paper bag and dried in a ventilated oven for 72 h at 100 °C to a constant weight to obtain the dry weight.

Relative water content on a percentage basis was calculated using the equation of Jones and Turner [46]:

\[ RWC (\%) = \frac{(FW-DW)}{(TW-DW)}\times100 \]

RWC: Relative water content
FW: Fresh weight
DW: Dry weight
TW: Turgid weight

<table>
<thead>
<tr>
<th>Tomato cultivars</th>
<th>Definition</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brigeor</td>
<td>Rootstock</td>
<td>Enza</td>
</tr>
<tr>
<td>Maxifort</td>
<td>Rootstock</td>
<td>De Ruiter</td>
</tr>
<tr>
<td>Treasury</td>
<td>Scion</td>
<td>Seminis</td>
</tr>
<tr>
<td>Pannovy</td>
<td>Scion</td>
<td>S&amp;G</td>
</tr>
</tbody>
</table>
For the measurements of osmotic potential 1 cm leaf disks were excised from young fully expanded leaves using a cork borer, wrapped in aluminium foil and immediately placed in liquid nitrogen. Osmotic potential was then measured at 22 °C with a psychrometer (C52-chamber; Wescor Corp., Logan, Utah). Water Use Efficiency (WUE) was calculated based on the transpired water which was derived from the quantity of water added daily to the pots minus losses estimated from a blank evaporation pot.

The general equation of WUE is described as follow:

\[
\text{WUE} = \frac{\text{dry matter produced (g)}}{\text{transpired water (kg)}}
\]

The equation was used for calculating the WUE referring to the fruit fresh matter (a) and the total plant dry matter (b).

\[
\begin{align*}
\text{WUE} &= \frac{\text{Fruit fresh matter (g)}}{\text{transpired water (kg)}} \quad (a) \\
\text{WUE} &= \frac{\text{Shoot dry matter (g)}}{\text{transpired water (kg)}} \quad (b)
\end{align*}
\]

**Statistical Analysis.** All measured biomasses and other morphological plant parameters were analyzed based on the correlations between the different scion-rootstock combinations at different water supply treatments. Analysis of variance (ANOVA) was performed using the SAS program (SAS Institute, Cary NC, USA). If ANOVA determined that the effects of the treatments were significant (P < 0.05 for F-test), then the treatment means were separated by Duncan’s Multiple Range Test.

**RESULTS AND DISCUSSION**

**Shoot and root dry matter, root: shoot ratio and shoot water use efficiency.** The results of shoot and root dry matter, root: shoot ratio and shoot water use efficiency (WUE) at the end of the growing cycle of graft combination and self grafted control plants in different water supply levels (WW: SWHC 85%; DS: SWHC 45%) were shown in Table 2. Shoot [except genotype (P < 0.01)] and root dry matter [except water stress (P < 0.01)], root: shoot ratio and shoot WUE were significantly (P < 0.001) affected by genotype, water stress (DS) and genotype x water stress (DS) interaction. Generally, plant growth decreased linearly in response to an increase in water stress. Shoot and root dry matter decreased by different rates in each genotype under water stress conditions. The results indicated that an increase existed in shoot dry matter particularly when Pannovy was grafted with Brigeor and Maxifort under WW condition. However, this increase was not significantly. Also similar increase under DS condition was found with Pannovy/Brigeor combination while a reduction occurred with Pannovy/Maxifort. However, the same response could not be detected with the scion variety of Treasury. It reduced the total plant dry matter under WW with two rootstock varieties. On the other hand, an increase existed in shoot dry matter particularly when Treasury was grafted with Brigeor and Maxifort under DS condition. Significantly higher shoot dry matter was observed under DS condition by the graft combinations of Pannovy/Brigeor and Treasury/Maxifort.

Similar to shoot dry matter, generally the root dry biomass of grafted and self-grafted plants decreased linearly in response to an increase in water stress. Among both grafted and self-grafted scion varieties, highly significant difference in root dry matter was observed under WW and DS conditions. The root dry matter was significantly higher when both scion varieties of Pannovy and Treasury were grafted on to Maxifort under WW condition. Maxifort can be characterized as an efficient rootstock with vigorous root system compared to Brigeor under WW condition. However, this efficiency was expressed only under WW condition with the scion variety of Pannovy. Since under DS condition Pannovy reduced its root dry matter when grafted on to Maxifort and Brigeor as compare to control plants. On the other hand, Treasury increased its root dry matter when grafted on to Maxifort and Brigeor as compare to control plants under DS condition. Significantly higher root dry matter was observed under DS condition by the graft combination of Treasury/Brigeor.

Concerning the root: shoot ratio, the results elucidated that the root to shoot (leaf, stem and fruit jointly) ratio of self-grafted tomato rootstocks and their scion-rootstock combinations was significantly higher under DS than WW condition. The root: shoot ratio increased significantly when two scion varieties were grafted with Maxifort under WW condition as compare to control plants. Though, Treasury increased its root: shoot ratio when grafted on to Maxifort and Brigeor as compare to control plants under DS condition. Significantly higher root: shoot ratio was existed under DS condition by the graft combination of Treasury/Brigeor.

Regarding shoot WUE, the results indicated that it was significantly higher under DS than WW conditions, irrespective of self-grafted scion varieties and scion-rootstock combinations. Treasury/Brigeor produced significantly higher shoot WUE as compare to control plants under WW condition. Conversely, under DS condition Treasury/Brigeor, Pannovy/Brigeor and Treasury/Maxifort produced significantly higher shoot WUE as compare to control plants.

Growth reduction is well established with increasing water stress [47]. Up to now it was observed in many crop species such as pepper (Capsicum annuum [48]), bell pepper (Capsicum annum L. [49]), potato (Solanum tuberosum [50]),
tomato (*Lycopersicon esculentum* L. [51, 52]), muskmelon (*Cucumis melo* L. [53]), eggplant [54], watermelon [55, 56], and cucumber (*Cucumis sativus* L. [57]). Growth reduction was also reported on grafted plants such as on tomato [58] and melon [59]. Plant yield was effected directly by grafting [60, 61, 28] by interactions of some or all of the following processes: enhance of nutrient and water uptake resulting from the vigorous root system of the rootstock [19, 61], increased production of endogenous hormones [62], or enhancement of scion vigor [38]. The plants that were grafted on to Brigeor produced higher root mass as compare to control plants under water stress. Similar results were also observed that grafted plants onto Cucurbita rootstocks develop a larger root system as compare to ungrafted plants, that should improve drought tolerance by enhancing root water extraction from the deeper soil layers [63]. Iacono et al. [64] observed that WUE increased in grafted grapevines (*Vitis vinifera* L. cv. Müller Thurgau) plants as compare to ungrafted plants under water deficit conditions. In our greenhouse experiment, root: shoot ratio was significantly higher under drought stress. Sharp et al. [65] also observed similar results for maize seedlings. Without drought stress the root/shoot dry weight ratio is 1.45 compared to 5.79 under drought stress. Rootstock variety of Maxifort decreased significantly the root/shoot ratio when it was grafted with both scion varieties, whereas a significantly increase was exhibited with Brigeor rootstock under drought stress. This result agreed with the study of Jefferies [66] who reported that the root/shoot ratio of grafted tomato plants is predominantly regulated by the scion. In the present study, shoot WUE increased under water stress conditions. Our results agree with Iacono et al. [64] who reported that WUE usually increases when irrigation is limited. Similar results were shown by Davies et al. [67] who reported that WUE expressed on a dry weight basis increased by 48% in tomato plants grown under partial root drying conditions. Increased WUE values under water stress conditions were also found in eggplant [54], bell pepper [68] and watermelon [56].

**Main stem length, fruit fresh weight, fruit diameter and fruit water use efficiency.** The results of main stem length, fruit fresh weight, fruit diameter and fruit water use efficiency (WUE) at the end of the growing cycle of graft combination and self grafted control plants in different water supply levels (WW: SWHC 85%; DS: SWHC 45%) were shown in Table 3. Main stem length, fruit fresh weight, fruit diameter [except Gen. x W.S (P < 0.05)] and fruit WUE were significantly (P < 0.001) [except Genotype (P < 0.05)] affected by genotype, water stress (DS) and genotype x water stress (DS) interaction, however there were no significant differences found regarding fruit WUE by Gen. x W.S. The grafted and self grafted plants grown in water stress conditions were noticeably smaller and less vigorous than the plants grown in the WW conditions. Under WW condition, the significantly longest plant main stem length was measured in self grafted control plants of Treasury, while the shortest was measured in Treasury/ Brigeor. Under adverse environmental conditions the growth of the plants are affected negatively as well as stem length. The significantly longest plant main stem length was measured in Treasury/ Brigeor graft combination under DS condition. Similar results were also observed by Yamac [69] who studied the effects of grafting on growth and yield of watermelon plants on to *Cucurbita* rootstocks under climate chamber conditions under saline conditions. He observed that the grafted plants had significantly taller plants as compare to ungrafted ones.

### TABLE 2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Shoot dry matter (g plant⁻¹)</th>
<th>Root dry matter (g plant⁻¹)</th>
<th>Root: shoot (g p⁻¹)</th>
<th>Shoot WUE (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S/ R)</td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
</tr>
<tr>
<td>Pan/Pan</td>
<td>185.6a</td>
<td>150.7B</td>
<td>10.6c</td>
<td>14.1B</td>
</tr>
<tr>
<td>Pan/Max</td>
<td>201.8a</td>
<td>147.4B</td>
<td>20.9a</td>
<td>12.8B</td>
</tr>
<tr>
<td>Pan/Brig</td>
<td>190.3a</td>
<td>166.0A</td>
<td>10.2c</td>
<td>12.4B</td>
</tr>
<tr>
<td>Treas/Treas</td>
<td>197.2a</td>
<td>144.0B</td>
<td>14.2b</td>
<td>12.1B</td>
</tr>
<tr>
<td>Treas/Max</td>
<td>190.8a</td>
<td>160.9A</td>
<td>19.4a</td>
<td>14.9AB</td>
</tr>
<tr>
<td>Treas/Brig</td>
<td>192.6a</td>
<td>146.2B</td>
<td>13.2b</td>
<td>17.1A</td>
</tr>
<tr>
<td>Genotype</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Wat. Stress</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Gen. x W.S</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Values denoted by different letters (lower and upper case letters for WW and DS, respectively) are significantly different between genotypes within columns at P < 0.05. ns, non-significant. *P < 0.05, **P < 0.01 and ***P < 0.001.
TABLE 3
Main stem length, fruit fresh weight, fruit diameter and fruit water use efficiency of six tomato graft combinations grown under well-watered (WW) and drought stress (DS)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Main stem length (cm plant⁻¹) WW</th>
<th>Fruit fresh weight (g plant⁻¹) WW</th>
<th>Fruit diameter (mm) WW</th>
<th>Fruit WUE (g L⁻¹) WW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan/Pan</td>
<td>133.0ab</td>
<td>851.3bc</td>
<td>3.61bc</td>
<td>14.8a</td>
</tr>
<tr>
<td>Pan/Max</td>
<td>129.5bc</td>
<td>951.3a</td>
<td>3.54c</td>
<td>13.6a</td>
</tr>
<tr>
<td>Pan/Brig</td>
<td>128.0bc</td>
<td>931.4ab</td>
<td>3.59bc</td>
<td>14.8a</td>
</tr>
<tr>
<td>Treas/Treas</td>
<td>140.0a</td>
<td>935.3a</td>
<td>4.26a</td>
<td>15.4a</td>
</tr>
<tr>
<td>Treas/Max</td>
<td>131.7bc</td>
<td>823.9c</td>
<td>4.18abc</td>
<td>13.8a</td>
</tr>
<tr>
<td>Treas/Brig</td>
<td>124.3c</td>
<td>838.3c</td>
<td>4.23ab</td>
<td>15.7a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Main stem length (cm plant⁻¹) DS</th>
<th>Fruit fresh weight (g plant⁻¹) DS</th>
<th>Fruit diameter (mm) DS</th>
<th>Fruit WUE (g L⁻¹) DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan/Pan</td>
<td>111.0 B</td>
<td>545.8AB</td>
<td>3.11BC</td>
<td>12.8A</td>
</tr>
<tr>
<td>Pan/Max</td>
<td>115.0 B</td>
<td>500.9BC</td>
<td>2.91C</td>
<td>13.6a</td>
</tr>
<tr>
<td>Pan/Brig</td>
<td>112.3 B</td>
<td>570.7A</td>
<td>3.21B</td>
<td>14.6A</td>
</tr>
<tr>
<td>Treas/Treas</td>
<td>127.7 A</td>
<td>558.7A</td>
<td>3.59A</td>
<td>13.6A</td>
</tr>
<tr>
<td>Treas/Max</td>
<td>114.7 B</td>
<td>493.0C</td>
<td>3.61A</td>
<td>13.1A</td>
</tr>
<tr>
<td>Treas/Brig</td>
<td>123.7 A</td>
<td>534.3ABC</td>
<td>3.18B</td>
<td>12.7A</td>
</tr>
</tbody>
</table>

Genotype ***Wat. Stress ***Gen. x W.S ***

Values denoted by different letters (lower and upper case letters for WW and DS, respectively) are significantly different between genotypes within columns at P < 0.05. ns, non-significant. *P < 0.05, **P < 0.01 and ***P < 0.001.

Regarding fruit fresh weight, the results indicated that the fruit fresh weight substantially decreased under DS compared to WW condition, irrespective of self-grafted scion varieties or scion-rootstock combinations. Self-grafted scion variety Pannovy considerably increased its fruit fresh matter when it was grafted with both rootstock varieties of Brigeor and Maxifort under WW condition. However, self-grafted scion variety Treasury considerably reduced significantly their fruit fresh matter when it was grafted with both rootstock varieties of Brigeor and Maxifort under WW condition. The graft combination of Pannovy/Maxifort produced significantly higher fruit fresh matter. Opposite to WW condition, different responses among varieties in terms of fruit fresh matter were shown under DS condition. Pannovy significantly increased its fruit fresh matter when it was grafted with rootstock Brigeor, but significantly reduced with Maxifort. This might be due to higher carbohydrate requirements of Maxifort rootstock to maintain higher root growth and actives under DS condition. Because, higher root dry weight and root length density (Table 2 and 5) are the indications that most of assimilates produced in shoot were allocated to the root of Maxifort rootstock. However, Treasury significantly decreased its fruit fresh matter with the two rootstock varieties of Brigeor and Maxifort. In general, at the beginning of growth, the water consumption of tomato crop is lower but then increases gradually until the start of flowering, after which it climbs to a maximum during the peak of fruit ripening [70]. Most of the results from these experiments elucidated that under undesirable environmental conditions the growth, yield and quality of tomatoes grafted with tolerant rootstocks were considerable higher than self-grafted and/or non-grafted plants [34, 71]. Several studies elucidated that the considerably increase of leaf area and thus enhancement in fruit yields in tomato [22]. Salter [72] reported that the flowering and fruit setting stages are the most sensitive to drought stress in terms of yield in tomato plants. Cuartero et al. [40] who reported that when the tomato scion variety Jaguar was grafted with the tomato rootstock variety of Radja and the tomato rootstock variety of Volgogradskij, fruit yield increased more than 60% in both grafted combinations as compared to self-grafted Jaguar tomato plants under drought stress conditions.

Concerning fruit diameter, the result indicated that either in self-grafted scion varieties or scion-rootstock combinations highest fruit diameter was achieved under non-limiting water supply condition. Compared to self-grafted variety of Pannovy/Pannovy, big fruits were produced by Treasury/Treasury because of its higher root dry weight and root length density. Further, significantly bigger fruits were produced by self grafted plants of Treasury and the graft combination of Treasury/Maxifort, but the yield of eggplant significantly increased when it was grafted with a tolerant tomato rootstock compared to non-grafted controls under greenhouse condition. Another study Giardini et al. [10] clearly showed that an application of lower irrigation during reproductive growth stage resulted in a reduction in yield and fruit size.

Regarding fruit WUE, the results indicated that the fruit WUE of grafted and self grafted plants decreased with increasing water deficit. There were no considerably differences found between self grafted and graft combinations under both WW and DS conditions. Iacono et al. [64] observed that WUE increased in grafted grapevines (Vitis vinifera L. cv. Müller Thurgau) plants as compare to ungrafted plants under water deficit conditions. In
another study by Rouphael et al. [73] who observed significantly higher yield and biomass WUE on grafted mini-watermelon plants on to *Cucurbita* rootstocks under water deficit conditions. Explanations for this different findings could be the different vegetable families used in these both studies.

**Leaf area, leaf relative water content, leaf water potential and leaf osmotic potential.** The results of leaf area, leaf relative water content, leaf water potential and leaf osmotic potential at the end of the growing cycle of graft combination and self grafted control plants in different water supply levels (WW: SWHC 85%; DS: SWHC 45%) were shown in Table 4. Leaf area, leaf relative water content [except Gen. x W.S (P < 0.05)], leaf water potential and leaf osmotic potential were significantly (P < 0.001) affected by genotype, water stress (DS) and genotype x water stress (DS) interaction, however there were no significant differences found regarding leaf area by W.S. The result indicated that the grafted plants produced higher leaf area as compare to self grafted plants under both WW and DS conditions. Leaf area formation decreased in response to an increase in water stress. Self grafted Pannovy and its graft combinations decreased the leaf area formation under drought stress (DS) conditions, whereas Treasury increased the leaf area formation when it was grafted on to rootstock genotypes of Maxifort and Brigeor. The graft combination of Pannovy/Maxifort produced significantly higher leaf area under WW conditions, on the other hand under DS, Treasury/Brigeor produced significantly higher leaf area as compare to self grafted control plants. Several studies elucidated that the considerably increase of leaf area and thus enhancement in fruit yields in tomato [22] were the results of positive interactions between scions and rootstocks. Similar results were also observed by Yamac [69] who studied the effects of grafting on growth and yield of watermelon plants on to *Cucurbita* rootstocks under climate chamber conditions under saline conditions. He observed that the grafted plants produced significantly higher leaf area as compare to ungrafted ones.

Regarding leaf relative water content, in both grafted and self grafted plants, it decreased linearly in response to an increase in water stress. There were no significant differences found among self grafted and grafted plants under WW conditions, while significant differences observed under DS conditions. Self grafted plants of Treasury showed significantly higher leaf relative water content under DS conditions.

Concerning leaf water potential and leaf osmotic potential, both parameters increased linearly in response to an increase in water stress. Leaf water potential and leaf osmotic potential was significantly higher under DS than WW condition, irrespective of self-grafted scion and scion-rootstock combinations. Grafted plants have significantly higher leaf water potential as compare to self grafted plants under DS conditions. There were no significant differences regarding leaf water potential found among self grafted and grafted plants under WW conditions, while significantly higher leaf water potential was observed when scion variety of Treasury was grafted on to two rootstock varieties and also in graft combination of Pannovy/Maxifort under DS conditions. Self grafted scion variety of Pannovy and its graft combinations have significantly higher leaf osmotic potential as compare to scion variety of Treasury under WW conditions. The best performance was shown by Pannovy/Brigeor under DS conditions.

Similar results were observed by Rouphael et al. [73] who observed that stomatal conductance and net assimilation CO₂ (leaf gas exchange) decreased quadratically in response to an increase in water stress in both grafted and ungrafted mini-watermelon plants. However, under severe water stress, the reduction in net assimilation CO₂ and stomatal conductance was more in ungrafted than in grafted plants. In both grafted and ungrafted plants, leaf relative water content, leaf water potential, osmotic potential, and turgor potential decreased linearly in response to an increase in water stress. Whereas in our study, in both grafted and self grafted plants, leaf water potential and osmotic potential increased in response to an increase in water stress. This might be the different vegetable families used in these both studies.

**Root fresh weight, total root length, root length density and root diameter.** The results of root fresh weight, total root length, root length density and root diameter at the end of the growing cycle of graft combination and self grafted control plants in different water supply levels (WW: SWHC 85%; DS: SWHC 45%) were shown in Table 5. Root fresh weight, total root length, and root length density were significantly (P < 0.001) affected by genotype, water stress (DS) and genotype x water stress (DS) interaction, however there were no significant differences found regarding root fresh weight by W.S. and root diameter by genotype, water stress (DS) and genotype x water stress (DS) interaction. Among both grafted and self grafted scion varieties, highly significant difference in root fresh matter was observed under WW and DS conditions. The root fresh matter was significantly higher when scion variety of Pannovy was grafted on to Maxifort under WW condition. Maxifort can be characterized as an efficient rootstock with vigorous root system compared to Brigeor under WW condition. However, this efficiency was expressed only under WW condition, since under DS condition Pannovy reduced its root dry matter when grafted on to Maxifort and Brigeor as com-
pare to control plants. On the other hand, Treasury increased its root fresh matter when grafted on to Maxifort and Brigeor as compare to control plants under DS condition. Significantly higher root fresh matter was observed under DS condition by the graft combination of Treasury/Brigeor.

Concerning total root length, the total root lengths of scion-rootstock combinations were higher under WW than DS conditions. The total root length was significantly higher when both scion varieties of Pannovy and Treasury were grafted on to Maxifort under WW conditions. On the other hand, slightly reduction or nearly similar root density was produced when Treasury was grafted on to Brigeor under DS condition compared to its expression under WW. From this result Brigeor can be characterized as drought tolerance rootstock. Under DS condition the graft combinations of Pannovy/Maxifort, Treasury/Maxifort and Treasury/Brigeor have significantly higher total root length than all graft combinations and self-grafted control plants.

Regarding root length density, it decreased linearly in response to an increase in water stress. Under both water supply levels, the graft combination of Treasury/Maxifort has significantly higher root length density than all graft combinations and self-grafted control plants.

Many studies reported that an interaction between rootstocks and scions exists resulting in high vigor of the root system and greater water and mineral uptake leading to an increased yield and to fruit growth enhancement [39, 74]. Because a vigorous rootstock under deficit water supply can increase the water absorption from soil due to a deep, widespread and much branched root system and maintains water supply to the shoot [41]. Differences in xylem diameter in the roots can also influence hydraulic conductivity and hence the water status of the shoot [42]. Similar results were observed by Yamac [69] that the grafted plants produced significantly higher root fresh weight, total root length, and root length density as compare to ungrafted ones under salt stress conditions.

**TABLE 4**

Leaf area, leaf relative water content (LRWC), leaf water potential (LWP) and leaf osmotic potential (LOP) of six tomato graft combinations grown under well-watered (WW) and drought stress (DS)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Leaf area (cm² plant⁻¹)</th>
<th>LRWC (%)</th>
<th>LWP (MPa)</th>
<th>LOP (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
</tr>
<tr>
<td>Pan/Pan</td>
<td>5860bc</td>
<td>5306D</td>
<td>84.7a</td>
<td>80.6AB</td>
</tr>
<tr>
<td>Pan/Max</td>
<td>6654a</td>
<td>6553C</td>
<td>85.7a</td>
<td>78.4B</td>
</tr>
<tr>
<td>Pan/Brig</td>
<td>5984abc</td>
<td>5604D</td>
<td>83.9a</td>
<td>78.7AB</td>
</tr>
<tr>
<td>Treas/Treas</td>
<td>6364abc</td>
<td>5606D</td>
<td>84.5a</td>
<td>84.0A</td>
</tr>
<tr>
<td>Treas/Max</td>
<td>6531ab</td>
<td>7012B</td>
<td>81.0a</td>
<td>79.0AB</td>
</tr>
<tr>
<td>Treas/Brig</td>
<td>5697c</td>
<td>7616A</td>
<td>81.0a</td>
<td>80.8AB</td>
</tr>
</tbody>
</table>

Values denoted by different letters (lower and upper case letters for WW and DS, respectively) are significantly different between genotypes within columns at P < 0.05. ns, non-significant. *P < 0.05, **P < 0.01 and ***P < 0.001.

**TABLE 5**

Root fresh weight, root length total (RL), root length density (RDL) and root diameter of six tomato graft combinations grown under well-watered (WW) and drought stress (DS)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Root fresh weight (g plant⁻¹)</th>
<th>Total RL (m plant⁻¹)</th>
<th>RLD (cm cm⁻³)</th>
<th>Root diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>DS</td>
<td>WW</td>
<td>DS</td>
</tr>
<tr>
<td>Pan/Pan</td>
<td>204.3c</td>
<td>298.3B</td>
<td>598.1b</td>
<td>466.2B</td>
</tr>
<tr>
<td>Pan/Max</td>
<td>392.9a</td>
<td>246.1C</td>
<td>919.9a</td>
<td>578.9A</td>
</tr>
<tr>
<td>Pan/Brig</td>
<td>192.8e</td>
<td>247.4C</td>
<td>695.1b</td>
<td>470.7B</td>
</tr>
<tr>
<td>Treas/Treas</td>
<td>278.6c</td>
<td>241.9C</td>
<td>645.6b</td>
<td>417.4B</td>
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<tr>
<td>Treas/Max</td>
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<td>905.0a</td>
<td>572.4A</td>
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<tr>
<td>Treas/Brig</td>
<td>248.9d</td>
<td>348.7A</td>
<td>617.5b</td>
<td>617.1A</td>
</tr>
</tbody>
</table>

Values denoted by different letters (lower and upper case letters for WW and DS, respectively) are significantly different between genotypes within columns at P < 0.05. ns, non-significant. *P < 0.05, **P < 0.01 and ***P < 0.001.
CONCLUSION

The results of this study clearly indicate that how the rootstock can strongly influence plant response to water stress in terms of leaf physiological and root morphological parameters of tomato plants. This influence, which is present only in some grafting combinations, therefore it should be taken in account in genotypic selection of the rootstocks. Self-grafted tomato scion varieties interact significantly different when they are grafted each other as a scion-rootstock combination under both water supply treatments. A positive interaction in terms of fruit fresh matter enhancement can be obtained with scion-rootstock combination particularly under drought stress (DS) conditions. Some of the combinations are well responsive under both DS and WW conditions. However, the greenhouse experiment indicates that not all scions and rootstocks are compatible to show a positive interaction regarding growth and fruit yield under well-watered (WW) and under DS conditions. Best performance regarding growth and fruit yield among scion-rootstock combinations relates with efficiency in water use. Higher fruit water use efficiency (WUE) and shoot WUE under DS condition, is the result of contributory root morphological and shoot physiological plant traits like an optimal root shoot partitioning, higher leaf conductance, thick leaf cuticle and short stem. Therefore, these traits should be studied deeply with more tomato scion and rootstock varieties. Moreover, this emphasizes the importance to study the adaptation of rootstock/scion combinations in the field. Consequently, due to both efficiency under DS and responsiveness under WW conditions, the tomato scion-rootstock combinations could be easily used to solve the drought stress problem and maintain acceptable yield where the water source is limited or increase the economic yield of tomato where the water source is not limited.

REFERENCES


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EFFICACY OF TWO ENTOMOPATHOGENIC FUNGI AND THEIR COMBINATION WITH SUMMER OIL AGAINST TOMATO MOTH, *TUTA ABSOLUTA*

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Ministry of Agriculture and Forestry, General Directorate of Food and Control, 06110, Ankara, Turkey

ABSTRACT

Tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is the major pest of tomatoes almost throughout Turkey. Most of the insecticides used against tomato moth in Turkey failed to control this pest due to being resistance to many pesticides. In this study, the efficacy of two “Entomopathogenic Fungi” (EPFs), *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.), and their combinations with “Summer Oil” (SO) at a rate of 0.5% was investigated against the pest under greenhouse conditions in two consecutive tomato growing periods (fall-winter-2014 and spring-2015). In each growing period only one trial was conducted. Application rates of the EPFs were expressed in quantity of product delivered in 1000 liters of water/ha. The *B. bassiana* product (min. 4 x 10⁹ conidia/ml) was applied at 1, 1.5, and 2 l/ha and the *M. anisopliae* product (5.5 x 10⁹ conidia/ml) at 0.75, 1, and 1.25 l/ha. The results of the spray applications proved that egg and larval mortality varies by increasing of duration. The differences in most of mortalities on each sampling date were generally significant among the different conidial concentrations of both EPF products. Compared to the untreated control plots, both EPF products alone and their combinations with SO were able to control of eggs and larvae. However, SO combinations of these two EPF products were more effective than each of EPF product application alone in controlling of both the egg and the larvae. The results also indicated that *B. bassiana* + SO combination was particularly effective against eggs, by causing 86.4% and 79.7% mortalities with the highest dose in 2014 and 2015, respectively, whereas *M. anisopliae* + SO combination was more effective against larvae of the pest, by causing 83.2% and 81.4% mortalities with the highest dose in the same years, respectively. Overall results suggest that both EPF products and their SO combinations can be used within an integrated pest management (IPM) program against *T. absoluta*.

KEYWORDS:
Tomato moth, *Tuta absoluta*, entomopathogenic fungi, summer oil, Turkey

INTRODUCTION

Tomato is the most produced crop with 40-42% of total annual vegetable production in Turkey. The annual tomato production of Turkey is around 12.5 million tons. Approximately 47% of the total greenhouse area of the country is situated in Antalya province (in south-western part of Turkey) where 2.5 million tons tomatoes grown per year [1].

Tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has recently become the most serious insect pest of tomatoes. The pest was first found in Turkey in Izmir province in 2009 [2] and then in Antalya (Kumluca district) in October 2010 [3] and in Southeast Anatolia Region in Diyarbakir and Şanlıurfa provinces in 2010 [4]. Since its initial detection in Turkey in 2009, the invasive destructive pest has started its new invasion journey to east and invaded most Asian countries [5]. The pest has spreaded exceptionally rapidly in Turkey, and it is now become present almost all over the areas where tomatoes are cultivated [6]. *T. absoluta* can feed on all of the plant parts, except the roots. It attacks the tomato plant at any growing stage and can cause 50-100% yield loss. Unless control measures are applied, heavy economical losses may occur [7, 8]. Its presence is one of the most serious obstacles for exporting tomato production as well [9].

Although pheromone traps have recently gained importance in control of *T. absoluta*, intensive insecticide programs are still applied against the pest especially in tomato cultivated greenhouses in Antalya and many other provinces. However, the desired level of control has not been obtained in the last years, due to its ability to develop rapidly resistance to many insecticides [10]. Increasing use of pesticides causes many other problems such as toxic residues on tomatoes and other vegetables, environmental contamination, birth defects; miscarriages, infertility, male sterility and other reproductive problems, etc. In contemporary agricultural production, alternative control strategies/materials are highly necessary to eradicate or to minimize the long-lasting undesired effects of synthetic chemical pesticides. This study aimed at testing some entomopathogenic fungal preparations and their combi-
nations with summer oil (SO) against the eggs and larvae of *T. absoluta*.

**MATERIALS AND METHODS**

**Test materials.** This study was carried out to evaluate the efficacy of emulsifiable concentrate (EC) formulations of two entomopathogenic fungi (EPF), *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) and *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes), and their combinations with SO (0.5%) in controlling *T. absoluta* under greenhouse conditions. The details of the test materials and their application doses are shown in Table 1.

**Study area.** The study was carried out in a plastic covered greenhouse (0.2 ha), located at Pinari (36°, 55' N; 30°, 48' E, altitude 39 m), near Antalya, Turkey for two consecutive growing seasons (fall-winter-2014 and spring-2015). Tomato seedlings were planted in greenhouse for both growing seasons.

**Spray applications.** In each growing season, only one trial was conducted for both fungal products and their combinations with SO. The tomato plants in the greenhouse were grouped in rows for treatments and the rows exposed to the treatments were separated from each other by guard rows. The treatments were applied in a completely randomized block design with 4 replications consisting of 60 plants in each plot. The treatments were designed as in Table 2.

Treatments were applied during larval population was at a peak level (towards the end of each growing season). The products were applied as dilute sprays (delivered in 1000 l of water/ha). Applications in both growing seasons were done by using motorized sprayer (Tarak® TR 100 E, Tarak Agricultural Equipment and Machinery Inc., Istanbul, Turkey), with a spray pressure of 14 bars (= 203.05 psi).

**Data collection and analysis.** Efficacy of the treatments was evaluated by counting eggs and larvae of the pest (live or dead) from leaf and fruit samples, taken periodically. Sampling times were 1 day before and 3, 7, 14 and 21 days after each application. At each sampling date, 40 leaves with larvae or eggs and 40 fruits with larvae (10 per plot) were collected from each treatment and examined under a stereo-microscope (zoom range: 0.63x - 15.75x) in the laboratory. During the data collection, infected (unhatched) eggs and larvae that had conspicuously shrunk and turned dark-brown due to fungal infection and could be easily distinguished from the living ones counted as dead.

Percent mortality rates obtained from each application were converted into Abbott’s corrected mortalities [11] and subjected to analysis of variance (ANOVA). Significant differences among the treatment means were separated using the Duncan’s multiple range test (DMRT), and a probability (*P*) of ≤ 0.05 was accepted as statistically significant [12].

**TABLE 1**

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Active substance</th>
<th>Company</th>
<th>Formulation</th>
<th>Dose (ml/100 l water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BroadBand®</td>
<td><em>Beauveria bassiana</em> strain PPRI 5339 (min. 4x10⁹ conidia/ml)</td>
<td>Backer Underwood</td>
<td>Emulsifiable concentrate (EC)</td>
<td>100, 150, 200</td>
</tr>
<tr>
<td>Met52®</td>
<td><em>Metarhizium anisopliae</em> strain F52 (min. 5.5x10⁹ conidia/ml)</td>
<td>Novozymes Biologicals</td>
<td>EC</td>
<td>75, 100, 125</td>
</tr>
<tr>
<td>Porkan®</td>
<td>Summer oil (700 g/l)</td>
<td>Syngenta</td>
<td>Liquid</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**TABLE 2**

Spray treatments tested in two successive growing periods (fall-winter-2014 and spring-2015) during the study

<table>
<thead>
<tr>
<th>Fungal products</th>
<th>Their combination with summer oil (SO)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BroadBand/100</td>
<td>BroadBand/100 + SO</td>
</tr>
<tr>
<td>BroadBand/150</td>
<td>BroadBand/150 + SO</td>
</tr>
<tr>
<td>BroadBand/200</td>
<td>BroadBand/200 + SO</td>
</tr>
<tr>
<td>Met52/75</td>
<td>Met52/75 + SO</td>
</tr>
<tr>
<td>Met52/100</td>
<td>Met52/100 + SO</td>
</tr>
<tr>
<td>Met52/125</td>
<td>Met52/125 + SO</td>
</tr>
<tr>
<td>Untreated control</td>
<td></td>
</tr>
</tbody>
</table>

*Summer oil was used at a rate of 0.5 l/100 l water at all the combinations.
RESULTS

The results of this study showed that egg and larval mortality rates were increased by increasing duration and dose, at each sampling date in both growing seasons. Significant differences were observed among the different conidial concentrations of both EPF products and their combinations with SO (Tables 3 and 6).

Compared to the untreated control, both EPF products alone and their combinations with SO provided highly significant control of eggs and larvae of the pest ($P \leq 0.05$). However, combined applications of these two EPF products were more effective than each of EPF product application alone for controlling both the egg and larval stages of the pest in both growing seasons. For instance, the single usage of the two EPF products, *B. bassiana* and *M. anisopliae*, caused 79.8% and 70.2% with the highest mean mortalities against the eggs in the first growing season, while their combinations with SO caused 86.4% and 74.8% the highest mean mortalities against the same biological stage of the pest, respectively (Table 3).

### TABLE 3
Mean percent mortalities of Tomato moth eggs 3 (A+3), 7 (A+7), 14 (A+14) and 21 (A+21) days after the application at the fall-growing season of 2014

<table>
<thead>
<tr>
<th>Treatments and Doses (ml/100 l water)</th>
<th>Mean percent mortality (±S.E.) after application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A+3</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>12.5</td>
</tr>
<tr>
<td>150</td>
<td>23.8</td>
</tr>
<tr>
<td>200</td>
<td>27.2</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em> + Summer oil</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>14.3</td>
</tr>
<tr>
<td>150</td>
<td>26.3</td>
</tr>
<tr>
<td>200</td>
<td>29.2</td>
</tr>
<tr>
<td><em>Metarhizium anisopliae</em></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>9.6</td>
</tr>
<tr>
<td>100</td>
<td>14.8</td>
</tr>
<tr>
<td>125</td>
<td>16.2</td>
</tr>
<tr>
<td><em>Metarhizium anisopliae</em> + Summer oil</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>11.2</td>
</tr>
<tr>
<td>100</td>
<td>16.3</td>
</tr>
<tr>
<td>125</td>
<td>21.3</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0</td>
</tr>
</tbody>
</table>

*: Means within a column followed by the same capital letter are not significantly different (DMRT, $P \leq 0.05$).

**: Means within a row followed by the same lower-case letter are not significantly different (DMRT, $P \leq 0.05$).

### TABLE 4
Mean percent mortalities of Tomato moth larvae 3 (A+3), 7 (A+7), 14 (A+14) and 21 (A+21) days after the application at the fall-growing season of 2014

<table>
<thead>
<tr>
<th>Treatments and Doses (ml/100 l water)</th>
<th>Mean percent mortality (±S.E.) after application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A+3</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>9.2</td>
</tr>
<tr>
<td>150</td>
<td>14.8</td>
</tr>
<tr>
<td>200</td>
<td>22.7</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em> + Summer oil</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>11.5</td>
</tr>
<tr>
<td>150</td>
<td>17.8</td>
</tr>
<tr>
<td>200</td>
<td>24.2</td>
</tr>
<tr>
<td><em>Metarhizium anisopliae</em></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>13.9</td>
</tr>
<tr>
<td>100</td>
<td>21.8</td>
</tr>
<tr>
<td>125</td>
<td>32.1</td>
</tr>
<tr>
<td><em>Metarhizium anisopliae</em> + Summer oil</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>16.2</td>
</tr>
<tr>
<td>100</td>
<td>24.2</td>
</tr>
<tr>
<td>125</td>
<td>36.2</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0</td>
</tr>
</tbody>
</table>

*: Means within a column followed by the same capital letter are not significantly different (DMRT, $P \leq 0.05$).

**: Means within a row followed by the same lower-case letter are not significantly different (DMRT, $P \leq 0.05$).
TABLE 5
Mean percent mortalities of Tomato moth eggs 3 (A+3), 7 (A+7), 14 (A+14) and 21 (A+21) days after the application at the spring-growing season of 2015

<table>
<thead>
<tr>
<th>Treatments and Doses (ml/100 l water)</th>
<th>Mean percent mortality (±S.E.) after application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A+3</td>
</tr>
<tr>
<td>Beauveria bassiana</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>9.3 B*a**</td>
</tr>
<tr>
<td>150</td>
<td>14.2 Ca</td>
</tr>
<tr>
<td>200</td>
<td>23.2 Ca</td>
</tr>
<tr>
<td>Beauveria bassiana + Summer oil</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>13.5 Ba</td>
</tr>
<tr>
<td>150</td>
<td>21.3 Ca</td>
</tr>
<tr>
<td>200</td>
<td>24.2 Ca</td>
</tr>
<tr>
<td>Metarhizium anisopliae</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>7.7 Ba</td>
</tr>
<tr>
<td>100</td>
<td>10.8 Ba</td>
</tr>
<tr>
<td>125</td>
<td>15.2 Ba</td>
</tr>
<tr>
<td>Metarhizium anisopliae + Summer oil</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>8.9 Ba</td>
</tr>
<tr>
<td>100</td>
<td>12.8 Ba</td>
</tr>
<tr>
<td>125</td>
<td>15.6 Ba</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0 Aa</td>
</tr>
</tbody>
</table>

*: Means within a column followed by the same capital letter are not significantly different (DMRT, P ≤ 0.05).
**: Means within a row followed by the same lower-case letter are not significantly different (DMRT, P ≤ 0.05).

B. bassiana + SO combination was particularly effective against eggs, causing 86.4% and 79.7% mortalities in the highest dose in 2014 and 2015, respectively (Tables 3 and 5).

M. anisopliae + SO combination was more effective against larvae of the pest, causing 83.2% and 81.4% mortalities in the highest dose in 2014 and 2015, respectively (Tables 4 and 6).

TABLE 6
Mean percent mortalities of Tomato moth larvae 3 (A+3), 7 (A+7), 14 (A+14) and 21 (A+21) days after the application at the spring-growing season of 2015

<table>
<thead>
<tr>
<th>Treatments and Doses (ml/100 l water)</th>
<th>Mean percent mortality (±S.E.) after application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A+3</td>
</tr>
<tr>
<td>Beauveria bassiana</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>7.6 B*a**</td>
</tr>
<tr>
<td>150</td>
<td>11.2 Ca</td>
</tr>
<tr>
<td>200</td>
<td>14.2 Ca</td>
</tr>
<tr>
<td>Beauveria bassiana + Summer oil</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>10.0 Ba</td>
</tr>
<tr>
<td>150</td>
<td>14.6 Ca</td>
</tr>
<tr>
<td>200</td>
<td>22.2 Ca</td>
</tr>
<tr>
<td>Metarhizium anisopliae</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>10.6 Ba</td>
</tr>
<tr>
<td>100</td>
<td>12.1 Ba</td>
</tr>
<tr>
<td>125</td>
<td>22.6 Ba</td>
</tr>
<tr>
<td>Metarhizium anisopliae + Summer oil</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>13.1 Ba</td>
</tr>
<tr>
<td>100</td>
<td>19.8 Ba</td>
</tr>
<tr>
<td>125</td>
<td>26.7 Ba</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0 Aa</td>
</tr>
</tbody>
</table>

*: Means within a column followed by the same capital letter are not significantly different (DMRT, P ≤ 0.05).
**: Means within a row followed by the same lower-case letter are not significantly different (DMRT, P ≤ 0.05).

DISCUSSION AND CONCLUSION

The results of this study revealed that both EPF products and their combination with SO at their highest doses and longer periods gave good results in the control of eggs and larvae of the pest, but the compatible combinations of two EPF products and summer oil were better than their single...
usage. These findings are compatible with those obtained in an earlier study which indicated that adding SO to sprays may be useful in pest management programs by providing a supplementary effect [13]. In addition, it was observed that the combination of EPF products and SO caused adults not to fly and eventually to die because of their wings covered with the oil. Another supplementary effect of adding oil to sprays was that the oil exhibited oviposition deterrent activity against adult females of *T. absoluta*. Oviposition deterrence of oils was also reported by some researchers against numerous pests [14, 15 and 16]. Erlers and Tosun [16] and Erler [15] reported that the mechanism of oviposition deterrence of oils or oily substances is based on the formation of an oily surface. The observations of this study on *T. absoluta* adults suggest that it was difficult for roaming on oily plant surfaces, so the pest preferred untreated parts of plants. Zwick and Westigard [17] and Larew [14] observed the same behavior in different insect pests.

Literature indicates that many EPFs have already been tested for the management of *T. absoluta*. For example, in a study by Shalaby et al. [18] *B. bassiana* and *M. anisopliae* were tested for their efficacy on *T. absoluta* larvae (Neonate “newly hatched”, 2nd and 3rd instars) and eggs under laboratory conditions. Their results showed that the estimated LC50 values of *B. bassiana* and *M. anisopliae* were 0.28 x 10⁶ and 0.11 x 10⁶, 0.45 x 10⁶ and 0.46 x 10⁶, and 0.32 x 10⁶ and 0.27 x 10⁶ conidia/ml for neonate, 2nd instar and 3rd instar *T. absoluta* larvae, respectively [18]. They also reported that *T. absoluta* eggs had a black appearance after the exposure to these two EPFs 5 days after exposure, and no hatching was observed at higher concentrations of both fungi compared to the control where the hatchability was 87.7%. Their results on egg mortality also indicated that *B. bassiana* was more effective against the eggs than *M. anisopliae* at lower conidial concentrations [18]. Similarly, egg mortalities caused by *B. bassiana* were higher than those caused by *M. anisopliae* in the present study. In another study, Tadele and Emana [19] evaluated the effect of three different concentrations of a local isolate of both *B. bassiana* and *M. anisopliae* against larvae of *T. absoluta* using the concentrations of 2.5 x 10⁶, 2.5 x 10⁵, and 2.5 x 10⁴ conidia ml⁻¹ under laboratory and greenhouse conditions and reported that mortalities caused by *B. bassiana* isolate at the different concentrations ranged from 79.17% to 95.83% in the laboratory and 73.0% to 84.04% in the greenhouse, and the highest mortality rate was observed at 2.5 x 10⁹ conidia ml⁻¹. The same as *B. bassiana* isolate, the *M. anisopliae* isolate also caused the highest mortality at the highest concentration. Similar results on larval mortality were found in the present study.

The other important point was that the EPF products tested had different efficacy rates against different larval stages of the pest. Although it is not given separately in this research, it was observed that the young (1st and 2nd instars) larvae are more susceptible to the EPF products than the older (3rd and 4th instars) larvae. That’s why, such applications should be made at a time when the majority of *T. absoluta* population are at a susceptible stage of development.

Based on the results of this study, it was concluded that both EPF products and their combination with SO have remarkable potential in the control of *T. absoluta* in greenhouses by reducing its egg, larval and adult populations as well. Furthermore, they appear to be safer than synthetic insecticides used for the control of *T. absoluta* and these results are promising for further studies.

REFERENCES

IDENTIFICATION OF MALTING BARLEY (HORDEUM VULGARE L.) GENOTYPES TOLERANT TO WATER STRESS

Ali Beyhan Ucak*, Burak Saltuk

Siirt University, Faculty of Agriculture, Department of Biosystem Engineering, Siirt, Turkey

ABSTRACT

The present research was carried out to determine water-stress tolerance of malting barley genotypes (Durusu, Firat, Atilir) grown under (winter planting), different water conditions [no water-stress (I00); strong water-stress (I0)] in the years 2016 and 2017. Variance analyses revealed significant differences between the genotypes (P<0.01). As the average of two years, the greatest yield was obtained from no water-stress x genotype interaction (I00xDurusu) with 5613.50 kg ha⁻¹, the lowest yield was obtained from strong water stress x genotype interaction (I0xAtilir) with 3848.82 kg ha⁻¹. Again as the average of two years, the greatest chlorophyll content was obtained from no water-stress x genotype interaction (I00xDurusu) with 45.23 spad, the lowest value was obtained from strong water stress x genotype interaction (I0xAtilir) with 35.80 spad. The greatest crop water stress index was obtained from strong water stress x genotype interaction (I0xAtilir) with 0.47, the lowest value was obtained from no water-stress x genotype interaction (I00xDurusu) with 0.24. The lowest protein content was obtained from no water-stress x genotype interaction (I00xDurusu) with 9.36 %, the greatest value was obtained from strong water stress x genotype interaction (I0xAtilir) with 12.98 %. The Durusu genotype with optimum water use efficiency and prominent with crop water stress index, chlorophyll, chlorophyll content and low protein content both in no water-stress and strong water stress treatments was identified as water stress-resistant and the genotype was considered to have reliable characteristics potentially to be used in further water stress-resistance studies. On the other hand Atilir (malting barley) genotype was identified as water stress-sensitive. As a result decreased protein content and total protein content in plants under no water-stress treatments.

KEYWORDS: Malting Barley, Drought, Protein content, Chlorophyll, Crop water stress index

INTRODUCTION

Barley is the third most important food legume after wheat in the world. It has considerable importance as a food, feed and fodder. Due to protein content, it has become an important component of human diet in developing world. Annual barley producing of Turkey is around 7.1 million tons. Turkey is one of the major barley producing countries [1]. Especially in malting barley, it is desirable to have at most 10% protein content in terms of quality. Although quality is very important in malting barley, it is always underrated.

Plants have been subjected to different stress conditions and developed proper defense mechanisms to survive and reproduce under stress conditions. However, today agronomists and plant breeders are focused on yields rather than survival of the plants. Breeding programs are mostly implemented to develop high-yield cultivars. However, recent global warming-induced abiotic stressors have negatively influenced agricultural production activities and such impacts compelled the researchers to take new measures against the negative impacts of climate change and resultant global warming. Among the abiotic stressors, water stress, insufficient nutrition, salinity and high temperature are the leading ones [2]. Recession in plant growth due to deficit moisture within the plant efficient root zone (through the soil profile of 0-90 cm) is defined as water stress. The initial symptoms of water stress realize at stomatal level and stomas close to prevent further moisture loss through transpiration [3]. Stomal closure reduces CO₂ availability in chloroplasts and negatively influences net photosynthesis rates [4]. Water stress is exerted on plant tissues under drought stress and this reduces photosynthesis rates significantly [5]. Neither the soil moisture content nor the atmospheric system can accurately put forth plant inherent water status as much as crop water stress index [6, 7]. Reginato [8] indicated that daily crop water stress index values varied based on atmospheric demands and soil moisture contents. Water stress is experienced when the plant cover temperature was equal or greater than the air temperature [9]. Canopy-air temperature difference (Tc-Ta) is a significant indicator of water stress [10]. Choudhury and Idso [11] carried out a water stress study on sunflower
and reported significant effects of air and dew temperatures on plant cover temperature under high soil moisture conditions. Plant resistance to droughts and water stress are the primary target of plant breeders. For plant, leaf canopy temperatures are the most significant parameters in measuring plant tolerance to water stress under stress conditions [12]. Moroni et al. [13] indicated the canopy (leaf-canopy) temperature as the fastest and the most accurate means of measuring water stress and pointed out that this parameter could be used as a selection criterion in breeding studies. Crop water stress index values vary based on plant genotypes, cultivars, environmental and climate conditions [14]. Water stress is among the most important factors restricting plant production activities and may result in significant changes in chlorophyll content and components through hindering photosynthetic activity in plants [15]. The parameters to be used in identification of drought or water stress may result in significant changes in chlorophyll content and components through hindering photosynthetic activity in plants [15]. The parameters to be used in identification of drought or water stress should be easy, rapid, cheap and repeatable [16, 13]. Oraki et al. [17] reported increased chlorophyll b levels, decreased chlorophyll a and yield levels with increasing water stress levels. Despite very the studies about drought (water stress) tolerance of wheat plants [18], the studies about plant responds to water stress in malting barley are quite limited. For malting barley, efficient selection criteria to be used in distinguishing potential status of the plants against water stress haven't been fully elucidated, yet. That is why in present study (2016-2017), 2 different irrigation treatments (I100, I0) were employed. The present study was conducted under field conditions in 2016 and 2017 to determine water stress resistance of 3 malting barley genotypes (Durusu, Firat, Attilir) grown under strong water-stress and no water-stress conditions by using kernel yield, crop water stress index and chlorophyll content values.

MATERIALS AND METHODS

Experiments were conducted under natural field conditions since it is quite hard to transfer the results of the studies carried out under controlled conditions like greenhouses or growth chambers into the practice. Sowing was performed late on 29th of November. Experiments were carried out over the experimental fields (Saipbeyli village in farmer conditions) of Siirt Province during the malting barley growing seasons of 2016 and 2017. The research site has an altitude of 894 m and is located on 37° 58’ N and 41° 50’ E. Durusu, Firat, Attilir malting barley genotypes were used as the plant material of the study. Long-term and annual climate data of the research site (during malting barley growing seasons) are provided in Table 1.

<table>
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<th>Years</th>
<th>Months</th>
<th>Mean maximum temperature (°C)</th>
<th>Mean temperature (°C)</th>
<th>Mean minimum temperature (°C)</th>
<th>Mean humidity (%)</th>
<th>Mean wind speed (m s⁻¹)</th>
<th>Mean daily sunshine (h)</th>
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<td>1.00</td>
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</tr>
</tbody>
</table>
Soil samples were taken before sowing from 0-90 cm soil profile (from three depth segments as 0-30, 30-60 and 60-90 cm). Soil moisture content at field capacity (33 kPa) was determined in accordance with Klute [19] and bulk density with Blake and Hartge [20]. Disturbed samples were subjected to organic matter, texture and permanent wilting point analyses. Water holding capacity at permanent wilting point (1500 kPa) was determined in accordance with Klute [19]. Soil physico-chemical characteristics are provided in Table 2.

**TABLE 2**

Some physical and chemical soil characteristics of the research site

<table>
<thead>
<tr>
<th>Properties</th>
<th>Soil layer (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-30</td>
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<tr>
<td>Clay (%)</td>
<td>62.00</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>20.00</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>18.00</td>
</tr>
<tr>
<td>Texture</td>
<td>Clay Clay Clay</td>
</tr>
<tr>
<td>Field capacity (Pfc)</td>
<td>33.52</td>
</tr>
<tr>
<td>Permanent wilting point (Pwp)</td>
<td>24.44</td>
</tr>
<tr>
<td>Bulk density (g cm⁻³)</td>
<td>1.42</td>
</tr>
<tr>
<td>pH (1.25 sw⁻¹)</td>
<td>7.50</td>
</tr>
<tr>
<td>Electrical conductivity (dS m⁻¹)</td>
<td>1.55</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>3.09</td>
</tr>
<tr>
<td>CaCO₃ (%)</td>
<td>6.40</td>
</tr>
</tbody>
</table>

Experimental soils were classified as brown forest soil with low electrical conductivity and salinity, low phosphorus content, high potassium content and medium level organic matter content and lime levels were not posing any problems for plant growth.

Irrigation water quality parameters were determined in accordance with the method specified by Tuzuner [21]. Irrigation water quality class was C₂S₁ with an average EC value of 0.34 dS m⁻¹ and a pH value of 7.21. Experiments were conducted in randomized blocks-split plots experimental design (4 L h⁻¹ drippers) was placed along each plant row. Soil infiltration rate was measured as 7 mm h⁻¹. Deep percolation and surface runoff were not considered. Each plot has a size of 6x1.8 m (10.8 m²). A buffer zone of 2 m was placed between the experimental plots as to prevent interactions.

All of the phosphorus fertilizer (pure 90 kg ha⁻¹ P₂O₅) and nitrogen (280 kg ha⁻¹ N) were supplied at sowing.

Gravimetric moisture content of each layer (0-30, 30-60 and 60-90) was converted into depth with Equation 1.

\[
d = \frac{(P_{wfc} - P_{waw}) \times A \times D}{100}
\]

Where; \(d\) is soil moisture content in depth (mm); \(P_{wfc}\) is field capacity (%); \(P_{waw}\) is moisture content of each layer (%); \(A\) is bulk density (g cm⁻³); \(D\) is layer depth (mm). Volume of water to be applied was calculated by using the following Equation 2.

\[
d_{f(0-90)} = d_{(0-30)} + d_{(30-60)} + d_{(60-90)}
\]

Where; \(d_{f(0-90)}\) is soil moisture at 0-90 cm soil profile (mm); \(d_{(0-30)}\) is soil moisture at 0-30 cm soil profile (mm); \(d_{(30-60)}\) is soil moisture at 30-60 cm soil profile (mm); \(d_{(60-90)}\) is soil moisture at 60-90 cm soil profile (mm).

Volume of water to be applied to each plot was calculated by Equation 3.

\[
V = d_f \times A \times U_0 \times P
\]

Where; \(V\) is volume of water to be applied (L); \(A\) is plot size (m²); \(U_0\) is deficit ratio (%) and \(P\) is cover ratio (%).

Plant canopy width was divided by row spacing to get cover ratios (CR). The ratio was taken as 0.30 and 0.80 for cover ratios of 30% and 80%. The principles specified in Sahin et al [22] were employed to find out the amount of water to be used in each plot.

Water budget method was used to calculate monthly and seasonal evapotranspiration values Sahin et al [22]. Water use efficiency (WUE) values were calculated by using Equation 4 [23].

\[
WUE = \frac{Y}{ET_o}
\]

Where; \(WUE\) is water use efficiency (kg da mm⁻¹); \(Y\) is yield; \(ET_o\) is evapotranspiration (mm).

Plant water consumptions were calculated by using Equation 5 [22].

\[
ET_c = P + I - R_s - D_p \pm \Delta S
\]

Where; \(ET_c\) is evapotranspiration (mm); \(P\) is precipitation (mm); \(I\) is amount of irrigation water (mm); \(R_s\) is surface flow (mm); \(D_p\) is deep percolation (mm); \(\Delta S\) is the change in soil moisture (mm).

Change in CWSI and CC values of Durusu, Firat, Atilir malted barley genotypes grown under \(I_{100}\) and \(I_0\) irrigation treatments were determined in one week intervals. CWSI and CC measurements were performed along the diagonals of each plot in four corners in three replications from the leaves close to head.

CWSI values were calculated by using Equation 6 as recommended by Idso [24].
\[
CWSI = \frac{(T_c - T_a) - LL}{UL - LL}
\]

Where; \(CWSI\) is crop water stress index; \(T_c\) is canopy temperature (°C); \(T_a\) is air temperature (°C); \(LL\) is lower limit of water stress; \(UL\) is upper limit of water stress.

The lower limit (LL) at which plants did not experience any water stresses was calculated by the equation provided by Idso [24] and using regression analyses between canopy-air temperature and vapor pressure deficit (VPD, kPa) (Equation 7);

\[
T_c - T_a = (a - b) \times VPD
\]

Where; \(a\) is intermediate section value (°C); \(b\) is slope of the line (kPa °C-1); \(VPD\) is vapor pressure deficit (kPa).

Vapor pressure deficit was calculated with basic psychrometric equations [25]. These equations are provided below;

\[
e_w = 0.61078 \exp \left( \frac{17.27T_w}{237.3 + T_w} \right)
\]

\[
e_a = e_w - [AP \times (T_a - T_w)]
\]

Where; \(e_w\) is saturated vapor pressure at wet-bulb temperature (kPa); \(e_a\) is actual vapor pressure at air temperature (kPa); \(T_w\) is wet-bulb temperature (°C); \(A\) is psychrometric constant (kPa °C-1); \(P\) is barometric pressure (kPa).

Psychrometric constant \((A)\) was calculated from the following equation;

\[
A = \left[ 0.00066(1 + 0.00115100066.0) \right]
\]

Saturated vapor pressure was calculated by using the following equation;

\[
e_a \times T_a = 0.61078 \exp \left( \frac{17.27T_a}{237.3 + T_a} \right)
\]

Vapor pressure deficit (VPD) was calculated as the difference of saturated vapor pressure at dry-bulb temperature from the actual vapor pressure at the same temperature;

\[
VPD = \left[ e_a \times T_a \right] - e_a
\]

Where; \(e_a \times T_a\) is saturated vapor pressure at dry-bulb temperature (kPa).

The upper limit (UL) at which plants experienced full-water stress was calculated by using the equations recommended by Idso et al [26];

\[
T_c - T_a = (a - b) \times VPG
\]

\[
VPG = \left[ e_a \times T_a \right] - e_a \times (T_a + a)
\]

Where; \(a\) and \(b\) are lower limits (LL) at which there are no water stress; \(VPG\) is slope of negative atmospheric vapor pressure required for the training of zero canopy-air vapor pressure.

Chlorophyll content (CC) of the genotypes was measured with a portable chlorophyll meter. Measurements were initiated when the plant cover ratio of the plots reached to 80% and performed throughout the growing season before and after the irrigations from the same plant and same leaves. Measurements were performed in days with clear sky and between 1200-1400 hours when the change in sun-ray angles the least. Chlorophyll-meter measurements were taken from the leaves just beneath the malting barley head, the device was oriented over the leaf as not to create a shade over it and 3 subsequent measurements (a total of 12 readings) were taken along the diagonal of the plot. CC increases as the value approaches to 1 and decreases as the value approaches to 0. Dried samples were ground in a 1 mm diameter mill and nitrogen assays were performed by using the Kjeldahl method to determine raw protein ratios (N x 6.25). Harvest was performed when the seed moisture content decreased to 12% to determine the yields. Side rows and 0.5 m space at top and bottom of inner two rows were omitted as to consider side effects.

Analysis of variance (ANOVA) was performed in accordance with randomized blocks-split plots experimental design. Significant treatments were then subjected to LSD (Least Significant Difference) multiple comparison tests. Correlation analyses were carried out to identify the relationships between the traits. The directions of the relationships (positive or negative) were determined. Analyses were carried out with JUMP 5.0.1a statistical software [27].

RESULTS AND DISCUSSION

Four irrigations were performed in all irrigation treatments. Irrigation water applied in 2016 and 2017 was measured as 182.31 and 172.21 mm in no water-stress treatments. Seasonal plant water consumptions varied between 373.43-362.33 mm in no water-stress treatments (Table 3). Higher ET, values of strong water-stress treatments were because plants continued to benefit from the residual moisture in soil from the winter precipitations even after termination of irrigations. Water consumptions of the same plant genotypes may vary based on climate and regions and such values may even vary within the same region. Relevant differences might be due to the differences in plant genotypes, climate parameters, soil properties, method of irrigation and irrigation schedules.

The variations in yield and physiological characteristics of Durusu, Firat, Atilir genotypes with irrigation water quantities are provided in Table 3, correlation coefficients between yield and other parameters are provided in Table 4. Significant differences were observed in yield, CWSI, CC, Protein content and WUE values of the genotypes (P<0.01) and such differences were then subjected to LSD test (grouping) (Table 3). In the first year of experiments, the greatest yield in strong water-stress treatments (4967.33 kg ha-1) was obtained from I0xDurusu interaction with a low CWSI (0.36)
and CC (38.13 spad) value and the lowest yield (3854.32 kg ha⁻¹) was obtained from IoxAttilir interaction. The highest yield in a no-water-stress treatments (5621.00 kg ha⁻¹) of the first year was obtained from I100xDurusu interaction with a low CWSI (0.23) and a high CC (45.40 spad) value and the lowest yield (4258.11 kg ha⁻¹) was obtained from IoxAttilir interaction with a high CWSI (0.48) and a low CC (41.70 spad) value. Genotypes had also significant impacts on yields (P<0.01). The greatest yield (5294.16 kg ha⁻¹) was obtained from Durusu genotype and the lowest yield (4069.66 kg ha⁻¹) was obtained from Attilir genotype. In the second year of experiments, the highest yield (4956.66 kg ha⁻¹) in strong water-stress treatments was obtained from IoxDurusu interaction with a low CWSI (0.37) and a high CC (37.36 spad) value and the lowest yield (3843.33 kg ha⁻¹) was obtained from IoxAttilir interaction with a high CWSI (0.48) and a low CC (35.30) value. The greatest yield (5606.66 kg ha⁻¹) in no-water-stress treatments of the second year was obtained from I100xDurusu interaction with a low CWSI (0.25) and a high CC (45.06 spad) value and the lowest yield (4273.33 kg ha⁻¹) was obtained from I100xAtilir interaction with a high CWSI (0.30) and a low CC (40.20) value. Variance analyses revealed that genotypes had significant effects on yields also in the second year of the experiment (P<0.01). Similar to the first year, the greatest yield (5281.66 kg ha⁻¹) was observed in Durusu genotype and the least (4058.33 kg ha⁻¹) in Attilir genotype. The other genotypes (Firat) was placed in between these two genotypes in both years. As to conclude, significant interactions were observed between irrigation treatments and genotypes. Complying with the present findings, Kassab et al. [28] also reported significant interactions between irrigation treatments and genotypes. Water deficits in flowering period may cause considerable yield losses [29]. In addition, Afkari [30], Kassab et al. [28] showed that water deficits significantly reduced plant heights, number of seeds per head, leaf area index and leaf relative water content of plant. Current findings comply with the results of Ali and Shui [29], Afkari [30] and Kassab et al. [28]. However, Alahdadi et al. [31] reported substantial yield losses at short-term water deficits. Moisture deficiencies may negatively influence plant regeneration since plant is quite sensitive to drought stress during pollination period [32]. Zeaifizade and Goliov [33] showed that deficit moisture levels from budding to the end of flowering had devastating impacts on yields. In addition, Chimenti et al. [34] indicated flowering and seed maturity stages as the sensitive stages of plants to water-stress. Current results are in line with the findings of [32, 33, 34]. Darvishzadeh et al. [35] carried out a selection study for water stress resistance of genotypes and reported that relevant genotypes exhibited similar performances both under water stress conditions and optimum conditions. Therefore in present study, the genotype Durusu with similar yield performance under both strong water-stress and no water-stress conditions were found to be prominent. Then, it was determined that this genotype could be used in studies to be carried out for the resistance or tolerance of malting barley genotypes to water stress and other abiotic stress factors. 

In the first year of experiments, the greatest CC (38.13 spad) in strong water-stress treatments was obtained from IoxDurusu interaction and the lowest value (36.30 spad) was obtained from IoxAttilir interaction. The greatest CC (45.40 spad) in no-water-stress treatments of the first year was obtained from I100xDurusu interaction and the lowest value (41.70 spad) was obtained from I100xAtilir interaction. Variance analyses revealed that genotypes had also significant effects on CC values. The greatest CC (41.76) was observed in Durusu genotype and the lowest value (39.00 spad) was observed in Attilir genotype. In the second year of experiments, the greatest CC (37.36 spad) in strong water-stress treatments was seen in IoxDurusu interaction and the lowest value (35.30) was observed in IoxAtilir interaction. In no-water-stress treatments of the second year, the greatest CC (45.06 spad) was seen in I100xDurusu interaction and the lowest value (40.20) was observed in I100xAtilir interaction. Variance analyses revealed also for the second year that genotypes had significant effects on CC values with the greatest value (41.21) in Durusu genotype and the lowest value (37.75 spad) in Attilir genotype. The decrease in CC values was low in drought-resistant genotypes and high in sensitive genotypes (Table 3). Plants have different resistances to stress conditions [36]. Robert et al [37] reported decreased chlorophyll a, b and total chlorophyll contents in plants under water stress. Several other researchers also reported decreased leaf chlorophyll contents under water stress conditions [38, 39]. It was also reported in previous studies that CC values might vary based on plant genotypes, cultivars, environmental and climate conditions [14]. Present findings comply with those earlier results. 

In the first year of experiments, the greatest CWSI (0.46) in strong water-stress treatments was seen in IoxAttilir interaction and the lowest (0.36) was observed in IoxDurusu interaction. In no water-stress treatments of the first year, the greatest CWSI (0.28) was seen in I100xAtilir interaction and the lowest (0.23) was observed in I100xDurusu interaction. Variance analyses revealed that genotypes also had significant effects on CWSI values with the greatest value (0.37) in Attilir genotype and the lowest value (0.29) in Durusu genotype. In the second of experiments, the greatest CWSI (0.48) in strong water-stress treatments was observed in
IoxAtilir interaction and the lowest value (0.37) was seen in IoxDurusu interaction. In no water-stress treatments of the second year, the greatest CWSI (0.30) was observed in IoxAtilir interaction and the lowest value (0.25) was seen in IoxDurusu interaction. Variance analyses again revealed that genotypes had significant effects on CWSI values with the greatest value (0.39) in Atilir genotype and the lowest value (0.31) in Durusu genotype. CWSI values of the second year were relatively higher than the CWSI values of the first year (Table 3). Drier conditions of the second year as compared to the first year increased evapotranspiration, thus CWSI values were found to be higher in the second year. Decreased CC and higher CWSI values were reported for water stress treatments [40]. Thusly, Khayatnezhad et al. [41] reported decreased chlorophyll contents and then reduced yields with water stress treatments in plants. Similarly, Kirmak and Gencoglan [42] and Ucak and Bagdatli [36] reported that there was a significant difference in plant growth, some plant physiological parameters, crop water stress index, chlorophyll contents, protein content and yield between irrigated and non-irrigated treatments. Current findings comply with those earlier findings. Durusu with high yield, CC and low CWSI and protein content values were identified as resistant and the others were identified as sensitive.

### TABLE 3

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (kg ha⁻¹)**</th>
<th>CWSI**</th>
<th>Chlorophyll content (spad)**</th>
<th>Irrigation water (mm)</th>
<th>ETA (mm)</th>
<th>Protein content (%)***</th>
<th>WUE (kg da⁻¹, mm)***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2016 (First year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irrigation treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I₁₀₀ (FT)</td>
<td>4911.22 a</td>
<td>0.41 a</td>
<td>43.22 a</td>
<td>182.31</td>
<td>373.43</td>
<td>9.86 b</td>
<td>1.31 b</td>
</tr>
<tr>
<td>I₁₀ (DI)</td>
<td>4326.44 b</td>
<td>0.25 b</td>
<td>37.28 b</td>
<td>0.00</td>
<td>211.50</td>
<td>11.90 a</td>
<td>2.04 a</td>
</tr>
<tr>
<td>Average</td>
<td>4618.83</td>
<td>0.33</td>
<td>40.25</td>
<td>91.15</td>
<td>292.46</td>
<td>10.88</td>
<td>1.68</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>14.0</td>
<td>0.013</td>
<td>0.58</td>
<td></td>
<td></td>
<td>0.24</td>
<td>0.065</td>
</tr>
<tr>
<td>Varieties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Durusu</td>
<td>5294.16 a</td>
<td>0.29 c</td>
<td>41.76 a</td>
<td>182.31</td>
<td>373.43</td>
<td>10.35 c</td>
<td>1.41 a</td>
</tr>
<tr>
<td>Fırat</td>
<td>4492.66 b</td>
<td>0.34 b</td>
<td>40.00 b</td>
<td>182.31</td>
<td>373.43</td>
<td>10.87 b</td>
<td>1.20 b</td>
</tr>
<tr>
<td>Atilir</td>
<td>4069.66 c</td>
<td>0.37 a</td>
<td>39.00 c</td>
<td>182.31</td>
<td>373.43</td>
<td>10.33 c</td>
<td>1.08 c</td>
</tr>
<tr>
<td>Average</td>
<td>4618.83</td>
<td>0.33</td>
<td>40.25</td>
<td>182.31</td>
<td>373.43</td>
<td>10.52</td>
<td>1.23</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>85.25</td>
<td>0.014</td>
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<td></td>
<td>0.45</td>
<td>0.037</td>
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<tr>
<td></td>
<td>2017 (Second year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irrigation treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I₁₀₀ (FT)</td>
<td>4988.88 a</td>
<td>0.27 b</td>
<td>42.17 a</td>
<td>172.21</td>
<td>362.33</td>
<td>10.02 b</td>
<td>1.35 b</td>
</tr>
<tr>
<td>I₁₀ (DI)</td>
<td>4315.55 b</td>
<td>0.43 a</td>
<td>36.36 b</td>
<td>0.00</td>
<td>201.50</td>
<td>12.06 a</td>
<td>2.14 a</td>
</tr>
<tr>
<td>Average</td>
<td>4607.22</td>
<td>0.36</td>
<td>39.27</td>
<td>86.10</td>
<td>281.91</td>
<td>11.04</td>
<td>1.75</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>8.30</td>
<td>0.010</td>
<td>0.42</td>
<td></td>
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<td>0.31</td>
<td>0.052</td>
</tr>
<tr>
<td>Varieties</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Durusu</td>
<td>5281.66 a</td>
<td>0.31 c</td>
<td>41.21 a</td>
<td>172.21</td>
<td>362.33</td>
<td>10.53 c</td>
<td>1.46 a</td>
</tr>
<tr>
<td>Fırat</td>
<td>4481.66 b</td>
<td>0.36 b</td>
<td>38.85 b</td>
<td>172.21</td>
<td>362.33</td>
<td>11.02 b</td>
<td>1.23 b</td>
</tr>
<tr>
<td>Atilir</td>
<td>4058.33 c</td>
<td>0.39 a</td>
<td>37.75 c</td>
<td>172.21</td>
<td>362.33</td>
<td>11.58 a</td>
<td>1.12 c</td>
</tr>
<tr>
<td>Average</td>
<td>4607.22</td>
<td>0.36</td>
<td>39.27</td>
<td>172.21</td>
<td>362.33</td>
<td>11.04</td>
<td>1.27</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>84.52</td>
<td>0.015</td>
<td>0.52</td>
<td></td>
<td></td>
<td>0.43</td>
<td>0.030</td>
</tr>
</tbody>
</table>

**ns,** not significant; **,** significant at P<0.01; *,** significant at P<0.05; means in the same column with the same letter are not significantly different; ETA, Evapotranspiration (mm); CWSI, Crop water stress index; WUE, Water use efficiency.
### Table 4
The correlation coefficients between yield and other parameters

<table>
<thead>
<tr>
<th></th>
<th>Yield</th>
<th>CWSI</th>
<th>Chlorophyll content</th>
<th>ETa</th>
<th>WUE</th>
<th>Protein content</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (2016)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>1.000</td>
<td>**</td>
<td>**</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>CWSI</td>
<td>-0.762</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>0.766</td>
<td>-0.952</td>
<td>1.000</td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>ETa</td>
<td>0.492</td>
<td>-0.920</td>
<td>0.914</td>
<td>1.000</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>WUE</td>
<td>-0.052</td>
<td>0.644</td>
<td>-0.665</td>
<td>-0.888</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Protein content</td>
<td>-0.770</td>
<td>0.969</td>
<td>-0.953</td>
<td>-0.897</td>
<td>0.628</td>
<td>1.000</td>
</tr>
<tr>
<td>b (2017)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>1.000</td>
<td>**</td>
<td>**</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>CWSI</td>
<td>-0.756</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>0.814</td>
<td>-0.931</td>
<td>1.000</td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>ETa</td>
<td>0.492</td>
<td>-0.915</td>
<td>0.8735</td>
<td>1.000</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>WUE</td>
<td>-0.063</td>
<td>0.643</td>
<td>-0.6087</td>
<td>-0.892</td>
<td>1.000</td>
<td>**</td>
</tr>
<tr>
<td>Protein content</td>
<td>-0.765</td>
<td>0.969</td>
<td>-0.9465</td>
<td>-0.902</td>
<td>0.645</td>
<td>1.000</td>
</tr>
</tbody>
</table>

**, significant at P≤0.01; *, significant at P≤0.05; ns, not significant

Significant correlations were observed between yield and CWSI, between yield and CC values, between yield and protein content values (P<0.01). The correlation coefficients (r) for the relationships of yield with CC, CWSI, protein content and WUE are presented in Table 4a and b respectively for the years 2016 and 2017. Significant correlations were observed between the investigated traits in 2016 (P<0.01). There was an increasing correlation between CC and yield (r= 0.766**). There was an decreasing correlation between protein content and yield (r= -0.770**). There was decreasing correlation between CWSI and yield (r= -0.762**). Significant correlations were also observed between all parameters in 2017 (P<0.01). There was a highly positive correlation between CC and yield (r= 0.814**). There was a highly negative correlation between protein content and yield (r= -0.765**). There was a negative correlation between CWSI and yield (r= -0.756**).

### CONCLUSIONS

As the average of two years, the greatest yield was obtained from $I_{ox}$Durusu interaction (5613.50 kg ha$^{-1}$) and the lowest yield was obtained from $I_{ox}$Atilir interaction (3848.82 kg ha$^{-1}$). The greatest CC was observed in $I_{ox}$Durusu interaction (45.23 spad) and the lowest value was seen in $I_{ox}$Atilir interaction (35.80 spad). The greatest CWSI was observed in $I_{ox}$Atilir interaction (0.47) and the lowest CWSI was observed in $I_{ox}$Durusu interaction (0.0.24). There was an inverse relationship between irrigation water and CWSI and a direct relationship between irrigation water and CC. CWSI values decreased and CC values increased with increasing irrigation water quantities. However, such increase or decreases were not constant and varied based on genotypes even in no water-stress treatments. The greatest WUE (1.10 kg da mm$^{-1}$) was observed in Atilir genotype. Therefore, the genotype Durusu was found to be prominent both in strong water-stress and no water-stress treatments and optimally converted applied irrigation water into the yield. The water stress-induced reduction in CC was low in water stress-resistant genotypes and high in sensitive genotypes. In brief, in strong water-stress and no water-stress treatments of the experimental years, yield, CWSI and CC values of Durusu malting barley genotype were above the averages. Therefore, Durusu (malting barley) genotype was identified as water stress-resistant and can be used in further studies to be carried out for resistance to abiotic stress factors. On teh other hand Atilir (malting barley) genotype was identified as water stress-sensitive. As a result decreased protein content and total protein content in plants under no water-stress treatments.

### ACKNOWLEDGEMENTS

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THE FOAMING STUDY FOR IMPROVING THE GAS PERMEABILITY IN ECOLOGICAL SYNTHETIC LEATHER BASE

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\textsuperscript{2}North China Institute of Aerospace Engineering, Langfang 065 000, P.R. China

ABSTRACT

Taking waterborne-polyurethane (WPU) with high solid content as resin slurry, ecological synthetic leather base with good air permeability was prepared through the foaming process. The micro-structure of the foaming base was characterized by the scanning electronic microscopy (SEM) and analyzed by the Image pro Plus software. The effects of dosages of foaming agents on the structure and properties of ecological synthetic leather base foaming film were studied. Azodicarbonamide (AC), a widely used agent with a highly foaming efficiency but insoluble in aqueous emulsion solution, was emulsified to perform as a valid foaming-agent. Dosage effect of such the agent on structure and mechanical properties of the foaming film in ecological synthetic leather base etc were studied. Results indicated that, by adding 0.3 % of AC (total weight of polyurethane) with foaming auxiliary: Urea [Urea/AC = 1:1 (w/w)], the optimized foaming film with uniform micro-pores can be worked out, which may improve the gas permeability of synthetic leather with excellently tensile strength. In addition, accompanied by the 0.3% AC, the value of specific surface area on the foamed film may increase to 1.0938m$^2$·g$^{-1}$, which was about 33 to 34 times of those without adding the AC. This was also corroborated with observations by means of the scanning electron microscopy (SEM).

KEYWORDS:
Waterborne-polyurethane, ecological synthetic leather, emulsification, the foaming film, the gas permeability

INTRODUCTION

Synthetic leather is becoming increasingly popular with an excellent mechanical strength, thermal and chemical resistances, and quite high quality of homogeneity, wearing comfort and shape stability etc that are comparable or even superior to genuine leather [1, 2]. However, there is the inevitable pollution of organic solvents such as N-dimethyl-formamide (DMF) etc to environment, which may limit the development of synthetic leather manufacturing [3, 4]. Based upon this, it is necessary to explore some good ways of reducing the pollution in this field. Nowadays, waterborne-polyurethane (WPU) may substitute for traditional organic solvents to reduce the pollution of volatile organic compounds (VOCs), so that it may protect the environment [5-8]. Moreover, such the WPU product shows an advantage over other traditional ones: non-toxicity, good coating property and abrasion resistance etc [9-12]. However, since the WPU coating can not perform as a highly micro-porous texture, the gas permeability and the tensile strength of the WPU coated synthetic leather are often worse than those coated with traditional solvent polyurethane [13-16].

In order to keep human body more comfortable, human garments should possess the higher gas permeability [17], especially when human bodies are under a very high temperature region, such the clothing should allow perspiration to evaporate rapidly into environment. Otherwise, human bodies will feel stuffy [18], and the resulting bacteria breeding can be very harmful to human health [19]. According to this, the gas permeability must be a significant quality index on the comfort of human garments.

During past tens of years, since the waterborne-polyurethane synthetic leather was becoming of great importance in the manufacturing of human garments, many researches have been employed to improve the gas permeability of such synthetic leather. For examples, Wang et al. [20-22] enhanced the gas permeability by adding micro-sphere shells which were heated and expanded at a high temperature region to form vesicle structures in synthetic leather. Shao et al. [23] applied a mechanical foaming method by using the Compact-Mix 600 Hansa-Mixer to improving the gas permeability of synthetic leather. Luo et al. [24] added the surfactant with fatty-acid salts, fatty-alcohol sulfates, and alkyl-benzene sulfonic acids into synthetic leather slurry, so that a large number of micro-pores can be generated after a powerful mechanical stirring [25-26], and the gas permeability was thus improved. Shi et al. [27] ran a physical foaming method by selecting many inorganic salts with small molecules. After the foaming-agent film was produced, inorganic salts were washed out to generate micro-pores in the film, and
the gas permeability was also improved. Wang et al. [28] decreased the foaming temperature by using Na2CO3, ZnO and zinc-stearate as auxiliaries, so that micro-pores were generated in synthetic leather to improve the gas permeability.

Comprehensively, each of above foaming methods has its own advantages and disadvantages. For instance, the physical foaming method is easily operated, but its foaming effect is quite weak. The mechanical foaming method can generate more micro-pores, but its operating equipment is very expensive. On the contrary, the cost of chemical method using traditional foaming-agent is quite low, but its foaming temperature must remain at a very high value. Therefore, if we can decrease the foaming temperature in a new chemical method, it would be valuable for leather synthesis.

The purpose of present work was to fabricate a new type of ecological synthetic leather with waterborne-polyurethane, which may possess an excellent gas permeability for human body and environment protection. Rather than increasing micro-pores in synthetic leather by traditionally physical and mechanical methods, here we fabricate uniform micro-pores in the ecological synthetic leather by using a new chemical manner, i.e., in order to obtain uniform micro-porous textures, different dosages of the AC foaming-agent were adopted. And then, influences of various AC dosages on fabricating micro-porous textures was investigated. Once these uniform micro-pores were generated in the ecological synthetic leather, we would test the gas permeability and the tensile strength of the samples.

MATERIALS AND METHODS

In this work, an aqueous emulsion solution with high concentration of solid waterborne-polyurethane (about 50%) was synthesized by ourselves [29]. Slurry of ecological synthetic leather was prepared by adding some foaming-agents, foaming auxiliaries, leveling agents, thickeners, the bubble adjusting agents, and the defoaming-agents etc. These solvents were evenly stirring by a magnetic agitator and a static defoaming machine for about 2 hours. The slurry was coated on the fibre fabrics. And then, such the base was put into the oven for drying. Micro-porous morphology was observed by the S-3400N scanning electron microscope (SEM). Specific Surface Area (SSA) on the foaming film was measured by the JW-BK112 Specific Surface & Aperture Analyzer. Elongation strain and Tensile strength of such synthetic leather base was tested by using the QB/T 2710-2005 - RGT-5 Tensile Tester.

RESULTS AND DISCUSSION

Selection of the foaming auxiliaries. It is well known that the foaming auxiliaries may obviously decrease the foaming temperature of the foaming agents and regulate the uniformity of micro-pores. As a result, in this experiment, some foaming auxiliaries were mixed with the AC in 1:1 ratio, which can represent a beneficial effect. In order to select the best foaming agent system (the AC foaming agent with the foaming auxiliaries) for synthesizing leather, series of the foaming auxiliaries were mixed for testing the foaming (decomposition) temperature: the AC only, the AC/Urea (1:1), the AC/Zinc-stearate (1:1), and the AC/ZnO&stearic acid (1:1), respectively. Values of the foaming temperature were tested through an analysis of Thermal Weight-differential Heat under the nitrogen protection, from a temperature range of 30 to 350°C. And, samples were warmed up at a speed of 10°C/min. Results are shown in Table 1.

From Table 1, it can be seen that, when the AC was adopted alone, it would lead to the foaming temperature at about 210°C. Nevertheless, not only would this remain a high foaming temperature, but bring about the degradation of the WPC resin before the temperature [21]. Therefore, other three foaming auxiliaries were added into the AC dosage by a 1:1 ratio, respectively, so as to play significant roles in decreasing the AC foaming temperature. As can be seen from Table 1, three foaming auxiliaries did decrease the foaming temperature, i.e., the Urea only may perform the best effect on decreasing the AC foaming temperature to 151.30°C; while the Zinc-stearate and the ZnO & stearic acid auxiliaries may decrease the AC foaming temperature to about 170°C, 180°C, respectively. Therefore, in the following section, the Urea can be selected for the best foaming auxiliary in experiment, accompanied with 1:1 rate of the AC dosage. In the following experiment, such the foaming temperature may represent the drying temperature of synthesizing ecological waterborne leather.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Comparison of the foaming temperature with the AC, and other foaming auxiliaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foaming system</td>
<td>AC</td>
</tr>
<tr>
<td>$T_d$ (°C)</td>
<td>209.91</td>
</tr>
<tr>
<td>$T_{max}$ (°C)</td>
<td>217.88</td>
</tr>
</tbody>
</table>

$T_d$: Initial decomposition temperature $T_{max}$: Final decomposition temperature
Figure 1 shows the Urea effect on decreasing the foaming temperature. In this Figure, both the pure Urea and the AC/Urea mixture [with 1:1 ratio (w/w)] became melting around 136°C with downward endothermic peaks, and decomposed around 159°C with steep upward exothermic peaks. However, the pure AC remained high values of these temperatures. Therefore, it may conclude that the Urea auxiliary would obviously decrease the decomposition temperature of the AC agent.

Till now, no definite literature has been reported on the chemical affinity of the AC foaming-agents with the foaming auxiliaries. So in our research, regarding this mechanism, we believe that, since the Urea (H₂N−CO−NH₂) and the AC (H₂N–CO–N=N–CO–NH₂) have similar molecular configurations, when they are approaching, the Urea may play an important catalytic role in promoting the AC decomposition, i.e., the stable AC molecular structure relying on a conjugated joint from its double bonds of carboxyl-group (>C=O) and its double bond of azo-group (−N=N−) would be collapsing, mainly due to the formation of inter-molecular hydrogen bonds of N and O (with electron lone pairs) ions in the Urea with H ions in the AC, respectively. Such the formation may decrease the barrier of activation energy for the AC dissociation, and thus decrease the AC decomposition (foaming) temperature.

Selection of the foaming-agent dosage. Effect of the foaming dosage on micro-pores. Since the AC is insoluble with water, it could hardly diffuse into aqueous emulsion solution with a highly solid polyurethane concentration. Therefore, at first, the AC and the Urea were ground into powders at a ratio of 1:1 (w/w), and then put into the water at about 60 °C. Then next, they were fully emulsified by a magnetic agitator, which may have the foaming bodies thoroughly diffuse into the waterborne-polyurethane, so as to be beneficial for uniformity of the foaming micro-pores in the matrix. Pictures of transversal section with different AC dosages are shown in Figure 2, which may reflect the uniformity of the foaming micro-pores. Please note that, in each of the foaming systems, the AC/Urea = 1:1 (w/w).

In this Figure, when the 0.20% AC was selected, only a few of micro-pores with different sizes were observed in Figure 2(a). And then, more micro-pores were observed when the AC dosage increased to 0.25%. When the AC dosage increased to 0.35%, micro-pores were connecting and merging into large bubbles, which may obviously damage the matrix continuity, and decrease the tensile strength of waterborne-polyurethane ecological synthetic leather. If the AC dosage still increased, such phenomena were even getting worse. Therefore, it was clear that the variation of AC dosage had a remarkable influence on uniformity and quantity of micro-pores. And, the optimal AC dosage should be 0.30%, which may have both the matrix continuity and the micro-pores homogeneity of the foaming film more excellent than others.

Effect of the foaming dosage on uniformity of micro-porous structures. In the experiment, the scanning electron microscope was applied to photograph of the foaming film of synthetic leather. Sizes of micro-porous texture can be clearly observed in Figure 3. Furthermore, a professional software, Image pro Plus Software [30], was applied to making more understandings of micro-porous mechanisms. Results are shown in Table 2 and Figure 3.
Table 2 represents the average size of micro-pores and variance ratio of micro-pores, which may reflect whether size and distribution of micro-pores are homogeneous or not, respectively. It was clearly to find that, the variation ratio of the AC dosage showed a remarkable influence on uniformity of micro-pores, as seen in Figure 3. That was, when the AC dosage was at 0.30%, both average size and variance ratio of micro-pores would reach to their minimal points, meaning that the 0.30% AC would behave the highest efficiency to realize the best uniformity of micro-pores. When the AC dosage was less than 0.30%, the number of micro-pores would be smaller, and the variance ratio would also become lower. When the AC dosage was at 0.20%, both the number and the variance ratio of micro-pores would reach to the highest points. When the AC dosage was higher than 0.30%, the number of micro-pores would increase with the increment of the AC amount, and micro-porous ratio would become just relative uniform. In addition, when the number of micro-pores increased to a certain value, distance between two micro-pores tended to be smaller and smaller, until it became only one thin wall, and pressure between two adjacent bubbles would become different. As a result, micro-porous wall would burst, and small micro-pores may merge into bigger bubbles. And then, the average diameter of bubbles would increase, till the continuity of the foaming resin was destroyed, which may decrease the tensile strength of synthetic leather.

![Images of micro-spheres corresponding to different dosages of the AC foaming-agent](a) 0.20% AC (b) 0.25% AC (c) 0.30% AC (d) 0.35% AC

FIGURE 2
Micro-spheres corresponding to different dosages of the AC foaming-agent

<table>
<thead>
<tr>
<th>Foaming Dosage (%)</th>
<th>Average size (PPI)</th>
<th>Variance</th>
<th>Micro-porous cluster ratio (%)</th>
<th>Single Micro-porous ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>81.23</td>
<td>1394.01</td>
<td>30.26</td>
<td>69.74</td>
</tr>
<tr>
<td>0.25</td>
<td>73.53</td>
<td>460.92</td>
<td>27.57</td>
<td>72.43</td>
</tr>
<tr>
<td>0.30</td>
<td>69.98</td>
<td>391.36</td>
<td>28.42</td>
<td>71.58</td>
</tr>
<tr>
<td>0.35</td>
<td>85.71</td>
<td>641.55</td>
<td>35.56</td>
<td>64.44</td>
</tr>
</tbody>
</table>
**Effect of the foaming dosage on specific surface area.** Specific Surface Area (SSA) can also reflect status of bubble holes in synthetic leather, i.e., the larger the SSA, the higher the foaming rate in the leather. Figure 4 shows the SSA versus the AC dosage in the waterborne-polyurethane ecological synthetic leather. It could be found that the SSA value indicated an upward trend with increment of the AC dosage. Particularly, after the 0.20% AC dosage, such the SSA value was significantly influenced by the AC concentration. At the 0.30% AC dosage, the SSA value was 1.0938 m^2·g^{-1}, which was about 33 to 34 times of those without adding the AC dosage. Therefore, it may conclude that more and more micro-pores in the AC foaming agent film may lead to gas-solid phase separation, and greatly increase overall values of Specific Surface Area.

![Chart showing SSA versus AC dosage](image_url)

**Figure 4**

**Effect of the AC foaming dosage on Specific Surface Area**

![Chart showing bubble size distribution](image_url)

**Figure 3**

**Effect of the foaming dosage on the uniformity of micro-pores**
Effect of the AC dosage on the tensile strength of synthetic leather base

![Figure 5: Tensile strength vs. AC dosage](Chart)

**FIGURE 5**

Effect of the AC dosage on the tensile strength of synthetic leather base

Influence of the AC dosage on the gas permeability of the waterborne-polyurethane ecological synthetic leather

![Figure 6: Permeability vs. AC dosage](Chart)

**FIGURE 6**

Influence of the AC dosage on the gas permeability of the waterborne-polyurethane ecological synthetic leather

**Effect of the foaming dosage on tensile strength.** Tensile strength of the ecological synthetic leather base versus the AC dosage is showed in Figure 5, with concentration of the AC dosage from 0.00% to 0.40%. In this Figure, tensile strength of the base decreased from 9.08 MPa to 3.87 MPa, with decrements of elongation from 1026% to 805%. So it was clear that the variation ratio of the AC dosage showed a remarkable influence on the tensile strength. Particularly, when the AC dosage was beyond 0.30%, such the decreasing trend would become more obvious. The reason was that, when the AC foaming dosage was very low, only a few of micro-pores were generated in the polyurethane resin, so the continuity of leather resin matrix would remain good, and so did the property of tensile strength. However, when the bubbles appeared more and more in the foaming base, the continuity of ma-
trix (polymer resin) would be destroyed, and the tensile strength was also decreased not to withstand the heavy load.

**Effect of the foaming dosage on gas permeability.** Figure 6 shows the distribution of gas permeability of waterborne-polyurethane ecological synthetic leather versus the AC dosage. Samples for testing the gas permeability were prepared by optimizing the AC dosage from 0.00% to 0.40%. It may conclude that the gas permeability appeared an upward trend with the increment of the AC dosage. Particularly, after the 0.35% AC, the gas permeability would be significantly influenced, i.e., many more micro-pores were generated in the synthetic leather base. Thus, the more micro-pores were generated, the better gas permeability of synthetic leather. However, too more micro-pores would lead to more large bubble holes in the base, which may decrease the tensile strength of the waterborne-polyurethane ecological synthetic leather, also see Figure 5.

**CONCLUSION**

In this paper, the AC foaming-agent was emulsified before putting into waterborne-polyurethane slurry, five different AC dosages: 0.20%, 0.25%, 0.30%, 0.35% and 0.40%, following by the 1:1(w/w) ratio of the Urea, were applied to synthesizing the waterborne-polyurethane ecological synthetic leather base, so as to generate the appropriate uniformity micro-pores to improve the gas permeability, the Specific Surface Area, and the tensile strength of synthetic film, respectively. Experimental observations indicated that, the gas permeability would improve according to the increment of the AC dosage. Particularly, in a sample with the 0.30% AC dosage plus the same ratio of the Urea auxiliary, uniform micro-pores in the foaming-agent film could be obviously generated, so that both gas permeability and tensile strength of the film could reach excellent values, which can be selected for the best optimized sample on the foaming film. Comprehensively, even if the Azodicarbonamide (AC), a traditional foaming agent for synthesizing the leather, was insoluble in aqueous emulsion solution, however, if it was emulsified by mixing with the same ratio of the Urea auxiliary, it may still perform a high efficiency as an excellent foaming agent in the waterborne-polyurethane ecological synthetic leather.

**ACKNOWLEDGEMENTS**

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EFFECTS OF DIFFERENT FOLIAR POTASSIUM TREATMENTS ON SEED YIELD AND QUALITY OF CAPIA PEPPERS

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ABSTRACT

This study was conducted to investigate the effects of different foliar potassium treatments on greenhouse seed yield and quality of BT BUR – KAP capia pepper (Capsicum annum) cultivar. In addition to soil available K2O content, 1, 2, 4 and 8 kg da⁻¹ K2O treatments were performed with 15% nitrogen-containing potassium nitrate fertilizer. The greatest seed yield (34.50 kg da⁻¹) was obtained from 2 kg da⁻¹ K2O treatment. As compared to the control treatment, 2, 4, 8 kg da⁻¹ K2O treatments yielded better seed germination ratios and mean germination times. For under-cover capia pepper seed production, seed yield and quality, the best outcomes were achieved with 2 kg da⁻¹ K2O treatments. Seed macro and micro elements and pre-harvest leaf amino acids were also investigated in this study. While all treatments yielded similar Mn, B, Fe, Cu, S and Na contents with the control treatment, K, Mg, Ca, P, N and Zn contents of potassium treatments were greater than the values of the control treatment. As compared to the control treatment, potassium treatments increased leaf total amino acid, cysteine, valine, tryptophan, leucine, proline, aspartate, glutamine, glycine, arginine and tyrosine contents. It was concluded based on present findings that in greenhouse capia pepper seed production, 2 kg da⁻¹ K2O treatments had the best outcomes for seed yield, germination and vigor.

KEYWORDS:
Capia pepper, foliar potassium, seed quality, seed yield

INTRODUCTION

Pepper is commonly consumed in fresh, pickled, paste, flaked and powder forms. It is the second largest undercover crop of Turkey after tomatoes. Undercover and open-field pepper productions are always increasing. While pepper production of Turkey was 2 127 000 tons in 2014, the value increased to 2 608 172 tons in 2017. The capia pepper production of 829 809 tons in 2014 increased to 1 107 713 tons in 2017. With these production quantities, pepper has the third place after tomato and watermelon [1]. World pepper production including undercover and open field production is about 36 143 113 tons [2]. Recently, Turkey is the world’s third largest pepper producer after China and Mexico [3].

Chemical fertilizers are among the most significant inputs in plant production. However, excessive fertilizers result in yield losses and soil degradation. Therefore, optimum fertilizer quantities should be determined in advance for every crop and plant to be cultured.

Plant yields are influenced by several factors. Plant nutrition is among the most important of them. Quite high yields are achieved with proper and sufficient fertilization program [4]. Among the plant nutrients, potassium play a great role in plant stoma quantities, water use efficiency, sugar transport and conversion into carbohydrates, enzyme activities, protein synthesis and seed quality [5, 6]. Potassium also improves plant resistance to biotic and abiotic stressors and thus improves both yield and quality [7, 8].

There are several studies indicating improved yield, quality and chemical composition with potassium fertilizer treatments. Potassium significantly increased yield and quality of peppers and tomatoes [9, 10, 11].

Soil and foliar potassium fertilizer treatments were reported to increase yield and quality in several plants. Greatest values were reported for plant height, number of leaves, plant fresh weight, yield, number of fruits per plant, plant sugar content and amino acid content with potassium treatments in spray forms [8, 11, 12].

In present study, effects of different potassium doses in spray forms on capia pepper seed yield and quality was investigated. Before the fruit harvest for seeds, the relationships between amino acid content and seed quality were also investigated.

MATERIALS AND METHODS

Study area. Experiments were conducted in a polycarbonate experimental greenhouse of Erciyes
University (38° 70’ 50” N, 35° 53’ 28” E) in the year 2016. Greenhouse indoor average temperature and relative humidity values are provided in (Table 1). Average temperatures varied between 20.14 - 27.24 °C and relative humidity values varied between 34.48 - 56.23%. Soil samples were taken from 0-30 cm soil profile and analyses were performed on these soil samples. Experimental soils were loamy in texture, unsaline and slightly alkaline potassium nitrate (13% N and 45.5% K₂O) was dissolved in water as to have 1, 2, 4 and 8 kg da⁻¹ K₂O and foliar sprays were performed with an hand pulverizer. All treatments were applied once above the plants at fruit formation period as to cover the entire plant. Control plants were treated only with water. Following the harvest, randomly selected seeds were subjected to macro-micro element analyses.

**Fertilizer Treatments.** To determine the effects of different doses of potassium fertilizers, pure potassium nitrate (13% N and 45.5% K₂O) was dissolved in water as to have 1, 2, 4 and 8 kg da⁻¹ K₂O and foliar sprays were performed with an hand pulverizer. All treatments were applied once above the plants at fruit formation period as to cover the entire plant. Control plants were treated only with water. Following the harvest, randomly selected seeds were subjected to macro-micro element analyses.

**Soil Analyses.** Soil texture was determined with the aid of Bouyoucos hydrometer method [15]. Soil pH values were measured potentiometrically in 1:2.5 soil-water solution with a pH meter with a glass electrode [16]. Soil lime content was determined volumetrically with a Scheibler calcimeter [17]. Soil organic matter contents were determined with the aid of Smith-Weldon method [18]. For available potassium contents, soil samples were shaken in ammonium acetate (1 N, pH=7.0) and <50% relative humidity for a week. Following the drying, moisture content of the seeds was determined in accordance with the principles specified by International Seed Tests Association with the aid of Constant Low Temperature Oven Method [13]. For accurate estimation of the effects of treatments on seeds, seed moisture contents were tried to be equivalent before the seed germination, micro and macro element analyses. Germination tests were performed on 200 seeds in 4 replications (50 seeds in each replication) to determine seed vigor [13]. Germinated seeds were counted and removed from the germination cups and countings were continued until the 21st day. The seeds with a rootlet length of 0.5 cm were accepted as germinated. Rooting media was irrigated as needed. Germination tests were conducted in climate cabins at 25 °C. Seed vigor was identified as percent germination at the end of 21st day. Seeds were placed in petri dishes with filter papers above and below the seeds. Mean germination time was calculated in accordance with [14].

**TABLE 1**

<table>
<thead>
<tr>
<th>Months</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Humidity (%)</td>
<td>56.23</td>
<td>51.64</td>
<td>47.13</td>
<td>42.17</td>
<td>36.48</td>
<td>43.64</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Soil Depth(cm)</th>
<th>Texture</th>
<th>pH</th>
<th>OM (%)</th>
<th>Lime (%)</th>
<th>EC (dS M⁻¹)</th>
<th>Available P₂O₅ (kg da⁻¹)</th>
<th>Available K₂O (kg da⁻¹)</th>
<th>Available P (kg da⁻¹)</th>
<th>Available N (kg da⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>Loam</td>
<td>7.75</td>
<td>2.96</td>
<td>13.26</td>
<td>0.18</td>
<td>56.65</td>
<td>10.25</td>
<td>10.78</td>
<td>Organic Matter</td>
</tr>
</tbody>
</table>

OM= Organic Matter

**Plant Material.** BT –BUR-KAP capia pepper seedlings of Bursa Seed Co. were used as the plant material of the study. BT –BUR-KAP cultivar is available open-field culture, fresh consumption, pepper paste production, frying and roasting. Fruits are flat and widened and get dark shiny red color when ripened. Fruit length is 16-18 cm, width is 6 cm and flesh thickness is 4-5 mm. Homogeneously grown potted seedlings produced by Akdeniz Seeding Co. were planted into greenhouse seed beds on 26.04.2016 when they reached to 5-6 true-leaf stage of growth. Experiments were conducted in randomized blocks design with 3 replications with 3 plants in each replication. Seedlings were planted in two rows at 100 x 60 x 60 cm. Seed fruits were harvested on 18.10.2016. Seeds were removed from the germination cups and countings were continued until the 21st day. The seeds with a rootlet length of 0.5 cm were accepted as germinated. Rooting media was irrigated as needed. Germination tests were conducted in climate cabins at 25 °C. Seed vigor was identified as percent germination at the end of 21st day. Seeds were placed in petri dishes with filter papers above and below the seeds. Mean germination time was calculated in accordance with [14].

**Fertilizer Treatments.** To determine the effects of different doses of potassium fertilizers, pure potassium nitrate (13% N and 45.5% K₂O) was dissolved in water as to have 1, 2, 4 and 8 kg da⁻¹ K₂O and foliar sprays were performed with an hand pulverizer. All treatments were applied once above the plants at fruit formation period as to cover the entire plant. Control plants were treated only with water. Following the harvest, randomly selected seeds were subjected to macro-micro element analyses.
Shelton, CT 06484-4794, USA) [24]. Fresh leaf samples were taken before the last harvest and they were kept in a freezer at -20 °C until the analyses for amino acids. Free amino acid composition of plant samples was determined in accordance with the methods (HPLC method) specified by [25] and [26]. Agilent 1200 model HPLC equipped with single detector (UV) and Zorbax Eclipse-AAA 4.6 x 150 mm, 3.5 μm (Agilent PN 963400-902) column was used to determine free amino acid composition of the samples.

Statistical Analyses. Experiments were conducted in randomized blocks design with 3 replications. Experimental data were subjected to variance analysis with “SSPS 13.0 for Windows” statistical software and significant means were compared with LSD test at 0.05 significance level.

RESULTS AND DISCUSSION

Effects of foliar potassium treatments on pepper seed yields. Considering the effects of foliar potassium treatments on seed yields, it was observed that 2, 4 and 8 kg da⁻¹ K₂O treatments increased seed yields, but the effects of only 2 kg da⁻¹ K₂O treatments on seed yields were found to be significant. The greatest seed yield (34.504 kg da⁻¹) was obtained from 2 kg da⁻¹ K₂O treatment and the lowest seed yield (22.308 kg da⁻¹) was obtained from 1 kg da⁻¹ K₂O treatment. Seed yield of control treatment was 22.532 kg da⁻¹ (Table 3).

The 2, 4 and 8 kg da⁻¹ K₂O treatments significantly increased seed germination ratios. The greatest germination ratio (95.5%) was observed in 8 kg da⁻¹ K₂O treatment and germination ratio of the control treatment was 77.5%. As compared to the control treatment, 2, 4 and 8 kg da⁻¹ K₂O treatments reduced mean germination times. The longest mean germination time (9.12 days) was observed in 1 kg da⁻¹ K₂O treatments and the shortest mean germination time (6.03 days) was observed in 4 kg da⁻¹ K₂O treatment. Mean germination time of the control treatment was 8.18 days.

Effects of foliar potassium treatments on seed nutrients. Potassium treatments yielded similar seed Na, Cu, Fe, Mn and B contents with the control treatment, but increased seed K, Mg, Ca, N, P and Zn contents as compared to the control. Potassium treatments increased seed potassium content. The greatest potassium content (13735 mg kg⁻¹) was obtained from 8 kg da⁻¹ K₂O treatments and the lowest potassium content (11234 mg kg⁻¹) was obtained from the control seeds. The greatest Mg content (2412 mg kg⁻¹) was obtained from 8 kg da⁻¹ K₂O treatments and the lowest Mg content (2170 mg kg⁻¹) was obtained from the control seeds. While 1, 2 and 4 kg da⁻¹ K₂O treatments increased seed Ca contents as compared to the control, 8 kg da⁻¹ K₂O treatment yielded similar Ca contents with the control treatment. The greatest N content (1.74 mg kg⁻¹) was obtained from 8 kg da⁻¹ K₂O treatments and the lowest N content (1.44 mg kg⁻¹) was obtained from the control seeds. The greatest P content (3022 mg kg⁻¹) was obtained from 8 kg da⁻¹ K₂O treatment and the lowest P content (2621 mg kg⁻¹) was obtained from the control seeds (Table 4).

### TABLE 3
Effects of foliar potassium treatments on pepper seed yields

<table>
<thead>
<tr>
<th>Seed yield kg/da</th>
<th>Yield per plant (gr)</th>
<th>Germination ratio (%)</th>
<th>Mean germination time (day)</th>
<th>Seed moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.53 b</td>
<td>10.82 b</td>
<td>77.5 b</td>
<td>8.18 a</td>
</tr>
<tr>
<td>1 kg K da⁻¹</td>
<td>22.31 b</td>
<td>10.71 b</td>
<td>76.5 b</td>
<td>9.12 a</td>
</tr>
<tr>
<td>2 kg K da⁻¹</td>
<td>34.50 a</td>
<td>16.56 a</td>
<td>88.0 a</td>
<td>6.31 b</td>
</tr>
<tr>
<td>4 kg K da⁻¹</td>
<td>26.85 ab</td>
<td>12.89 ab</td>
<td>89.5 a</td>
<td>6.03 b</td>
</tr>
<tr>
<td>8 kg K da⁻¹</td>
<td>31.29 ab</td>
<td>15.02 ab</td>
<td>95.5 a</td>
<td>6.79 b</td>
</tr>
</tbody>
</table>

* Different letters indicate significant differences at P < 0.05.
** Different letters indicate significant differences at P < 0.01.

### TABLE 4
Effects of foliar potassium treatments on seed macro nutrients

<table>
<thead>
<tr>
<th></th>
<th>K**</th>
<th>Mg**</th>
<th>Ca**</th>
<th>N**</th>
<th>P**</th>
<th>S**</th>
<th>Na**</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11234 c</td>
<td>2170 b</td>
<td>2435 b</td>
<td>1.44 c</td>
<td>2621 c</td>
<td>1266</td>
<td>878</td>
</tr>
<tr>
<td>1 kg K</td>
<td>12145 b</td>
<td>2234 ab</td>
<td>2657 a</td>
<td>1.56 b</td>
<td>2712 b</td>
<td>1244</td>
<td>912</td>
</tr>
<tr>
<td>2 kg K</td>
<td>12760 b</td>
<td>2322 a</td>
<td>2788 a</td>
<td>1.67 a</td>
<td>2878 b</td>
<td>1312</td>
<td>887</td>
</tr>
<tr>
<td>4 kg K</td>
<td>13100 a</td>
<td>2344 a</td>
<td>2612 a</td>
<td>1.72 a</td>
<td>2934 a</td>
<td>1324</td>
<td>812</td>
</tr>
<tr>
<td>8 kg K</td>
<td>13735 a</td>
<td>2412 a</td>
<td>2446 b</td>
<td>1.74 a</td>
<td>3022 a</td>
<td>1233</td>
<td>789</td>
</tr>
</tbody>
</table>

* Different letters indicate significant differences at P < 0.05.
** Different letters indicate significant differences at P < 0.01.
Considering seed micro element contents, differences observed only in seed Zn contents. The greatest Zn content (40.19 mg kg\(^{-1}\)) was obtained from 2 kg da\(^{-1}\) K\(_2\)O treatment and the lowest Zn content (30.99 mg kg\(^{-1}\)) was obtained from 4 kg da\(^{-1}\) K\(_2\)O treatment. Seed Zn content of the control treatment was 34.12 mg kg\(^{-1}\) (Table 5).

There was a negative correlation between seed germination ratio and mean germination time. Such a correlation revealed that mean germination times shortened, thus seed vigor increased with increasing germination ratios. While germination ratios positively correlated with seed N, K, Mg and P contents, there was a negative correlation between germination ratio and seed Na content (Table 6).

Seed germination ratios and mean germination times did not have any significant correlations with Ca, Zn, Cu and Fe contents.

**Effects of foliar potassium treatments on plant amino acid contents.** Plant stress status was assessed through analyzing the effects of foliar potassium treatments on leaf amino acid contents. As compared to the control, all treatments significantly increased total amino acid content of leaf samples. The greatest total amino acid content (100.42 nmol ul\(^{-1}\)) was obtained from 4 kg da\(^{-1}\) K\(_2\)O treatment and the west value (71.65 nM ul\(^{-1}\)) was obtained from the control plants. It was observed when the amino acid contents were assessed in detail that 1, 2, 4 and 8 kg da-1 K\(_2\)O treatments significantly increased leaf aspartate, asparagine, glutamine, glycine, tyrosine, cysteine, tryptophan and proline contents as compared to the control. It was also observed that 2, 4 and 8 kg da\(^{-1}\) K\(_2\)O treatments increased leaf arginine, valine and leucine contents as compared to the control (Table 7).

### TABLE 5

| Effects of foliar potassium treatments on seed micro nutrients |
|------------------|-----------|-----------|-----------|-----------|
| Zn** | Fe* | Cu* | Mn* | B* |
| mg/kg |  |  |  |  |
| Control | 34.12 b | 25.16 | 2.27 | 13.30 | 8.79 |
| 1 kg K | 36.57 b | 25.57 | 2.82 | 14.76 | 7.68 |
| 2 kg K | 40.19 a | 23.45 | 2.44 | 15.67 | 9.12 |
| 4 kg K | 30.99 c | 24.55 | 2.34 | 14.56 | 8.55 |
| 8 kg K | 36.22 b | 28.77 | 2.12 | 15.89 | 8.70 |

* Different letters indicate significant differences at P <0.05

### TABLE 6

| Correlations of germination ratio and mean germination time with seed macro and micro nutrients |
|--------------------------------------------------|----------------|----------------|----------------|----------------|
| GR | MGT | N | K | Mg | P | Na | Mn |
| 1.00 |  |  |  |  |  |  |  |
| -0.838* | 1.00 |  |  |  |  |  |  |
| 0.906* | -0.765 | 1.00 |  |  |  |  |  |
| 0.923* | -0.677 | 0.979** | 1.00 |  |  |  |  |
| 0.960** | -0.738 | 0.975** | 0.993** | 1.00 |  |  |  |
| 0.969** | -0.783 | 0.979** | 0.986** | 0.997** | 1.00 |  |  |
| -0.845* | 0.654 | -0.693 | -0.743 | -0.768 | -0.789 | 1.00 |  |
| 0.727 | -0.461 | 0.817* | 0.846* | 0.839 | 0.804 | -0.327 | 1.00 |

* Correlations are significant at P <0.05.
** Correlations are significant at P <0.01.

### TABLE 7

| Effects of foliar potassium treatments on leaf amino acids, pmol ul\(^{-1}\) |
|----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                                  | Aspartate | Glutamate | Asparagine | Serine | Glutamine | Histidine | Glycine | Thionine | Arginine | Alanine | Tyrosine |                      |
| Control                          | 2.64 b    | 1.11      | 4.99 b     | 5.54 b  | 4.02 b    | 3.08      | 1.76 b  | 3.92      | 10.15 b   | 11.69    | 0.67 c   |                      |
| 1 kg K                           | 3.32 a    | 1.50      | 7.13 a     | 6.08 b  | 6.14 a    | 4.08      | 2.73 a  | 4.98      | 12.80 ab  | 12.98    | 0.82 b   |                      |
| 2 kg K                           | 3.54 a    | 1.74      | 8.11 a     | 6.76 a  | 6.34 a    | 3.94      | 2.98 a  | 5.54      | 13.64 a   | 12.51    | 0.85 b   |                      |
| 4 kg K                           | 3.77 a    | 1.85      | 8.05 a     | 7.11 a  | 7.20 a    | 4.39      | 3.21 a  | 6.39      | 14.54 a   | 13.96    | 0.96 a   |                      |
| 8 kg K                           | 3.40 a    | 1.74      | 9.28 a     | 6.87 a  | 7.10 a    | 4.00      | 3.55 a  | 5.75      | 13.11 a   | 12.72    | 0.95 a   |                      |
|                                  | Cysteine  | Valine    | Methionine | Threonine | Tyrosine | Lysine | Sarcosine | Proline | Total amino acid, nmmol ul\(^{-1}\) |
| Control                          | 1.30 c    | 0.70 b    | 1.25       | 0.93 b  | 1.11      | 2.23      | 1.62 b  | 3.10      | 8.49      | 0.05 d   | 71.65 e  |                      |
| 1 kg K                           | 1.70 b    | 0.83 ab   | 1.69       | 1.87 a  | 1.35      | 2.23      | 1.99 b  | 3.72      | 8.77      | 0.10 c   | 88.17 d  |                      |
| 2 kg K                           | 1.85 b    | 0.92 a    | 1.80       | 1.80 a  | 1.39      | 2.48      | 2.17 a  | 4.14      | 8.45      | 0.20 a   | 92.47 c  |                      |
| 4 kg K                           | 1.99 b    | 1.06 a    | 1.92       | 2.01 a  | 1.58      | 2.61      | 2.34 a  | 4.35      | 9.43      | 0.15 b   | 100.42 a |                      |
| 8 kg K                           | 2.21 a    | 0.96 a    | 1.73       | 1.83 a  | 1.56      | 2.52      | 2.58 a  | 4.21      | 8.60      | 0.10 c   | 96.38 b  |                      |
TABLE 8
Correlations of germination ratio and mean germination times with leaf amino acids

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TABLE 9
Correlations of germination ratio and mean germination times with the rest of leaf amino acids

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With increasing K2O doses, seed germination ratios increased, thus correlations were observed among leaf Tyrosine, Phenylalanine, Isoleucine, Cysteine, Leucine, Lysine, Asparagine, Serine, Glycine contents together with increasing germinations (Table 8).

Since a positive correlation was observed between leaf Tyrosine, Phenylalanine, Isoleucine, Cysteine, Leucine, Lysine, Asparagine, Serine, Glycine contents and seed germination, it was thought that seed germination could be estimated ahead (Table 9). A germination test in pepper seeds takes about 21 days. There was a negative correlation between mean germination time, which can be taken as an indicator of seed vigor and serine, and isoleucine. Increasing leaf serine and isoleucine contents were observed with decreasing mean germination times. Therefore, it is possible to estimate seed vigor ahead by measuring leaf Serine and Isoleucine contents.

CONCLUSION

In this study, potassium treatments increased total amino acid contents as compared to the control treatment. Present findings comply with the results of earlier studies [8, 11, 12, 27]. Together with K, 13% N treatments also were found to be effective in increasing leaf amino acids. N exists in structure of Tryptophan amino acid. Therefore, leaf Tryptophan contents increased with increasing N treatments (Table 7).

Germination ratios increased with increasing K doses (Table 3). Such findings comply with the results of earlier studies [28, 29, 30] indicating that potassium retarded early plant development, phosphorus resulted in early ripening, thus prevented insufficient seed fills.

Pre-harvest leaf Isoleucine and Serine contents positively correlated with seed germination and negatively correlated with seed vigor. Further research is recommended to investigate similar correlations of Isoleucine and Serine with the seed germination and vigor of the other pepper cultivars and vegetable species cultured in open-fields.

REFERENCES


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ONE-POT SYNTHESIZED Fe₃O₄/PMMA MAGNETIC NANOADSORBENTS FOR FAST Cd(II) REMOVAL

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ABSTRACT

Cd(II) has detrimental effects on the environment and human health, and is commonly identified as a main contaminant. Therefore, Cd(II) removal from wastewater is of great significance. Without any surfactant, crosslinking agent or organic stabilizer, the core-shell Fe₃O₄/polymethyl methacrylate (PMMA) magnetic nanoadsorbents were fabricated using PMMA wastes. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Brunauer–Emmet–Teller surface area analysis (BET) showed that the adsorbents had good dispersion with rich functional groups on the adsorbent surface. The effects of the adsorbents concentration, initial Cd(II) concentration, pH value, and treatment time on the adsorption were investigated in detail. For operation at T=298 K and pH=1.5 ± 0.05, high Cd(II) removal efficiency of >95% was observed. The kinetic studies revealed that these nanoadsorbents followed a pseudo-first-order model. The calculated thermodynamic parameters from isothermal studies indicated that the maximum adsorption capacities of Cd(II) calculated from Freundlich model was 42.37 mg/g. Furthermore, the absorbents could be regenerated by just using magnet and the adsorption capacity was from original 95.5% to 91.2% after five consecutive cycles. This novel method would be of great importance to the industries for the reuse of E-wastes and the removal of heavy metal Cd(II) from polluted water.

KEYWORDS:
polymethyl methacrylate, magnetic nanoadsorbents, Cd(II) removal.

INTRODUCTION

Cadmium pollution has attracted more and more attention since cadmium compounds have been widely used in lead-zinc mine, non-ferrous metal smelting, and electroplated factories. Cd(II) has detrimental effects on the environment and human health, and is commonly identified as a contaminant because of its high toxicity and mobility[1]. “Industrial enterprises design and health standards” states that the highest allowable concentration of cadmium in ground water is 0.01 mg/L in USA [2]. “Industrial waste discharge standard” requires that the cadmium content in wastewater shall not exceed 0.1 mg/L in China [2]. Cadmium in industrial wastewater can be typically treated by various physicochemical processes such as electrochemical reduction [3], precipitation [4], membrane/reverse osmosis [5, 6], ion exchange [7, 8], and adsorption [9, 10, 11, 12, 13, 14, 15, 16, 17]. However, precipitation produces a large amount of precipitate sludge that requires additional processes for further treatment [4]. The disadvantages of membrane treatment is the high operational and maintenance cost [5]. Ion exchange is a convenient method to treat wastewater, but strict pretreatment is needed and the operation cost is also higher than that of other methods [7, 8]. When it comes to Cd(II) removal, the nanostructured TiO₂ particles [2] and green synthesized Fe₃O₄ nanoparticles [18] have been used and the removal capacity is satisfactory with an extended treating time. However, the separating and recycling of these materials turn out to be a challenge especially when the particle size goes down to nanoscale. At the same time, reducing the operation time in each cycle is urgently required in industrial applications. Consequently, adsorption is an alternative favorable and feasible approach because of its low cost and high efficiency [8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18]. Besides, adsorption can effectively remove heavy metals in the wastewater at a low concentration [19]. Activated carbon and iron minerals are common adsorbents to purify polluted water [20, 21], but they still fail to remove the contaminants at ppb levels. Iron and iron oxide nanostructures have been proved as higher efficient materials for the heavy metal removal by reduction or adsorption [22, 23, 24, 25]. However, there are two major challenges when using these nanomaterials. One comes from the easy oxidation of pure nanoparticles, especially in acidic solutions. The other is the difficulty to recycle these nanoparticles with such a small size, especially in a continuous flowing system. To protect the magnetic nanoparticles against oxidation, a noble shell structure is introduced [26].
Due to low toxicity, high magnetic property and low cost, Fe$_3$O$_4$ has been synthetically applied to research magnetic materials [27, 28]. Thus, magnetic nanoparticles have been prepared using Fe$_3$O$_4$ to introduce magnetic property for convenient recycling. For example, Cao etc. have fabricated magnetic composite particles by a simple one-pot hydrothermal method in the presence of commercial polymethyl methacrylate [29]. Fe$_3$O$_4$ nanoparticles are dispersed well in PMMA to form bigger magnetic composite particles. These composite particles have special functional groups on the surface, which can improve the interaction between suspension particles and matrix. In addition, China is the world’s second largest electronic waste producer, producing all kinds of E-waste, Figure 1. PMMA, which can improve the interaction between suspension particles and matrix. Therefore, using waste PMMA to prepare magnetic powders were dispersed uniformly. The black material was protonated and HCl solution was drained. The above three neck flask was placed in ultrasonic cleaning machine at 5 °C. Then (NH$_4$)$_2$S$_2$O$_8$ (0.54 g) were added assisted with intermittent ultrasonication (5 min ultrasonication at intervals of 10 min) in the condition of constant stirring. The products were separated with a magnet and washed by ethanol. This operation was also repeated for 2-3 times. Finally, the final products were dried at 40 °C for 6 h under the condition of vacuum (The detailed preparation of nano-sized Fe$_3$O$_4$ magnetic powders was presented in the Supporting Information (SI), Figure S1).

**Fabrication of Fe$_3$O$_4$/PMMA magnetic nanoadsorbents**: The prepared nano-sized Fe$_3$O$_4$ magnetic powders were dispersed in 70 mL H$_2$O under sonication, and then PMMA (0.1 g) in ethanol (15 mL) and HCl (0.1 mol/L) were added into a three-neck flask. The mixture was dispersed under ultrasonication at room temperature until the magnetic powders were dispersed uniformly. The black material was protonated and HCl solution was drained. The above three neck flask was placed in ultrasonic cleaning machine at 5 °C. Then (NH$_4$)$_2$S$_2$O$_8$ (0.54 g) were added assisted with intermittent ultrasonication (5 min ultrasonication at intervals of 10 min) in the condition of constant stirring. The products were separated with a magnet and washed by ethanol. This operation was also repeated for 2-3 times to remove the residual methyl methacrylate monomers and HCl acid. The final products were dried at 40 °C for 6 h under the condition of vacuum, Figure S2.

The same synthetic method was used to fabricate Fe$_3$O$_4$/PMMA magnetic nanoadsorbents, just changing the waste PMMA into pure PMMA (the product is named as “PFPMN”) as a reference substance.

The adsorption process of adding PMMA and the protonation processing of adding HCl acid have played a vital role in the early stage of the preparation. And using intermittent ultrasonication can effectively improve the dispersity of the material. Therefore, three conditions are discussed to illustrate the reaction mechanism in Figure 2. The sample was protonated without adsorption processing, Figure 2(a). The sample had the adsorption processing...
without protonation, Figure 2(b). The other conditions were the same as before, intermittent ultrasoundation was not used in the process in Figure 2(c). As shown in Figure 2(a), the prepared adsorbents have a core-shell structure after the protonation. However, the average thickness of the shell is only 13 nm in the same conditions. Compared with Figure 2(b), the shell in Figure 2(a) is obviously thinner. This indicates that if the protonated Fe₃O₄ nanoparticles don’t have the adsorption processing in ethanol solution, the core-shell structure of the FPMN can also be fabricated. Unfortunately, the thickness of the formed shell is thinner with the same treatment time. Therefore, the adsorption process of PMMA has contributed to the formation of a thicker shell. When the sample have the adsorption processing, the magnetic core is exposed and the shell can’t be seen clearly in Fe₃O₄ surface, Figure 2(b). The results have shown that the protonation is a key step for the formation of the core-shell structure. Ultrasonation plays an important role in the preparation of core-shell adsorbent. Figure 2(c) is the image of prepared samples without using ultrasonation as auxiliary conditions. The dispersion is poor and the magnetic particles have been reunited together. To sum up, protonation and ultrasonation are necessary in the fabrication of FPMN.

Characterizations. XRD analysis of the samples were carried out using a Philips X’Per powder X-ray diffractometer with Cu-Ka radiation (λ=1.5406 Å). The samples were investigated by Fourier infrared analyzer (FTIR, Bruker Tensor 27). The morphology of the FPMN was characterized by scanning electron microscopy (SEM, JMS 6700). The SEM samples were prepared by drying a drop of FPMN on carbon-coated SEM sample stage. The morphology, particle size and dispersion of the samples were measured by transmission electron microscopy (TEM, Hittach H-800). The specific surface area, pore volume, and pore diameter of nanoparticles were measured by Brunauer–Emmett–Teller surface area analysis (BET, Micromeritics ASAP 2020).

![FIGURE 2](image)

The TEM microstructures of the samples prepared with different conditions: (a) without adsorption processing (b) without protonation (c) without intermittent ultrasonation.

![FIGURE 3](image)

XRD patterns of (a) Fe₃O₄ (b) PFPMN and (c) FPMN.
Cd(II) Removal. Batch experiments were conducted in a three-neck flask (100 mL), each of which contained 50 mL target solution. A given amount of adsorbents was introduced to each experiment. To avoid oxidation of the samples in the preparation of magnetic nanoadsorbents, all the experiments were carried out under the protection of nitrogen, and the data was recorded. The pH values of solutions were adjusted using solutions of HCl and NaOH. Then the experiments were carried out under different initial solution concentrations of Cd(II), pH values, time and different dosages of FPMN to better reveal the impacts of different experimental conditions on the removal efficiency. The above experiments all were repeated above three times at room temperature.

RESULTS AND DISCUSSION

Characterizations of Fe₃O₄/PMMA magnetic nanoadsorbents. Figure 3 shows the XRD patterns of FPMN. Because the purity of waste PMMA was so high, the almost overlap, Figure 2(b&c), shows that the recycled PMMA could replace pure PMMA completely. The well-defined main peaks at 30.13, 35.76, 37.71, 54.01, 55.13, 57.08, 62.63 were related to Fe₃O₄ (JCPDS No. 01-1121) [33,34]. The peak positions corresponded to the crystal plane of (220), (311), (400), (422), (511) and (440) of the cubic spinel structure of Fe₃O₄, Figure 3(a). No other characteristic peaks observed. Figure 3 confirmed that the coated PMMA is amorphous and Fe is not leached. The Fe₃O₄ was not oxidized and the crystal shape remained unchanged in the process of fabrication.

To further determine the chemical structure of the sample, the FTIR spectra of PMMA and FPMN were obtained, Figure 4. The characteristic peaks of 1320 cm⁻¹ was attributed to the C-O stretching vibration absorption of PMMA, Figure 4(a). The characteristic absorption peaks at 3124, 2976 and 920 cm⁻¹ corresponded to the C-H stretching vibration absorption and the peak shift of the C-H vibration peak was the in-plane bending and out of plane bending. Furthermore, the characteristic absorption peaks at 1706 cm⁻¹ corresponded to the C=O stretching vibration absorption, Figure 4(b). All the characteristic PMMA absorption peaks observed in the composite powders indicated further that the FPMN were successfully fabricated.

To further characterize the particle size and morphology of FPMN, the samples were tested by SEM, Figure 5(a) and (b). The surface of the sample was observed to be attached to a number of smaller nanoparticles, which was also identical to the observation of TEM image. The whole morphology showed a blackberry shape. Figure 5(c) and (d) shows the TEM microstructures of the synthesized nanoparticles. The inner darker area was nano-sized Fe₃O₄ magnetic powders [26]. The Fe₃O₄ and PMMA were not a simple cross-linking or blending, Fe₃O₄ was coated and a typical core shell structure was formed. The TEM observation also indicated that the prepared adsorbents had a better dispersion and the thickness of package was uniform. The particle size of the sample was about 350 nm and the coating thickness was about 28 nm, Figure 5(d).

FTIR spectra of (a) PMMA and (b) FPMN.
BET analysis on surface area and porosity of the samples was measured using a Micromeritics ASAP 2020. The BET characterization showed that the specific surface area, pore volume, and pore diameter of nanoparticles were 53 m²/g, 0.6 cm³/g, and 4.8 nm, respectively.

**Nanoadsorbents formation mechanism.** The magnetic powders coated by PMMA are acidizing at a low pH by dilute solution of cheap inorganic proton-acid (HCl). When the magnetic powders are in the weak acid environment, H⁺ are adsorbed by interaction of static electricity, thus settling on the surface. For one thing, a lot of positive charges on the surface of the shell can prevent the agglomeration. For another, the PMMA containing unpaired electrons is more easily adsorbed on the surface of magnetic powders. The intermittent ultrasonation favors the dispersion of the sample. Finally, the core-shell structure is formed, Figure 6. And the proton can be recycled and reused. Therefore, this method can greatly reduce the environmental pollution.

**FIGURE 5**
(a) and (b) the SEM images of FPMN; (c) and (d) the TEM images of FPMN.

**FIGURE 6**
The possible synthetic mechanism of core-shell structure.
The effects of initial Cd(II) concentration on the Cd(II) removal were studied. The percentages of the adsorbed Cd(II) increased with increasing the dosages of FPMN over the range from 0.2 to 2.0 g/L, Figure 7(A). The Cd(II) was removed from the solution at the highest testing concentration of 1.2 g/L, which had a much lower removal efficiency than 0.8 g/L solution of Cd(II). Almost 100% Cd(II) was removed while using 2.0 g/L FPMN within 90 min for CdCl₂ solution, which exhibited the highest removal efficiency, Figure 7(B).

Figure 7(C) shows the effects of initial pH on the Cd(II) removal. The initial pH was a critical parameter for the adsorption of Cd(II) on the solid adsorbents in aqueous solutions [38]. The pH of the solution significantly affected the surface charges and the protonation degree of adsorbents. The influence of pH on the Cd(II) adsorption capacity by FPMN was studied over the pH range from 0 to 10.4. At a fixed adsorbent concentration, the higher Cd(II) removal was achieved under acidic condition rather than in neutral or basic conditions. The percentage of the adsorbed Cd(II) was observed to decrease slightly when the pH was increased. When the pH ranges was changed from 1.5 to 8.8, the removal percentage of Cd(II) decreased from 98.5% to less than 1%.

Figure 7(D) shows the effects of adsorption time on the removal of Cd(II) at an initial concentration of 2.0 g/L FPMN. The adsorption rate of Cd(II) was low during the first few minutes. The adsorbents exhibited the highest removal efficiency and the adsorbed amount of Cd(II) reached its equilibrium value in about 90 min. Compared with the reported materials of nanostructured TiO₂ particles [2] that the hydroxyl groups on the surfaces of the dandelions were responsible for Cd(II) adsorption, the active functional groups on the FPMN surface played a leading role in the process of absorption. The regenerative metal-organic framework with reversible Cd(II) uptake was not stable and fast [17], the Cd(II) removal was instead really stable and fast by the FPMN adsorbents.

![Graphs and images showing Cd(II) removal percentage vs various parameters](image-url)
Adsorption kinetics and isotherms. The pseudo-first-order kinetic [2] and pseudo-second-order kinetic model [32] were tested. The linear equations of these kinetic models were given as follows:

\[
\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t
\]

(1)

\[
\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}
\]

(2)

where \(q_e\) and \(q_t\) are the amount of Cd(II) adsorbed on the surface of adsorbents at equilibrium and at any time, respectively; \(k_1\) and \(k_2\) are the rate constants of pseudo-first-order and pseudo-second-order kinetic model. The validity of each kinetic model was checked by the fitness of the straight lines (Figure 8). It manifested that the pseudo-first-order kinetic model presented a better fit to all of the experimental data according to \(R^2\). This indicates that these adsorbents followed a physical absorption involving Vander Waals Force through the electrostatic interaction. The rate constant of pseudo-first-order \(k_1\) of 0.0913 min\(^{-1}\) is greater than the reported wool (0.0396 min\(^{-1}\)) and D354 anion-exchange resin (0.0670 min\(^{-1}\)) [31].

Adsorption isotherms are of importance in the design of adsorption systems because they can reflect the surface characteristics of the adsorbent at a micro level as well as the adsorption characteristics of adsorption capacity at a macro level [32]. The adsorption isotherm is based on the assumption that every adsorption site is equivalent and independent of whether or not adjacent sites are occupied. The data obtained from Figure 9 was evaluated by Freundlich model\(^{11}\) and Langmuir isothermal model [32]. The stronger binding sites were occupied first and the binding strength decreased with increasing the occupied site of the Freundlich model. The Freundlich equation could be expressed as Equation (3):

\[
\log q_e = \log K_f + \frac{1}{n} \log C_e
\]

(3)

where \(q_e\) (mg/g) is the amount of Cd(II) adsorbed on the surface of adsorbents at equilibrium and \(K_f\) and \(n\) are constants of the Freundlich model.

The Langmuir equation can be expressed as Equation (4):

\[
\frac{C_e}{q_e} = \frac{C_0}{q_0} + \frac{1}{q_0 K_L}
\]

(4)

where \(q_0\) is the maximal sorption capacity at equilibrium (mg/g); \(K_L\) is Langmuir constant related to the affinity of the binding sites.

The Freundlich adsorption isotherm and Langmuir isotherm model can be used successfully for modeling the equilibrium data. The maximum adsorption capacities of Cd(II) calculated from Freundlich model were 42.37 mg/g, which was much higher than that of multiwalled carbon nanotubes (10.86 mg/g) [2].

![FIGURE 8](image)

Linear fit of experimental data obtained using (a) pseudo-first order model and (b) pseudo-second order model (T= 25 °C, pH= 1.2, t= 90 min).
FIGURE 9
Cd(II) adsorption isotherm by FPMN fitted by the (a) Langmuir and (b) Freundlich models.
(T= 25°C, pH= 1.2, t= 90 min).

FIGURE 10
The reuse of the FPMN for Cd(II) removal.

FIGURE 11
The possible role mechanism of Cd(II) removal.
Regeneration of Fe3O4/PMMA magnetic nanoadsorbents. The desorption experiments of magnetic nanocomposite particles were to reuse the adsorbents for cadmium removal. The FPMN could be desorbed only by NaOH solution (0.5 mol/L) at room temperature [35, 36, 37]. The FPMN were reused for Cd(II) adsorption after desorption. The above experiments were repeated for five times to determine the maximum adsorption capacity. The regeneration and utilization of adsorbents was the key index to improve the cost efficiency [31, 32]. Compared with the first reused experiment, the adsorption of the second and the third tests did not show an obvious difference. The adsorption performance of the fourth was slightly lower, from original 95.5% to 91.2%. The results for this study implied that about 95% Cd(II) was desorbed from the adsorbents. Moreover, in order to evaluate the reusability of the adsorbents, the desorption experiment was repeated for many times. The adsorption efficiency remained constant (91% - 95%) after five cycles of adsorption and desorption, Figure 10.

Removal Mechanism. FPMN are new functional materials consisting of magnetic and non-magnetic materials with core-shell structure. The magnetic core is mainly responsible for easy collection and the performance of the shell plays a leading role in the process of absorption. FPMN has the special properties of the PMMA that the surface of the adsorbents is full of active functional groups. This can effectively improve the dispersivity of the adsorbents. Furthermore, the functional groups can selectively adsorb metal ions from aqueous solution by electrostatic attraction. Figure 11 shows the schematic diagram for the adsorption of Cd(II) by FPMN. The surface of the PMMA shell can provide copious active adsorption points, and facilitate the Cd(II) transportation in the adsorption process. The obvious increase of the surface area and porosity of the adsorbents can be ascribed to the unique morphology of the nanoadsorbents.

CONCLUSION

Without any surfactant, crosslinking agent or organic stabilizer, core-shell Fe3O4/PMMA magnetic nanoadsorbents have been fabricated using waste PMMA (polymethyl methacrylate). This method can both reduce E-waste pollution and environment pollution. Based on the characterization analysis by XRD, FTIR, SEM and TEM, the adsorbents have showed a core-shell structure, and their surface is rich in functional groups which are helpful to the Cd(II) removal. Furthermore, the adsorbents demonstrate a great performance for Cd(II) removal, and they are able to remove Cd(II) within 90 min. The adsorbents have a higher adsorption capacity at pH 1.5. In addition, the Cd(II) removal percentage decreased with increasing the initial Cd(II) concentration. Moreover, the adsorption kinetic was better fit with pseudo-first-order. The maximum adsorption capacity of 42.37 mg/g is calculated following Freundlich model for the magnetic polymer nanoadsorbents. The prepared adsorbents are not only efficient for environmental cleanup, but also can be recycled more easily and rapidly. The absorbents can be regenerated by just using a magnet and the adsorption capacity is nearly unchanged after three consecutive cycles. These demonstrated that the adsorbents are an efficient, stable, less toxic and magnetically separable adsorbents for fast Cd(II) removal.

ACKNOWLEDGEMENTS

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The detailed preparation of nano-sized Fe3O4 magnetic powders; The detailed preparation of Fe3O4/PMMA magnetic nanoadsorbents; Brunauer-Emmett-Teller surface area analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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DETERMINATION OF ENERGY EFFICIENCY IN AFTER GROUNDNUT PRODUCTION (OSMANIYE CASE)

Burak Saltuk

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ABSTRACT

This study aims to determine the energy use efficiency of groundnut production for the season of 2017 in Osmaniye province. Data for this study such as the economic life of machine and tools, work performance and fuel-oil consumption, machine weight and amount of fertilizer and seed are provided from in-situ measurements, relevant studies, various sources, and catalogs. As a result of the evaluations, energy output/input ratio was calculated as 1.94; specific energy value was 12.88 MJ kg⁻¹, energy productivity was 0.08 kgMJ⁻¹, net energy efficiency was 44.853.37 MJ ha⁻¹. In after crop groundnut production, irrigation energy was found to be the highest with %33.73 share. About normal production of groundnut, 48.69% of the total energy inputs were found to be irrigation energy this was followed by fuel-oil energy with 31.02%, fertilizer energy with 17.59%, seed energy with 7.87%, plant protection material energy with 6.59%, machine energy with 2.96% and human energy with 0.22%.

KEYWORDS:
Osmaniye, energy balance, groundnut, energy rate

INTRODUCTION

Groundnuts are used in large quantities in various fields of industry as well as being human food. Groundnut oil is used as solid and liquid food cooking oil, as well as in fish preserving, biscuits, pastry, confectionery and soap making. Groundnut residue is a precious feed material after the oil extraction from groundnuts [1].

After oil extraction process, groundnut residues contain nearly %45 crude protein, %24 essences, and %5.5 minerals. Therefore, developed countries, groundnut residues are being used in the formation of compound feeds. Groundnut seeds contain about 18% carbohydrate and plenty of nutrients such as K, Ca, Mg, P and S. Also, groundnuts are rich in A, B and E vitamins. Because it is a legume plant, it is fed directly to animals as green fodder, dried and the bale is used as winter feed. Groundnut’s hay contains 11% protein, 5% fat, 22% crude cellulose, 42% undissolved extract, 10% ash and 10% water [2-3-4-5].

Due to the insufficient production of oilseed plants in Turkey, certain amounts of oilseeds are imported every year. According to 2016 data, 555 million tons of oilseeds are produced in the world, and 43.9 million tons of oil is provided from groundnuts. In Turkey, 2.6 million tons of oilseed and 780 thousand tons of crude vegetable oil produced. Vegetable crude oil production in the world is 187 million tons [5].

Because it is a legume plant, groundnut utilizes the free nitrogen of the air and provides a soil rich in nitrogen and organic matter to the after crop. Groundnut is a hoe crop that is tilled during vegetation period; it leaves a clean and ventilated soil. So groundnut is an ideal crop-rotation product, it will contribute to more effective use of plant nutrients at different depths and enrich the soil microbiology [6]. As a result, the determination of the land use quality of agricultural land during agricultural practices is a priority regarding sustainable production instead of production capability and income level [7]. Groundnuts can be grown as primary crops in some parts of Turkey as it could be an after crop of cereals in other parts [8].

<table>
<thead>
<tr>
<th>Years</th>
<th>Area (da)</th>
<th>Production (Tonne)</th>
<th>Yield (kg/da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>73790</td>
<td>24793</td>
<td>336</td>
</tr>
</tbody>
</table>

Osmaniye has favorable climate and soil conditions for groundnut farming. In Turkey, nearly %90 of groundnuts are processed and marketed in Osmaniye. Groundnut farming in the province of Osmaniye has shown great improvement in the past ten years. Especially the production as an after crop increased its importance for the region. Since there is not much difference in yield between the main product and the after-product; It has become a good source of income for a wide range of people from groundnut production to processing and marketing [5].

Energy analysis of agricultural production is an essential approach in defining and grouping the agricultural systems concerning energy
consumption. In order to increase efficiency in production and reduce inputs, the inputs and outputs used in production should be carefully analyzed [10]. In order to increase the value of energy efficiency, it is necessary to increase the efficiency or to reduce the inputs. In particular, fuel, chemical fertilizers, plant protection materials, machinery, and tractor inputs need to be reduced. Efficiency can be increased within certain limits. However, the energy use efficiency value can be reduced by making the inputs conscious (spraying, mechanization, and fertilization) [11].

In some studies conducted to determine the energy use efficiency of production, energy use activities are determined in the production of; Lavender [12], barley [13], sunflower [14], sugar beet [15], walnut [16, 17, 18], pistachcio [19, 20, 21], groundnuts [21, 22, 23, 24], almond [25, 26, 27].

In this study, the energy balance of groundnut production in Osmaniye in 2017 was investigated.

MATERIALS AND METHODS

Osmaniye is located to the east of the Mediterranean Region. It is surrounded by Gaziantep in the east, Hatay in the south, Adana in the north and Kahramanmaraş in the north. The province has a surface area of 3,767 square kilometers, of which 45% is forested, 37% is planted agricultural area, 16% is unfavorable for agriculture, and 2% is for other lands. The province is 118 m above sea level and 21 km to the Mediterranean Sea. The land gains elevation from south to north and east. In the western part of Osmaniye, the plains of the Adana plain extend to the east. Amanos Mountains (Gavur Mountains) extending eastward from Iskenderun Bay to the south, the Taurus Mountains to the northwest, Dumanlı Mountains to the east and Dülşül Mountains to the east are present in the area. There is lightly rough terrain between the mountains and the plains. The plains are mostly located in the districts of Toprakkale, Kadirli, and Düziçi [28]. The Osmaniye climate shows variations from the mountainous parts and plains but is influenced by the Mediterranean climate. Summers are hot and dry, and winters are mild and rainy. The surface area is 3.279.9 km², and it is 121 m. above sea level, 20 km far from the Mediterranean Sea. The precipitation is much higher in winter and autumn than in other months, and the average annual rainfall is 767.6 mm. [29].

In this study, various input quantities and output values obtained from groundnut production were gathered from different sources, technical data of agricultural tools and machinery were taken from the applications and catalogs in the region. The cultural practices and maintenance procedures for the production of groundnuts in Osmaniye are given in Table 2.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>After Crop Groundnut Production Cultural Applications and Maintenance Operations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Practices</td>
<td>Features of Practices</td>
</tr>
<tr>
<td>Soil Cultivation</td>
<td>After the harvest of the pre-plant, the soil is plowed at a depth of 20-25 cm with the plow. Then the disc harrow is applied two times. Finally, the soil is fixed/pressed with the roller or worshiper 2-3 times according to the moisture condition of the soil. With the 4-row pneumatic seed drill, the seeding is carried out as the row is 70 cm and the row is 8-10 cm. The amount of seed to be used is 10-20 kg/da depending on the seed size. Groundnut seeds should be sprayed against sub-soil disease and pest before planting. If sowing is done in the dry form, sprinkler irrigation is applied 1 or 2 times after sowing to ensure germination of seed and appropriate output. 8-10 kg/da of pure phosphorus, approximately 3 kg/da of pure nitrogen should be given with sowing. In addition to the amount of nitrogen supplied with sowing, about 10-15 kg/da of pure nitrogen is given in the form of ammonium sulfate. In case of iron deficiency in some soils, iron is applied from the leaf.</td>
</tr>
<tr>
<td>Seeding</td>
<td>During the cultivation of groundnuts, a total of 4-5 times hoeing machine is applied. Depending on the soil condition, throat filling may be required. In the weed control, herbicides are used, instead of hand hoe; 1 or 2 times depending on the weed condition.</td>
</tr>
<tr>
<td>Fertilization</td>
<td>In groundnut production, depending on the condition of the air and the environment 3-4 times aphid, red spider and fungicide are applied. Most of the groundnut plant in Osmaniye is irrigated as sprinkler irrigation. The groundnut plant has watered an average of 7 times, including the water for the seed germination, depending on the climate and soil conditions during the growing period. In case of excessive temperature or sandy soil, the number of irrigation is increased. Groundnut harvest time is the time where the plant leaves start to turn yellow, the grains get pink, or the shell interior turns from white to brown, and the capsules are full. Groundnut plants are dismantled and inverted from the ground with a groundnut ejector machine with 2 or 3 rows of the 1-row barrel. After 3-5 days in the sun, it is collected by the collecting and blending machine. Collected products are moved to the laying place and finally subjected to screening.</td>
</tr>
</tbody>
</table>
Sowing norm ranges from 10-20 kg/da depending on the frequency of crops, the distance between the rows and the seed size. Before sowing, every 100 kg of seed is treated with 0.150 kg of insecticide and 0.5 kg of fungicides according to the sub-ground disease and pest and the type of chemical used. The amount of fertilizer used was calculated as 10 kg/da pure phosphorus and 12 kg/da pure nitrogen in the primary product and the after product. Two herbicides were applied for the weed control in both the primary product and the after product. Fungicides and insecticides were applied for various diseases and pests three times. Sprinkler irrigation was calculated as seven times in the after product. Fuel-Powered energy supply was used in sprinkler irrigation. The after product groundnut plant in the region has an average water consumption of 382.7 mm/da [30].

In order to calculate the energy efficiency of groundnut production in Osmaniye, it is necessary to calculate the energy inputs and energy outputs first. Energy inputs consist of workforce energy, mechanical energy, fuel-oil energy, seed energy, water energy, fertilizer energy and energy of the energy.

According to the measurements made for the after product groundnut in Osmaniye province, the values of the energy inputs and outputs are determined. Tractor production energy as energy inputs (machine-made energy for tractors was calculated as 35,216 MJ kg⁻¹ for Turkey [31]. Machine-made energy, fuel-oil energy, fertilizer energy, pesticides energy, human labor energy, seed energy and transport In order to determine the energy usage analysis in groundnut production, the second product was used in energy ratio, specific energy, energy efficiency and net energy efficiency in formulas 1, 2, 3 and 4 [32].

**Energy units are referred as MJ/ha in equation 1, 2 and 3.** Equal energy values of inputs and outputs used in the crop production are shown in Table 3. Energy output consists of yield and sub-yield obtained from unit area. Equivalent values of energy inputs and outputs must be known to calculate the energy input and output. Prior studies are benefited to identify the energy equivalents.

### RESULTS AND DISCUSSION

In Osmaniye province, energy balance in after product groundnut peanut production is shown in Table 4 and general energy inputs and distributions are given in Table 5. When we examine Table 4, 105.07 MJ / ha human energy is consumed per unit area; this value is the lowest input with 0.22%. 1412.57 MJ energy was consumed for 1 ha field in tool/machine energy, which corresponded to 2.96% of total energy. In all the inputs, irrigation energy was consumed with 16073.40 MJ / ha and the highest with 33.73%. The fuel-oil energy input corresponds to 31.02% by consuming 14781.80 MJ / ha and the fertilizer energy input to 8382.00 MJ / ha and 17.59%. So pest control energy input was 6.59% with 3141.80 MJ / ha, while the seed energy input was 7.87% with a value of 3750 MJ / ha.

<table>
<thead>
<tr>
<th>Energy Equivalents of Inputs and Outputs in Agricultural Production</th>
<th>Energy Equivalents Coefficient (MJ/unit)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Input</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Labor (h)</td>
<td>1.96</td>
<td>[33]</td>
</tr>
<tr>
<td>Tractor</td>
<td>158.3</td>
<td>[34]; [35]</td>
</tr>
<tr>
<td>Soil Cultivation Equipment</td>
<td>121.3</td>
<td>[34]; [35]</td>
</tr>
<tr>
<td>Fuel (L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diesel</td>
<td>35.69</td>
<td>[36]</td>
</tr>
<tr>
<td>Oil</td>
<td>6.51</td>
<td>[36]</td>
</tr>
<tr>
<td>Chemical Fertilizers (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>60.6</td>
<td>[37]; [38]</td>
</tr>
<tr>
<td>Phosphorus (P2O5)</td>
<td>11.1</td>
<td>[37]; [38]</td>
</tr>
<tr>
<td>Pest Control Material (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbicide</td>
<td>269</td>
<td>[39]; [10]; [36]</td>
</tr>
<tr>
<td>Insecticide</td>
<td>214</td>
<td>[10]; [36]</td>
</tr>
<tr>
<td>Fungicide</td>
<td>278</td>
<td>[40]</td>
</tr>
<tr>
<td>Seed (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groundnut</td>
<td>25</td>
<td>[41]; [42]</td>
</tr>
<tr>
<td>Irrigation</td>
<td>4.2</td>
<td>[43]</td>
</tr>
<tr>
<td>Output</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groundnut</td>
<td>25</td>
<td>[41]; [42]</td>
</tr>
</tbody>
</table>

Energy rate = \( \frac{\text{Energy output}}{\text{Energy input}} \) (1)

Specific energy = \( \frac{\text{Energy input}}{\text{Yield}} \) (2)

Energy productivity = \( \frac{\text{Yield}}{\text{Energy input}} \) (3)

Net energy productivity = Energy output – Energy input (4)
According to Table 6, the total energy input from the after crop groundnut production was 47646.63 MJ / ha, the total energy output was 92500.00 MJ / ha, and the energy rate was 1.94. In this study, the energy rate was 1.94, while [23] found the energy rate of groundnut production in Kiashahr region in the north of Iran 3.93. [20] found energy rate 2.73 in the production of groundnuts in the region of Guilan in the north of Iran. [19] found 4.53 energy ratio in 120 enterprises producing groundnuts in three different cities in the Guilan region in the north of Iran.

In Osmaniye conditions, energy productivity in the production of groundnuts was determined as 0.08 kg / MJ only when the seed amount taken from the unit production area (ha) was taken into consideration. In the after crop groundnut production under Osmaniye conditions, 0.08 kg groundnut seed is produced for 1 MJ energy consumption. This value in the production of groundnuts; was found as 0.212 kg / MJ in the Kiashahr region in the north of Iran by [23]. This value in groundnut production was found to be 0.18 kg / MJ by [19], [20] found 0.14 kg / MJ in their study in the north of Iran in the region of Guilan.

The difference between the total amount of energy gained during production and the total amount of energy used in production operations is defined as net energy efficiency (MJ / ha) [13].

### TABLE 4

Osmaniye After Product Energy Balance in Groundnut Production

<table>
<thead>
<tr>
<th>Input</th>
<th>Amount per hectare</th>
<th>Total energy input (MJ/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human labor (h)</td>
<td>53.61</td>
<td>105.07</td>
</tr>
<tr>
<td>Soil preparation</td>
<td>5.40</td>
<td>10.59</td>
</tr>
<tr>
<td>Seeding and Other processes</td>
<td>37.87</td>
<td>74.23</td>
</tr>
<tr>
<td>Harvest</td>
<td>10.33</td>
<td>20.25</td>
</tr>
<tr>
<td>Machine (h)</td>
<td>47.22</td>
<td>1412.57</td>
</tr>
<tr>
<td>Tractor</td>
<td>23.61</td>
<td>342.64</td>
</tr>
<tr>
<td>Soil preparation</td>
<td>5.40</td>
<td>87.87</td>
</tr>
<tr>
<td>Seeding and Other processes</td>
<td>14.54</td>
<td>221.91</td>
</tr>
<tr>
<td>Harvest</td>
<td>3.67</td>
<td>760.15</td>
</tr>
<tr>
<td>Fuel +Oil (L)</td>
<td>429.29</td>
<td>14781.80</td>
</tr>
<tr>
<td>Soil preparation</td>
<td>73.15</td>
<td>2518.81</td>
</tr>
<tr>
<td>Seeding and Other processes</td>
<td>293.44</td>
<td>10104.01</td>
</tr>
<tr>
<td>Harvest</td>
<td>62.70</td>
<td>2158.98</td>
</tr>
<tr>
<td>Chemical Fertilizers (kg)</td>
<td>220.00</td>
<td>8382.00</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>100.00</td>
<td>1110.00</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>120.00</td>
<td>7272.00</td>
</tr>
<tr>
<td>Chemicals (kg)</td>
<td>12.05</td>
<td>3141.80</td>
</tr>
<tr>
<td>Herbicide</td>
<td>2.50</td>
<td>672.50</td>
</tr>
<tr>
<td>Fungicide</td>
<td>6.65</td>
<td>1848.70</td>
</tr>
<tr>
<td>Insecticide</td>
<td>2.90</td>
<td>620.60</td>
</tr>
<tr>
<td>Seed (kg)</td>
<td>150.00</td>
<td>3750.00</td>
</tr>
<tr>
<td>Irrigation (m³)</td>
<td>3827.00</td>
<td>16073.40</td>
</tr>
<tr>
<td>Total energy input</td>
<td></td>
<td>47646.63</td>
</tr>
</tbody>
</table>

### TABLE 5

After Product Groundnut general energy inputs and distributions

<table>
<thead>
<tr>
<th>Energy input</th>
<th>MJ ha⁻¹</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuel oil energy</td>
<td>14781.8</td>
<td>31.02</td>
</tr>
<tr>
<td>Machine energy</td>
<td>1412.57</td>
<td>2.96</td>
</tr>
<tr>
<td>Human Labour Energy</td>
<td>105.07</td>
<td>0.22</td>
</tr>
<tr>
<td>Seed Energy</td>
<td>3750.00</td>
<td>7.87</td>
</tr>
<tr>
<td>Pest Control/ Chemicals</td>
<td>3141.80</td>
<td>6.59</td>
</tr>
<tr>
<td>Fertilizer Energy</td>
<td>8382.00</td>
<td>17.59</td>
</tr>
<tr>
<td>Irrigation Energy</td>
<td>16073.40</td>
<td>33.73</td>
</tr>
<tr>
<td>Total</td>
<td>47646.63</td>
<td>100.00</td>
</tr>
</tbody>
</table>

### TABLE 6

After Product Groundnut total energy input-output and ratio values

<table>
<thead>
<tr>
<th>Total Energy Input (MJ/ha)</th>
<th>47646.63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Productivity</td>
<td>3700.00</td>
</tr>
<tr>
<td>Total Energy Output (MJ/ha)</td>
<td>92500.00</td>
</tr>
<tr>
<td>Energy Rate</td>
<td>1.94</td>
</tr>
<tr>
<td>Specific Energy (MJ/kg)</td>
<td>12.88</td>
</tr>
<tr>
<td>Energy Productivity (kg/MJ)</td>
<td>0.08</td>
</tr>
<tr>
<td>Net Energy Efficiency (MJ)</td>
<td>44853.37</td>
</tr>
</tbody>
</table>
After Crop Groundnut Energy Input (MJ ha⁻¹) Rate (%)

<table>
<thead>
<tr>
<th></th>
<th>Energy Input (MJ ha⁻¹)</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect Energy b</td>
<td>16686.36</td>
<td>35.02</td>
</tr>
<tr>
<td>Total</td>
<td>47646.63</td>
<td>100.00</td>
</tr>
<tr>
<td>Renewable Energy c</td>
<td>19928.47</td>
<td>41.52</td>
</tr>
<tr>
<td>Non renewable Energy d</td>
<td>27863.10</td>
<td>58.48</td>
</tr>
<tr>
<td>Total</td>
<td>47646.63</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**CONCLUSION**

In this study, the energy use efficiency in the after crop groundnut production for the 2017 season was determined in Osmaniye province in Turkey. As a result of the calculations, it is seen that the highest share of the production inputs is taken by the irrigation energy, followed by oil-fuel, fertilizer, seed, medicine, machinery and human labor energies, respectively. In order to reduce the irrigation energy input, the irrigation needs to be done as much as the need of the plant on necessary time. It can be said that the after crop groundnut in Osmaniye is an efficient production considering the energy output / input ratio in groundnut agriculture.

**REFERENCES**


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POLLUTION AND HUMAN HEALTH RISK ASSESSMENT OF HEAVY METALS IN KINDERGARTEN SURFACE DUST OF BENGBU CITY, CHINA

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2School of Environment, Nanjing Normal University, Nanjing, Jiangsu 210023, China

ABSTRACT

The concentrations of six heavy metals (Pb, Cu, Zn, Cr, Cd and Ni) from the surface dust of kindergartens in Bengbu have been investigated. The pollution degree of heavy metals and the level of children health risk are evaluated by the geo-accumulation index and the human health risk assessment method of the United States Environmental Protection Agency (USEPA). The results show that the average contents of Pb, Cu, Zn, Cr, Cd and Ni in the surface dust of kindergartens in Bengbu are 52.73, 58.19, 228.31, 39.56, 1.15 and 26.67 mg/kg, respectively. These values are 1.98, 2.85, 3.68, 0.59, 11.86 and 0.89 times of the soil background values in Anhui Province. The results of geo-cumulative index show that Cd is moderately polluted, Zn is light-moderately polluted, Cu and Pb are slightly polluted, Ni and Cr are clean. The results of human health risk assessment show that the exposure dose of heavy metals from high to low is Zn, Cu, Pb, Cr, Ni, Cd in turn. Hand-mouth intake is the main way of heavy metal exposure invading human bodies. The total non-carcinogenic risk value is 0.211 and the total carcinogenic risk index is 1.24×10^-8, which are lower than the risk standard recommended by USEPA, and will not cause obvious health hazards to children.

KEYWORDS: Surface dust, heavy metal pollution, health risk assessment, index of geo-accumulation, Bengbu

INTRODUCTION

Surface dust, which is an important component of surface particulate matter, can be treated as an indicator for the state of urban ecosystems. Surface dust is a source and a sink of many matters, including heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls [1]. In recent decades, heavy metals have become one of the most important pollutants endangering the environment and the human health for their high toxicity, concealment, easy enrichment and refractory degradation [2]. With the improvement of people’s attention to this problem, the health risk of heavy metals in urban surface dust has become to be a hot field. Note that previous researches often focus on city regions, such as industrial areas, business districts and traffic arteries [3-7]. However, studies about kindergartens, primary and secondary schools, universities and other special areas are still lacking. Especially, the human health risks caused by heavy metals in the surface environment of these areas are still lack of systematic understanding [8-9].

Kindergarten is the place where children live and study with a long time for the daily life. Children are naturally playful and hyperactive, and these features make their clothes, hair, hands and faces adhere to more dust. Heavy metals in the dust will enter bodies of children through exposure ways, such as breathing, hand-mouth intake and skin contact [10]. It should also be noted that children’s respiratory and digestive systems are immature, more sensitive to pollutants and less able to withstand risks. Thus, the health hazards from heavy metals in surface dust are more serious for children than adults [11]. Considering above factors, it is very useful to study the pollution characteristics and health risks of heavy metals in surface dust of kindergartens.

Bengbu, which is located in the northeastern part of the Anhui Province of China, covers an area of 5952 square kilometers. In 2017, the total population of Bengbu was 3812500. Bengbu is an important industrial center in the Anhui Province, and has a series of industrial systems, including machinery, chemical industry, medicine, electronics, and building materials. For several decades, its total economic output has located in the forefront of Anhui Province. In recent decades, the content, spatial distribution, pollution and potential ecological hazards of heavy metals from surface dust in Bengbu have been studied and published in previous literatures [12]. However, the health risk of heavy metals in surface dust in this region is still not studied adequately.

In this study, samples of surface dust from kindergartens in different regions of Bengbu are collected. Based on these samples, the spatial differences and pollution characteristics of heavy metals are analyzed by statistical methods. We focus on the health hazard of heavy metals exposure to young children, in order to improve the quality of...
kindergarten environment and ensure the health of young children. It is hoped that this study can provide reference for urban environmental pollution prevention and ecological city planning.

MATERIALS AND METHODS

Sample collection and pretreatment. The date for sampling is at May 2018 with a sunny, windless and dry weather after a rain process for 7 days. Plastic dustpans and brushes are used to collect dust in kindergarten playgrounds, recreational facilities and inside areas. The sampling time is set at noon to afternoon, in order to avoid the interference of cleaning activities. Dust samples from each kindergarten were mixed into one sample, with a weight in the range between 80g and 100 g. The 37 samples were put into polyethylene self-sealing bags. After these dust samples were brought back to the laboratory, tweezers were used to remove small stones, hair and broken leaves. These samples were dried in the shade for 2 weeks. After drying, the samples were grinded with agate mortar and passed through 100 mesh nylon sieve, bagging and standby. The locations of kindergartens investigated in this study are shown in Figure 1.

Sample analysis and quality control. After the treatment with the HCl-HNO3-HF-HClO4 digestion system [13], the mass concentrations of Pb, Cu, Zn, Cr, Cd and Ni in these samples were determined by the inductively coupled plasma atomic emission spectrometry (ICP-OES). The reagents used in the experiment are all high-grade pure. The experimental glassware are washed and placed in 10% HNO3 solution for more than 24 hours before using. The glassware were washed with ultra-pure water for 3 times before using. The national soil standard substance (GSBZ50012-88) was used for quality control in the determination process. The recovery rate of heavy metals is between 96.72% to 105.68%, reaching the precision requirement specified by the national standard.

Evaluation method of heavy metal pollution in surface dust. Several methods can be used to evaluate the degree of pollution of heavy metals in surface dust [14-18]. The geo-accumulation index can be used for assessing the influence from both natural factors and human activities [17, 19]. The formula of geo-accumulation index can be expressed in the form:

$$I_{geo} = \log_2 \left( \frac{C_n}{k \times B_n} \right)$$

where $C_n$ is the content of heavy metal, mg·kg$^{-1}$; $B_n$ is the reference value of heavy metal; $K$ is the correction factor (the general value is 1.5). In this study, soil background value of Anhui province is used as the reference value [20]. The corresponding relationship between geo-accumulation index and heavy metal pollution grade is stated as follows [21]: $I_{geo} < 0$, clean; $0 \leq I_{geo} < 1$, mild pollution; $1 \leq I_{geo} < 2$, mild to moderate pollution; $2 \leq I_{geo} < 3$, moderate pollution; $3 \leq I_{geo} < 4$, moderate to severe pollution; $4 \leq I_{geo} < 5$, severe pollution; $I_{geo} \geq 5$, very serious pollution.
TABLE 1
Exposure parameters for the health risk assessment models

<table>
<thead>
<tr>
<th>parameter</th>
<th>physical significance</th>
<th>unit</th>
<th>value</th>
<th>data sources</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>heavy metal content</td>
<td>mg/kg</td>
<td>mean</td>
<td>this paper</td>
</tr>
<tr>
<td>EF</td>
<td>exposure frequency</td>
<td>d/a</td>
<td>180</td>
<td>[23]</td>
</tr>
<tr>
<td>ED</td>
<td>exposure years</td>
<td>a</td>
<td>3</td>
<td>[23]</td>
</tr>
<tr>
<td>AT</td>
<td>average exposure time</td>
<td>d</td>
<td>EDx365 (non-carcinogens) 70x365 (carcinogens) [22, 24]</td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>average weight</td>
<td>kg</td>
<td>15.9</td>
<td>[25]</td>
</tr>
<tr>
<td>Rin</td>
<td>dust intake rate</td>
<td>mg/d</td>
<td>200</td>
<td>[25]</td>
</tr>
<tr>
<td>Rinh</td>
<td>respiration rate</td>
<td>m/d</td>
<td>7.5</td>
<td>[25]</td>
</tr>
<tr>
<td>PEF</td>
<td>emission factors of particles</td>
<td>m³/kg</td>
<td>1.36x10⁹ [22, 24]</td>
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<tr>
<td>SL</td>
<td>skin adhesion</td>
<td>mg/(cm²·d)</td>
<td>0.2 [25]</td>
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<tr>
<td>SA</td>
<td>exposed skin area</td>
<td>cm²</td>
<td>1150</td>
<td>[26]</td>
</tr>
<tr>
<td>ABS</td>
<td>absorption factor of skin</td>
<td>-</td>
<td>0.001</td>
<td>[27]</td>
</tr>
</tbody>
</table>

Calculation model and parameter selection of exposure quantity. In this study, the health risk assessment model from USEPA is used to estimate the exposure dose of heavy metals to surface dust in kindergartens in Bengbu [22]. This model considers the fact that heavy metals in surface dust are harmful to human body mainly through hand-mouth contact, breathing inhalation and skin contact. All the six heavy metals studied in this study have the non-carcinogenic risk to human body. Among these metals, Cr, Cd and Ni have both of the non-carcinogenic risk and the carcinogenic risk [22]. For the carcinogenic risk of heavy metals, the USEPA model only gives the data of carcinogenic slope factor through respiratory pathway, so only the exposure dose of carcinogenic risk through this pathway is considered in this study. The average daily exposure dose for different exposure routes is:

Average daily exposure of hand-mouth intake pathway (ADD<sub>ing</sub>):

\[ ADD_{ing} = C \times \frac{EY \times ED}{AT \times BW} \times R_{in} \times 10^{-6} \]  

Average daily exposure of respiratory inhalation pathway (ADD<sub>inh</sub>):

\[ ADD_{inh} = C \times \frac{EY \times ED}{AT \times BW} \times R_{inh} \times PEF \]  

Average daily exposure of skin contact pathway (ADD<sub>derm</sub>):

\[ ADD_{derm} = C \times \frac{EY \times ED}{AT \times BW} \times SL \times SA \times ABS \times 10^{-6} \]

Exposure parameters are important technical parameters for the health risk assessment of heavy metals, and the selection of these values will impact the evaluation results. Based on previous researches at home and abroad and the actual situation of children’s growth and development in China, the physical meanings and values for each parameter in the formulas are shown in Table 1.

Representation method of human health risk. The health risks of heavy metals in surface dust can be divided into the non-carcinogenic risk and the carcinogenic risk, which can be calculated by formula (5) and formula (6) respectively [22, 28]:

\[ HI = \sum HQ_i = \sum \frac{ADD_{ij}}{RfD_{ij}} \]  

\[ R_{Total} = \sum R_i = \sum LADD_{ij} \times SF_{ij} \]

where HI is total risk of non-carcinogenic heavy metals, HQ<sub>i</sub> is single risk of non-carcinogenic heavy metals, ADD<sub>ij</sub> is average daily exposure to non-carcinogenic heavy metals, RfD<sub>ij</sub> is reference dose, RTotal is total risk of carcinogenic heavy metals, R<sub>i</sub> is single risk of carcinogenic heavy metals, LADD<sub>ij</sub> is average daily exposure of carcinogenic heavy metals in lifetime, SF<sub>ij</sub> is slope factor for carcinogenic metal. When HI or HQ < 1, the non-carcinogenic health risk of heavy metals is small or negligible. When HI or HQ > 1, heavy metals present non-carcinogenic health risks [22, 28]. The R<sub>Total</sub> criterion recommended by US EPA for carcinogenic risk is 10⁻⁶.

RESULTS AND ANALYSIS

Analysis of heavy metals content in surface dust. The statistical indicators of heavy metals in surface dust of kindergartens in Bengbu City are shown in Table 3. Table 3 shows that the average contents of Pb, Cu, Zn, Cr, Cd and Ni in surface dust of kindergartens in Bengbu City are 52.73, 58.19, 228.31, 39.56, 1.15 and 26.67 mg·kg⁻¹, respectively. Except that the average contents of Cr and Ni are lower than the background values of soil in Anhui Province, the contents of other four heavy metals are all higher than their corresponding environmental background values. The heavy metal with the largest excess multiple is Cd, whose average content is 11.86 times the background value. The contents of Zn, Cu and Pb are 3.68 times, 2.85 times and 1.98 times of background value, respectively. Compared with the results of heavy metals in surface dust of kindergartens in Xi’an, Kaifeng and Huainan, the content of Cu in surface dust of kindergartens in...
Heavy metals in surface dust mainly come from anthropogenic sources such as industrial sewage, automobile exhaust and tire wear, as well as natural sources. Whether the sources of heavy metals are the same is of interest. The correlation between contents of different heavy metal elements is not obvious, and the content of heavy metals in surface dust is helpful to speculate the degree of sample dispersion. The intensity of human disturbance to the environment directly affects the magnitude of CV. When the coefficient of variation is greater than 30%, it is strong variation, otherwise they may come from more than one source [12].

The Pearson correlation coefficients matrix of the six heavy metals is calculated and shown in Table 4. It can be seen that the correlation coefficients between Pb and Cu, Zn and Cu, and between Pb and Zn are 0.503, 0.515 and 0.659, respectively, indicating that there are significant correlations between them. Moreover, the contents of Pb, Cu and Zn in surface dust are 1.98, 2.85 and 3.68 times of the background values of soil in Anhui Province. This result indicates that they are likely to come from the same source, otherwise they may come from more than one source [12].

The Pearson correlation coefficients matrix of the six heavy metals is calculated and shown in Table 4. It can be seen that the correlation coefficients between Pb and Cu, Zn and Cu, and between Pb and Zn are 0.503, 0.515 and 0.659, respectively, indicating that there are significant correlations between them. Moreover, the contents of Pb, Cu and Zn in surface dust are 1.98, 2.85 and 3.68 times of the background values of soil in Anhui Province. This result indicates that they are likely to come from the same pollution source. The correlation coefficient between Cr and Cu, Ni is relatively strong, and the contents of Cr and Ni are lower than their environmental background values, so they may have similar sources. The correlation between Cd and other five heavy metal elements is not obvious, and the content of Cd in surface dust has its certain source, and it may come from the parent material of soil formation.

Table 2 shows that the variation coefficients of Pb, Cu, Zn, Cr and Ni are 81.78%, 54.77%, 67.70%, 53.04%, 42.52%, respectively. In other words, all six heavy metals belong to the class of moderate variation when it is between 10% and 30%, and weak variation when it is less than 10% [33]. Table 4 shows that the correlation coefficients between Pb and Cu, Zn, Cr, Cd and Ni are 81.78%, 54.77%, 67.70%, 53.04%, 42.52%, respectively. In other words, all six heavy metals belong to the class of strong variation. This result shows that the spatial distribution of heavy metals in surface dust of kindergartens in Bengbu is quite different, and the sources of heavy metals are obviously disturbed by human activities.

Bengbu is higher than that in Huainan and Kaifeng, but is lower than Xi’an. The contents of Pb, Zn, Cr and Ni are also lower than those of other cities. The Pearson correlation coefficients matrix of the six heavy metals is calculated and shown in Table 4. It can be seen that the correlation coefficients between Pb and Cu, Zn and Cu, and between Pb and Zn are 0.503, 0.515 and 0.659, respectively, indicating that there are significant correlations between them. Moreover, the contents of Pb, Cu and Zn in surface dust are 1.98, 2.85 and 3.68 times of the background values of soil in Anhui Province. This result indicates that they are likely to come from the same pollution source. The correlation coefficient between Cr and Cu, Ni is relatively strong, and the contents of Cr and Ni are lower than their environmental background values, so they may have similar sources. The correlation between Cd and other five heavy metal elements is not obvious, and the content of Cd in surface dust has its certain source, and it may come from the parent material of soil formation.
According to the geographical locations of kindergartens in Bengbu, kindergartens investigated in this study are divided into three categories, i.e., old urban kindergartens, new urban kindergartens and suburban kindergartens. The average contents of heavy metals in surface dust of kindergartens for each category are calculated, which is shown in Figure 2. It can be seen that the content of heavy metal Ni in kindergartens in suburbs is higher than that in new districts and smaller than that in old districts. Furthermore, the content of other five heavy metal elements shows the same characteristic, i.e., old districts > new districts > suburbs.

**Heavy metal pollution level of surface dust.**

According to the statistics of heavy metals content in surface dust of kindergartens, the geo-accumulation index for different regions was calculated by formula (1) and the result is shown in Table 5.

According to Table 5, the average geo-accumulation index of six heavy metals in surface dust is Cd (2.97), Zn (1.27), Cu (0.92), Pb (0.39), Ni (-0.74) and Cr (-1.34), respectively. Specifically, Cd is moderate pollution, Zn is mild to moderately pollution, Cu and Pb are mild pollution, while Ni and Cr are clean.

Based on the geo-accumulation index in different regions, the geo-accumulation index in old districts is the largest (0.78), followed by that in new districts (0.57), and that in suburbs (0.38). Note that all districts are slightly polluted. Overall, the average geo-accumulation index of six heavy metals is 0.58, which is already at the level of slight pollution and should be paid attention to.

**Average daily exposure of heavy metals.**

The daily exposure doses of six heavy metals in surface dust of kindergartens in Bengbu City were calculated by formula (2), (3) and (4). The results are shown in Table 6.

Table 6 shows that the exposure doses of six heavy metals studied in this study show the same feature through different intake routes, i.e., respiratory inhalation < skin contact absorption < hand-mouth intake. For the same heavy metal, hand-mouth intake is about 1000 times of the skin contact absorption and 100,000 times of the breath inhalation. It can be seen that hand-mouth intake is the main way for heavy metal exposure to invade the human body. The exposures of heavy metals from high to low were Zn, Cu, Pb, Cr, Ni and Cd.

### TABLE 5

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Old districts</th>
<th>New districts</th>
<th>Suburbs</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Igeo</td>
<td>Grades</td>
<td>Igeo</td>
<td>Grades</td>
</tr>
<tr>
<td>Pb</td>
<td>0.65</td>
<td>mild pollution</td>
<td>0.44</td>
<td>mild pollution</td>
</tr>
<tr>
<td>Cu</td>
<td>1.18</td>
<td>mild to moderate pollution</td>
<td>0.87</td>
<td>mild pollution</td>
</tr>
<tr>
<td>Zn</td>
<td>1.63</td>
<td>mild to moderate pollution</td>
<td>1.20</td>
<td>mild to moderate pollution</td>
</tr>
<tr>
<td>Cr</td>
<td>-1.22</td>
<td>clean</td>
<td>-1.35</td>
<td>clean</td>
</tr>
<tr>
<td>Cd</td>
<td>3.15</td>
<td>moderate to severe pollution</td>
<td>3.06</td>
<td>moderate to severe pollution</td>
</tr>
<tr>
<td>Ni</td>
<td>-0.69</td>
<td>clean</td>
<td>-0.78</td>
<td>clean</td>
</tr>
<tr>
<td>Mean</td>
<td>0.78</td>
<td>mild pollution</td>
<td>0.57</td>
<td>mild pollution</td>
</tr>
</tbody>
</table>
The main aim of this study is to assess the pollution level of heavy metals in surface dust from kindergartens in Bengbu. We have shown that heavy metals in surface dust are generally at a slight pollution level for the study area. Furthermore, the spatial variation of heavy metal content in surface dust is influenced by functions of areas, intensities of human activities and past usages of lands [23]. Among the surface dust of kindergartens in Bengbu, the heavy metal content in the old urban area is the highest, and the corresponding geo-accumulation index is twice as much as the value in suburbs. The reason is that factories exist in these regions with the past decades. Nowadays, the dense population, the developed commerce, the large traffic volume and the small green space are also potential factors. In addition, there are still a large number of metal processing industries in the old urban areas, leading to non-carcinogenic health risks. The order of non-carcinogenic health risk from different exposure routes was hand-mouth intake > skin contact > breathing inhalation. The health risk index of hand-mouth intake routes was 1 to 2 orders of magnitude higher than that of skin contact routes, and 2 to 4 orders higher than that of breathing inhalation routes. Children's non-carcinogenic health risk from hand-mouth intake accounted for 96.68% of the total non-carcinogenic risk. Hand-mouth intake is the most important way for health risk from human exposure to surface dust.

**DISCUSSION**

**Human health risk assessment.** Formulas (2) to (6) were used to calculate the non-carcinogenic health risk index of six heavy metals in surface dust of kindergartens in Bengbu through hand-mouth intake, breathing inhalation and skin contact. The results are shown in Table 7.

As can be seen from Table 7, the HQ and HI values of non-carcinogenic health risks of six heavy metals in different exposure routes are all less than 1, which is lower than the recommended standard of USEPA. It shows that the above six heavy metals in surface dust of kindergartens in Bengbu do not cause obvious non-carcinogenic health hazards to children. The single non-carcinogenic health risk index of heavy metals is Pb > Cr > Cu > Ni > Cd > Zn from large to small, and the non-carcinogenic health risk index of Pb and Cr is one order of magnitude higher than that of other heavy metals. It can be seen that Pb and Cr are the main pollutants of non-carcinogenic health risk caused by heavy metals in surface dust of kindergartens in Bengbu City. They may have some adverse effects on children's health. Therefore, effective control of Pb and Cr pollution is one of the key factors to solve the health risk of surface dust in kindergartens in Bengbu City.

The carcinogenic health risk index of heavy metals such as Cr, Cd and Ni is $5.31 \times 10^{-11}$ to $1.22 \times 10^{-8}$, and the total carcinogenic risk index is $1.24 \times 10^{-8}$. $R_i$ and $R_{Total}$ are lower than the acceptable risk threshold recommended by USEPA. The results showed that the carcinogenic risk of heavy metals such as Cr, Cd and Ni in surface dust of kindergartens in Bengbu City was low, and there was no obvious carcinogenic risk for children. The carcinogenic toxicity of different heavy metals varied greatly. The carcinogenic risk of Cr was 3 orders of magnitude higher than that of Cd and 2 orders higher than that of Ni at the same dose of inhalation. The carcinogenic risk of Cr accounted for 98.39% of the total carcinogenic risk. The order of non-carcinogenic health risk of heavy metals in surface dust under different exposure routes was hand-mouth intake > skin contact > breathing inhalation. The health risk index of hand-mouth intake routes was 1 to 2 orders of magnitude higher than that of skin contact routes, and 2 to 4 orders higher than that of breathing inhalation routes. Children's non-carcinogenic health risk from hand-mouth intake accounted for 96.68% of the total non-carcinogenic risk. Hand-mouth intake is the most important way for health risk from human exposure to surface dust.

**TABLE 6**

Average daily exposure of heavy metals under different exposure routes [mg/(kg·d)]

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Hand-mouth intake</th>
<th>Respiratory inhalation</th>
<th>Skin contact</th>
<th>Carcinogenic exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>$3.27 \times 10^{-4}$</td>
<td>$9.02 \times 10^{-5}$</td>
<td>$3.76 \times 10^{-7}$</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>$3.61 \times 10^{-3}$</td>
<td>$9.95 \times 10^{-4}$</td>
<td>$4.15 \times 10^{-7}$</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>$1.42 \times 10^{-4}$</td>
<td>$3.91 \times 10^{-4}$</td>
<td>$1.63 \times 10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>$2.45 \times 10^{-3}$</td>
<td>$6.77 \times 10^{-4}$</td>
<td>$2.82 \times 10^{-7}$</td>
<td>$2.90 \times 10^{-10}$</td>
</tr>
<tr>
<td>Cd</td>
<td>$7.13 \times 10^{-6}$</td>
<td>$1.97 \times 10^{-5}$</td>
<td>$8.20 \times 10^{-9}$</td>
<td>$8.43 \times 10^{-12}$</td>
</tr>
<tr>
<td>Ni</td>
<td>$1.65 \times 10^{-3}$</td>
<td>$4.56 \times 10^{-5}$</td>
<td>$1.90 \times 10^{-7}$</td>
<td>$1.96 \times 10^{-10}$</td>
</tr>
<tr>
<td>Total</td>
<td>$2.53 \times 10^{-1}$</td>
<td>$6.96 \times 10^{-5}$</td>
<td>$2.90 \times 10^{-6}$</td>
<td>$4.94 \times 10^{-10}$</td>
</tr>
</tbody>
</table>

**TABLE 7**

Heavy metal health risk index of surface dust in kindergartens in Bengbu City

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Hand-mouth intake</th>
<th>Respiratory inhalation</th>
<th>Skin contact</th>
<th>Carcinogenic exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>$9.34 \times 10^3$</td>
<td>$2.56 \times 10^5$</td>
<td>$7.16 \times 10^4$</td>
<td>$9.41 \times 10^2$</td>
</tr>
<tr>
<td>Cu</td>
<td>$9.03 \times 10^3$</td>
<td>$2.48 \times 10^5$</td>
<td>$3.46 \times 10^4$</td>
<td>$9.06 \times 10^3$</td>
</tr>
<tr>
<td>Zn</td>
<td>$4.73 \times 10^3$</td>
<td>$1.30 \times 10^5$</td>
<td>$2.72 \times 10^4$</td>
<td>$4.76 \times 10^3$</td>
</tr>
<tr>
<td>Cr</td>
<td>$8.17 \times 10^2$</td>
<td>$2.37 \times 10^5$</td>
<td>$4.73 \times 10^4$</td>
<td>$8.66 \times 10^{-2}$</td>
</tr>
<tr>
<td>Cd</td>
<td>$7.13 \times 10^3$</td>
<td>$1.97 \times 10^5$</td>
<td>$8.20 \times 10^4$</td>
<td>$7.95 \times 10^{11}$</td>
</tr>
<tr>
<td>Ni</td>
<td>$8.25 \times 10^3$</td>
<td>$2.21 \times 10^5$</td>
<td>$3.52 \times 10^4$</td>
<td>$8.29 \times 10^{-3}$</td>
</tr>
<tr>
<td>HI</td>
<td>$2.04 \times 10^3$</td>
<td>$2.40 \times 10^5$</td>
<td>$6.33 \times 10^3$</td>
<td>$2.11 \times 10^{-1}$</td>
</tr>
<tr>
<td>$R_{Total}$</td>
<td>$2.53 \times 10^{-1}$</td>
<td>$6.96 \times 10^{-5}$</td>
<td>$2.90 \times 10^{-6}$</td>
<td>$4.94 \times 10^{-10}$</td>
</tr>
</tbody>
</table>
particulate matter containing Pb, Cu and Zn produced in production activities. Particulate matter will eventually settle to the surface in different ways. This is why the heavy metal content in the surface dust of kindergartens in the old districts is higher than the new districts and suburbs.

The values of the heavy metal carcinogenic risk index R_i and R_total indicate the probability of cancer occurrence, which is expressed by the number of cancer patients in a certain population. The USEPA recommended Total standard is 10^{-6}, which means one additional cancer patient per million people. It is considered that the substance with content lower than this value has no carcinogenic risk [29-30]. Results in this study show that six heavy metals in surface dust of kindergartens in Bengbu do not cause obvious non-carcinogenic health hazards to children, while Cr, Cd and Ni do not cause obvious carcinogenic risk to children. There are significant differences in the health risks of children exposed to heavy metals in surface dust in different ways. Hand-mouth intake is the most important way for health risks of human exposure to surface dust. Therefore, for children, it is necessary to wash hands and face frequently in daily life. Because children have more hand-mouth activities than adults, and their own immunity is weak, children are more vulnerable to various pollutants in surface dust compared with adults. Therefore, we should pay attention to the pollution from surface dust.

Although the results show that the carcinogenic risk of heavy metals in surface dust of kindergartens in Bengbu City is generally low and there is no obvious carcinogenic risk for children, it should be noted that the carcinogenic pollutants in urban surface dust are not limited to the heavy metals Cr, Cd and Ni considered in this study. There are a large number of other carcinogenic pollutants, such as polycyclic aromatic hydrocarbons, heavy metals As and Hg. Therefore, the carcinogenic risk of heavy metals in surface dust of kindergartens in Bengbu City shown in this study might be underestimated. In addition, the ecological toxicity of heavy metals is not only related to the content of heavy metals, but also closely related to their occurrence forms. In this study, the health risk of heavy metals is estimated only from the total amount of heavy metals, and the chemical morphological toxicity of heavy metals is not involved. To judge the health risk of heavy metals better, the influence of the occurrence form of heavy metals will be considered in the future studies.

**CONCLUSIONS**

In this study, the average contents of Pb, Cu, Zn, Cr, Cd and Ni in surface dust of kindergartens in Bengbu City are 52.73, 58.19, 228.31, 39.56, 1.15 and 26.67 mg/kg, respectively. Except that the average contents of Cr and Ni are lower than the soil background values of Anhui Province, the other four heavy metals are higher than the environmental background values. In the surface dust of kindergartens in Bengbu City, Cd is moderately polluted, Zn is mildly to moderately polluted, Cu and Pb are mildly polluted, Ni and Cr are in a clean level. Heavy metals in surface dust of kindergartens in Bengbu have caused a certain degree of pollution, which should be paid attention to. The total non-carcinogenic risk index and total carcinogenic risk index of heavy metals in surface dust of kindergartens in Bengbu are lower than those recommended by USEPA, which will not cause obvious health hazards to children.

**ACKNOWLEDGEMENTS**

This work was supported by the Key Natural Science Foundation of the Anhui Higher Education Institutions of China (KJ2017A570, KJ2016A459), the National Spark Program Project (2015GA710068), the Excellent Top Talents Project in Anhui Higher Education Institutions (gxyq2018106) and the Key Natural Science Research Projects of Bengbu University (2018ZR04zd).

**REFERENCES**


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THE ANTIFUNGAL AND PHYTOTOXIC EFFECT OF DIFFERENT PLANT EXTRACTS OF SALVIA VIRGATA JACQ

Yusuf Bayar*, Melih Yilar
Kirsehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, 40200 Kirsehir, Turkey

ABSTRACT

This study was conducted in order to investigate the phytotoxic and antifungal activity of the methanol and n-hexane extracts obtained from the surface parts of the Salvia virgata Jacq. plant collected in Kirsehir province. In the experiments, S. virgata extracts were prepared and used in 125, 250, 500, 1000 ppm doses. The seeds of Lactuca sativa L., Lepidium sativum L., and Triticum vulgare L., plants were placed as 25 seeds for each of them. The seeds were placed in a 9-cm diameter Petri dish with two layers of filter paper, and then they were left for incubation for 3 weeks at 24°C±1 conditions. At the end of the period, the germination percentages, root-shoot developments and wet and dry weights of the seeds were determined. In the antifungal studies, the plant methanol and hexane extracts were added to the PDA environments prepared in the way for their final concentration to be 125, 250, 500, 1000 ppm doses. In the study, Thiram (80%) fungicide was used as the negative control (only PDA) and positive control. The extract and Thiram-added PDA were transferred to the petri dishes and left to incubation for 7 days. According to the trial results, the seed germinations of the S. virgata methanol and n-hexane extracts inhibited garden cress, wheat, and lettuce at the rate of 79.45%, 18.67%, 88.57%, and 78.08%, 82.86%, respectively. The mycelium developments of the pathogens were inhibited by 1000 ppm dose of methanol and n-hexane extracts: 0%, 28.17%, 38.77%, 0% and 2.43%, 36.04%, 37.0%, and 72.22%.

INTRODUCTION

Due to the severe problems that occurred as a result of the chemical control against the plant diseases performed for long years, the studies to find alternative methods especially starting in developed countries have accelerated. As a result of using pesticide intensely, the natural balance was deteriorated and it has begun to threaten the environment and human health [1].

It is important to find environment-friendly methods that can substitute chemicals in the control of plant diseases and are suitable for the integrated control principles. Also, in order to maintain the agriculture, it becomes a necessity to research methods alternative to chemical control and transfer them into applications. One of these alternative methods is to determine the herbal compounds and use them in the control of the plant diseases, pests, and weeds. In the previous studies, there are many studies on the fungicidal, herbicidal, and insecticidal effects of the compounds and essential oils in plants and their biological activities [2-5].

Lamiaceae family with its 250 genera and 7133 species has a wide spreading area [6-7]. Salvia L., one of the largest genera of the Lamiaceae family includes approximately 1000 species [8]. Salvia virgata Jacq., included in the Salvia genus, is a perennial species and its length reaches to 160 cm. The plant can spread in various areas like the empty fields and roadsides and in all regions of Turkey [8]. S. virgata is an important plant with high-quality used in the medical practices and it is used as wound-healing and against the skin disorders among people [9-10]. It also has antioxidant, antimicrobial, and antibacterial effect [11-12].

This study was conducted to determine the antifungal activity of different extracts obtained from Salvia virgata Jacq. plant on Rhizoctonia solani Kühn, Alternaria solani (Ell. And G. Martin), Fusarium oxysporum f sp radicis lycopersici, and Verticillium dahliae pathogens were inhibited by 1000 ppm dose of methanol and n-hexane extracts: 0%, 28.17%, 38.77%, 0% and 2.43%, 36.04%, 37.0%, and 72.22%.

KEYWORDS:
Antifungal activity, extract, phytotoxic effect, Salvia virgata
MATERIALS AND METHODS

Plant material. *Salvia virgata* Jacq. plant used in the trial was collected in Kırşehir province in 2018 vegetation period. The plant materials collected in the blooming stage were dried in the shade in the laboratory environment and they were ground in the electrical grinder. The plant diagnosis was performed by Faculty Member Dr. Melih Yılar.

Fungus cultures. In the study, *Rhizoctonia solani* Kühn, *Alternaria solani* (Ell. And G. Martin), *Fusarium oxysporum* f sp radicis lycopersici (Jarvis & Shoemaker), and *Verticillium dahliae* (Kleb) plant pathogens were used. These plant pathogens were obtained from the stock cultures in Ahi Evran University, Faculty of Agriculture, Plant Protection Department, laboratory of Phytopathology.

Preparation of Extracts. 500 gr ground plant material was put into 2-liter Erlenmeyer and 1000 ml sterile distilled water was added on it. This solution was put into the shaker for extraction at room temperature for 24 hours. After the extraction, the solution was filtered from the filter paper. Methanol and hexane in the solutions were removed using a rotary evaporator. The final solution was kept at +4°C until it was used in the trial.

The antifungal activity of the plant extract under in vitro conditions. The materials obtained were dissolved by acetone-water mixture and the stock solution was obtained. Among the original solutions, the ones with the final concentration of 125, 250, 500, and 1000 ppm were added to the PDA environments cooled until 45-50 °C [14]. For control, fungi were only inoculated in the petri dishes containing PDA. In addition, a fungus with Thiram active ingredient was used as the positive control in the trials. These PDA media in different concentrations (250, 500, and 1000 ppm) and pure water for control purpose were added in 5ml to Petri dishes. The plant extracts with different concentrations (250, 500 and 1000 ppm) and pure water for control purpose were added in 5ml to Petri dishes and humidified. The petri dishes were left to incubation for 3 weeks at averagely 24°C ±1°C for 12-hour light and 12 hour- dark. At the end of this period, the seed germination rates of the test plants and the root and shoot height were determined. The trial was repeated twice [16].

Statistical Analysis. The significance levels of the differences between the treatments were determined by using the analysis of variance (ANOVA) during the trials. The means were compared using the DUNCAN test. The statistical analyses were performed using SPSS -15 computer program.

RESULTS AND DISCUSSION

Phytotoxic effect study. These studies were triplicated in the petri dishes with a 90-mm diameter. The seeds of the test plants were distributed homogeneously (25 for each) in the petri dishes in which 2-layer blotting paper was placed. The plant extracts in different concentrations (250, 500, and 1000 ppm) and the distilled water for control purpose were humidified by adding 5 ml in the petri dishes. The plant extracts with different concentrations (250, 500 and 1000 ppm) and pure water for control purpose were added in 5ml to Petri dishes and humidified. The petri dishes were left to incubation for 3 weeks at averagely 24°C ±1°C for 12-hour light and 12 hour- dark. At the end of this period, the seed germination rates of the test plants and the root and shoot height were determined. The trial was repeated twice [16].

It was determined that *Salvia virgata* plant methanol and hexane extracts had a phytotoxic activity on garden cress, wheat, and lettuce and an antifungal activity on *A.solani, V.dahliae, F. oxysporum f sp. Radicis lycopersici*, and *R.solani* pathogens. Tables 1, 2, 3, and 4 and Figures 1 and 2 summarize the results obtained.

The phytotoxic effect of *S. virgata* extract on the cultivated plants varied depending on the extract, dose and test plant. The hexane extract of *S. virgata* reduced the seed germination and the root and shoot development of garden cress, wheat, and lettuce at a statistically significant level compared to the control (Table 1). Accordingly, the hexane extract inhibited the seed germinations of garden cress, wheat, and lettuce at the rate of 78.08%, 82.86%, and 100%, compared to the control. The hexane extract inhibited the root and shoot lengths of garden cress and lettuce at the rate of 100%, it inhibited the root length of wheat at the rate of 83.88% and the shoot length of wheat at the rate of 82.86% compared to the control. Although the effect in the plant methanol extract realized at a lower level compared to the hexane extract, it had a high level of phytotoxic effect on the test plants. Accordingly, the methanol extract reduced the seed germination of garden cress, wheat, and lettuce at the rates of 79.45%, 18.67%, and 88.57%, respectively, reduced the root elongation at the rates of 94.77%, 70.18%, and 83.21%, respectively and reduced the shoot elongation at the rates of 94.26%,...
51.45%, and 71.77%, respectively compared to the control. In the studies conducted on the Salvia species, it has been reported that *S. officinalis* water extract inhibits the seed germination and development of lettuce [17] and corn [18]. Also, it has been reported that *S. miniata* inhibits the germination of *Papaver rhoeas* and *Avena sativa* [19] and *S. leucophylla* essential oil inhibits the seed germination of *Brassica campestris* [20]. The phenolic compounds that were determined to have phytotoxicity and biological activity effect include *S. virgata* plant [21]. The methanol extract of this plant has a total phenolic content of 195.22 (mg GAE/g extract) [22].

In the results of the present study, it was also determined that *S. virgata* hexane and methanol extracts had a phytotoxic effect on the cultivated plants. Previous studies revealed that the salvia species displayed biological activity and contained phenolic compound, which supports this study. In accordance with the present study and previous studies, *S. virgata* plant has the potential to demonstrate high allelopathic effect and therefore, it may be a natural herbicide.

It was determined that *Salvia virgata* methanol and hexane extracts varied according to the extract, application dose, and plant pathogen and it had an antifungal potential. *S. virgata* methanol extract inhibited significantly the mycelium developments of *V. dahliae* and *F. oxysporum f sp radicis lycopersici* pathogens compared to the control. However, it was found that it had no effect on the mycelium development of *A. solani* and *R. solani* (Table 3, Figure 1).

### TABLE 1

<table>
<thead>
<tr>
<th>Doses</th>
<th>The phytotoxic effect of the <em>Salvia virgata</em> hexane extract on the test plants.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lepidium sativum L.</td>
</tr>
<tr>
<td></td>
<td>GR (%)</td>
</tr>
<tr>
<td>Control</td>
<td>97.33a ± 2.66</td>
</tr>
<tr>
<td>250ppm</td>
<td>57.33 ± 5.75</td>
</tr>
<tr>
<td>500ppm</td>
<td>38.66 ± 7.06</td>
</tr>
<tr>
<td>1000ppm</td>
<td>21.33 ± 3.52</td>
</tr>
</tbody>
</table>

*GR: Germination; SL: Shoot length; RL: Root length; *a* the means with different letters in the same column are different at the significance level of *p*<0.05 according to DUNCAN.

### TABLE 2

<table>
<thead>
<tr>
<th>Doses</th>
<th>The phytotoxic effect of the <em>Salvia virgata</em> methanol extract on the test plants.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lepidium sativum L.</td>
</tr>
<tr>
<td></td>
<td>GR (%)</td>
</tr>
<tr>
<td>Control</td>
<td>97.33a ± 2.66</td>
</tr>
<tr>
<td>250ppm</td>
<td>66.66b ± 4.80</td>
</tr>
<tr>
<td>500ppm</td>
<td>40.00c ± 4.62</td>
</tr>
<tr>
<td>1000ppm</td>
<td>20.00d ± 2.30</td>
</tr>
</tbody>
</table>

*GR: Germination; SL: Shoot length; RL: Root length; *a* the means with different letters in the same column are different at the significance level of *p*<0.05 according to DUNCAN.

### TABLE 3

<table>
<thead>
<tr>
<th>Doses</th>
<th>The mycelium development effect of <em>Salvia virgata</em> methanol extract on plant pathogenic fungi.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. solani</td>
</tr>
<tr>
<td>Control</td>
<td>0.00b ± 0.00</td>
</tr>
<tr>
<td>125ppm</td>
<td>60.00a±0.00</td>
</tr>
<tr>
<td>250ppm</td>
<td>60.00a±0.00</td>
</tr>
<tr>
<td>500ppm</td>
<td>42.69b±0.88</td>
</tr>
<tr>
<td>1000ppm</td>
<td>36.74d±2.77</td>
</tr>
</tbody>
</table>

*The means with different letters in the same column are different at the significance level of *p*<0.05 according to DUNCAN.
As a result of the percentage effect study, 1000 ppm dose of the methanol extract inhibited the mycelium development of *V. dahliae* and *F. oxysporum f. sp. radicis lycopersici* pathogens at the rates of 38.77% and 28.17%, compared to the control. However, it was determined that it had no effect on the mycelium development of *A. solani* and *R. solani* pathogens (Figure 1).

### FIGURE 1

The % effect of *Salvia virgata* methanol extract on the mycelium growth inhibitions (MGI) of the plant pathogenic fungi.

### TABLE 4

<table>
<thead>
<tr>
<th>Doses</th>
<th><em>A. solani</em></th>
<th><em>V. dahliae</em></th>
<th><em>F. oxysporum f. sp. radicis lycopersici</em></th>
<th><em>R. solani</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00c±0.00</td>
<td>0.00d±0.00</td>
<td>0.00c±0.00</td>
<td>0.00c±0.00</td>
</tr>
<tr>
<td>Control</td>
<td>60.00a±0.00</td>
<td>60.00a±0.00</td>
<td>51.18a±0.00</td>
<td>60.00a±0.00</td>
</tr>
<tr>
<td>125ppm</td>
<td>57.72a±2.28</td>
<td>46.21b±1.62</td>
<td>38.45b±1.06</td>
<td>60.00a±0.00</td>
</tr>
<tr>
<td>250ppm</td>
<td>53.52b±2.08</td>
<td>37.10c±1.61</td>
<td>36.68bc±0.79</td>
<td>60.00a±0.00</td>
</tr>
<tr>
<td>500ppm</td>
<td>47.52c±0.44</td>
<td>36.14c±6.16</td>
<td>34.70c±0.51</td>
<td>60.00a±0.00</td>
</tr>
<tr>
<td>1000ppm</td>
<td>38.16d±0.20</td>
<td>16.67d±2.60</td>
<td>32.21d±0.73</td>
<td>58.54b±10.16</td>
</tr>
</tbody>
</table>

*The means with different letters in the same column are different at the significance level of p<0.05 according to DUNCAN.*

### FIGURE 2

The % effect of *Salvia virgata* hexane extract on the mycelium growth inhibitions (MGI) of the plant pathogenic fungi.
S. virgata hexane extract inhibited the mycelium development of all the pathogens compared to the control. It was determined that R. solani was the most tolerant pathogen to the hexane extract and V. dahliae was the most sensitive pathogen to the hexane extract (Table 4, Figure 2).

1000 ppm dose of the hexane extract inhibited the mycelium development of V. dahliae, F. oxysporum f. sp. radicis lycopersici, A. solani, and R. solani pathogens at the rates of 72.22%, 37.01%, 36.04%, and 2.43%, respectively, compared to the control (Figure 2).

In previous studies, it was reported that the plant extracts and their essential oils had a significant effect on the plant pathogens [24–25]. Salvia species exhibit many activities such as antifungal, antibacterial, antioxidant, herbicidal ones [18, 26–31]. However, it was not determined that Salvia virgata had an effect on A. solani, V. dahliae, F. oxysporum f. sp. radicis lycopersici and R. solani pathogens, which were the plant pathogenic fungus. Also, the present study revealed the results parallel with the ones reported in previous similar studies.

CONCLUSION

Consequently, it was revealed that Salvia virgata methanol and hexane extracts had a phytotoxic effect on garden cress, wheat, and lettuce plants and an antifungal effect on A. solani, V. dahliae, F. oxysporum f. sp. radicis lycopersici, and R. solani, which were significant plant pathogenic fungi. The effect of Salvia virgata methanol and hexane extracts on the plant pathogens was determined with this study for the first time.

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INVESTIGATION OF HEAVY METAL CONTAMINATION AND MICROBIOLOGICAL QUALITY OF TURNIP JUICE IN WESTERN TURKEY

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²Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Afyon Kocatepe University, 03200 Afyonkarahisar, Turkey
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ABSTRACT

Turnip juice is red, turbid and sour soft drink, which is especially produced in the South Anatolia Region and consumed in large quantities. In this study, 30 different turnip juice samples (n=15 regular and n=15 hot) were bought from different manufacturers and analyzed in term of microbiological, physicochemical and heavy metals. Soluble solid contents were found between 2.0- 3.2 % in regular samples and 2.3-3.4% in hot samples with pH levels of 3.1- 6.1. The counts of total mesophilic aerobic bacteria were found to be between 3.45 – 6.51 log cfu/ml in regular samples and 3.00-4.48 log cfu/ml in hot samples. The counts of lactic acid bacteria were found to be between 5.26 – 7.62 log cfu/ml in all samples. The mold was found to be 2.51-4.51 log cfu/ml in regular samples and 2.36-4.34 log cfu/ml in hot samples. Salmonella was not found. Coliform bacteria were found in three samples of turnip juice (3.04; 3.11 and 3.11 log cfu/ml). On the other hand, E. coli was found only in two regular samples (2.48 and 2.34 log cfu/ml). Arsenic was found in the amounts of 1.836 and 0.685 μg/g in hot samples. No Pb and Cd were detected in any of the turnip juice samples. Cu, Zn, Sn, Fe and Cr were detected at the levels of 0.023-0.298; 0.16-1.506; 0.147-0.217; 0.623 - 4.635 and 0.011- 0.025 μg / g in regular turnip juice samples, while these levels were 0.035-0.258; 0.188 - 0.692; 0.142 -0.238; 0.656 - 4.467 and 0.014 - 0.021 μg / g in hot turnip juice samples respectively. The results of our study show that the collected samples are in compliance with the standards in terms of some parameters and not compliant in some others. These results manifest the necessity of frequent analysis of such food products in our country and continuous monitoring during their production.

KEYWORDS:
Turnip juice, heavy metal, microbiology

INTRODUCTION

Turnip juice is a traditional fermented Turkish beverage, a tasty sour and crimson drink cloudy in texture, which is manufactured with the help of lactic acid bacteria. It is produced with bulghur flour, water, black carrots, salt, sour dough and turnip radishes [1-2]. In general, turnip juice production is carried out in a controlled way with spontaneous fermentation with the natural microflora found in vegetables or by adding starter culture [3]. Regular and hot turnip juices are manufactured and consumed in many regions in Turkey. Currently the amount of turnip juice consumption has reached significant amounts and is at least equal in popularity with other beverages. Turnip juice is presented in the open or in bottles and plastic containers for consumption and in time has become available almost everywhere in Turkey where beverages are sold. Therefore, turnip juice production has gained an industrial character in recent years and that it is currently being sold in markets in some European countries [4]. The aim of this study has been to research the heavy metal contamination and microbiological quality of turnip juices marketed in western Turkey.

MATERIALS AND METHODS

Material. A total of 30 types of turnip juice (15 regular and 15 hot) belonging to 15 different producers were obtained according to a random sampling method and brought to our laboratory in this study. Microbiological, physico-chemical and heavy metal measurement analyzes were carried out on the collected turnip juice samples.

Methods. The determination of the soluble solids in the collected turnip juice samples was carried out with the refractometric method [5] while pH was determined using a pH meter with a direct glass electrode and Hanna instruments brand pH meter [6].
The microbiological analysis of samples was carried out with mesophilic aerobic bacteria count [7], yeast and mold count [8], coliform group bacteria count [9], Escherichia coli count [10] and Salmonella spp analysis [11].

Heavy metal analyses of the collected turnip juice samples were subjected to the incineration procedure according to Fruit Juice Using the Avivo 200 ICP-OES method. 8 ml of nitric acid, 2 ml of hydrogen peroxide and 5 ml of the sample was taken and incinerated in a microwave. In the initial phase of the incineration process the microwave temperature was raised to 100°C for 10 minutes, in the second phase it was kept on standby for 15 minutes at a temperature of 100°C (Milestone Application Note HPR-CL-03). Heavy metal levels of the samples acquired with the incineration procedure were determined on the Perkin Elmer Optima 8000 ICPOES device.

RESULTS AND DISCUSSION

When pH levels of turnip juice samples were examined, pH values ranged from 3.1 to 6.1 (Figure 1). According to TS 11149 turnip juice standard, the pH value of turnip juice is between 3.3 and 3.8. [1]. However, in this study it was found that 4 of the regular turnip juice sample and 6 of the hot turnip juice samples did not conform to the standards in terms of pH. A study carried out by Canbaş and Fenercioğlu [12] displayed pH values between 3.35-3.85 for turnip juice while Arıcı [13] noted pH values which varied between 3.16-3.60 and Utuş [14] reported values of 3.45-3.53 as pH values after fermentation of samples, Güneş [15] reported values of 3.39-3.49, Öztürk [16] reported pH values between 3.26-3.86 and Çakır [17] reported pH values ranging between 3.31-4.13 in the analyzed turnip juices. In general, the turnip juices were found to comply with pH standards and those that were non-compliant had not strayed far from the standard. The pH values found in our study were higher than those reported by other studies.

The amount of soluble dry matter detected in analyzed turnip juice samples is shown in Figure 2. While the amount of soluble solids was 2.0 – 3.2% in regular turnip juice, this figure was determined to be between 2.3% and 3.4% for hot turnip juices. According to Turnip Juice Standard no. TS 11149, the amount of soluble solids in turnip juice has to be at least 2.5% [1]. Accordingly, 5 of the regular turnip juice samples and 3 of the hot juice samples had values below standard.

The total number of mesophilic aerobic bacteria detected in the analyzed turnip juice samples is shown in Figure 3. It was discovered that the number of mesophilic aerobic bacteria in regular turnip juice was between 3.45-6.51 log cfu/ml while the commensurate amount in hot turnip juice was between 3.00-4.48 log cfu/ml. According to Turnip Juice Standard no. TS 11149 the maximum limit for mesophilic aerobic bacteria in turnip juice is 5.00 log cfu/mL [1]. The analyses carried out revealed that only 1 regular turnip juice sample had a value exceeding the standard (6.51 log cfu/ml). Öztürk [16] carried out a study in which the number of mesophilic aerobic bacteria was found to be between 1.8x10^3-4.6x10^7. Other studies carried out on turnip juice have determined a total number of mesophilic aerobic bacteria that varied between 2.6x10^5-6.1x10^7 cfu/mL [18-13]. These results are commensurate with the results of the current study.

The number of lactic acid bacteria detected in the analyzed turnip juice samples is shown in Figure 4. The number of lactic acid bacteria in regular turnip juice was between 6.00 – 7.62 log cfu/ml while the commensurate amount in hot turnip juice was between 5.26-6.79 log cfu/ml. In a study carried out by Öztürk [16], the number of lactic acid bacteria in analyzed turnip juice varied between 2.1x10^5-9.3x10^7 cfu/mL while Aydar [18] and Arıcı [13] reported lactic acid bacteria amounts of between 1.2x10^4-4.8x10^7 cfu/mL in turnip juice. Turnip juice is a fermented product and lactic acid bacteria have a role in this fermentation. The difference in the level of lactic acid bacteria in each sample can be attributed to the different raw material used in production, differences in the place and conditions of production, storage time and temperatures.
FIGURE 2
Soluble solid contents of Turnip Juice Samples

FIGURE 3
Mesophilic Aerobic Bacteria counts of Turnip Juice Samples

FIGURE 4
Lactic Acid Bacteria Counts of Turnip Juice Samples
The number of yeasts/molds detected in turnip juice samples in the study is shown in Figure 5. While yeast numbers of regular turnip waters were between 2.51-4.51 log cfu/ml, these figures for hot turnip juice were between 2.36-4.34 log cfu/ml. According to Turnip Juice Standard no. TS 11149 the allowable limit for mold in turnip juice is given as 20 cfu/ml [1]. In the study, one sample was found to be above the limit. Öztürk [16] reported that the total number of yeasts in the analyzed turnip juice samples ranged from 5.2x10^5-1.4x10^8 cfu/mL. In other studies, the total number of yeasts in turnip juice was reported to be 3.5x 10^5-1.1x 10^7 cfu/ml [19-18-13].

Two of the regular turnip juice samples one hot sample in the study were determined to contain Coliform bacteria (3.04; 3.11 log cfu/ml and 3.11 log cfu/ml respectively). E. coli was found in two regular turnip juice samples (2.48, 2.34 log cfu/ml) while hot turnip juice did not reveal such a presence (<log2.00). No Salmonella spp. was detected in any of the turnip juice samples.

According to TS 11149 standard, the limit for coliform bacteria is 1100 EMS/mL and E. coli and Salmonella are banned altogether [1]. In this study, 3 samples were found non-compliant for E. coli while all samples were compliant for Salmonella.

Although there is no definite definition of heavy metals, in general definitions, "density" and "negative effects on health" are emphasized. Food contaminants are defined as substances that contaminate during production, processing, preparation, storage, packaging, transport or cause environmental pollution if they are not willingly added to food [20]. Arsenic is an element with significant impact on human health [21]. Arsenic, which is among the pollutants that disrupt the natural balance, is important for human and animal health due to both being free in nature as well as the toxic effects it manifests in a living structure [22]. Çakır [17] carried out a study in which 13 of the 29 different turnip juice samples collected from the market revealed amounts of arsenic that were in excess of the limit specified in Turnip Juice Standard no. TS 11149. The remaining samples revealed arsenic levels ranging between 0.001-0.2 mg / kg. In this study, the arsenic levels of turnip juice samples were found to be 1.836 and 0.685 µg/g in two samples, respectively while the remainder of the samples had contents below the detectable limit.

The cadmium in air and water is colorless and odorless and therefore undetectable. Cadmium can be incepted orally, through respiration or the skin. Cadmium intoxication may cause damage to the kidneys, bones and lungs [23, 24, 25]. Cadmium levels were found below the detectable limit in this study. Even trace amounts of lead are absorbed from the digestive tract and transmitted to the tissues by blood. It is more valid children. However, disregarding the importance of food safety and the prevalence of unconscious eating habits generalize the problem [26]. In this study, the lead levels of turnip juice samples were found to be below the detectable limit. According to TS 11149 Turnip Juice Standard, the maximum amount of lead in turnip juice is 0.05 mg / kg [1]. Şahin [27] reported in his study that the amount of Pb in turnip juices was 0.058 mg / kg on average. Çakır [17] reported that two samples he analyzed did not conform to the standard. In this study, all specimens were found to be in accordance with the turnip juice standard in terms of lead.
Chrome is the seventh most common element in the world [28]. Cr is naturally present in air, soil and water ecosystems. Its natural availability in soil is between 10 and 50 mg / kg. The concentration in fresh water is usually between 0.1-117 µg / L and the concentration in the seas is between 0.2-50 µg/L [29]. Chrome enters the body through the mouth and through breathing. It causes liver and kidney disorders. Chromium is a carcinogenic substance [30]. In three of the samples of regular turnip juice, between 0.011 and 0.025 µg/g of chrome and in 4 hot turnip juice samples between 0.014 and 0.021 µg/g of chrome were found in this study.

Although copper is one of the trace elements required for many cellular enzymes to function, it is one of the unwanted metal contaminants in food [17]. Copper is a metal that can accumulate in mammalian tissues and show toxic effects when its concentration in tissues reaches critical values. Pathological changes in many tissues, especially liver and kidney, have been reported as a result of exposure to this metal [31-32]. Çakır [17] reported copper amounts between 0.013-0.62 mg/kg in a turnip juice sample. According to TS 11149 Turnip Juice Standard, the standard value of copper in turnip juice is 5.0 mg / kg [1]. The copper level was between 0.023-0.298 (µg/g) in regular turnip juice samples and between 0.035 and 0.258 (µg/g) in hot turnip juice samples in this study (Figure 6). According to the findings, the turnip juice samples were in compliance with the standard.

Zinc has many functions that are important for living organisms. The main tasks of these enzymes are essential in the activation of many enzymes and hormone production and the necessary minerals in terms of brain development and functionality [33]. Zinc excess causes negative results such as the excess of other minerals. Zinc metal and many compounds show low toxicity compared to other heavy metals [34]. Çakır [17] carried out a study in 2011 in which the amount of zinc was found to be 0.14-2.2 mg / kg. According to TS 11149 Turnip Juice Standard, the standard of zinc in turnip juice is maximum 5.0 mg / kg [1]. The level of zinc in regular turnip juice samples in this study was determined as 0.16 and 1.506 (µg/g) while the level for hot turnip juice samples varied between 0.188 and 0.692 (µg/g) (Figure 7).

One of the undesirable metal contaminants in food is tin. According to TS 11149 turnip Juice Standard, the amount of tin in turnip juice can be up to 200 mg / kg [1]. It was determined that the level of tin in regular turnip juice in this study varied between 0.147 and 0.217 (µg/g) while the hot turnip juice samples varied between 0.142 and 0.238 (µg/g) (Figure 8). The results of the study carried out by Çakır [17] and the results of this study are compliant with Turnip Juice Standard number TS 11149.

Iron is an essential element for normal cell metabolism. Iron is an element that has a vital impact on health when taken in the required amount whereas if taken in excess it can reduce the amount of calcium and zinc intake and cause liver and heart disease [35]. When taken in high doses, it affects almost all organs, can be cytotoxic or even lethal.

In the analysis, the iron level was found to be 0.623 to 4.635 (µg/g) in regular turnip juice samples while the results for hot turnip juice varied between 0.656 and 4.467 (µg / g) (Figure 9). Çakır [17] reported an iron level of 0.25-3.4 mg/kg in his study. According to TS 11149 Turnip Juice Standard, iron in turnip juice should be at most 15.0 mg / kg [1]. According to the obtained data, the iron levels were found to be in accordance with TS 11149 Turnip Juice Standard.
FIGURE 7
Zn levels of Turnip Juice Samples

FIGURE 8
Sn levels of Turnip Juice Samples

FIGURE 9
Fe levels of Turnip Juice Samples
CONCLUSION

Our study revealed that some parameters of the collected samples complied with the standards while others did not. This result reveals that the analysis of such food products in our country should be done frequently and the production should be monitored continuously.

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ABSTRACT

The concentration and molecular weight of HPAM (HPAM) solutions, temperature, and pH were investigated in order to investigate the effects of HPAM on the amount of silicate scaling in the gathering line with the widely reuse of the Al-kali/surfactant/polymer (ASP) flooding. Heteropoly yellow method for spectrophotometer determination of silicon is applied to quantitative analysis of silicon in ASP-flooded produced water to obtain the acknowledgement that the HPAM have the maximum effect on silicate scale quantity under any conditions. The research provides a reliable basis for the mechanism research of the scaling problem in the oil extraction process of asp flooding and the adoption of scale inhibition and scale inhibitor.

KEYWORDS:
HPAM, silica scaling, Asp flooding wastewater

INTRODUCTION

With the exploitation of oil field continuing, the production ability of old oil field in our nation is decreasing year by year. It has entered a stage of high water content and high recovery. Alkali-Surfactant-Polymer (ASP) Flooding is a significant way to improve oil recovery [1-2]. It is an effective way to suppress yield reduction and maintain stable production [3]. Mineral scale deposits such as calcium carbonate and phosphate, calcium oxalate, barium and strontium sulfate, magnesium silicate and others and colloidal inorganic species such as silica present important challenges for ASP flooding oilfield. ASP flooding will cause scaling in reservoir rock and production well. Scaling may cause reservoir block and stuck well pump. Scaling effects the application of ASP flooding seriously [4]. Alkaline cause reservoir rock corrosion. Productions from corrosion may form scale such as silicate and carbonate. In order to study law of scaling and corrosion of reservoir rock [5]. When silica is left uncontrolled it forms hard and tenacious deposits that are difficult and hazardous to remove. Conventional phosphonate mineral scale inhibitors do not inhibit silica formation and deposition [6]. The ASP flood has high pH of 11 or above. As it moves through the reservoir, quartz silica is dissolved and the dissolved silica becomes stable in the high pH alkaline flood [7-9]. Scaling problems substantially cause financial losses.

In our experimental system, the systematic study of magnesium silicate scale growth in the presence of HPAM was analyzed under different conditions in order to investigate the effect of the HPAM in the Asp flooding wastewater on the scaling.

EXPERIMENTAL

Materials. The anhydrous NaOH, HPAM, LAS, NaCl, sodium silicate, ammonium molybdate and HPAM were adopted to investigate the effect of HPAM on silicate scaling in the test, and all of the above agents were analytical pure agents.

The chemical formula of HPAM is:

\[ -\left(\text{CH}_2 - \text{CH}\right)_{m}\left(\text{CONH}_2 - \text{COOH}\right)_{n} \]

Assays for factors. In order to investigate the effects of HPAM on the amount of silicate scaling, the concentration and molecular weight of HPAM (HPAM) solutions, temperature, and pH were investigated. And all kinds of situation have been made the detailed analysis.

This experiment investigated the scaling cation with given ion concentration and the corresponding concentration of anion mixed solution. Under different reaction conditions, we inspected the changes of the concentration of the scaling cation with the HPAM added in ASP flooding gathering pipeline to get the acknowledgement of the common scale formation quantity and the influence of the formation process.
RESULTS AND DISCUSSION

Result of the phenomenon analysis. During the experiment, the phenomenon of the experiment was observed by the blank system and the contrast by adding the HPAM, as shown in Fig.1 and Fig.2. The magnesium silicate scale solution without HPAM is turbidity and tends to flocks (Fig.1). Whereas, with the HPAM added the reaction system solution is clarify and there is none precipitation formation (Fig.2). The viscosity of test solution becomes large with HPAM. The experimental results show that the addition of HPAM can affect the formation of silica scale to a certain extent. In order to make a detailed analysis, we have carried out a large number of experimental analysis of the factors affecting the formation of silicon scale and simulated the ASP flooding system to investigate the effect of the components of ASP flooding system on the concentration of residual silicon ions in the solution, and the influence of the components of ASP flooding on fouling was obtained.

Result of factors analysis. Influence of the molecular weight of HPAM on magnesium silicate formation. In this study, the effect of partially HPAM with different molecular weights on the formation of magnesium silicate was investigated. In order to study the effect of different molecular weight HPAM on the formation of magnesium silicate. The effect of HPAM solution at 60°C on the concentration of residual silicon ions in the solution was investigated. The experimental results are shown in Fig.3.

It indicated that different molecular weight of HPAM has a certain impact on silica fouling production from Figure 3. First, the addition of partially HPAM increased the concentration of residual silicon ions in the solution, which was confirmed by the addition of partially HPAM to a certain extent, inhibited the formation of silica scale. With the increase of molecular weight, the remaining concentration of silicon ions in the solution increases, HPAM on the inhibition of silica scale formation of the stronger. However, due to high molecular weight HPAM to a certain extent, will affect the quantitative analysis of silicon ions, so we selected The HPAM in 300 million molecular weight to launch other experiment.

FIGURE 1
Magnesium silicate without HPAM

FIGURE 2
M silicate generated with HPAM

FIGURE 3
The concentration of silicon ion in the solution with different molecular weight of HPAM

FIGURE 4
The concentration of silicon ion in the solution with HPAM in different concentration
The influence of HPAM concentration on magnesium silicate formation. This experiment investigated the influence on the concentration of silicon ion under different concentrations of HPAM in 60 centigrade and the remaining concentration of silicon ion under the blank situation using distilled water instead of HPAM. The results were shown in Fig.4 through measuring the concentration of silicon ion in the solution when added different concentration of HPAM, such as 0ppm to 100ppm, with molecular weight is 3 million.

Fig.4 showed that adding HPAM has a certain inhibitory action to the magnesium silicate scales formation and changes with the concentration changing. First, the addition of HPAM at different concentrations increased the residual silicon ion concentration higher than that in the blank (without HPAM), but the increase was little. Secondly, the concentration of residual silicon ions in the solution decreased first and then increased with the increase of the concentration of HPAM. Based on the analysis of the experimental data, it was concluded that the ability of HPAM to inhibit scaling was best when the concentration reached 100 ppm, and the scale was most likely to be fouled when the concentration of HPAM in the solution was 30 ppm.

The influence of the reaction time on the effect of HPAM on magnesium silicate formation. We worked on the influence of HPAM on magnesium silicate formation under the different reaction time through the investigation on changes of the remaining silicon ion concentration in the solution under different reaction time. We first select the concentration of 3 million molecular weight HPAM at 60°C under the conditions of reaction time were 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 14 h. The distilled water instead of HPAM was chosen as the blank group at the same condition (Fig.5) to get a comparison.

From Fig.5, we can see that at 2 h, 4 h, 6 h, the residual silicon ion concentration in the solution changed little, this is may be due to the reaction is not enough. After the reaction was stabilized, the residual silicon ion concentration in the solution increased with the reaction time. The difference between the two curves shows that the longer the reaction time, the greater the effect of HPAM on the scale formation. According to the analysis of the experimental data, we conclude that the longer the reaction time is, the greater the inhibitory effect of HPAM on scale formation is.

The influence of pH on the effect of HPAM on magnesium silicate formation. In this study, the effect of HPAM on the residual silicon ion in the solution was investigated in order to investigate the effect of HPAM on the silica scale formation at different pH values. The experimental results are shown in Fig.6.

It can be seen from Fig.6 that the addition of HPAM at different pH values has an effect on the concentration of the remaining silicon ions in the solution. The experimental results showed that the content of silicon ions in the blank group and HPAM group decreased first and then increased at pH 8, which was the most likely to cause silicon scaling at pH 8. At the same time, the difference between the blank group and the HPAM group also showed that the addition of HPAM, the condition of over-acid or over-base will weaken the impact of HPAM on the residual silicon ion content in the solution. The difference between HPAM and blank group also shows that the influence of HPAM on the formation of silica scale increases first and then decreases with the increase of pH value. When the pH value is neutral, forming the greatest impact.

The influence of temperature on magnesium silicate scaling was investigated. We also chose the distilled water instead of HPAM as the blank group at the same condition (Fig.7) to get a comparison.
It can be seen from Fig. 7 that the concentration of residual silicon ions in the solution decreases first and then increases with increasing temperature while the addition and reduction of HPAM is slow at 40°C to 70°C. The remaining silicon ion concentration in the blank group solution was not changed until the temperature increased and then decreased at high temperature. From the difference between the two curves, it can be seen that HPAM has little effect on the formation of silicon scale when the temperature is low. Only when the temperature is about 90°C, HPAM has the greatest influence on the scale formation.

CONCLUSIONS

(1) The magnesium silicate scale solution without HPAM is turbidity and tends to flocks. Whereas, with the HPAM added the reaction system solution is clarifying and there is no precipitation formation, and at the same time the test solution with HPAM has bigger viscosity.

(2) HPAM with different molecular weight may influence silica scale formation. HPAM in all molecular weight improves the concentration of the residual silica ion better than the blank sample. The concentration of the residual silica ion in solution decreases with the concentration of HPAM at first, then increases until tends to be the same. The concentration of the residual silica ion in solution decreases at first and then increases with reaction time extension; The concentration of the residual silica ion in solution with HPAM decreases at first and then increases with the pH rising and has the minimum when pH = 8; The concentration of the residual silica ion in solution with HPAM decreases at first and then increases with the temperature rising.

(3) The research provides a reliable basis for the mechanism research of the scaling problem in the oil extraction process of asp flooding and the adoption of scale inhibition and scale inhibitor.

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STUDY ON EFFECT OF WORKING FLUID ON FRACTURE PROPAGATION AND MECHANICAL PROPERTIES OF SHALE: BASED ON RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Shale wellbore instability is a major cause of high cost and long time drilling and completion for shale gas. The effect of working fluid soaking on shale crack propagation and mechanical properties was analyzed to optimize drilling technology and protect wellbore stability. Response surface methodology is applied to study the influencing factors under working fluid soaking. The results show that radial holes play a certain guiding role in the directional propagation of hydraulic fractures in low permeability reservoir. Fractures start to crack and propagate a certain distance along radial holes, and then gradually divert to the maximum principal in-situ stress direction of reservoir.

The expansion of shale cracks after fluid immersion can be divided into two stages: the rapid formation of cracks and the slow extension of existing fractures; the shale rock strength is reduced after soaking of different fluids, and the shale rock mechanical parameters are immersed by drilling fluid. Significant changes have occurred, showing a decrease in elastic modulus and an increase in Poisson’s ratio. After soaking with the same drilling fluid, the longer the immersion time, the greater the reduction in shale rock strength.

KEYWORDS:
Shale, rock strength, Working Fluid, Fracture Propagation, Mechanical Properties, response surface methodology

INTRODUCTION

As an important unconventional natural gas, shale gas and its development have been paid increasingly attention in recent years [1-4]. Hydraulic fracturing treatment is one of the essential technologies to develop shale gas in an economical way [5], because induced fractures can increase the shale matrix permeability and provide flow passages for shale gas. To clarify the propagation mechanism of induced fractures, lots of fracturing stimulation studies have been conducted in the field and laboratory. Complicated fractures rather than typical symmetrical fractures were apparently observed when conducting mine back experiments [6]. Breakdown pressure and fracture propagation were investigated in granite fracturing using different fracturing fluid, which indicates the breakdown pressure is lower and the induced fractures are more complex using supercritical CO2 compared with water and liquid CO2 [7]. Also, the effect of applied stress on fracture extension was discussed in granite fracturing experiment. Furthermore, the hydraulic fracture initiation was studied in sandstone under different viscosity and flow rate of fracturing fluid, and the results could be matched with the predictions of a simple hydraulic fracture initiation model [8]. Similarly, Sandstone was also utilized to research on fracture geometry under varying injection rate conditions and it is found that lower injection rate can cause simpler fractures [9]. In addition, numerous hydraulic fracturing experiments have been performed in artificially medium blocks to discuss fracture propagation. Many researchers investigated the interaction between natural and hydraulic fractures. And the interactions mainly include approaching, activation, crossing, diversion, offsetting and bypassing. Layered medium blocks were employed to conduct hydraulic experiments, and the layer cohesion and approaching angle have a great effect on the fracture extension [10]. The influence of the flow rate and stress on the fracture propagation was analyzed using multi-fractured Medium, which reveals the fracture interaction is the smallest with high flow rate and the fractures are smoother with less branches under higher horizontal stress difference [11]. However, in these studies above, the mechanical properties of granite, sandstone and artificial blocks differ from the shale, and they cannot represent shale with complex natural fractures and bedding planes.

In researches on shale fracture propagation, the hydraulic fracture-natural fracture interaction was firstly discussed in 1982. Afterwards, the importance of natural fractures in shale for hydraulic fracturing was specifically investigated [12]. Physical and numerical simulation of hydraulic fracture
initiation and propagation were carried out in shale, which implies the hydraulic fracture geometry is complicated and like network in shale due to the existence of beddings. Moreover, the effects of fracturing fluid viscosity and flow rate on the fracture propagation were studied, and it was expressed by the parameter $q$, $\mu$, and the too small or too large values are both harm for fracture network generation \[13\]. Also, Variable flow rate fracturing with low viscosity slick water fluid of 2.5 mPa s was proved to be an effective treatment to improve the connectivity of hydraulic fracture with natural fractures \[14\]. Large-scale shale hydraulic fracturing experiments were conducted, and it is suggested that lower horizontal stress difference and natural fracture are two indispensable conditions to generate the complicated fractures \[15\]. Shale cylinder samples from the Longmaxi Formation, Silurian were used to perform hydraulic fracturing experiments, and except for extending along bedding planes, a long main fracture basically vertical to beddings is generated when axial stress is more than confining stress \[16\].

The soaking experiment is an image-wise simultaneous recording of rock-fluid reaction phenomena such as particle dispersion, collapse, and cracking along weak faces/layers/edges. After the shale and fluid contact each other, the rock will usually be brittle or collapse. Traditional test methods, such as dispersion testing, hardness testing, and expansion testing, do not fully reflect the effects of rock structures on fracture propagation and wellbore instability, while rock sample soaking experiments can visually observe rock-fluid interactions and fracture extensions. This means can make up for the shortcomings of traditional methods. The soaking test is one of the most effective methods for evaluating rock-fluid interaction and crack elongation. The change in petrophysical and chemical properties during the experiment reflects the combined effects of rock properties on potential wellbore instability.

**EXPERIMENTAL**

To study continental shale hydraulic fracturing in the laboratory, a scientific research well called JK4 well was drilled to get shale cores. JK4 well is situated in Jin Suoguan, Shanxi province and the south of the Ordos Basin. And the depth of JK4 well is about 124 m and the target formation is Chang 7–2 which is located at about 107 m–120 m. The cores are naturally fractured black shale which contains pyrite and asphaltene. The shale beddings are developed well and they have nearly $10^\circ$–$15^\circ$ angles to horizontal direction. Besides, the shale is characterized by silt interbeds with varying thickness and they are nearly horizontal to beddings. The natural fractures are mostly along bedding planes and interbeds, but a few are at a certain angle to bedding planes. The interbeds with various thickness are measured macroscopically and microscopically. Meanwhile, the mechanical property of shale cores were tested under triaxial compression states.

In the 5 containers of the roller heating furnace, add 200 mL of 5 working fluids respectively, and place the rock samples into the working fluid separately; the temperature of the roller heating furnace is constant at the core temperature of the coring section, soaking 24, 72, After 168h, the sections were taken out to take photos, the surface crack propagation was observed, and the mechanical properties after immersion for 168 h were tested.

**FIGURE 1**

Surface Plot of Stress (MPa) vs D, C

**FIGURE 2**

Surface Plot of Stress (MPa) vs D, B

**RESULTS AND DISCUSSION**

After the working fluid is immersed, the crack propagation on the shale surface can be divided into two stages: the rapid formation of the fracture and the slow extension along the existing fracture. The rapid formation of cracks refers to the formation stage of most cracks, which occur within 72 h of fluid soaking, in which new cracks are formed and extended along the direction of crack extension; the slow extension along existing cracks refers to the length of shale After the time is immersed in the fluid, the dispersion of the clay minerals gradually
weakens, causing the shale to slowly extend along the existing cracks. In this stage, the crack width becomes wider and the cracks become more pronounced (Fig. 1-3).

For Fig. 4-6, after immersion in alkaline working fluid, the triaxial compressive strength of shale decreased significantly from 221.2 MPa before immersion to 167.7 MPa after immersion, with a decrease of 24%; the shale elastic modulus was $2.58 \times 10^4$ MPa. It fell to $2.39 \times 10^4$ MPa, a decrease of 7%; the shale Poisson’s ratio increased from 0.182 to 0.240, an increase of 32%.

The reduction in strength after the rock is saturated with water has been confirmed by a large number of experiments. After water invades the rock soaking mineral particles, weakening the connection between mineral particles and reducing rock strength.

For rocks that do not contain or contain small amounts of water-sensitive minerals, their strength can still recover to some extent when the rock is dry. However, for shale, after the water intrusion, the clay minerals hydrated and expanded, and the distance between the crystal layers became larger, which led to a decrease in the compressive strength and elastic modulus of the shale.

The analysis of directional propagation of hydraulic fracture guided by radial holes is based on physical parameters of a low permeability sandstone reservoir of fossil hydrogen energy. Both numerical simulation results and physical experiment results show that hydraulic fracture will extend along the radial holes direction at a certain distance. Based on the above research studies, radial holes can be used to achieve directional propagation of fractures during fracturing and to increase the stimulated reservoir volume in oilfield.

For other reservoir lithology, whether radial holes play a guiding role still requires further study. It is recommended to analyze the induced stress.
field produced by radial holes in shale reservoir with high clay content. Clay minerals swelling in contact with water will lead to different induced stress and different hydraulic fracture propagation laws.

**CONCLUSION**

Radial holes play a certain guiding role in the directional propagation of hydraulic fractures in low permeability reservoir. Fractures start to crack and propagate a certain distance along radial holes, and then gradually divert to the maximum principal in-situ stress direction of reservoir.

The expansion of shale cracks after fluid immersion can be divided into two stages: the rapid formation of cracks and the slow extension of existing fractures; the shale rock strength is reduced after soaking of different fluids, and the shale rock mechanical parameters are immersed by drilling fluid. Significant changes have occurred, showing a decrease in elastic modulus and an increase in Poisson's ratio. After soaking with the same drilling fluid, the longer the immersion time, the greater the reduction in shale rock strength.

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PALEOENVIRONMENTAL CONDITIONS, GEOCHEMISTRY AND HYDROCARBON POTENTIAL OF KIZILDERE FORMATION (HATAY-TURKEY)

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ABSTRACT

The study area is in Arsuz - HATAY region and the formation characteristics of the clay rocks of Kizildere Formation (Middle - Upper Miocene) and sedimentation environment of formation were investigated in this study.

The petroleum-rich formations formed during the Miocene, these formations; Kalecik Formation with conglomerates, Horu Formation with reefal limestones and Kizildere Formation. The Middle-Late Miocene aged Kizildere Formation is typically observed in the Konacik (Arsuz) region. Sand and clay layers are common in the formation, bedding thickness of the gray-yellow colored sandstones are 10-50 cm (medium-thick), and gray claystones are 2-30 cm (thin-medium). Nodular and stratified gypsum layers are observed at the top of the succession of sandstone and claystone and gypsum layers thickness are 5-15 cm. Tectonic cracks in the formation are widely observed by virtue of tectonism that is effective after Middle Miocene in the region. Plant residues in marl that is layered with gypsum layers are macro-scale.

The average Total Organic Carbon (TOC) values in the claystones are 0.56 which indicates that there is a potential for oil production in claystones. The averages of the concentrations of trace elements are as follows: V, Ni, Cr, Co, Cu, Rb, As, Zn, Sb, Mo, Cd, Pb, U, Ba, Li, Cs and S are respectively 1180.65, 219.83, 149.26, 19.45, 22.63, 42.96, 8.40, 54.47 ppm, 0.61, 1.28, (-0.71), 5.68, 1.35, 171.15, 24.64, 3.06, 325.48 ppm. Increases in vanadium concentrations and V/Ni, Ni/Co, V(V+Ni), V/Cr ratios indicate that claystones are deposited in the marine and anoxic environment that is suitable for protection of organic matter. According to trace elements and TOC values, classification was made in cluster diagram and it was determined that the transition of environment conditions. The classification of trace elements and TOC values in the region and the environment transitions in which organic matter preservation increases were determined.

KEYWORDS:
Kizildere Formation, Source rock, hydrocarbon potential, TOC, trace elements.

INTRODUCTION

The term source rock is used for rocks producing oil and/ or natural gas and in order for a rock to be the source rock; its thermal maturity and the amount of organic matter must be higher than the other rocks. Firstly, organic matter rich claystones are determined in the source rock [1].

Petrography and geochemical analyzes are made to determine the source rock potential and environmental conditions of a region [2]. Some of these analyzes are carried out to determine the amount of organic matter, it is searched trace element enrichment and environmental conditions. The organic matter content of the rock samples is determined by the analysis of Total Organic Carbon (TOC) and its quantities determine the hydrocarbon production potential in the area. It is necessary to investigate the accumulation conditions of hydrocarbon in reservoir rock for production. Many methods are used to obtain this information, and the determination of the conditions of the trace elements and the environment are among these methods. XRF (X-Ray Fluorescence Spectrometry), ICP-MS (Inductive Matched Plasma Mass Spectrometry) and AAS (Atomic Absorption Spectroscopy) analysis methods are used to determine trace elements [3].

In the many source rock potentials study, it is seen that trace element enrichments are also directly related to the high organic material production in the region. The trace characteristics can be used to determine the environmental characteristics and the environment in which the source rock is formed is important for oil production and organic matter conservation. Some carbon derivatives are used as environment markers [4, 5]. As an alternative to the biomarker studies, trace element studies are also carried out to determine the environment of the source rock [6, 7]. The amount of trace elements in crude oil is similar to the amount of trace elements in the source rock [8-10]. The trace element studies
in crude oil are carried out to determine the environmental conditions of the formation where the oil is formed [11]. Determining the type of kerogen in the source rock and measuring the thermal maturity is one of the oldest known methods, and many of the source rocks are analyzed by this method [12-16]. The process of determining the characteristics of the source rock during the acquisition of well logs is an interesting and explored subject and new methods are developed in this issue [17, 18].

The amount of trace elements in the environment is determined in the samples taken from the study areas and these data are interpreted and it is concluded that the environment where hydrocarbon is available/ unfavorable for production of source rock. The study area is located in the Arsuz (Hatay-TURKEY), north of the Amanos Mountains and the southern part of Iskenderun Bay.

MATERIALS AND METHODS

In the study, 20 claystone and sandstone samples from Arsuz region in the northwest of Hatay. In the laboratory, total organic carbon analyzer, Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and Atomic Absorption Spectroscopy (AAS) devices were analyzed. Experiments were made in Adiyaman University Central Research Laboratory.

ICP-MS and AAS analyzes were aimed to determine the enrichment of trace elements in the region and to determine the enrichment environments of these elements and to investigate the hydrocarbon potential. Rb, Cu, Cr, As, Zn, Sb, Co, Mo, Cd, Pb, U, Ba, Li, Cs, S, Ni, and V elements are analyzed on ICP-MS in the samples taken from the study area. The concentrations of Ca, K, Mg and Na were determined by AAS. The results were mapped and cluster analysis was performed with hierarchical cluster analysis, Ward’s method.

LOCAL GEOLOGY OF ARSUZ (HATAY)

The research region is the Arsuz in the northwest of Hatay - TURKEY (Fig 1). Kozlu [19], Tekin et al. [20] have done studies in Arsuz, while Selçuk [21], Yıldız and Taptık [22], Şafak [23] (1993) and Boulton et al. [24] have studied Samandağ – Sinanlı region that is seen the similar features of the geology of the Arsuz region. Miocene and Pliocene units were deposited on the Kızıldağ ophiolite (Late Cretaceous). These units consist of Kalecik, Kızıldere, Haymaseki and Aktepe Formations.

Toros Mountains are located north of Iskenderun basin while Amanos Mountains are located to the east of the basin. The Yumurtalık Fault associated with Misis uplift is to the east. The stratigraphic section of the region is given in the Fig 2. The Kızıldağ ophiolite was formed during the Paleozoic and Mesozoic periods, and the Neogene sequence consisting of Kalecik Formation, Horu Formation, Kızıldere Formation, Mezelet Formation, Haymaseki Formation and Aktepe Formation is formed on the Kızıldağ ophiolite. The Neogene sequence starts with the Middle Miocene transgression [25, 26].
The Miocene sequence unconformably overlies the ophiolites and coarse clastics were firstly deposited of the Kalecik Formation. The reef limestones (Horu Formation) were lenticularly deposited on the Kalecik Formation and the Kızıldere Formation was observed on the sandstone-shale sequence [25-27]. Evaporitic levels on the Kızıldere Formation (Haymaseki Member) deposited due to the regression have situated during the Messinian period and then the marine units (Aktepe Formation) have deposited in the transgression zone. During the Lower Pliocene period, the sea was transgressively covered the land and the basin was closed by the sediments dominated river facies in the Upper Pliocene-Quaternary time period.

Kızıldere Formation showing the source rock characteristics was named by Schmidt [27]. The thickness of the formation which is composed of gray-colored thick-medium layer sandstone-claystone sequences is 800-2500 m. It is seen conformity with the Menzelet Formation which consists of light gray sandy marls with transition lithology between Kızıldere Formation and Haymaseki Formation.

Petroleum producing rocks were formed during the Miocene and formations; Kalecik Formation (Middle Miocene) consisting of conglomerates, Horu Formation consisting of reef limestones (Middle Miocene) and Kızıldere Formation. The Horu Formation and the Kızıldere Formation, which are the reservoirs, cover rocks and source rocks are of great importance. Middle-Late Miocene aged formation is typically observed in the Konacık (Arsuz-HATAY) region.

Sandstone-claystone sequences in the formation are common, the thickness of gray-yellow colored sandstones is between 10-50 cm, medium-thick bedded (Fig 3.A-B) and gray colored claystones between 2-30 cm (Fig 3.C). Layered gypsum levels are observed above the sandstone and claystone sequence and the layer thicknesses are between 5-15 cm. The deeper claystone layers thicken to 102 cm. Tectonic cracks have been observed widely in the formation due to post-Miocene tectonism is effective (Fig 3.D). Plant remains in the marls along the sequential marls are visible to the gypsum.

**AMOUNT OF ORGANIC MATERIAL**

The amount and type of organic matter in a rock determines the rock potential of the rock. Total organic carbon content in the rock samples is related to the kerogen in the rock, and the organic carbon is made of kerogen but could not be taken out of the
rock. The values obtained by TOC analysis are the percentage of organic carbon content [28]. It is known from the previous studies that the rocks with Total Organic Carbon (TOC) value may be Source rock if the amount of carbon obtained is more than 0.50% by weight [29]. The rocks with TOC value of less than 0.50% do not a source rock (Table 1).

**TABLE 1**
Source rock potential according to TOC values [29]

<table>
<thead>
<tr>
<th>Total Organic Carbon (% TOC)</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.5</td>
<td>Poor</td>
</tr>
<tr>
<td>0.5 – 1.0</td>
<td>Fair</td>
</tr>
<tr>
<td>1.0 – 2.0</td>
<td>Good</td>
</tr>
<tr>
<td>2.0 – 4.0</td>
<td>Very Good</td>
</tr>
<tr>
<td>&gt;4</td>
<td>Excellent</td>
</tr>
</tbody>
</table>

The results of TOC analyzed on rock samples in the Kızildere formation are given in Table 2. The total organic carbon average of the claystones is 0.56 % [30]. The amount of organic matter must be at least 0.5% for the lithological unit to be a source rock.

### GEOCHEMICAL DATA

Main and trace element contents of Kızildere claystones are given Table 3. The environmental parameters of V, Ni, Co, Mo, Cr and Cd values were investigated in detail. V, S, Ni, Br and Cr abundance is observed in the spider diagram drawn according to the concentration of trace elements (Fig 4A).

**Vanadium.** Vanadium can be enriched by adhering to the surface of the sediments in marine areas and can also be enriched by forming organometallic complexes [31-33]. Vanadium is partially less reactive in seawater; V element also tends to be more enriched in sediments deposited under anoxic conditions [32].

![Field observations of Kızildere Formation](image-url)
The mean V amount of claystone samples taken from the study area was determined as 1180 ppm. The enrichment of vanadium element in the study area indicates that the environment is anoxic.

**Molybdenum.** Molybdenum (Mo) is associated with organic matter and humic acids and it can be used as a marker for precipitation conditions. Mo concentrations have been suggested to increase with anoxic conditions [34] and the enrichment of Mo depends on the amount of organic material and the sulfidic conditions of the environment. Mo is enriched by forming organometallic compounds with organic substances or by forming metal sulfide compounds (such as MoS₂).

Mo content is between 0.29-3.33 ppm in the clay samples; the average value is 1.29 ppm. According to Scott and Lyons [35], H₂S may be present in the environment but it is poor in oxygen. According to the results of the analysis, there is no enrichment of Mo and the presence of S element in claystone samples shows that the environment is poor in oxygen.

**Cobalt.** Cobalt (Co) can be used as an indicator for precipitation conditions. Cobalt (Co) is usually enriched with nickel (Ni) in oxic conditions and the Ni/Co ratio are used as a marker of oxygen levels. The Ni/Co ratio below 5 is theoxic environment and the values above 5 indicate the suboxic/dioxic environment [34, 36].

The average Co Value in the clay samples of Kızıldere formation is 19.44 ppm. Ni/Co ratio is 11.45 and the values in all samples are above 5 and the environment is determined to be in anoxic redox conditions.
Chromium. The chromium (Cr) element is known to be not directly affected by the redox conditions, and Cr is thought to come from detrital origin and the V/Cr ratio is considered to be an environmental marker. If V/Cr values are greater than 2, it shows anoxic conditions and small oxic conditions [34, 36, 37].

The V/Cr ratios of the claystones of Kızıldere Formation ranged from 6.8 to 9.4. Jones and Manning [36] from Ni/Co and V/Cr values, according to environmental indicator all clay samples of Kızıldere formations form anoxic environment.

Cadmium (Cd). Unlike redox-sensitive elements such as Mo, U, V and Re, the cadmium element is the only naturally oxidized element. Cadmium is distributed in oxic environment conditions and enriched in sulphidic sediments [32].

The average value of Cd in the samples is 0.70 ppm. Pattan and Pearce [32] according to the study in the sample of the amount of Cd minus results in the environment gives an idea about the result that the environment is not anoxic.

Environment Indicators. The ratio of trace element ratios of paleoredox environment markers determined by various researchers is shown in Table 4. V/(V+Ni) is a good deposition environment indicator [6, 38, 39]. V/(V+Ni) values higher than 0.5 indicates an organic material deposited in the anoxic environment. Values below 0.5 indicate oxic deposition environment [6].

V/Cr ratio is another redox environment indicator and vanadium element is adsorbed on the surface of clay minerals while Cr element is only related to detritic fraction [40]. Therefore, high V/Cr ratios (> 2) indicate anoxic conditions [41].

The replacement of Cr with Al in clays is only related to the detritic fraction and is not affected by redox conditions [36]. V/Cr ratio in the paleoenvironment interpretation and V/Cr values are greater than 2 anoxic conditions, less than 2 values indicate the oxic conditions [34, 37].

Ni/Co ratio is one of the redox environment markers. Ni and Co may be found in pyritohione but the high Ni/Co ratio is related to anoxic conditions. If the Ni/Co ratio is less than 5, the paleo-environment is oxic, the value between 5 and 7 is disoxic, and if it is higher than 7.5, it is anoxic conditions of environment [36]. The Ni/Co ratios of clay samples are given in Fig. 4B and the average is 11.45 ppm. According to different researchers, this ratio is greater than 7 and this value indicates anoxic environment. The claystones from Kızıldere Formation were deposited in anoxic environment according to environmental marker table from Ni/Co and V/Cr values (Fig. 4B). As a result of the paleochemical data given in Fig 4C, V/Ni ratio is shown to be marine anoxic.

The V/(V + Ni) ratios of the samples ranged from 0.82 to 0.88 and the average was 0.84. The average V/Ni ratio in the samples is 5.5 ppm. It is observed that the rate of nickel increases as the ratio of vanadium increases in claystone samples. In Fig. 4C these values were evaluated in the environmental difference graph and it was observed that the environment was marine anoxic. The differences in the cluster analysis show that the concentrations of these elements also change according to the environment.

The mean V value in claystones is 1180.65 ppm, the mean value of Cr is 149.26 ppm and the V/Cr ratio is 7.44 ppm. V/Cr value ranges to be the marker of paleoredox environment, all of the ratios are > 4.5. V/Cr ratios are also compared with Ni/Co ratios to determine the environment, accordingly all claystones are formed in anoxic environment.

There is a relationship between Ni, Co and V representing the sedimentary rock origin, and Ni is positive correlation with Co and V. This relationship between elements shows that Ni, Co and V elements can be used as environment markers. In addition, as a result of the paleochemical data given the V/Ni ratio is shown to be marine anoxic.

DATA CLASSIFICATION OF KIZIDERE CLAYSTONES

Cluster analysis was analyzed to determine the similarities in the statistical evaluation of Kızıldere Formation claystones (Fig. 5 C). Dendrogram was performed by using TOC and density data of all trace elements and two groups were evident. When the map positions of these two groups are examined, it is located in the southwest of the class region that shows the distinction between NE and the other class. The locations of the samples are shown in the map, satellite map and block diagram (Fig. 5 A, B, D).
Contour maps have been drawn to determine the environmental differences of claystones from the region. The change of Co, Ni, Cr and V elements due to the anoxicity of the environment is in the northwest and southeast direction (Fig. 6A, B, C and D). Anoxicity growth environment of the region is the north east.

**FIGURE 4**
Paleoenvironmental graphs of trace elements (A) Trace element concentrations in Kizildere claystones, (B) Ni/Co and V/Cr graph of environment classification (C) V-Ni graph of samples [36]
FIGURE 5
(A) Kizildere claystones locations in the satellite map, (B) sample locations in the block diagram, (C) dendrogram of trace elements and TOC in claystones, (D) sample location

FIGURE 6
Contour maps of trace elements (A) cobalt contour map, (B) nickel contour map, (C) chromium contour map and (D) vanadium contour map
RESULTS

The aim of the study is to investigate the units in Kizildere Formation in Arsuz region and to determine paleo environment and source rock characteristics. The widespread occurrence of sandstone-claystone sequences and this sequence is covered with evaporitic cover rock supports geologically support of hydrocarbon potential in the region.

Petroleum prospect areas were formed during the Miocene and formations; Kalecik Formation (Middle Miocene) consisting of conglomerates, Horu Formation consisting of reef limestones (Middle Miocene) and Kizildere Formation. Among these units, the Kizildere formation which provides the reservoir rock and the Horu formation and rock reservoir rock source rock and rock characteristics is important.

Total organic carbon average in claystone samples is 0.56 [43]. This value is Ruble et al. [29] shows that claystones are appropriate for oil production. It is seen that it has the potential to be the source rock producing medium organic carbon. Vanadium is an anoxic tendency and V enrichment in the study area indicates that the environment is anoxic. The mean Ni / Co ratio in the samples is 11.45 ppm, indicating that the study area is under anoxic conditions. The ratio of V/Cr values used in [36] as a marker of paleoredox environment was found to be > 4.5 in the study area. This data is determined that the studying area is under anoxic environment conditions. The average V/(V+Ni) ratio of the samples is 0.84 ppm and it is under the anoxic conditions of oxygen-poorly oxic conditions.

V/Ni values and TOC values are compared and there is also an increase in TOC values due to the increase of V/Ni values. According to the V/Ni study of Galarraga et al. [44] the Ni concentration in the study area is above 90 ppm in all samples, indicating that the formation may be in an environment with low oxygen. It is observed that there is a relationship between Ni oldugin Co and V representing the origin of sedimentary rock. The relationship between these elements indicates that Ni, Co and V are enriched/depleted due to environmental changes. The elements showed regionally enrichment and consumption together with TOC and the concentration data of all trace elements. When the map positions of these two groups are examined, they are located in the southwest and northwest. The location of the samples showed that the anoxic environment developed in the southeast direction.

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EVALUATION OF ANIMAL DRINKING WATER QUALITY OF CATTLE ENTERPRISES

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ABSTRACT

Though availability of good and optimal quality water is essential to animal health, a basic necessity and a component for effective farm operations, it is often the most neglected factor. Lack of water hygiene depresses production of livestock rapidly and seriously. In many rural areas, farms and livestock enterprises’ water quality is poor, affected by pollution or slightly tolerable. There is remarkably insufficient recent information on many aspects of water quality issues in cattle livestock. Without comparative research using today’s high performance techniques, interpretation of water quality data may be incorrect. For this reason, it is essential that these and similar studies be carried out carefully, that the water quality and its effects can be monitored regularly. The ultimate goal of the study undertaken here is to provide information on the quality, problems and pollutants of drinking water in livestock using various original research and resources, along with the research findings presented here.

KEYWORDS:
Drinking water quality, cattle, livestock drinking water, water pollution

INTRODUCTION

Good nutrition of animals depend essentially on a non-nutrient factor; water. Water is an important part of all project designs and processes, as it supports not only digestion, metabolism, excretion, balancing the body fluid and heat, but also formation of amniotic fluid for fetus and synthesis of milk for the offspring. Despite the importance of water to livestock, research has been conducted particularly on breeding, feeding and struggle with diseases but shelter design and drinking water problems usually have not been taken into consideration in the livestock studies about stock breeding especially in Turkey.

Water being not a nutrient for animals is one of the basic project criteria. But yet, existence and non-existence of water is more important than other food stuff as it not only keeps body fluid balance, discharges the waste nutrients, regulates heat, performs body activities such as digestion, absorption and metabolism but also forms liquid amniotic for fetus, and carries the necessary nutrients to other tissues and breasts for milk synthesis [1]. In addition to increasing the yield potential of cattle by feeding and genetic improvements, the key factors of increasing the animal production are improving and optimising the environmental circumstance of their living and sheltering spaces. Control of water consumption by cattle also serves this purpose.

Livestock systems differ in the amount of water used per animal and in how these requirements are met. There is no single drinking water requirement for a species or an individual. The amount of water ingested depends on a number of factors, such as body weight, temperature, physiological state (stage of pregnancy, lactation etc.), diet, frequency of water provision, type of housing and environmental stress [2]. For ruminant bovine, 4-6 litres of water is required per 1 kg of dry food. The water must be clean and 10-15 oC [3] It is essential to know about the minimum daily amount of drinking water as well as average amount of water. The daily amount of drinking water must not be below 46 liter in summer and 10 liter in winter for cattles [4]. Except drinking water, the livestock use water for different activities (washing or cleaning of equipment, transport of product, isolution of ingredients, water remaining in the final product etc.) A characteristic of process water is that it comes into contact with product directly or indirectly. Therefore, process water also should meet drinking water quality [5].

Physiochemical properties of water can be a useful way of helping to determine water quality. These expressions are usually characterized by measurement of pH, sodium chloride, bicarbonate, sulfate, calcium, magnesium, iron, potassium, phosphorus, fluoride and nitrate [6]. Studies investigating the effect of TDS (Total dissolved solids) on dairy cattle claim that TDS has an impact on the intake, feeding and milk production of cattle. Research has shown that the high amount of TDS in drinking water, combined with increasing ambient temperature, milk production in dairy cattle is highly harmful [7, 8, 9]. High amounts of sodium in the water may cause sodium poisoning in the spinal cord fluid or brain tissue, which is caused by a high
concentration of sodium in the plasma [10]. Magnesium, sodium, chloride and calcium are ions that contribute to the salt content of water, and they may have harmful effects due to their toxic effects or their interaction with other elements. However, these minerals are generally not toxic in the group. Symptoms caused by nitrate, difficulty in breathing, blue spots on the nose of the animal, lack of coordination, vomiting and fever, tremors and difficulty in standing can be counted. If the animals showing these symptoms do not die, nitrate-containing water can be avoided and the negative effects of the nitrate may be completely eliminated when careful care is taken [11].

A dairy cow can detect unpleasant smell and taste and also the colour of the water has importance if the water resource is unpalatable or has an odour, the cow will not drink adequate amount of water to meet the production demands. Bad taste or odour could be caused by the presence of bacteria or metabolic by products [12].

Water distribution systems may contain microorganisms because of water treatment disturbances or deriving from leaks, cross-connections and back-flows. Bacterial growth may also occur at or near the pipe surfaces as biofilms, the interface with suspended particulates and within the water itself. Furthermore, inorganic and organic contamination may also occur along water distribution systems [13]. The microorganisms caused by diseases and the feces and urines of disease carrier animals and humans pollute the hygene of the water. Direct contact with these wastes or the water contaminated by them cause infection. The presence of microorganisms is the indicator of the direct or indirect contact of sewer with water to during water transportation at one or more stages [14].

Taken into consideration of all the points mentioned above, the aims of this study are to evaluate the animal drinking water characteristics of the chosen livestock enterprise, compare the data with the related standards and point out the present threats. The paper is presented with related case studies and literature reviews. It was considered that the case studies which are based on the grounded this area and are deepening the knowledge that and refer to specific livestock sectors.

**MATERIALS AND METHODS**

The study has been carried out in Adana province. Rainfall in Adana is completely as rain or hail in the autumn, winter and spring months are seen. There is no snowfall in the area and the summer months are hot and arid. In the region, rains mostly in November, December, January, February, March and April, while the minimum rain falls in June, July, August and September.

The study is conducted in three stages as initially fields visits and sample collection, then analysis of the samples and finally evaluation and interpretation of the results of the analysis. In the study, an animal farm is focused on representing the livestock enterprises in Adana and nearby provinces in terms of production types, capacity, shelter types, planning systems and water resources. In order to determine the quality and effects of water used in livestock enterprises necessary measurements conducted and the data have been evaluated with scientific methods.

The farms that constitute the research framework have been determined by using the purposive sampling method among all livestock enterprises (197 livestock enterprises) which could be reached in the Lower Seyhan Plain.

Best representative animal farm were determined with questionnaire and detailed studies then experimental implementation stage was conducted. Permission of the owner of the farm was a prerequisite in the selection process of the livestock enterprise where the research was conducted and implemented. One of the major obstacles was the owners were unwilling to give specific information about their enterprises. Some characteristics of the chosen enterprise was explained but the name of the farm and some of the specific information about the enterprise that must be kept confidential was not explained because of the owner’s request. The enterprise is one of the leaders in livestock sector and well-known for long years in Adana province.

**Enterprise.** It is a free open commercial enterprise with 3500 acres of land and approximately 9000 bovine animals present and have semi-open feeding paddocks, quarantine paddocks, forage and silage storages. Preparation of the ration, feeding of the animals and all the other activities are conducted by experts in the enterprise. The need for roughage feed is met by the fodder crops grown in their own land. The water is provided from deep well and the continuity of water is controlled by buoy systems in watering troughs where animals drink water. There are open manure silos in the enterprise and manure is drained in varying periods. Part of the manure is used in the lands of the enterprise while the rest of may be given to other farms. There is a slaughterhouse in the enterprise. Slaughtering and other meat processing activities are carried out by known food establishments throughout the country. The enterprise has modern, technological equipments and completely professional business ethics. The water troughs within the enterprise are concrete-rectangular cut, metal-semicircular and over-covered concrete-rectangular cut.

**Sampling and Analyzing Methods.** After fulfilling the required conditions of study in the enter-
prise, samples were taken from the different water sources providing drinking water for the animals in the enterprise. Macellan handheld gps device was used to determine the coordinates of the location. PET bottles and plastic bags, cleaned in distilled water, were used to take water samples, glass bottles sterilised by heat application under certain pressure in autoclaves, used for the samples taken for microbiological analyzes, the devices in the laboratories where the analysis of the samples have been conducted were used. While taking samples, the conditions stated in basic water analyze techniques were implemented. During sampling process, ultimate attention was paid not to contaminate the sterile glass bottles. The samples taken for chemical analyzes were put into prepared bottles which had been washed in distilled water and the samples taken for microbiological analyzes were put into sterile holders by the same method. The sample bottles were carefully labelled in order not to cause any mistakes while taking samples and they were preserved unspoiledly.

Nitrate (NO₃) analysis has been conducted according to Kjeldahl method [15, 16]. The electrical conductivity (EC) of the samples was measured at 25 °C and value is expressed in dS/m unit [17]. pH was measured by pH-meter with glass electrode [18]. Calcium (Ca) and magnesium (Mg) was calculated by the titration method [17]. Carbonate and bicarbonate were be titrated with acid [18]. For Chlorine (Cl) detection, samples were titrated with standard Silver Nitrate AgNO₃ solution using Potassium Chromate (K₂CrO₄) indicator. Chlorine (Cl) was calculated from the spent solution [17]. After the other cation and anion analyzes were removed, the anion totals were removed from cation sum and the value was written as Sulfate (SO₄) [18]. For Sodium (Na), flame photometry was evaluated with the standard NaCl (Sodium Chloride) solution and expressed as me/L [17]. As in the determination of Sodium (Na), after reading on the flame photometer, Potassium (K) was determined as me/L from the standard graph [17].

Taking into consideration that thousands even millions of microorganisms might exist in one mL of samples, the samples examined for microbiological analysis were subjected to serial dilution with 1/4 Ringer solution (The dilution rate in the last tube was 10⁷). 90 mL ringer solution was added into 10 mL water samples and they become homogenous after being mixed in vortex for 1 minute. 0.1 mL was taken from the appropriate dilution and Plate Count Agar was implemented for total aerobic mesophilic viable count. Mac-Conkey Mug Agar was implemented for E-coli count, Violet Red Blue Agar culture procedure was implemented for Total Coliform count. The incubation period of bacteria 48 hours at 30 °C for Plate Count Agar, 24 hours at 37 °C for Mac-Conkey Mug Agar and Violet Red Blue Agar. The colonies produced in MacConkey Mug Agar were identified with Kovacs Indole Reagent for E. Coli count. Counting the colonies obtained after incubation, total colony formation unit (kob) was measured in each 1 mL water sample. Division of total colony number by the multiplication of instillation number with dilution factor units was the method followed to calculate the total viable count.

The data taken from the studies of land and laboratory were evaluated by appropriate standard tables and computer packaged software. Data were subjected to ANOVA analysis to evaluate the effect of the characteristics among samples. The test of significance was determined on the basis of Duncan’s test at p<0.05 probability using the SPSS.16 statistics software.

Period for gathering water samples were determined as January - September and August - April for bovine breeding enterprises according to the precipitation frequency of the city and preliminary survey. Samples were taken from 2 different sources as the source of drinking water and point of animals drinking water. The source of drinking water was labeled as S (source; well pump in the enterprise), animal drinking water point was labeled as T (trough) while datas were put into tables. Due to the excessive number of troughs in the animal enterprises, the average values of water samples taken from waterers in different points inside the same enterprise are presented. In order to make comparisons, water samples were taken from the clearest throughs and the throughs which have the highest probability of contamination of animal waste.

**RESULTS**

It is possible to classify bovine breeding enterprises examined in the provinces of Adana as dairy farming enterprise, cattle enterprise and combined dairy and cattle enterprise. It was observed that separate arrangements were not made according to the cultivation species in combines dairy and cattle enterprise and animal are kept in the same yard. The enterprise produce low amounts of animal product and they either sell these products or use them for their own consumption [19]. It is known that the races in livestock enterprises in Adana are classified according to culture, hybrid and local races.

When the cattle population and their racial distribution are examined, animals from each three races are observed to be present in the enterprise. Almost all the culture races cattle are Holstein animals. Local race is at quite a low level and hybrid races animals are generally Holstein Southern Red Local raced hybrids. Imported Limousin, Simmental, Hereford, Angus animals were also observed in the enterprise in where have many animals. The
great portion of the forage was obtained from the enterprises’ own land but small and medium scaled enterprises without lands buy the forage. The reason of the big amount of forage obtained from enterprises own lands is because enterprises are mostly on the plain. The enterprises in the plain grow forage plants in the lands around the enterprise and attain their animal fodder from these places. The purchased fodder is mostly concentrate feed and the fodder attained from enterprise is roughage. Concentrate feed is provided mostly from factory. Bran and cottonseed cossette are also used. Straw and corn silage is used in roughage. Ration of the animals might show difference in summer and winter months. Animals fed with barley, bran and hay in winter are grazing in nearby lands in summer. The enterprises with many animals do not prefer to graze their animals outside their enterprise.

There is electricity wiring in all enterprises. There are different drinking water and water resources in the enterprises. Some of the enterprises we research obtain their water from the wells (%12), most of them use the city water (%74) and rest of them (%14) get their water both from wells and city water. The amount of water animals drink daily differ according to enterprises, type of animals, the forage and the season. Most of enterprises we research (%77) has no record of the amount of drinking water utilization and they even can’t make guess about the daily amount of the water by animals consume. The amount of drinking water obtained from the cattle enterprise where we carried out the measurement was recorded as average, 29 lt/day in January, 57 lt/day in August, 48 lt/day in September, 52 lt/day in April in order for 550 kg animal. These results were found compatible with the many literatures. But not accurate animal water consume records in much of enterprises we research make the water control process difficult and caused them to ignore the importance and amount of drinking water. Drinking water quality parameters are of great importance for an animal that consumes such a large amount of water. Physical characteristics of the drinking water in two seasons for selected parameters were determined as presented in Table 1 and Table 2.

The results indicate that the physical characteristics of the drinking water varied according to the site in enterprise and probably to the waiting process in water points and lines and to the water quality at local water and different contaminations with water.

The results of our study were evaluated with a large literature review. The obtained physical characteristics of the animal water as withdrawn from the different water points along the year were within the permissible limits required by the given standards and the WHO guidelines. Analysis of variance revealed the presence of significant effects (p<0.05) of pollution sources, waterer points and water storage conditions and seasons on water physical properties. In regard to physical characteristics of the drinking water the highest values were found in water through which nearest the wastes; while the lowest were determined in water through which farthest from wastes and also there is significant different between water points. There were significant differences between animal drinking waters along year between winter and spring season (p<0.05).

The pioneering studies on this subject and the values compiled from various worldwide institutions working on water quality are presented in a table (Table 3).

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample Supply</th>
<th>n</th>
<th>X ± S</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg (mg/L)</td>
<td>S</td>
<td>9</td>
<td>29.53±3.15</td>
<td>26.14</td>
<td>33.05</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>12</td>
<td>79.51±40.31</td>
<td>26.43</td>
<td>139.36</td>
</tr>
<tr>
<td>Na (mg/L)</td>
<td>S</td>
<td>11</td>
<td>27.09±2.03</td>
<td>24.34</td>
<td>29.14</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>13</td>
<td>131.80±143.64</td>
<td>26.90</td>
<td>435.37</td>
</tr>
<tr>
<td>Ca (mg/L)</td>
<td>S</td>
<td>7</td>
<td>60.21±3.69</td>
<td>57.11</td>
<td>64.47</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>11</td>
<td>94.03±57.77</td>
<td>47.43</td>
<td>229.77</td>
</tr>
<tr>
<td>K (mg/L)</td>
<td>S</td>
<td>9</td>
<td>1.83±0.48</td>
<td>1.14</td>
<td>2.19</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>12</td>
<td>12.92±17.44</td>
<td>3.11</td>
<td>54.32</td>
</tr>
<tr>
<td>CO3 (mg/L)</td>
<td>S</td>
<td>8</td>
<td>14.66±5.42</td>
<td>9.98</td>
<td>19.97</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>10</td>
<td>16.04±6.32</td>
<td>11.14</td>
<td>29.96</td>
</tr>
<tr>
<td>HCO3 (mg/L)</td>
<td>S</td>
<td>7</td>
<td>222.69±11.98</td>
<td>211.45</td>
<td>233.54</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>11</td>
<td>308.07±162.51</td>
<td>118.43</td>
<td>656.57</td>
</tr>
<tr>
<td>Cl (mg/L)</td>
<td>S</td>
<td>8</td>
<td>22.25±2.37</td>
<td>20.91</td>
<td>25.81</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>13</td>
<td>172.71±357.78</td>
<td>24.99</td>
<td>1056.75</td>
</tr>
<tr>
<td>SO4 (mg/L)</td>
<td>S</td>
<td>9</td>
<td>81.55±10.31</td>
<td>73.12</td>
<td>96.31</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>13</td>
<td>138.97±98.92</td>
<td>76.10</td>
<td>336.29</td>
</tr>
<tr>
<td>NO3 (mg/L)</td>
<td>S</td>
<td>15</td>
<td>7.49±0.37</td>
<td>7.00</td>
<td>7.99</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>21</td>
<td>19.92±28.39</td>
<td>9.18</td>
<td>125.64</td>
</tr>
<tr>
<td>EC (mg/L)</td>
<td>S</td>
<td>5</td>
<td>0.58±0.01</td>
<td>0.57</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>8</td>
<td>2.68±1.23</td>
<td>1.30</td>
<td>4.70</td>
</tr>
<tr>
<td>pH (mg/L)</td>
<td>S</td>
<td>5</td>
<td>7.97±0.27</td>
<td>7.64</td>
<td>8.24</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>8</td>
<td>7.65±0.32</td>
<td>7.12</td>
<td>8.23</td>
</tr>
</tbody>
</table>
### TABLE 2
Drinking water concentrations for selected parameters (August 2015 - April 2016)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample Supply</th>
<th>n</th>
<th>± S</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg (mg/L)</td>
<td>S</td>
<td>7</td>
<td>24.91±4.08</td>
<td>21.12</td>
<td>29.12</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>11</td>
<td>45.60±14.01</td>
<td>28.44</td>
<td>66.18</td>
</tr>
<tr>
<td>Na (mg/L)</td>
<td>S</td>
<td>9</td>
<td>25.59±7.75</td>
<td>23.45</td>
<td>27.65</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>13</td>
<td>66.44±14.93</td>
<td>29.98</td>
<td>128.44</td>
</tr>
<tr>
<td>Ca (mg/L)</td>
<td>S</td>
<td>7</td>
<td>2.16±0.16</td>
<td>1.99</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>11</td>
<td>80.39±20.36</td>
<td>51.21</td>
<td>103.16</td>
</tr>
<tr>
<td>K (mg/L)</td>
<td>S</td>
<td>7</td>
<td>7.36±2.63</td>
<td>4.55</td>
<td>12.33</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>11</td>
<td>18.94±3.02</td>
<td>16.23</td>
<td>21.87</td>
</tr>
<tr>
<td>CO₃ (mg/L)</td>
<td>S</td>
<td>7</td>
<td>22.10±4.08</td>
<td>17.14</td>
<td>26.48</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>12</td>
<td>104.20±172.20</td>
<td>17.33</td>
<td>523.24</td>
</tr>
<tr>
<td>Cl (mg/L)</td>
<td>S</td>
<td>7</td>
<td>97.79±2.61</td>
<td>95.11</td>
<td>100.78</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>11</td>
<td>130.08±99.41</td>
<td>2.68</td>
<td>345.80</td>
</tr>
<tr>
<td>SO₄ (mg/L)</td>
<td>S</td>
<td>7</td>
<td>7.20±0.41</td>
<td>6.77</td>
<td>7.99</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>11</td>
<td>17.75±23.57</td>
<td>9.05</td>
<td>105.74</td>
</tr>
<tr>
<td>HCO₃ (mg/L)</td>
<td>S</td>
<td>9</td>
<td>231.65±38.05</td>
<td>198.11</td>
<td>272.28</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>13</td>
<td>261.18±154.55</td>
<td>100.75</td>
<td>582.11</td>
</tr>
<tr>
<td>NO₃ (mg/L)</td>
<td>S</td>
<td>7</td>
<td>22.10±4.08</td>
<td>17.14</td>
<td>26.48</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>11</td>
<td>104.20±172.20</td>
<td>17.33</td>
<td>523.24</td>
</tr>
</tbody>
</table>

### TABLE 3
Drinking water standards compiled from different sources

<table>
<thead>
<tr>
<th>Upper levels for livestock a</th>
<th>Maximum upper levels b</th>
<th>Expected Human Standards</th>
<th>European Legislation Directive 98/EC and NRC</th>
<th>Canadian Task Force, 1987</th>
<th>Possible cattle problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate</td>
<td>1,000</td>
<td>1,000</td>
<td>GL: 100f MAC: 200f</td>
<td>&lt;1000</td>
<td>&gt;500f</td>
</tr>
<tr>
<td>Calcium</td>
<td>100</td>
<td>200</td>
<td>&lt; 43b &lt;250 (Secondary) f</td>
<td>&lt;300-400</td>
<td>&gt;125c</td>
</tr>
<tr>
<td>Chloride</td>
<td>100</td>
<td>300</td>
<td>GL: 30f MAC: 50f</td>
<td>&lt;200f</td>
<td>&gt;20 for veal calves c</td>
</tr>
<tr>
<td>Magnesium</td>
<td>50</td>
<td>100</td>
<td>GL: 20f MAC: 175f</td>
<td>&lt;500 calves f and &lt;1000 adult cows g</td>
<td>&lt;1000</td>
</tr>
<tr>
<td>Sodium</td>
<td>50</td>
<td>300</td>
<td>&lt; 29b &lt;250 (Secondary) f</td>
<td>&lt;500 calves f and &lt;1000 adult cows g</td>
<td>&lt;1000</td>
</tr>
<tr>
<td>Sulfates</td>
<td>50</td>
<td>300</td>
<td>&lt; 250b &lt;250 (Secondary) f</td>
<td>&lt;500 calves f and &lt;1000 adult cows g</td>
<td>&lt;1000</td>
</tr>
<tr>
<td>Nitrate-Nitrogen</td>
<td>20</td>
<td>100</td>
<td>&lt;44b &lt;10 (Legal) f</td>
<td>50</td>
<td>&lt;10f</td>
</tr>
<tr>
<td>Potassium</td>
<td>20</td>
<td>20</td>
<td>&lt;20b GL: 10f MAC: 12f</td>
<td>&lt;5-8</td>
<td>&gt;8:11d</td>
</tr>
<tr>
<td>EC</td>
<td></td>
<td></td>
<td>A1 7.8-5f</td>
<td>&lt;5-8</td>
<td>&gt;9.0c</td>
</tr>
<tr>
<td>pH</td>
<td>6.0 to 8.5</td>
<td>8.50</td>
<td>&lt;6.8 to 7.5 (secondary) f</td>
<td>&lt;5.1 to &gt;9.0f</td>
<td></td>
</tr>
<tr>
<td>Coliform, kob/100 mL</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>over 1 for calves; over 15-50 for cows c over 1 for calvescover for cows g</td>
<td>&lt; 1 million f</td>
</tr>
<tr>
<td>Fecal coliform, kob/100 mL</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Bacteria, kob/100 mL</td>
<td>1000.0</td>
<td>1000.0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mg/L except pH and EC (ppm, which is equal to parts per million)

a. Adapted from Socha et al. (2003) [20]
b. Adapted from Adams and Sharpe (1995) based primarily on criteria for drinking water for human consumption [21].
c. Adapted from Adams and Sharpe, 1995 [21].
d. Adapted from Canadian Task Force, 1987 [22].
e. US Environmental Protection Agency (EPA), 2009 [23].
f. Adapted from Turkish Drinking Water Standards [24].
g. [25]
When the pH values of the water in the enterprise are examined, it is seen that 95% is compatible with the limits given in the literature. It was observed that 5% pH values approached the limits that could pose a risk. EC values of the waters were also evaluated together with the salinity and found compatible with the literature. Excessive alkalinity can cause physiological and digestive upsets in livestock. [26]

Sodium sulphate is known to cause diarrhea of animals, magnesium and calcium also pose a risk for cattle breeding, but their association with sulphate is an important problem. Waters containing more than 800 mg/L sodium cause a drop in milk production [11]. As a result of the reaction with chlorine sulfates will cause bad smell and taste as well as oxygen reduction. It is known that sulphate has diarrhoea effect in humans and some farm animals at high sulphate levels (600 mg/L and above). Therefore, it is recommended that health authorities be informed if the levels of sulphate in excess of 500 mg/L are detected in drinking water sources [27]. The amount of salt in the body accumulates, so the total amount of salt is very important. In addition, the salts cause different psychological effects. Sulfate salts are known to cause more health problems than chlorine and carbonates. In a study in this subject, a high risk of polioencephalomyelitis (PEM) with a serious cattle nerve and health problems than chlorine and carbonates. In a study conducted Iowa, in the quality analysis of drinking water collected from 128 cattle farms, coliform, nitrate, total dissolved salts and 26 different parameters were measured and compared these parameters with the productions [35]. As a result of the study, it was found that the high nitrate concentration prolonged the breeding period. The nitrate values obtained from the samples gathered from the drinking water resources in the enterprise were found compatible with the standards (% 83.9), however, especially in rainy period, they exceeded the given border values. It was concluded that the troughs might have been contaminated with wastes or other contaminant may due to the increasing rain in January.

It is known that the microbiological control of water is needed to provide its safety for drinking and to avoid cross contamination during dairy and food processing at livestock sectors. Microbiological values of drinking water of the enterprise emphasize on the importance of bioload in the drinking water. Microbiological results of the drinking water are given in Table 4 and Table 5.

### Table 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample Supply</th>
<th>n</th>
<th>( x \pm Sx )</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ecoli</em> (ort. log kob/ml)</td>
<td>S</td>
<td>7</td>
<td>0.002±0.002</td>
<td>0.000</td>
<td>0.004</td>
</tr>
<tr>
<td><em>Fecal Coliform</em> (ort. log kob/ml)</td>
<td>T</td>
<td>11</td>
<td>1.296±0.932</td>
<td>0.156</td>
<td>3.120</td>
</tr>
<tr>
<td><em>Total Coliform</em> (ort. log kob/ml)</td>
<td>T</td>
<td>11</td>
<td>1.616±0.991</td>
<td>0.000</td>
<td>2.910</td>
</tr>
</tbody>
</table>

### Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample Supply</th>
<th>n</th>
<th>( x \pm Sx )</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ecoli</em> (ort. log kob/ml)</td>
<td>S</td>
<td>7</td>
<td>0.001±0.001</td>
<td>0.000</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Fecal Coliform</em> (ort. log kob/ml)</td>
<td>T</td>
<td>11</td>
<td>1.129±0.806</td>
<td>0.023</td>
<td>2.156</td>
</tr>
<tr>
<td><em>Total Coliform</em> (ort. log kob/ml)</td>
<td>T</td>
<td>11</td>
<td>0.79±0.600</td>
<td>1.444</td>
<td>2.019</td>
</tr>
</tbody>
</table>
As seen from the tables above, 87.4% of values are compatible with the information in the literature and below the standard limits. However, values increased in some measurements. The rising temperature in summer, increasing microorganisms and its accelerating activity provide a suitable environment. This situation can be shown as the cause of the increase in summer season. As the reason of dropped values are might be rains in September and January. Even if the troughs are covered with barn roofs, especially troughs in the edges might fill with rain water. In microbiological measurements, 96.3% of the results in the limit values were obtained from the troughs found in the closest points to the animal wastes.

As a result of the statistical analysis of the data obtained from enterprise, the differences of the results in the drinking water SO₄, Mg, Na, K, NO₃ and microbiological results in these months (summer and rainy periods) are considered meaningful (p<0.05). The assumption that animal drinking water values may differ in summer and winter months is supported by our study data. As a result of the statistical analyses, it was possible to evaluate the values in the rainy months as a group and the values in the arid months as another group and this situation also found meaningful (p<0.05). No significant relationship was found between trough species and water analysis results. The results of drinking water analysis show a wide scale especially in some parameters. This situation can be seen from the high standard deviation values given in Table 1 and Table 2, we can see that the respective series are not spread.

DISCUSSION

When the analysis of the water samples we took from the drinking waterlines of bovine animal shelters is evaluated, some values are observed to be much different from each other. The main and apparent causes of the differences are the current state of drinking water net in the enterprise and their possibility of being affected by different organic substances such as animal wastes. However, the secondary causes for drinking water pollution may be different, variable and be specific in any enterprises. The drinking water can be preserved safely for a limited period especially in open waterers of time even if clean water is provided from the net which we consider as a safe source. The analysis results especially the bacterial contamination content in our study support this assumption. The drinking water sources can be used as cheap and reliable source provided that they are properly treatments after their analyze.

In some cases, pollutants can cause one time or temporary contamination in the troughs or water sources, which can be observed in case of heavy rain or uncontrolled water systems. Since contamination is failed to be recognized, contagion occurs and spread in enterprises where regular water analyses aren’t made even this situation can result in animal deaths. The regular control of the drinking water systems will reveal the change in the quality parameters of drinking water and support the necessity to make periodic drinking water controls in bovine enterprises. Besides all these, common criteria provide a general guide for quality of water acceptable for most livestock but different quality waters may be also acceptable or harmful because of differences in age, physiological state, species, conditions or feed components. It is therefore recommended that a water management plan be developed to seasonal and annual trends of water quality and be conducted regular observation and testing before damage or loss of production occurs.

REFERENCES


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THE EFFECT OF WOOD VINEGAR PRODUCED FROM NUTSHELLS ON THE SOIL NEMATODES IN WHEAT AGRO-ECOSYSTEMS

Ibrahim Koc*

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ABSTRACT

This study was carried out in order to determine the effects of the wood vinegar produced from nutshell on the free-living nematodes in the soil in the wheat agro-ecosystem. The research was done in 2014-2015 and 2015-2016, according to the Randomized Block Experimental Design and as fixed in the field experiment tried out by four repetitions. To the parcels in the experiment area, different doses of wood vinegar (0.5%, 1.0%, 2.0%, 3.0%, 4.0% and 5.0% mL) were treated by means of backpack sprayer, and to the control group, water was provided only. As a result, from the experimental area, nematodes at the level of 18 genera and 1 order were detected from 5 trophic groups. In consequence of Simple Correspondence Analyses carried out, significant relations between wood vinegar treatments and nematode groups were determined. It is thought that wood vinegar doses and their use frequencies and the years (2014-2015 and 2015-2016) can have an effect on the numbers of nematode by trophic groups.

KEYWORDS:
Agro-ecosystem, nutshell, nematodes, soil, wheat, wood vinegar

INTRODUCTION

An optimum pesticide is expected to be effective against the pests targeted and also harmless to the other living creatures including human beings. However, it is realized from the results arising that the case is not in this way [1]. It has been detected that pesticides pose long-termed hazard to the environment and people, and also they are ecologically unacceptable [2]. In order that agriculture can be sustained and environment can be protected, it is inevitable for eco-friendly methods and techniques to be developed [3]. Wood vinegar (WV) and other slightly pyrolysis liquids are produced as a by-product of carbonization processes, and it has been found out that they were used in the Neanderthal’s era [4]. [5] in their studies, with reference to Jang, stated that the 80 to 90% of WV is composed of water and the rest 10 to 20% of it is from more than 200 organic components, and its annual production has been around 14.000 tons lately. WV consists of acetic acid and of organic acids, phenolic, alkane, alcohol and ester compounds, as well [6]. In the literature reviews made, some certain studies pointing out that WV has characteristics to enhance the quality of fertilizer, plant and soil in the area of agriculture [4, 6, 7, 8, 9, 10] acts as an insecticide or with a repellant effect [6, 11, 12, 13, 14, 15, 16, 17] or without it [5, 18] has antibacterial effect [19, 20, 21, 22, 23, 24], and has antifungal effect [6, 8, 17, 19, 24, 25, 26, 27, 28, 29] have been encountered with. In addition to these studies, Koç et al. (2018b) stated that they had detected significant relations between WV treatments produced from poultry manure and nematode groups. Nematodes play a significant role in basic soil treatments and/or practices [31]. Within the scope of the researches so far, although it is seen that wood vinegar has been tested for various purposes, any critical studies in which it is investigated for nematodes that are essential for soil biota has not been seen. The objective in this study is to determine the effect of wood vinegar obtained from nutshell on the nematodes at trophic level in wheat agro-ecosystem.

MATERIALS AND METHODS

WV used in this study has been obtained from a firm which develops the bio-coal and wood vinegar products from hazelnut shells by a gasifier [27]. The study was carried out in Krasunia odessa winter wheat field (height: 1276, lateral: 380 47’ 33” 1815”, long: 410 32’ 45. 700”) belonging to BERCE Alparslan Agricultural Administration, at a distance of 12 km to the central part of the province Muş (in Turkey). The climatic data for the area of study, i.e., total rainfall, mean temperature and mean relative humidity values of 2014-2015, 2015-2016 and the last decade (long period average), respectively, are presented in Table 1 [32]. The classification by the texture of soil in the area of study is clay soil with the rate of clay by 63.29%, silt ratio by 25.8% and sand ratio by 10.9% [33]. The field experiment has been established as fixed, in the production seasons of 2014-2015 and 2015-
2016, according to Randomized Block Experimental Design and with four repetitions. Each parcel has the size of 5m×5m=25m² and at least a 2-meter-gap was spaced out between blocks and parcels [34]. The treatments made in the experiment was realized by following-up the schedule for applying fertilizer and agricultural spraying, which was determined by BERCE Alparslan Agricultural Administration. Spraying (WV treatment) was applied by a backpack sprayer. WV treatment was carried out by applying different doses of WV at 0.5%, 1.0%, 2.0%, 3.0%, 4.0% and 5.0% mL, and the control group was provided with tap water only. WV treatment was realized once in 2014-2015 and four times in 2015-2016.

For soil sampling process, soil samples were taken from 8 different parts of each parcel [35] by means of nematode sampling instrument (from a depth of 10 to 30 cm), and those samples taken were blended well to be aggregated and were collected in sterile polyethylene nylon bags. Afterwards, they were taken to the laboratory in the shortest time and preserved at +4°C [36, 37]. Soil sampling was carried out as to be three times for each year: in 2014-2015, before treatment (2015, May 19th), after treatment (2015, June 25th) and at the end of harvesting (2015, July 25th); and in 2015-2016, before treatment (2016, April 21st), after treatment (2016, June 25th) and at the end of harvesting (2016, August 09th). Nematodes were extracted from the soil by “Advanced-Baermann Funnel Technique” [38] and enumerated as based on their trophic levels on the objective lens (×10) of microscope [36, 37]. Nematode trophic groups, according to [39, 40], were counted on the morphological characteristics of their head structures and identified to genus. In the statistical analysis of gathered data, Simple Correspondence Analysis was used, and for carrying out the mentioned statistical analysis, Minitab (Ver. 17) statistical package was used [41, 42].

RESULTS AND DISCUSSION

From the experimental area, nematodes from 5 trophic groups were detected at 18 genera and 1 order level (Table 2).

For the year 2014-2015, it is estimated that 0.5% and 5% mL WV, 1% and 3% mL WV treatments are interrelated with or similar to each other (Figure 1a). It is considered that plant parasitic nematodes and omnivore-predator nematodes are relational (Figure 1b).

**TABLE 1**

<table>
<thead>
<tr>
<th>Total rainfall (mm)</th>
<th>Mean temperature (°C)</th>
<th>Mean relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014-15</td>
<td>740.4</td>
<td>790.1</td>
</tr>
<tr>
<td></td>
<td>55.02</td>
<td>54.00</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Trophic group</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungivore nematode</td>
<td>Cephalobus spp.</td>
</tr>
<tr>
<td></td>
<td>Aphelenchoides spp.</td>
</tr>
<tr>
<td></td>
<td>Aphelenchus spp.</td>
</tr>
<tr>
<td></td>
<td>Acrobeloides spp.</td>
</tr>
<tr>
<td>Bacterivore nematode</td>
<td>Eucephalobus spp.</td>
</tr>
<tr>
<td></td>
<td>Cervidellus spp.</td>
</tr>
<tr>
<td></td>
<td>Plectus spp.</td>
</tr>
<tr>
<td></td>
<td>Acrobeles spp.</td>
</tr>
<tr>
<td></td>
<td>Wilsonema spp.</td>
</tr>
<tr>
<td></td>
<td>Monhystera spp.</td>
</tr>
<tr>
<td>Plant parasitic nematode</td>
<td>Pratylenchus spp.</td>
</tr>
<tr>
<td></td>
<td>Ditylenchus spp.</td>
</tr>
<tr>
<td></td>
<td>Merlinus spp.</td>
</tr>
<tr>
<td></td>
<td>Tylenchus spp.</td>
</tr>
<tr>
<td></td>
<td>Tylenchorhynchus spp.</td>
</tr>
<tr>
<td></td>
<td>Pratylenchus spp.</td>
</tr>
<tr>
<td></td>
<td>Tylenchorhynchus spp.</td>
</tr>
<tr>
<td></td>
<td>Troplus spp.</td>
</tr>
<tr>
<td>Omnivore nematode</td>
<td>Dorylaimida (order)</td>
</tr>
<tr>
<td>Predator nematode</td>
<td>Achromadorea spp.</td>
</tr>
</tbody>
</table>

3537
Additionally, it is estimated that plant parasitic nematodes and omnivore-predator nematodes are related to the control treatment; Fungivore nematodes and the 2% mL dose of WV are relational (Figure 2a). For the year 2015-2016, it is estimated that control and 0.5% mL WV are relational (Figure 2b).
As for nematode groups, it is estimated that they are not related to each other (Figure 3a). It is presumed that Bacterivore nematodes, Control and 0.5% mL WV are relational to one another, and other group nematodes are not related to the treatments made (Figure 3b).
According to the Control, no significant difference is seen between bacterivore nematodes in average and the other applications except 3% mL WV treatment. In general, when compared to the year 2014-2015, there has been a decrease in the number of bacterivore nematodes for the year 2015-2016, and it is noticed that the maximum decrease is at WV doses of 1% and 5% mL (Figure 4a).

FIGURE 3
Relations graphic for nematode groups (a), relations graphic of nematode groups with WV doses (b)
In the treatments made, according to the control, it is understood that there has been a decrease in the average number of plant parasitic nematodes in general. When compared to 2014-2015, in 2015-2016, the number of plant parasitic nematodes has decreased at the WV doses of 1%, 2% and 5% mL (Figure 4b). It is seen that the average fungivore nematodes, in comparison with the control, are more in number, even a little, in WV treatments of 2% and 3% mL doses. In 2015-2016, as compared to 2014-2015, it is noticeable that there has been a decrease at 0.5%, 1%, 2% and 4% mL WV doses (Figure 4c). It has been found out that the average number of omnivore-predator nematodes, to the control, is less except 1% mL WV. When compared to 2014-2015, in 2015-2016, it has been detected that there is an increase in the number of nematodes, excluding the dose of 5% mL WV, and the maximum increase is at 1% mL WV (Figure 4d).

On one hand, the pesticides used in agro-ecosystems are effective in terms of enhancing the yield; on the other hand, they can affect everyone and everything. For this reason, it is of vital importance for us to turn towards alternative products which do not leave residue on the crops, do not harm the bio-chain and environment, and will be able to contribute to the sustainability of agriculture in a positive way.

The case that 0.5% and 5% mL and 1% and 3% mL treatments for the year 2014-2015 are relational is considered as possibly related to the fact that the treatments made for this year have had similar effects on nematode groups. It is anticipated that plant parasitic nematodes and omnivore-predator nematodes are in relation separately with the control treatment to which any WV treatment has not been applied, and therefore, in which the balance of prey-predator relation is not disturbed. It has been estimated that fungivore nematodes are interrelated as based on having the highest number of nematodes by 2% mL WV.

For the year 2015-2016, it is assumed that the case in which the control group and 0.5% mL WV treatment are relational may result from the point at which 0.5% mL WV treatment has created an effect approximate to the control treatment on nematode groups, in the sense of affecting. It is also considered that the case in which nematode groups are not relational for this year (2015-2016) may arise from that more WV treatments have been carried out, compared to the previous year, (WV treatment was made once in 2014-2015; four times in 2015-2016). That bacterivore nematodes, control and 0.5% mL WV are interrelated is considered as possibly related to the fact that this group of nematodes reacts similarly to the control and 0.5% mL WV treatment is interrelated is considered as possibly related to the fact that this group of nematodes reacts similarly to the control and 0.5% mL WV treatment or 0.5% mL WV has affected bacterivore nematodes more.

In consideration of the findings obtained within the scope of this study (Figure 2 and 3); significant relations have been detected between WV treatments and trophic groups. Moreover, compared
to the control, it is considered that WV treatments have created an effect in some way, more or less, on nematodes. Thus, according to the control treatments, it is estimated that the years (2014-2015 and 2015-2016) and WV treatments could have different effects on nematode groups. The results obtained from this study correspond to the results attained in the study by [30]. It is seen that the results of this study are more or less in line with the findings of studies featuring the WV properties as bactericide, fungicide, insecticide, bio-fertilizer and so on. For instance, when compared to the studies bringing the WV’s biocide characteristics (such as bactericide, insecticide, fungicide) into the forefront [6, 8, 11, 12, 13, 14, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29], it is realized that the findings (indicating that bacterivore nematodes are less found at 2% mL WV treatment; plant parasitic nematodes are found less in number in all WV treatments, and etc.) are supported. Furthermore, by some year and WV treatments, compared to the control, it is seen that the number of nematodes is more (e.g., for bacterivore nematodes, 1%, 4% and 5% mL WV treatments in 2014-2015; for fungivore nematodes, 1%, 2% and 4% mL WV treatments in 2014-2015; for omnivore-predator nematodes, 1% mL WV treatment in 2015-2016). This situation is also supported by the studies pointing out that WV can be considered as fertilizer, soil and plant enhancer in agricultural area [4, 6, 7, 8, 9, 10].

In conclusion, it is considered that the dose and use frequency of wood vinegar treatments used in this study will be able to have an effect on nematodes (positive-negative / more or less) at the level of trophic groups.

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APPLICATION OF HIGH-RESOLUTION SEQUENCE STRATIGRAPHY ON A BEACH-BAR SYSTEM, LAYER B0 OF THE MEDIUM SUB-MEMBER OF SHAHEJIE FORMATION, MD OILFIELD, CHINA

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ABSTRACT

To enhance the resolution of reservoir recognition and predict the remaining oil in the MD Oilfield, high-resolution sequence of layer B0 of the Medium Sub-member of Shahejie Formation in MD oilfield was divided on the basis of high-resolution sequence stratigraphy, theory of base level cycle, cores and electrical logging data. As a result, higher base level cycles were recognized as a long-term base level cycle, 3 intermediate base level cycles and 9 short-term base level cycles. In addition, the regional stratigraphic framework was established. Two kinds of short-term base level cycles were identified: shallowing-upward non-symmetry (type B) and symmetry which first deepen upward then shallow upward (type C). On this basis, a sedimentary model of beach-bar was built.

KEYWORDS:
High-resolution sequence stratigraphy, base level cycle, MD oilfield, beach-bar, medium sub-member of Shahejie Formation

INTRODUCTION

High resolution sequence stratigraphy theory is put forward by professor Cross, institute of mining in Colorado [1]. On the basis of core, two-dimensional outcrops, logging and high-resolution seismic reflection profile, on the meanwhile, fine stratigraphic division which can translate one-dimensional drilling information into three-dimensional strata relationship prediction is applied. The theory is aimed to establish stratigraphic framework of the regional oilfield reservoir, as a result, the distribution of reservoir, caprock, inter-layer and source rock is evaluated and predicted [2-4]. The main content: during the change of base level, due to the change of ratio of accommodate space and the supply of sediments (A/S), size distribution of sediment in the same sedimentary system or facies system occurs, and the preservation of the sediments, accumulation pattern of the stratigraphy, facies association, facies types, rock structure and composite type change accordingly [5-8]. Zheng R.C. et al. [9] uses their experience on sequence division of different kinds of continental level cycle, class division and isochronic correlation, putting forward the division of base level cycle into megacycle, ultra long-term, long-term, medium-term, short-term and ultra short-term. Then the main characteristics of each class cycle, boundary type, time range, identification mark and the main control factors were analyzed in detail. Recently, the application of high-resolution sequence stratigraphy is mainly focused on fluvial sedimentary system, delta sedimentary system, fluvial-delta-shallow lacustrine sedimentary system [10-12], but little on the beach-bar sedimentary system. In this paper, according to the theory and technology of high-resolution sequence stratigraphy, the division scheme of base level cycle, structure types and stacking patterns of layer B0 of the Medium Sub-member of Shahejie Formation in MD oilfield were studied.

Over the past 30 years, MD oilfield has witnessed five stages: productivity construction and production rise, production decline, low-speed and low-efficiency development, rolling development and production rise, and production decline. At present, the study area is in the stage of low-speed and low-efficiency development, low reservoir implementation, and uneven distribution of remaining oil. In this setting, it is particularly important to carry out high-resolution sequence division and
stratigraphic correlation for the reservoir in this area, establish isochronal stratigraphic framework based on the theory of high-resolution sequence stratigraphy, and describe the development law of the reservoir in the stratigraphic framework [3, 13-14].

GEOLOGICAL SETTING AND METHODS

Geological setting. MD oilfield is located in the east beach of Mapengkou village of Qikou sag in Huanghua depression of Bohai Bay Basin (Fig.1) [15], the depth is 0 ~ 3.5 m. The tectonic location is located in the south limb of Beidagang buried hill structure zone in Huanghua depression, being in the transition zone of two tectonic system (north to east onshore and east to west offshore).

Methods. To identify the boundary of sequence, high-resolution sequence, outcrop profiles in the study area were studied and recorded by photos, logging data of more than 36 wells was used to analyze sedimentary types, and sedimentary facies types. The identification and division of high-resolution sequence, build of sequence framework and sedimentary model, the theory of high-resolution sequence stratigraphy of Cross [1] was used.

RESULTS AND DISCUSSION

Identification of sequence boundary. Sequence division and correlation is the key to study high-resolution sequence stratigraphy, and the identification of sequence boundary and marker bed are the key step to sequence division and correlation [19-22]. In this paper, macro-control and micro-adjustment were applied to stratigraphic correlation. Firstly, the regional marker bed was determined, and the stratigraphic boundary of each member was defined by analyzing seismic data, then the local marker bed was determined within each member. On the basis of "cycle correlation, class control", the sequence was divided. Layer B0 mainly deposits mudstone and sandstone. The combination of different logging curve (natural gamma, spontaneous potential, apparent resistivity and acoustic time difference) showed a good response of the lithological and sedimentary cycle, actually natural gamma and self-potential were particularly sensitive to sand and mudstone.

Identification of top and bottom boundary. Layer 1 in the study area mainly develops mudstone. And it has continuous strong amplitude reflection on seismic profile, so it can be viewed as a regional marker. Therefore, the bottom boundary of layer B0 can be identified on the seismic profile. Layer 1 has a good logging response, with high acoustic time difference value, high spontaneous potential value, high natural gamma value, low density and jagged electrical resistivity. Layer B0 has box-shaped and
funnel-shaped spontaneous potential with moderate to low value, which indicates a stable water energy, sedimentary rate and abundant source supply. Apparent resistivity is box-shaped and funnel-shaped with low to moderate value, showing that layer has vertically reverse rhythm and was well-sorted. The top of layer B0 (called B0-1 layer) mainly deposits mudstone, with high acoustic time difference value, high spontaneous potential value, high natural gamma value and low density. Upper Sub-Member of First Member of Shahejie Formation is about 170~200m, with gray sandstone and dark gray mudstone interbedding. The well-developed mudstone is on the top, as a result, the top and bottom boundary of layer B0 can be easily recognized by analyzing logging curve.

Identification of boundary of sub-layer. The sand bodies of the bottom of B0-2, B0-3, B0-7 and B0-8 sub-layers of layer B0 of MD oilfield are well-developed, and have good logging response. For example, the sand bodies of B0-8 sub-layer are relatively developed, with the spontaneous potential curve and natural gamma curve being negative box-shaped.

Boundary identification according to core data. Compared with seismic and logging data, core (outcrop) has higher resolution, which is the basis of identifying higher order base level cycle. According to the change of base level reflected by lithofacies association, transition point was firstly identified. Then the change of base level direction was determined by the change of water depth within sequence, the preservation of sedimentary landforms and the tendency of sediment erosion in rock sequences [23,24]. Through detailed observation and description of well cores in the study area, two core boundaries can be identified. The transition boundary of lithofacies type or facies association: the change of ratio of accommodate space and the supply of sediments (A/S). There are mainly abrupt boundaries of mudstone and sandstone in the study area. Due to the change of water depth and hydrodynamic condition, the rhythmic bedding of mudstone and sandstone form in the study area, this boundary was very common.

The bedding change boundary within lithofacies: the reflection of hydrodynamic change when sediments form. For example, wavy bedding-sand lamination-parallel bedding-low angle cross-beding is a shallowing-upward progradational cycle, with the accommodate space gradually decreasing. Then the changeable feature of bedding can be used to indicate the rise and fall of base level. Generally, the sequence, which indicates hydrodynamic condition is weakening and water depth is increasing, was defined the base level rising semi-cycle. Meanwhile, the sequence, which indicates hydrodynamic condition is strengthening and water depth is the decreasing, was commonly defined the base level falling semi-cycle. The boundary of base level falling semi-cycle is commonly developed.

![FIGURE 2](image)

**Shallowing-upward asymmetric short-term cycle sequence in layer B0 of the Medium First Member of Shahejie Formation in MD oilfield (well GS8-1)**
Identification of short-term base level cycle. Short-term base level cycle sequence is the basic genetic stratigraphic unit divided according to the outcrop, drilling, core and logging data. It is a small-scale rhythmic lacustrine transgressive boundary or erosion boundary or conformity boundary that can be compared with several single lithologic layer or multiple rhythmic lithological layers [25]. Two main short-term base levels cycle develop in the study area.

The first one is shallowing-upward unsymmetrical short-term cycle sequence (type B). The short-term base level cycle of high accommodate space is mainly the base level falling semi-cycle, with a shallowing-upward sequence. Type B only develops in base level falling semi-cycle. Base level rising semi-cycle is under-compensation deposits, and it is away from the source supply area with sufficient source, showing gradually decrease of accommodate space due to the falling in base level and the transition from aggradation to progradation. Type B is commonly seen in beach-bar sedimentary system in the study area, consisting of lake mud-sand beach-sand bar, lake mud-sand bar, lake mud-sand beach (Fig.2).

The second one is deepening-upward then shallowing-upward symmetric short-term base level cycle sequence (type C). The short-term base level cycle of moderate accommodate space is symmetric and relatively developed in all facies zones [26,27]. Type C mainly develops in the condition of A/S>1. The top and bottom boundary is generally conformity. Due to the progradation - aggradation - retrogradation (aggradation), the first upward-deepening then shallowing-upward symmetric sequence forms. Type C is commonly composed of sand bar-lake mud-sand beach/bar, sand beach/bar-lake mud-sand beach/dam (Fig.3).

Identification of long-term and medium-term base level cycle. Medium-term base level cycle is the result of secondary lake transgression and lake regression of long-term base level cycle, mostly developing complete cycle [27,28]. Dividing short-term base level is the basis of dividing medium-term base level. Assembling short-term base level into different stacking patterns, and the top and bottom boundaries of different stacking patterns are regarded as transitional boundary of medium-term base level cycle.

Layer 1 in MD oilfield is composed of 3 medium-term base level cycles (Fig.4), namely MSC1, MSC2 and MSC3. On the basis of medium-term base level cycle, 3 medium-term base level cycles are stacked as the falling semi-cycle of long-term base level cycle (LSC1).

Medium-term base level cycles MSC1, MSC2 and MSC3 are in falling semi-cycle of long-term base level cycle. MSC1 is the rising semi-cycle, consisting of two short-term base level cycles SSC1 and SSC2 (equal to B0-1, B0-2 sub-layer respectively); MSC2 is a complete cycle, with rising semi-cycle being composed of short-term base level cycles SSC6 and SSC7 (equal to B0-6, B0-7sub-layer respectively) and falling semi-cycle being composed of SSC3, SSC4 and SSC5(equal to B0-3, B0-4, and B0-5 sub-layer respectively); MSC3 is the falling semi-cycle, consisting of two short-term base level cycles SSC8 and SSC9 (equal to B0-8, B0-9 sub-layer respectively).

FIGURE 3
Symmetric short-term cycle sequence in layer B0 of the Medium First Member of Shahejie Formation in MD oilfield (well GS8-1)
FIGURE 4
Sequence division in layer B0 of the Medium First Member of Shahejie Formation, MD oilfield, Huanghua depression (well GS63-2)

**Sequence framework.** Multi-order base level as reference is the basis of high-resolution stratigraphic correlation. The identification of sequence boundary and the division of base level cycle are aimed to have stratigraphic correlation, build sequence framework, analyze characteristics and rules of sequence, and provide evidence for sand body distribution and oil or gas location [29]. Building sequence stratigraphic framework is to take strata formed in the same age into the stratigraphic correlation framework in a certain order.

Different sequences or different parts in the same sequence within layer B0 in Medium Sub-member have different characteristics, with the overall distribution following the volume distribution rules of sediments (Fig.5). The thickness of base level cycles in layer B0 in the study area changed little and the preservation degree of stratigraphy was relatively complete. The development degree of short-term or medium-term base level cycles depended on their geographical location and the relative size of accommodate space in medium-term base level cycles. It was mainly controlled by the volume distribution of sediments limited by the accommodate space and the preservation degree of sediments determined by A/S value.

During the sedimentary period of layer B0, long-term base level was falling. Short-term base level cycle SSC9 and SSC8 made up the falling semi-cycle of medium-term base level cycle MSC3. During the SSC9 sedimentary period, due to high base level and the increase of accommodate space, mudstone mainly developed in the study area. During the SSC8 sedimentary period, due to the decrease of accommodate space, abundant beach-bar deposited in the study area. The rising semi-cycle of...
medium-term base level cycle MSC2 equals to sub-layer B0-6 and B0-7, at that time, accommodate space was large and sand body do not develop. The falling semi-cycle of medium-term base level cycle MSC2 equals to sub-layer B0-3, B0-4 and B0-5, and the sand body of sub-layer B0-3 and B0-4 develop, but mudstone of sub-layer B0-1 was good stability. This high-resolution isochronal framework provided an important evidence for the study of the space and time distribution of reservoir flow units.

**Sedimentary model.** On the basis of highly resolution sequence stratigraphy, core, logging data, the single well facies analysis, and well-tie sequence correlation, the sedimentary model of beach-bar of layer B0 in the study area was built (Fig.6). During the sedimentary period, the geological setting of depression, slope and uplift provided favorable conditions for the formation of shallow beach-bar reservoir.

![Sedimentary model in layer B0 of the Medium First Member of Shahejie Formation, MD oilfield, Huanghua depression](image-url)
The beach-bar sedimentary system mainly developed in the water recession period, with the lake mud-sand beach-sand bar developing from bottom to top successively. Sand beach was formed on a relatively flat terrain by the lake wave and lake currently. It was thin and frequently interbedded with lake mud, distributing along shorelines. The self-potential curve was finger-shaped. Sand bar was caused by the accumulation of coarse debris shore formed in the broken wave zone of the lake waves, mostly was irregular oval. The thickness of a single layer was large, and it was lens-shaped on the section. The self-potential curves were mainly funnel-shaped and box-shaped. The beach bar sand body does not occupy the main position in the lake basin, but because the sand body was close to source area, it was often surrounded by the source rock. As a result, the porosity and permeability were often high, it was preferable oil and gas reservoir. The beach bar sand body reservoir not only has high yield, but also has the highest waterflood recovery among the lake basin sandstone reservoirs [30].

**CONCLUSION**

1. Two types short-term base level cycle develop in study area: shallowing-upward non-symmetry (type B) and symmetry which firstly deepen upward then shallow upward (type C). The layer B0 was divided into 9 short-term base level cycles and 3 medium-term base level cycles.
2. During the layer B0 sedimentary period, the long-term base level was falling. MSC1, MSC3, and MSC3 medium-term base level cycles developed in the study area. MSC1 was composed of SSC8-SSC9, MSC2 was a complete cycle, consisting of SSC3-SSC7, and MSC3 was composed of SSC1-SSC2.
3. The beach-bar sedimentary system mainly developed in the water recession period, with the lake mud-sand beach-sand bar developing from bottom to top successively.

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INFLUENCE OF HOLDING SOLUTIONS ON VASE LIFE OF CUT HYDRANGEA FLOWERS (HYDRANGEA MACROPHYLLA THUNB.)

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ABSTRACT

The vase life of cut flowers is the most important factor affecting the market value popularity of hydrangeas as cut flowers has increased steadily in recent years. However, the vase life of cut hydrangea flowers is short depends on wilting and sepal browning. In this study SUA (succinic acid) (50 mg L\(^{-1}\) and 100 mg L\(^{-1}\)), GA (glycolic acid) (38 mg L\(^{-1}\) and 76 mg L\(^{-1}\)), BC (benzethonium chloride) (25 mg L\(^{-1}\)), CA (citric acid) (100 mg L\(^{-1}\)) and 8-HQS (8-hydroxyquinoline sulfate) (250 mg L\(^{-1}\)) were used to determine the effects on vase life of cut hydrangea flowers (Hydrangea macrophylla 'Ankong Rose'). Distilled water was used as the control. Vase life, relative fresh weight, daily and total solution uptake were measured. The experiment was a completely randomized design with ten replications and there was one flower in each replication. The longest vase life of hydrangea flowers was 12.10 days with 8-HQS (250 mg L\(^{-1}\)), followed by 76 mg L\(^{-1}\) of GA (10.70 days) and 38 mg L\(^{-1}\) of GA (9.70 days). 8-HQS significantly increased the vase life of hydrangeas and also determined that same treatment increased the total solution uptake and delayed relative fresh weight loss. These results indicated that 8-HQS can be used to prolong the vase life of cut hydrangea flowers.

KEYWORDS:
Hydrangea, cut flower, vase life, 8-hydroxyquinoline sulfate, benzethonium chloride

INTRODUCTION

Hydrangea is an ornamental plant for using in the garden and as potted plant, and also for cut flower production [1]. Hydrangea macrophylla is the most widely grown among the Hydrangea species [2]. This plants are “an ideal cut flower” and are ranked 8th among the most sold flowers in the Holland flower auction [3]. The popularity of cut hydrangea flowers is increasing every year.

Vase life of cut hydrangeas are usually short and it ranges from 7 to 15 days [4]. Hydrangeas vase life is related to physiochemical processes as in all cut flowers and reduces through ethylene production (freshly-harvested flowers are ethylene sensitive but flowers harvested in the classic stage are not) [5] and bacterial contamination in vase solution [6]. The reason of vascular occlusion is the air emboli and microorganisms caused by the blockage of the xylem vessels [7]. Microorganisms, in paticularly bacteria and fungi, have a negative effect on the longevity of the cut flowers. These microorganisms block the stem end and shorten the vase life by restricting the uptake of water [6, 7]. Some applications have been applied to prolong the vase life of cut flowers by adding various germicides to the water [6, 8]. In cut flowers, several agents have been used for extending vase life by improving water uptake and diminish the bacterial growth [9]. In many studies, 8-hydroxyquinoline (8-HQ), 8-HQS and 8-hydroxyquinolinium citrate (8-HQC) are the most commonly used fungicides. They has prevented the growth of more than 40 bacteria and fungus species in cut flowers [10]. Benzethonium chloride (BC) is one of the most important quaternary ammonium compounds (QACs) which have a surfactant ability which is substantially compatible with adhesion BC is used as a protective and antimicrobial agent for antibacterial hand wipes, anitseptic creams and antipruritic ointments [11]. Succinic acid (SUA) is an antimicrobial agent that affects various physiological and biochemical functions in plants. SUA is a carbohydrate produced in the creps cycle in all living things. Citric acid (CA) is an organic acid and provides carbon and energy source for cellsand also used in the respiratory cycle and some other biochemical pathways [12, 13]. CA reduces the microorganism population in vase solution and improve the water conductivity of cut flowers in xylem [7] [11, 13, 14]. Glycolic acid (GA) is an alpha hydroxy carboxylic acid. Although some plants appear naturally, their trade is produced synthetically. Glycolic acid is a surfactant and has anti-microbial activity. GA is used due to its antimicrobial effect against many bacteria and fungi. Although the positive effects of 8-HQS [6] and citric acid (CA) out of
these compounds on the vase life of cut flowers have been revealed, research on succinic acid, glycolic acid and Benzethonium chloride is limited [11]. Therefore, the effects of succinic acid, glycolic acid, benzethonium chloride as well as 8-HQS and citric acid on the vase life of cut hydrangea flowers were investigated in this study.

MATERIALS AND METHODS

This study was carried out in 2017 in the Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara-Turkey. Hydrangea (Hydrangea macrophylla ‘Ankong Rose’) plants were grown greenhouse (39°57'40.2"N 32°51'51.7"E) located in Ankara University. Hydrangea’s harvested when the petals opened 25%, immediately placed in tap water and taken to the laboratory of the same department. The flower stems were recut a 45 cm and then taken in a temperature-controlled room at tempeture of 21±2 °C and relative humidity of 60±5% and fluorescent lamps provided by 1000 lux light for 12 hours photoperiod. Cut flowers were placed individually in a into measuring cylinder filled with 100 ml of vase solutions (Table 1). All solutions were freshly prepared at the begining of experiment and solution was added to the vases when solution ended during the experiment.

![Table 1](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cas No</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (Control)</td>
<td></td>
<td>Distilled water</td>
</tr>
<tr>
<td>GA1 (Glycolic acid, Merck)</td>
<td>S7172386703</td>
<td>38 mg L⁻¹</td>
</tr>
<tr>
<td>GA2 (Glycolic acid, Merck)</td>
<td></td>
<td>76 mg L⁻¹</td>
</tr>
<tr>
<td>SUA1 (Succinic acid, Merck)</td>
<td>110-15-6</td>
<td>50 mg L⁻¹</td>
</tr>
<tr>
<td>SUA2 (Succinic acid, Merck)</td>
<td></td>
<td>100 mg L⁻¹</td>
</tr>
<tr>
<td>BC (Benzethonium chloride, Sigma Aldrich 8-HQS (8-hydroxyquinoline sulfate, Sigma-Aldrich, CA (Citric acid, Carlo Erba Reagents))</td>
<td>207386-91-2</td>
<td>250 mg L⁻¹</td>
</tr>
<tr>
<td></td>
<td>5949-291-1</td>
<td>100 mg L⁻¹</td>
</tr>
</tbody>
</table>

Measured Factors. Vase Life (Day). Vase life was terminated when withering, sepal browning, or sepal desiccation became apparent on approximately 80% of decorative florets in an inflorescence based on daily observations [15].

Solution Uptake. For measuring the amount of solution absorption, control bottles (distilled water) with the same volume of the test solution containing measuring cylinder (100 ml) were used to find the evaporative loss by weight. This was subtracted from the weight reduction in flower containing bottles and the values were expressed g stem⁻¹ day⁻¹. This process was done daily for 13 days for each replication. Additional solutions were added to the applications where the solutions were finished.

Water Uptake and Relative Fresh Weight. The daily amount of vase solution taken by hydrangeas, vases and flower stems were calculated by weighing each day separately. The results were substracted and stated in mL. For fresh weight of flower stems, they were measured daily and individually and the values were stated as a % of the initial fresh weight. Fresh weight of cut flowers was measured daily through the experiment from day 1 to 13.

Statistical analysis. The experiment with seven treatments was conducted in completely randomized design (CRD) with ten replicates and one flowers for each replication. Statistical significance between mean values was stated using analysis of variance (ANOVA) and Duncan’s Multiple Range Test at p≤0.05 using IBM SPSS Statistics (20.0).

RESULTS AND DISCUSSION

Vase Life (Day). There were statistically significant differences in the vase life of the hydrangea flowers. The longest vase life was 12.10 days with 8-HQS followed by 76 mg L⁻¹ of GA2 (10.70 days) and 38 mg L⁻¹ of GA1 (9.70 days) respectively. The lowest value was recorded with CA (7.20 days) and the control was measured 8.50 days (Fig. 1).

This result can be explained by the fact that 8-HQS controlled the development of microorganisms, prolonging the vase life and prevented the vascular occlusion of acidic solutions and increased the vase life by eliminating the obstructions of water uptake [16]. Similarily positive effects of HQS on the vase life of gerbera jamesonii and affects the bio-chemical changes related to senescence, reduces the leakage of ions were reported [17, 18]. While in parallel with the increasing doses of GA, the vase life of the flowers increased compared to the control. Moreover, CA did not provide any increase in vase life. This is due to the fact that the dose used in the CA treatment had a toxic effect on stems of the flowers.
Solution Uptake. In all treatments, including the control daily uptake of solutions decreased by certain rates until the end of the vase life (Fig. 3). At the end of the vase life, no statistically significant difference was found between the control, 38 mg L\(^{-1}\) of GA\(_1\), 76 mg L\(^{-1}\) of GA\(_2\) and 100 mg L\(^{-1}\) of SUA\(_2\). The daily uptake of 8-HQS was higher than the other treatments and this treatment followed by BC. The lowest value of the daily solution uptake was of 50 mg L\(^{-1}\) of SUA\(_1\) (Fig. 2).

Total Vase Solution Uptake. According to statistical analysis, differences between applications were found to be significant. 8-HQS had the highest...
value in total vase solution uptake with 88.08% while SUA1 had the lowest value with 69.61% (Table 2).

As a result of the findings, the vase life was closely correlated to the vase solution. There has been a positive relationship between the water uptake and the prolongation of the vase life of the hydrangeas, showed that is associated to the plugging of water flow in the stem. The cut hydrangeas treated with 8-HQS had a higher uptake that resulted in a longer vase life and prolonging is positively corelated with water uptake. Reddy et al. (1996) reported that 8-HQS reduces microorganisms and cause an increase in water uptake with cut flowers [19]. BC is used as a protective and antimicrobial agent and plays a role in facilitating the water uptake. Blockage of vascular by microorganism causes reducing water uptake and finally results in breaking of stem or bending and wilting of petal in hydrangeas, roses, gladious and dendrobium flowers. However, these problems are eliminated with the use of 8-HQS [11, 20, 21, 22, 23].

**Relative Fresh Weight.** There is a significant difference were found among the treatment in relative fresh weight. Generally, the relative fresh weight of all treatment constantly decreased from the third day after the beginning of the experiment until the death while 8-HQS treated flowers relative fresh weight gradually increased over the first seven days (Fig 3). The highest relative fresh weight was obtained in cut flowers kept in 250 mg L\(^{-1}\) of 8-HQS, 38 mg L\(^{-1}\) of GA and 76 mg L\(^{-1}\) of GA vase solutions respectively. However The lowest relative fresh weight was obtained in cut flowers kept in 100 mg L\(^{-1}\) of SUA.

In parallel with this study; especially 8-HQS, increasing the vase life and being very positive effect in reducing respiratory rate and physiological weight loss in dendrobium [22] and gladiolus flowers [21]. Similarly, the positive effects of 8-HQS on the fresh weight of the carnation, astroemeria and zinnia [24] were reported. Because of the fact that GA has antimicrobial activity, it is thought to be effective in reducing weight loss. Although BC treatment has a positive effect on the water uptake of flowers, it was found to be inversely proportional to the relative fresh weight. It has been thought that this may be due to the destruction of nutrients in parallel with the increase in respiratory rate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Vase Solution Uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.68 bc</td>
</tr>
<tr>
<td>GA(_1) (38 mg L(^{-1}))</td>
<td>71.62 bc</td>
</tr>
<tr>
<td>GA(_2) (76 mg L(^{-1}))</td>
<td>71.19 bc</td>
</tr>
<tr>
<td>SUA(_1) (50 mg L(^{-1}))</td>
<td>69.61 c</td>
</tr>
<tr>
<td>SUA(_2) (100 mg L(^{-1}))</td>
<td>71.39 bc</td>
</tr>
<tr>
<td>BC (25 mg L(^{-1}))</td>
<td>87.28 b</td>
</tr>
<tr>
<td>8-HQS (250 mg L(^{-1}))</td>
<td>88.08 a</td>
</tr>
<tr>
<td>CA (100 mg L(^{-1}))</td>
<td>74.69 ab</td>
</tr>
</tbody>
</table>

**FIGURE 3**
Effect of different holding solutions on relative fresh weight of cut ‘Ankong Rose’
CONCLUSION

In this study was set to determine the potential role of GA, SUA, BC, 8-HQS and CA in maintaining good marketable quality of cut hydrangea flowers and in extending flowers vase life. In conclusion compared to control, 8-HQS (250 mg L-1) and 76 ers and in extending flowers vase life. In conclusioning good marketable quality of cut hydrangea flowers. However 100 mg L -1 of citric acid may be involved in the regulation of acid treatment on physiochemical attributes in cut flowers, Hortic. Rev. 18, 1-85.


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REGRESSION MODELS PERFORMANCE IN WATER QUALITY ASSESSMENT

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ABSTRACT

Water samples were taken for the 65-km drainage area of the Mudurnu River Basin and analyses were carried out. Changes were observed in the quality of water from upstream to downstream. The aim of this study is to determine the water quality of Mudurnu River through water samples taken from 6 stations placed on Mudurnu River for 6 months twice a month. The water quality parameters were analyzed in the water samples. Moreover, the correlations among water quality parameters were assessed using Regression Models. The Regression Model obtained in the regression analysis explained the Total Kjeldahl Nitrogen parameter with the highest rate. It was also understood that there was a highly significant positive correlation between Chemical Oxygen Demand and Biochemical Oxygen Demand.

KEYWORDS:
River Basin, Water Quality Assessment, Model

INTRODUCTION

Surface water quality get gradually polluted by anthropogenic influences, industrial and agricultural waste materials. Aquatic ecosystems are in a much greater danger in the areas in which agricultural, energy industrial activities are concentrated and in urban residential areas. Large-scale infrastructure projects (highways, urbanization, etc.) and mining activities have a direct impact on pollution. Rivers, as sources of drinking water, irrigation, fishery and energy production, are the first fluvial aquatic systems to be affected by this pollution. Therefore, it is essential to understand the concentration, distribution and sources of pollutants and water quality in order to protect water resources and control water pollution [1, 2, 3].

In Turkey, water pollution and its effects are frequently observed particularly in the river basins, where intensive industrial activities take place. The heavy industrial activities in the basin lead to pollution. Therefore, water resources need to be protected very well. Turkey, whose population will reach about 100 million in 2030, will become a country suffering from water scarcity. For this reason, it is necessary that water resources be conserved very well [4].

The effective management of these water resources requires knowledge on the quality of river water and its variability. The general approach to assessing water quality is based on the comparison of experimentally determined parameters with current guidelines. Traditional monitoring approach in includes determining ecological and chemical status. Recently, multivariate statistical approach has become popular in understanding the water quality and the ecological situation [5, 6, 7, 8, 9, 10, 11, 12, 13, 14].

The purpose of this study is to determine the correlations between the data set obtained in the Mudurnu River Basin at the monitoring program and pollutant parameters using regression models.

MATERIALS AND METHODS

The study area: Mudurnu River Basin. The Mudurnu River Basin rises from the southern slopes of the Abant Mountains, and is formed by small springs and streams that gather together. The section of Mudurnu River, whose total length is 130 km, within the borders of the Sakarya province in Turkey is 65 km long and its total drainage area is 1720 km². The study area covers the area of this 65 km-long basin of the Mudurnu River polluted by industrial and agricultural activities.

The sampling stations were selected by identifying six stations that could represent the system the best. There are 25 production plants in this area, which include agricultural fields, residential areas, and plants for textile, food, aluminum, PVC, automotive, beverages, and water refilling facilities. The Mudurnu River sampling stations are shown in Figure 1.

The first sampling station (S1) is in the town of Taşburun, in a site where wastewater discharges of the factories located in the area has not started yet. Wastewater discharge from treatment facilities of the factories starts after the second sampling station (S2). The third sampling station (S3) is a tributary, the Küçücek Creek. Agriculture and livestock breeding are extensively practiced in the area in which this sampling station is located. The fourth sampling station (S4) is in the area where the Mudurnu River and the Küçücek Creek join. The
fifth and sixth sampling stations (S5, S6) are located in the downstream of the river basin where the sampling is carried out.

**Methods.** Water samples were collected twice a month for 6 months (May 2016–October 2016) in the Mudurnu River Basin. The analyses of Temperature, pH, Ammonium Nitrogen (NH$_3$-N), Nitrite Nitrogen (NO$_2$-N), Nitrate Nitrogen (NO$_3$-N), Total Kjeldahl Nitrogen (TKN), Total Nitrogen (TN), Total Phosphorus (TP), Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) were carried out on the water samples that were collected in accordance with standard methods [15].

All the mathematical and statistical computations were conducted using the SPSS 21.0 [16].

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**RESULTS AND DISCUSSION**

**Averages and Deviations of Chemical Parameters.** In this study, a total of 72 samples were gathered from the sampling stations for in a period of 6 months. The average values and standard deviations of the parameters measured in the Mudurnu River Basin are given in Table 1.

As it can be seen, the average values of the measured parameters are Temperature (18.6℃), pH (7), TKN (0.3 mg/L), NH$_3$-N (0.1 mg/L), NO$_2$-N (0.07 mg/L), NO$_3$-N (1.2 mg/L), TN (1.6 mg/L), TP (0.1 mg/L), TSS (257 mg/L), BOD (3.8 mg/L) and COD (7.9 mg/L).

---

**FIGURE 1**
The Mudurnu River Sampling Stations

**TABLE 1**
The Averages and Deviations of Chemical Parameters

<table>
<thead>
<tr>
<th></th>
<th>°C</th>
<th>pH</th>
<th>TKN</th>
<th>NH$_3$-N</th>
<th>NO$_2$-N</th>
<th>NO$_3$-N</th>
<th>TN</th>
<th>TP</th>
<th>TSS</th>
<th>BOD</th>
<th>COD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid N</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
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<tr>
<td>Loss</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Average</td>
<td>18.653</td>
<td>7.014</td>
<td>.383278</td>
<td>.154653</td>
<td>.074528</td>
<td>1.20514</td>
<td>1.62569</td>
<td>.132361</td>
<td>257.36</td>
<td>3.808</td>
<td>7.918</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>3.0909</td>
<td>.3137</td>
<td>.2801115</td>
<td>.1371402</td>
<td>.0579042</td>
<td>.297809</td>
<td>.232105</td>
<td>.0624949</td>
<td>231.025</td>
<td>.3863</td>
<td>1.3764</td>
</tr>
</tbody>
</table>
Correlations between Chemical Parameters. According to the Correlation Matrix given in Table 2, there is a positive relationship between the parameters of TKN and TN (0.509) and a negative one between TKN and NO$_3$-N (-0.744) and TSS (-0.436). Moreover, positive correlations are seen between the parameters of NO$_3$-N and NH$_4$-N (0.236) and between TP and NO$_2$-N (0.521). On the other hand, it is understood that there is a negative correlation between TSS and TN (-0.292). A positive relationship is observed between TP and TSS (0.334) and a negative one between TP and COD (-0.237). It is understood that there is also a significant positive relationship between COD and BOD (0.682).

Regression Analysis between Parameters. The purpose of regression analysis is to detect to what extent the determined parameters are affected by each other and the extent to which the parameters are determinant for one another.

Regression Analysis between the Parameter of TKN and the Other Chemical Parameters. The TKN parameter can be explained by the other parameters by 87%.

### TABLE 2
Correlation Analysis of Chemical Parameters

<table>
<thead>
<tr>
<th></th>
<th>°C</th>
<th>pH</th>
<th>TKN</th>
<th>NH$_4$-N</th>
<th>NO$_2$-N</th>
<th>NO$_3$-N</th>
<th>TN</th>
<th>TP</th>
<th>TSS</th>
<th>BOD</th>
<th>COD</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>TKN</td>
<td></td>
<td>.321**</td>
<td>-.229</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NH$_4$-N</td>
<td>.092</td>
<td>-.012</td>
<td>-.131</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>NO$_2$-N</td>
<td>-.068</td>
<td>-.231</td>
<td>.139</td>
<td>-.071</td>
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<td></td>
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<tr>
<td>NO$_3$-N</td>
<td>-.090</td>
<td>.198</td>
<td>-.744**</td>
<td>.236*</td>
<td>-.172</td>
<td>1</td>
<td></td>
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<td>TN</td>
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<td>.330**</td>
<td>-.073</td>
<td>.509**</td>
<td>.146</td>
<td>.259*</td>
<td>.037</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td>.157</td>
<td>-.102</td>
<td>-.021</td>
<td>-.031</td>
<td>.521**</td>
<td>-.082</td>
<td>-.009</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSS</td>
<td></td>
<td>-.191</td>
<td>.109</td>
<td>-.436**</td>
<td>.054</td>
<td>.154</td>
<td>.216</td>
<td>-.292*</td>
<td>.334**</td>
<td>1</td>
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</tr>
<tr>
<td>BOD</td>
<td></td>
<td>.228</td>
<td>.127</td>
<td>.043</td>
<td>-.001</td>
<td>.060</td>
<td>.106</td>
<td>.175</td>
<td>-.195</td>
<td>-.094</td>
<td>1</td>
</tr>
<tr>
<td>COD</td>
<td></td>
<td>.054</td>
<td>.288</td>
<td>.721</td>
<td>.991</td>
<td>.615</td>
<td>.375</td>
<td>.141</td>
<td>.101</td>
<td>.431</td>
<td></td>
</tr>
</tbody>
</table>

**. Correlation is significant at 0.01 level (2-tailed).  
*. Correlation is significant at 0.05 level (2-tailed).

### TABLE 3
Model Summary of Regression Analysis of the TKN Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Estimated Standard Error</th>
<th>Durbin–Watson Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.932*</td>
<td>.869</td>
<td>.861</td>
<td>.104348</td>
<td>2.002</td>
</tr>
</tbody>
</table>

a. Determiners: (Constant): TSS, NO$_2$-N, NO$_3$-N, TN  
b. Dependent Variable: TKN
According to the result of the variance analysis, the significance level for the parameters was found to be 0.000 and since 0.000<0.05, there is a significant difference between the averages of the parameter groups.

The parameters of NO₂-N, NO₃-N and TSS affect the TKN parameter in a negative direction and the TN parameter in a positive direction. When the beta coefficients are taken into consideration, it can be seen that the parameters of NO₃-N and TN highly affect the TKN parameter. The explanatory power of the regression model is considerably high at the level of 86.1%.

**Regression Analysis between the NO₃-N Parameter and the Other Chemical Parameters.**
The NO₃-N parameter can be explained by the other parameters by 87%.

According to the result of the variance analysis, the significance level for the parameters were found to be 0.000 and since 0.000<0.05, there is a significant difference between the averages of the parameter groups.

**TABLE 4**
Anova of the Regression Analysis of the TKN Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>Total Squares</th>
<th>df</th>
<th>Mean Squares</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4.841</td>
<td>4</td>
<td>1.210</td>
<td>111.141</td>
<td>.000b</td>
</tr>
<tr>
<td>Residue</td>
<td>730</td>
<td>67</td>
<td>.011</td>
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<td></td>
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<tr>
<td>Total</td>
<td>5.570</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: TKN  
b. Determiners: (Constant): TSS, NO₂-N, NO₃-N, TN

**TABLE 5**
Coefficients of the Regression Analysis of the TKN Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>Non-Standardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>.262</td>
<td>.104</td>
<td>2.515</td>
<td>.014</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>-.566</td>
<td>.237</td>
<td>-.117</td>
<td>-2.387</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>-.718</td>
<td>.044</td>
<td>-.764</td>
<td>-16.185</td>
</tr>
<tr>
<td>TN</td>
<td>.651</td>
<td>.060</td>
<td>.540</td>
<td>10.856</td>
</tr>
<tr>
<td>TSS</td>
<td>-.000116</td>
<td>.000061</td>
<td>-.096</td>
<td>-1.916</td>
</tr>
</tbody>
</table>

a. Dependent variable: TKN

**TABLE 6**
Model Summary of Regression Analysis of the NO₂-N Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Estimated Standard Error</th>
<th>Durbin–Watson Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.906a</td>
<td>.820</td>
<td>.812</td>
<td>.128982</td>
<td>1.890</td>
</tr>
</tbody>
</table>

a. Determiners: (Constant): NO₂-N, TKN, TN  
b. Dependent Variable: NO₂-N

**TABLE 7**
Anova of the Regression Analysis of the NO₃-N Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>Total Squares</th>
<th>df</th>
<th>Mean Squares</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5.166</td>
<td>3</td>
<td>1.722</td>
<td>103.503</td>
<td>.000b</td>
</tr>
<tr>
<td>Residue</td>
<td>1.131</td>
<td>68</td>
<td>.017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.297</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: NO₃-N  
b. Determiners: (Constant): NO₂-N, TKN, TN

**TABLE 8**
Coefficients of the Regression Analysis of the NO₃-N Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>Non-Standardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Std. error</td>
<td>Beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>.425</td>
<td>.115</td>
<td>3.686</td>
<td>.000</td>
</tr>
<tr>
<td>TKN</td>
<td>-1.094</td>
<td>.063</td>
<td>-1.029</td>
<td>-17.223</td>
</tr>
<tr>
<td>TN</td>
<td>.781</td>
<td>.079</td>
<td>.609</td>
<td>9.947</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>-.958</td>
<td>.274</td>
<td>-.186</td>
<td>-3.499</td>
</tr>
</tbody>
</table>

a. Dependent variable: NO₃-N
The NO$_3$-N parameter is explained by the parameters of TKN, TN and NO$_2$-N by 81%. According to the model, the NO$_3$-N parameter is negatively affected by the parameters of TKN and NO$_2$-N and positively by the parameter of TN. When the beta coefficients are taken into consideration, the NO$_3$-N parameter is affected by the TKN parameter the most.

**Regression Analysis between the TN Parameter and the Other Chemical Parameters.**

The TN parameter can be explained by the other parameters by 71%.

According to the result of the variance analysis, the significance level for the parameters was found to be 0.000 and since 0.000<0.05, there is a significant difference between the averages of the parameter groups.

The TN parameter can be explained by the parameters of TKN, NO$_2$-N, and NO$_3$-N by 70%. It is understood that the effect of all these parameters on TN is positive. When the beta coefficient is considered, it is seen that the TKN parameter is the most effective on TN.

**Regression Analysis between the BOD Parameter and the Other Chemical Parameters.**

The BOD parameter can be explained by the other parameters by 55%.

<table>
<thead>
<tr>
<th>TABLE 9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model Summary of the Regression Analysis of the TN Parameter</strong></td>
</tr>
<tr>
<td>Model</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>a. Determiners: (Constant): NO$_3$-N, NO$_2$-N, TKN</td>
</tr>
<tr>
<td>b. Dependent Variable: TN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anova of the Regression Analysis of the TN Parameter</strong></td>
</tr>
<tr>
<td>Model</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Regression</td>
</tr>
<tr>
<td>Residue</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>a. Dependent Variable: TN</td>
</tr>
<tr>
<td>b. Determiners (Constant): NO$_3$-N, NO$_2$-N, TKN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coefficients of the Regression Analysis of the TN Parameter</strong></td>
</tr>
<tr>
<td>Model</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>(Constant)</td>
</tr>
<tr>
<td>TKN</td>
</tr>
<tr>
<td>NO$_2$-N</td>
</tr>
<tr>
<td>NO$_3$-N</td>
</tr>
<tr>
<td>a. Dependent Variable: TN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model Summary of the Regression Analysis of the BOD Parameter</strong></td>
</tr>
<tr>
<td>Model</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>a. Determiners (Constant): COD, $^0$C</td>
</tr>
<tr>
<td>b. Dependent Variable: BOD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 13</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anova of the Regression Analysis of the BOD Parameter</strong></td>
</tr>
<tr>
<td>Model</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Regression</td>
</tr>
<tr>
<td>Residue</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>a. Dependent Variable: BOD</td>
</tr>
<tr>
<td>b. Determiners (Constant): COD, $^0$C</td>
</tr>
</tbody>
</table>
According to the result of the variance analysis, the significance level for the parameters was found to be 0.000 and since 0.000<0.05, there is a significant difference between the averages of the parameter groups.

The BOD parameter can be explained by the parameters of Temperature and COD by about 54%. Both parameters are positively effective on the BOD parameter. When the beta coefficient is taken into consideration, it is understood that the effect of the COD parameter on BOD is higher.

Regression Analysis between the Parameter of COD and the Other Chemical Parameters. The parameter of COD can be explained by the other parameters by 63%.

According to the result of the variance analysis, the significance level for the parameters was found to be 0.000 and since 0.000<0.05, there is a significant difference between the averages of the parameter groups.

The COD parameter is explained by the parameters of BOD, NO₂-N, TKN, pH, Temperature, TP and NO₃-N by about 59.3%. The parameters of Temperature and TP affect the COD parameter negatively, whereas all the other parameters have a positive effect on COD. According to the beta coefficients, it is understood that the BOD parameter affect COD the most.

### TABLE 14
Coefficients of the Regression Analysis of the BOD Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>Non-Standardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>Linearity Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>1.549</td>
<td>.274</td>
<td>5.646</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>1 °C</td>
<td>.037</td>
<td>.010</td>
<td>.293</td>
<td>3.618</td>
<td>.001</td>
</tr>
<tr>
<td>COD</td>
<td>.199</td>
<td>.023</td>
<td>.709</td>
<td>8.752</td>
<td>.000</td>
</tr>
</tbody>
</table>

a. Dependent Variable: BOD

### TABLE 15
Model Summary of the Regression Analysis of the COD Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Estimated Standard Error</th>
<th>Durbin–Watson Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.796</td>
<td>.633</td>
<td>.593</td>
<td>.878396</td>
<td>2.042</td>
</tr>
</tbody>
</table>

a. Determiners (Constant): NO₃-N, TP, °C, pH, BOD, NO₂-N, TKN

b. Dependent Variable: COD

### TABLE 16
Anova of the Regression Analysis of the COD Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>Total Squares</th>
<th>df</th>
<th>Mean Squares</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>85.125</td>
<td>7</td>
<td>12.161</td>
<td>15.761</td>
<td>.000³</td>
</tr>
<tr>
<td>Residue</td>
<td>49.381</td>
<td>64</td>
<td>.772</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>134.507</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: COD

b. Determiners (Constant): NO₃-N, TP, °C, pH, BOD, NO₂-N, TKN

### TABLE 17
Coefficients of the Regression Analysis of the COD Parameter

<table>
<thead>
<tr>
<th>Model</th>
<th>Non-Standardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>Linearity Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>-6.296</td>
<td>2.715</td>
<td>-2.319</td>
<td>.024</td>
<td></td>
</tr>
<tr>
<td>BOD</td>
<td>2.281</td>
<td>.293</td>
<td>.640</td>
<td>7.797</td>
<td>.000</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>4.695</td>
<td>2.236</td>
<td>.198</td>
<td>2.100</td>
<td>.040</td>
</tr>
<tr>
<td>TKN</td>
<td>1.485</td>
<td>.626</td>
<td>.302</td>
<td>2.374</td>
<td>.021</td>
</tr>
<tr>
<td>pH</td>
<td>.952</td>
<td>.365</td>
<td>.217</td>
<td>2.610</td>
<td>.011</td>
</tr>
<tr>
<td>°C</td>
<td>-.165</td>
<td>.039</td>
<td>-.370</td>
<td>-4.244</td>
<td>.000</td>
</tr>
<tr>
<td>TP</td>
<td>-4.843</td>
<td>2.047</td>
<td>-.220</td>
<td>-2.365</td>
<td>.021</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>1.361</td>
<td>.551</td>
<td>.294</td>
<td>2.471</td>
<td>.016</td>
</tr>
</tbody>
</table>

a. Dependent Variable: COD
CONCLUSIONS

This study was carried out in order to determine the pollutant sources in the area in the Mudurnu River Basin in which the factory discharges were severe and also in the downstream and upstream of that area and to identify the correlations between the pollutant parameters of the Regression Models.

The mean values of the parameters measured were Temperature (18.6°C), pH (7), TKN (0.3mg/L), NH₄-N (0.1 mg/L), NO₂-N (0.07 mg/L), NO₃-N (1.2 mg/L), TN (1.6 mg/L), TP (0.1 mg/L), TSS (257 mg/L), BOD (3.8 mg/L) and COD (7.9 mg/L).

The NO₂-N parameter was measured at high concentrations at all the sampling stations. According to the Inland Water Resources Quality Criteria of the Water Pollution Control Regulations, the Mudurnu River Basin is placed in Class IV when the NO₂-N parameter is taken into consideration.

It was determined that all the sampling locations that were measured had second quality water with respect to the parameter of BOD, while the first sampling station (S1) had second quality water and the other sampling stations had third quality water in terms of TP. In terms of the TKN parameter, only the third sampling station (S3) had second quality water but the other stations had first quality water, and for the parameter of NH₄-N, the first and third sampling stations (S1, S3) showed the characteristics of first quality, while the other stations that of second quality. In terms of the parameters of Temperature, pH, COD and NO₂-N, all the sampling stations have first quality water.

It was also understood that there was a highly significant positive correlation between COD and BOD (0.682). These are the highest correlation specified in the correlation matrix. The Regression Model obtained in the regression analysis explained the TKN parameter with the highest rate (86.1%). In contrast, it was observed that the Regression Model had a lower explanation rate for the parameters of NH₄-N, TP and TSS.

The NH₄-N parameter was explained by the TKN and TN parameters by 5.2%. The NO₂-N parameter was affected by the parameters of pH, TKN and NO₃-N negatively and by TN, TP and COD positively. The explanatory power of the Regression Model generated was 44% and it was observed that the greatest amount of pollution leaked into the Mudurnu River through plant zones. The sectors of textile, food, aluminum and automotive caused high levels of pollution.

High levels of nitrite in rivers stations to a sudden domestic/industrial wastewater discharge. Even low concentrations of the nitrite parameter are known to be toxic for aquatic life. Therefore, this parameter in particular needs to be paid attention in the studies carried out in rivers and rehabilitation operations should be performed in order to lower high concentrations.

REFERENCES


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AN ESTIMATION OF IN-CYLINDER PRESSURE BASED ON LAMBDA AND ENGINE SPEED IN HCCI ENGINE USING ARTIFICIAL NEURAL NETWORKS

Seyfi Polat1, Ozgur Ozdilli2, Huseyin Cizmeci3

1Department of Mechatronics Engineering, Faculty of Engineering, Hitit University, Turkey
2Department of Machine and Metal Technology, Hitit University, Turkey
3Department of Computer Technology, Hitit University, Turkey

ABSTRACT

In this study, the in-cylinder pressure predicted based on lambda and engine speed with the ANN method for HCCI engine. In-cylinder pressures obtained at different lambda and engine speeds, constant inlet air temperature (80°C), RON40 (40% iso-octane/60% n-heptane) fuel in a single cylinder, four-stroke, naturally aspirated, port injection HCCI engine. MATLAB ANN program was used for training, validation and testing of inputs. The crank angle, engine speed, and lambda were used as input values and the in-cylinder pressure was used as the target value. The Levenberg-Marquardt training algorithm was used for the training of inputs. Also, three layers and 10 neurons were used for the training process. The best validation performance was obtained at epoch 535 as 0.000043691 MSE value. The correlation factor of training, validation, and testing between the targets to outputs were obtained at 0.99912, 0.99905 and 0.99893 respectively. The total correlation factor was found at 0.99908. It is observed that there is a high degree of accuracy between the estimation of results and experimental data using the developed ANN model.

KEYWORDS:
Artificial neural network, ANN, HCCI, engine, in-cylinder pressure, lambda

INTRODUCTION

Fossil fuel sources, which are used extensively in the automotive industry, are decreasing day by day. Fossil fuels used in internal combustion engines cause environmental pollution and it results in global warming due to the greenhouse gas effects. Researchers are constantly trying to develop to internal combustion engines to reduce fuel consumption and the harmful effects of exhaust gases. Gasoline and diesel engines are widely used in the automotive industry. In gasoline engines, the air/fuel mixture taken into the cylinder with throttling causes losses. HC and UHC emissions of gasoline engines are very high. In addition, the thermal efficiency is low because high compression rates are not applied in gasoline engines. In diesel engines, the homogeneity of the mixture isn’t sufficiently so the combustion efficiency reduces. The high NOx and PM emissions are a major disadvantage in diesel engines [1-6].

Homogeneous Charge Compression Ignition (HCCI) engines are compared with conventional spark ignition (SI) engines and compression ignition (CI) engines, they have advantages such as high thermal efficiency, very low NOx and PM emissions and low heat losses. In HCCI engines, the air/fuel mixture self-ignites simultaneously throughout the entire cylinder by compressing the air/fuel mixture. Thus, it is an advantage in terms of NOx and soot emissions. But, this situation leads to the formation of knocking due to the higher pressure rise rates in the cylinder, especially under high load conditions. As a result, HCCI engines cannot be used commercially because of these problems. One of the biggest challenges in HCCI engines is the inability to directly control the start of combustion and other stages of combustion. In order to control the combustion process in HCCI engines, the researchers used many different applications such as heating the intake inlet air, variable valve timing, EGR (Exhaust Gas Recycling), changing the compression ratio, increasing the suction pressure. In addition, the physical and chemical properties of the fuel directly affect the combustion process in HCCI engines. Therefore, researchers are trying to expand the operating range of HCCI engines by using fuels which have different properties [7-11].

Studies on internal combustion engines are predominantly experimental because the results are much more reliable. As a result of the experiments, the engine operating parameters and performance parameters are used to improve engine efficiency. However, the engine operating conditions must be the same in order to compare the combustion and performance parameters reliably. In order to determine the operating range of the HCCI engine between the knock and misfire, it is necessary to carry out the tests at different engine speeds and at different lambda values. As a result of the experiments, it is possible to make calculations related to
combustion parameters by using the measured in-cylinder pressure values. The lambda value can be determined by the most common two methods during the experiments. The first method is that lambda value can be calculated by measuring the amount of air and fuel taken into the cylinder. The second method is that lambda value can be calculated by measuring the oxygen in exhaust gas using an exhaust gas analyzer. In both methods, there are serious measurements of error because HCCI engines cannot be operated stably. In order to obtain data during the experiments, it is not always possible to operate the HCCI engine stably at the same lambda value at different engine speeds. In order to compare the in-cylinder pressure values with the same lambda values at different engine speeds, the lambda must be constant. Otherwise, a correct comparison cannot be made and the results cannot be interpreted correctly. In such problems, artificial neural networks (ANN) methods can be used to estimate the in-cylinder pressure values of the regions that cannot be measured during the experiments. Experimental costs can be reduced by decreasing the number of experiments using the ANN method. Furthermore, the test data can be more easily compared.

Positive results have been obtained by using ANN to solve problems in which traditional algorithms or mathematical formulas cannot be calculated in many areas such as education, health and defense [12]. Artificial neural networks have been developed for use in solving complex problems [13]. ANN is a parallel and distributed processor designed on the basis of the human brain, simulating the working principle of the simple nervous system. Learning in artificial neural networks is achieved by adjusting the weights of synaptic connections between cells to achieve the desired goal [14].

Ismail et al. [15] reported artificial neural networks (ANN) modelling program for a light-duty diesel engine powered by using blends of various biodiesel fuels with conventional fossil diesel. They used the ANN method to predict nine different engine-out responses, namely carbon monoxide (CO), carbon dioxide (CO2), nitrogen monoxide (NO), unburned hydrocarbon (UHC), maximum pressure (Pmax), location of maximum pressure (CAD Pmax), maximum heat release rate (HRRmax), location of maximum HRR (CAD HRRmax) and cumulative HRR (CuHRR). For the input parameters, they used four pertinent engine operating parameters which are engine speed, output torque, fuel mass flow rate and biodiesel fuel types and blends. As a result of the study, it is seen that the results of the model developed using the ANN method are consistent with the experimental data.

Rezai et al. [16] made a performance prediction of HCCI engines with oxygenated fuels using artificial neural networks. In the study, they used HCCI experimental data to characterize variations in seven engine performance metrics including indicated mean effective pressure (IMEP), thermal efficiency, in-cylinder pressure, net total heat released, nitrogen oxides (NOx), carbon monoxide (CO), and total hydrocarbon (THC) concentrations. They developed two types of ANNs including radial basis function (RBF) and feedforward (FF) to predict the seven engine performance metrics. The validation results indicate both RBF and FF models can predict HCCI engine performance metrics with less than 4% error for butanol and ethanol-fueled engines. They determined that the FF neural network models are advantageous in terms of network simplicity with fewer required neurons but need twice as much training time compared to the RBF models.

Bahri et al. [17] developed a real-time model of ringing in HCCI engines using artificial neural networks. They used HCCI experimental data to investigate combustion noise and ringing operation in a 0.3 L converted-diesel HCCI engine. In the study, the combustion noise level (CNL) was investigated along with the main HCCI combustion parameters and emissions. They found that a strong correlation between the CNL and variation of in-cylinder pressure at 10, 15, 20 CAD aTDC (P10, P15 and P20) and maximum in-cylinder pressure (Pmax). They used the experimental data design ANN model to predict CNL for identifying normal and ringing regions. The results indicate that the real-time ANL model can predict CNL with less than 0.5% error for the HCCI engine. The ANL model is of utility for identify engine operating limits to avoid the ringing operation.

Mohammadhassani et al. [18] predicted NOx emissions from a direct injection diesel engine using an artificial neural network. They used the experimental data which are various engine speeds, mass fuel injection rates, and intake air temperatures obtained from a 6-inline-cylinder, four-stroke, diesel test engine for training and testing the network. In the study, 80% of 144 obtained experimental data were used for the training process, 10% of the data (randomly selected) were used for validation and the remaining data were used for testing the accuracy of the network. They used the mean square error (MSE) function for evaluating the performance of the network. The results showed that the artificial neural network can efficiently be used to predict NOx emissions from the tested engine with about 10% error.

Noor et al. [19] predicted the performance of a marine diesel engine such as the brake power (BP), brake specific fuel consumption (BSFC), brake thermal efficiency (BTE), volumetric efficiency (VE), exhaust gas temperature (EGT) and nitrogen oxide (NOX) emissions using artificial neural network method. They obtained the input data for network training from laboratory engine testing operated at various speed, load and fuel blends. They used the Levenberg-Marquardt training algorithm for the ANN prediction model. The results showed that the ANN model provided a good agreement to the
experimental data with the coefficient of determinations (R2) of 0.99. Mean absolute prediction error (MAPE) of ANN and the mathematical model is between 1.57-9.32% and 4.06-28.35% respectively. They reported that the ANN approach can be used to predict the performance of a marine diesel engine with high accuracy.

In this study, the in-cylinder pressure predicted based on lambda and engine speed with ANN method for HCCI engine. In-cylinder pressures obtained at different lambda and engine speeds in a single cylinder, four-stroke, naturally aspirated, port injection HCCI engine.

MATERIALS AND METHODS

**Experimental Setup.** A single cylinder, four stroke, port injection, naturally aspirated gasoline engine was converted to an HCCI engine. The tests were performed at constant inlet air temperature (80°C), different engine speeds and lambda values with RON40 (40% iso-octane/60% n-heptane) fuel. Technical specifications of the test engine are given in Table 1.

In the inlet line, an electrical heating system was mounted in order to increase the inlet air temperature. DC dynamometer which is rated 30 kW/6500 rpm was used in order to measure the load test engine. In-cylinder pressure was measured using a Kistler 6121 piezoelectric transducer with a resolution of 0.36 crank angle degrees. Also, an encoder which is 2000 pulses was mounted in the crankshaft to measure engine speed and determine the top dead center (TDC). Raw in-cylinder pressure data amplified using Cussons P8160 combustion analysis device. Amplified in-cylinder pressure data were converted to digital data using National Instrument data acquisition card. UEGO sensor is mounted in the exhaust line to measure lambda. In addition, the air/fuel ratio was changed with potentiometer from the dynamometer control panel. The schematic view of the experimental setup is seen in Fig. 1.

**ANN Method.** (1) Structure of ANN. There are many learning methods used in artificial neural networks. One of these methods is back propagation. The back-propagation learning method is one of the algorithms used in the multi-layered network structure. In the ANN method, firstly the training data (input) is sent to the network, then the output data (output) produced by the network is compared with the expected value (target). The difference between these two values gives the error value. At the next

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Engine Specification</th>
<th>Value/Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engine Model</td>
<td>Ricardo Hydra</td>
</tr>
<tr>
<td>Bore x Stroke [mm]</td>
<td>80.26 x 88.9</td>
</tr>
<tr>
<td>Cylinder Number</td>
<td>1</td>
</tr>
<tr>
<td>Displacement Volume [L]</td>
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</tr>
<tr>
<td>Compression Ratio</td>
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</tr>
<tr>
<td>Connecting Rod Length [mm]</td>
<td>158</td>
</tr>
<tr>
<td>Max. Power [kW]</td>
<td>15</td>
</tr>
<tr>
<td>Fuel Injection System</td>
<td>Port Type</td>
</tr>
<tr>
<td>Valve System</td>
<td>SOHC 2 Valves</td>
</tr>
</tbody>
</table>

---

**FIGURE 1**

Schematic view of the experimental setup
iteration, the error value is distributed to the weight values of the network and the error value is reduced at the end of the process [20]. In this study, the crank angle, engine speed and lambda were used as input values and the in-cylinder pressure was used as the target value. Multilayer network structure can be seen in Fig. 2.

(2) **LM Training algorithm.** In this study, the Levenberg-Marquardt training algorithm which is widely used in artificial neural networks was used. The Levenberg-Marquardt algorithm, which is derived from Newton’s algorithms, performs parameter calculation processes with the error vector and Jacobian matrix created for all inputs. The Levenberg-Marquardt algorithm uses system resources (memory, etc.) more than other algorithms. Thus, the training of the network takes place in a shorter time. Training finishes when the generalization stops healing [21, 22]. The weight values of the network are calculated using Eq. (1).

\[
\Delta w = (J^T J + \mu I)^{-1} J^T e
\]  

Where, ‘w’, ‘I’ and ‘\( \mu \)’ are weight vector, unit matrix and combination coefficient respectively. ‘J’ is Jacobian matrix which is \([ (P \times n), N ]\), ‘e’ is error vector which is \([ (P \times n), 1 ]\), ‘P’ shows the number of training samples, ‘n’ shows the number of outputs and ‘\( N \)’ is the number of weight [23].

(3) **MSE (Mean Squared Error).** The most important performance criterion in (ANN) is the accuracy of the estimate. This accuracy measure is calculated as the difference between the actual value and the estimated value from the network. One of the most commonly used criteria for accuracy is the Average Square Error (MSE). The most important feature of this criterion is that the error can be decomposed into the sum of variance. The lower the MSE value obtained (close to zero) is shown the better the performance criterion [24]. MSE value can be calculated using Eq. (2).

\[
MSE = \frac{1}{n} \sum_{i=1}^{n} (Y_i - \hat{Y}_i)^2
\]  

Where, n is the number of data points, \( Y_i \) is the observed values and \( \hat{Y}_i \) is the predicted values.

(4) **Implementation of the ANN to predict in-cylinder pressure.** The in-cylinder pressure estimation was performed with the MATLAB ANN Toolbox using data obtained from the HCCI engine test setup at five different engine speeds and different air/fuel mixture ratios. Experimental parameters and data can be seen in Table-2. Also, training data (input) of the in-cylinder pressure can be seen in Fig. 3 without hidden in-cylinder pressure data which are experimental numbers of 3, 7, 12 and 16.
In this study, a summary of the neural network design that was showed in Table-3. The ANN method consists of three types of neuron layers which are input, hidden and output as generally. Three suitable engine control parameters determined as input neurons were engine speed, crank angle and lambda. One engine-out determined as output neuron was in-cylinder pressure. The in-cylinder pressure was modelled as a function of engine speed, crank angle and lambda values using the MATLAB ANN model as illustrated in Fig. 2.

The artificial neural network is used to predict the in-cylinder pressure from an HCCI engine using the LM training algorithm. The crank angle, engine speed and lambda were used as input values and the in-cylinder pressure was used as the target value. MATLAB ANN Toolbox was used to calculate the weights of layers for training, validation, and testing stages of the network. After that, the accuracy of the network is tested by comparing the predicted values of in-cylinder pressure to measured experimental data which are not trained to the network. In this study, 19 experiments were performed with the HCCI engine. In each experiment there are 2000 in-cylinder pressure values. Thus, 38000 in-cylinder pressure values are obtained with experiments. 15 experiments were used as training data and four experiments were hidden for comparing the output of ANN.

Fig. 4 shows the performance of the ANN for training, validation and testing depends on MSE. 1000 iterations were used in the training process. Best validation performance was obtained at epoch 535 as 0.000043691 MSE value.

Fig. 5 shows the regression analysis between the network responses (outputs) based on the corresponding targets for training, validation and testing. The R value is an indication of the relationship between the outputs and targets. If R is equal to 1, this indicates a complete linear relationship between outputs and targets. If R is close to zero, there is no linear relationship between outputs and targets. The correlation factor of training, validation and testing between targets to outputs were obtained at 0.99912, 0.99905 and 0.99893 as respectively. The total correlation factor was found at 0.99908 as shown in Fig. 5. It can be seen that appropriate results were obtained when the regression analysis was evaluated for the accuracy of the network.
The comparison of the predicted and measured in-cylinder pressure for different engine speeds and lambda values are shown in Fig. 6. It is observed that there is a high degree of accuracy between the estimation of results and experimental data using the developed ANN model. As a result, the developed ANN method with Levenberg-Marquardt training algorithm is an appropriate technique to estimate in-cylinder pressure for HCCI engines as shown in Fig. 6. In HCCI engines, the engine operating range is reduced when the engine speed increases. Therefore, the lambda range is reduced at high engine speeds. In the experiments, two different lambda values can be measured at 1600 rpm while five different lambda values can be measured at 800 rpm. Because at high engine speeds, misfire occurs at poor mixture and the knocking occurs due to rich mixtures. The accuracy of the network was reduced due to the number of training data was limited at high engine speeds the compare to low engine speed.

The variation of in-cylinder pressure at different engine speeds (from 800 rpm to 1600 rpm at 100 intervals) and constant lambda value (2.30) is shown in Fig. 7. In the experimental studies, it is not possible to take measurements at the constant lambda value at different engine speeds due to the engine could not be operated as steadily. The in-cylinder pressure values can be estimated with high accuracy using the developed ANN model at different engine speeds and constant lambda value. The combustion and performance parameters of the HCCI engine can be calculated using these in-cylinder pressure values and the results can be compared for the same experimental conditions as successfully.
In this study, the in-cylinder pressure estimation was performed with the MATLAB ANN Toolbox using data obtained from the HCCI engine test setup at five different engine speeds and different air/fuel mixture ratios. The crank angle, engine speed and lambda were used as input values and the in-cylinder pressure was used as the target value. The Levenberg-Marquardt training algorithm was used for training of input data to ANN. Also, three layers and 10 neurons were used for the training process. Best validation performance was obtained at epoch 535 as 0.000043691 MSE value. The correlation factor of training, validation and testing between targets to outputs were obtained at 0.99912, 0.99905 and 0.99893 as respectively. The total correlation factor was found at 0.99908. It is observed that there is a high degree of accuracy between the estimation of results and experimental data using the developed ANN model. However, the accuracy of the network was reduced due to the number of training data was limited at high engine speeds the compare to low engine speed. As a result of this study, experimental costs can be reduced by decreasing the number of experiments using the developed ANN method in HCCI engines and the test data can be more easily compared for combustion and performance analysis.

CONCLUSIONS

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PREDICTION OF INDOOR TEMPERATURE IN A GREENHOUSE: SIİRT SAMPLE

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ABSTRACT

Greenhouses are agricultural structures which allow indoor conditions to be controlled. Food demand is increasing as the population increases. Therefore, creating new areas for food production and making perpetual agricultural production without interruption is a key-stone necessity to meet demands. Increasing the crop production could only be possible with constant cultivation period. Greenhouses are the capital ships of feeding the population and fighting poverty. Due to the climate changes and increasing population, greenhouses will gain more and more significance in the years to come. However, greenhouses will cause harms instead of benefits if they are applied in wrong climatic conditions. In this study, in a greenhouse having floor area of 11220 m², indoor and outdoor temperatures are quantified for two years, after modelling and simulating the energy efficiency, indoor temperature values are estimated by artificial neural networks. This study shows that artificial neural networks could accurately estimate the indoor temperature of greenhouses and relative humidity 6 hours in advance, and the temperature could be estimated 3 days in advance.

KEYWORDS:
Neural network, outdoor temperature, relative humidity, production, sensor

INTRODUCTION

No matter how broad agricultural areas are spread, the agriculture sector seems distant from answering the needs of the human population. Therefore about 795 million people are undernourished globally [1].

In order to meet the food needs of today, more production and more efficiency are needed. By increasing the greenhouse agricultural areas, it is possible to continue food production for 12 months. The food production can be done throughout the year, keeping the climatic conditions under control. It is called "greenhouse" in practice where climate conditions are kept under control and production is done all year round. Greenhouses can be used to produce various cultivated plants, seeds, and seedlings throughout the year without completely or partially adhering to the environmental, climatic conditions with the control of factors such as temperature, light, and humidity.

Greenhouses are various vegetable production structures covered with glass, plastic and light-permeable materials [2].

Air temperature as well as solar radiation and air relative humidity is one of the most important variables of the greenhouse climate that can be controlled. It conditions not only crop development and production but also energy requirements, which can account for up to 40 percent of the total production costs. The majority of plants grown in greenhouses are warm-season species, adapted to average temperatures in the range 17–27 °C, with approximate lower and upper limits of 10 and 35 °C. If the average minimum outside temperature is <10 °C, the greenhouse is likely to require heating, particularly at night. When the average maximum outside temperature is <27 °C, ventilation will prevent excessive internal temperatures during the day; however, if the average maximum temperature is >27–28 °C, artificial cooling may be necessary. The maximum greenhouse temperature should not exceed 30–35 °C for prolonged periods [3].

Greenhouses provide a suitable environment for the intensive production of various crops. They are designed to control solar radiation, temperature, humidity, and carbon dioxide levels in the aerial environment. The availability of solar radiation and it's daily and yearly distribution has a tremendous influence on productivity and quality of plant growth and also on comfort living [4]. Decreasing and pollution of natural water resources gradually as a result of global warming forces growers to use marginal quality waters in irrigated agriculture [5].

Since air temperature and humidity are the two significant parameters affecting thermal comfort significantly, and an evaporative cooling system can handle the only sensible load, the conventional evaporative cooling system is suitable for the dry and temperate climate where the humidity is low [6].

While there was greenhouse cultivation in 51 provinces in Turkey in 2011, by the year 2014 this number is 70 provinces. The increase rate in the last
4 years is above 15%, which is the annual average rate of increase with a rate of 72% [7].

Heating in greenhouses is only applied in the form of measures for the prevention of frost damage (such as roof sprinkler system, stove). The cover material can be PE (Polyethylene) or glass, but the use of insect net in ventilation openings is limited [8].

Indoor greenhouse conditions must continuously provide the temperature and humidity values suitable for the plants. Daily radiation requirements are approximately 6 hours for the plants to have sufficient photosynthesis in the greenhouse. In the northern hemisphere (November, October, January) and in the southern hemisphere, 500 - 550 hours of sunshine is needed in May, June, and July. For the growth, yield and quality of fruit and vegetables in the greenhouse, the greenhouse interior temperatures should not be below 12 °C and above 32 °C [9].

Heating is done in greenhouses built in recent years in Turkey. Heating in greenhouses increases product yield, quality, and quantity. Especially in the greenhouses, which are heated in the Mediterranean coastline, twice the yield increase can be achieved. However, the need for heat energy is increased in the greenhouses where heat saving measures are not taken and an average of 100 kWh.m⁻².a⁻¹ heat energy is needed depending on the climate values of the region during the production period. Increased energy costs cause the producer to begin questioning the profitability of heating. The reason for the conservation of greenhouses in heated greenhouses, as well as heating in the greenhouse, has been determined to be important in terms of profitability and energy efficiency [10].

A study conducted in Australia; researchers worked on the estimation of indoor temperatures by using artificial neural networks in the field of architecture. In order to find the most suitable network architecture, the outputs and inputs of the indoor audits were determined. Then, estimation models were developed to generate indoor temperature using the actual data in the spring [11].

Today's world agriculture sector is using plastics intensively (plastic cover materials, chemical fertilizers, and pesticide boxes, post-harvest wastes and drip irrigation laterals etc. Through conscious use and disposal of materials, especially like plastics the damages that agricultural wastes will pose on nature, soil and water resources will be minimized [12].

The indoor air temperature of greenhouses is an important parameter in environmental monitoring. The mathematical models for greenhouses do not apply to the characteristics of the dry and cold northern regions. In this study, the convection heat transfer coefficient was analyzed by mathematic modeling according to in-house weather conditions. If the weather conditions are known, the solution of the indoor temperature can be done using computer programming [13].

The intensive cultivation and excessive inorganic fertilizer application in greenhouse agriculture have caused serious soil environmental problems, such as high salinity, low nutrient availability, etc. To test whether drip irrigation combined with buried straw layer can improve the soil environment, an experiment was conducted from May to October in 2016 in Yunxiao, Fujian province, south of China. The overall results suggested that drip irrigation combined with buried straw layer could create a beneficial growth environment for crops under the greenhouse [14].

Researchers have explored the relations between outside and inside temperatures for several hundred houses in the Pacific Northwest. The analysis was initially considered as an effective tool for detecting inaccurate data, but afterward unusual elevations of internal temperatures are evaluated. As a result, analysis and modeling provide more realistic heating estimates for houses [15].

Greenhouses are high energy consuming structures and can carry on producing operations in cold seasons. Energy consumption in greenhouses constitutes 50% of greenhouse production costs. High energy consumption has become an important factor that prevents the development of greenhouses. It is important to estimate the energy consumption to increase the energy efficiency of the greenhouse.

Depending on results of a study in the basis of the energy-saving principle, the energy consumption of the Venlo greenhouse was developed. Three optimization algorithms have been used to define the parameters that are difficult to determine in the energy consumption model. By combining the greenhouse energy consumption estimation model with relatively accurate weather forecast data next week, the energy consumption of the greenhouse in different weather conditions is estimated. As a result, the average daily temperatures in the greenhouse have been reduced by 9% for the energy used for heating [16].

Indoor ambient temperature conditions are vital for people because people spend most of their time in the home environment. [17], a study conducted in Seoul, Korea, indoor temperature and relative humidity (RH) were measured continuously for 1 year in 14 houses. The relationship between the interior and exterior conditions of the house was determined by four meteorological parameters. These are Temperature, sensed temperature (AT), RH and absolute humidity (AH). In the study, the regression models between the indoor and outdoor temperature determined a heating threshold at 15.0 °C for the outdoor temperature. The indoor temperatures in the apartments were lower in the summer months and higher in the winter months than in the detached houses.
In various building applications, the use of artificial neural networks is gaining great popularity in predicting climate data such as internal temperature, heating load, and ventilation rate. Artificial neural networks have been developed in order to anticipate indoor air quality and energy efficiency of buildings. [18], the non-linear Autoregressive and ex-ternal entry (NNARX) model and genetic algorithm were used to perform external environment estimations. The comparison between the two methods was made and the applicability of some important mathematics and the validity criteria for practical reality were examined. Correlation coefficients of 0.998 and 0.997 were obtained with appropriate results [18].

A research conducted in a glass greenhouse having a Venlo natural ventilation system; researchers measured indoor and outdoor greenhouse temperatures. Accordingly, the difference between indoor and outdoor greenhouse temperature is strongly associated with incoming solar radiation and wind speed. From these results, a simplified model was established to estimate the greenhouse air temperature by knowing the greenhouse characteristic and external climate variables. The model is based on the energy balance of the greenhouse. It allowed the researchers to estimate the temperatures in the greenhouse [19].

In this study, in a greenhouse cited in Turkey’s Southeastern Anatolia Region, in Tigris Basin, Siirt province with a continental climate, (polyethylene) covered with 11220 m² area, indoor and outdoor meteorological values were measured every 30 minutes. All values were analyzed by statistical methods and predictions are made simultaneously with ANN (neural network) method on indoor temperature.

**MATERIALS AND METHODS**

This study is conducted in a greenhouse sited in Southeastern Anatolia, Tigris Basin in Siirt Province, City Center, Kilici Village, coordinated as 37°59’18.22”N and 41°46’15.43”E.

Climate Measure Device PCE-FWS 20-1 (Figure 2). This touchscreen device PCE-FWS 20-1, wind direction, wind speed, temperature, relative humidity, and precipitation in a sensitive way. The device was composed of two units, both inside and outside the greenhouse, and the measurements were taken every 30 minutes in the days of greenhouse production during the year. Data collected on a day by month basis was used for the months and the parts corresponding to the production periods were used. In order to determine the effect of minimum temperatures in greenhouses, cucumber plant used in production was taken, and information on climate requirements was obtained from literature [21].

It allows the system to recognize a predetermined output when inputs are changed in a system whose ANN inputs and outputs are considered as a computer program. Such problems are common in patterns, examples, characters, and so on. The basis of the ANN is the electrical model of the human nerve network. That is, the data included in one of the neural network inputs is multiplied by the weight of each input and then collected. If this total value exceeds a certain limit value, it is then transmitted to this total output. The problem here is to regulate the weights of the inputs to obtain the desired output at the end. The arrangement of the weights of inputs is carried out in many ways. Figure 3 shows the model of a simple ANN. In the figure $X_i$ input values of the network, $W_i$ weight of suitable input, $Y_i$ output values, $\sum$ showing the total value of input values added together and $\lceil$ shows the border function.
Generally, in ANN multiple layered programs are used. In such a model, there is an input layer, sub or hidden layer, and output layers [22]. Ann is the information system of many processors or neurons are connected solidly and non-linear layers. Most of the times ANN consists of three layers: the input layer, in input layer entries, are introduced into the network, hidden layer or layers, in this layer inputs are processed, and output layer, in the output layer, outputs are produced suitable for inputs.

The main advantage of the ANN technique from traditional methods is that it does not demand a clear, distinctive presentation of information about the complexity of the subject matter being investigated.

Usually, a sigmoidal function is used as a transport function to provide non-linear data transmission:

$$f(x_i) = \frac{1}{1 + e^{-(\lambda x_i)}}$$

Equality is (1) and shows the thresholding gain [23].

The results of the prediction model are analyzed by the coefficient of determination ($R^2$) and the mean squared error (MSE) (Equations (2) and (3), respectively):

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - \bar{y})^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2}$$

$$MSE = \frac{\sum_{i=1}^{n} (y_i - \bar{y})^2}{n}$$

Where $n$ is the number of observations, $y_i$ is the target, $\bar{y}$ is the average indoor temperature, $\tilde{y}$ is the predicted indoor temperature. The highest value of the $R^2$ and the lowest value of the MSE were considered in determining the best model.

RESULTS

Spring cultivation in Siirt province starts in the first week of January and the harvest period lasts until the end of June. The planting stage of seedlings covers a one-week period. Autumn cultivation; starts in the third week of August and continues until the first week of February. Siirt province shows terrestrial and arid climate characteristics. When the average temperature and day-to-day radiation values of the two provinces with different geographic features are examined, the need for heating in greenhouses for six months emerges because the average temperature values in Siirt province are below 10 °C in November-April period. In the spring production season of the 3-month heating period during Siirt province’s autumn production cycle, by June production is ended. It is seen that even if natural ventilation and shading materials are used in July and August, production cannot continue. The lowest temperature in October was October 11 at 04.35 am as 6 °C. The minimum temperature value in the greenhouse was realized as -2 °C in the second week of March. (Figure 4).

In this period, it was the fruit setting period for the cucumber plant, and it was outside the reference values of 15-25 °C. During the month the temperature value in total of 4 days decreased by 12-14 °C.
The period did not last long and there was no harm to the plants. In Siirt province, it is required to operate heating systems in greenhouses starting from the 2nd week of November. As the temperature values in the greenhouse are kept to a constant degree, the costs of the heating load will increase the cost of production. On the basis of autumn cultivation, the temperature in the months of November, December, especially for fruit set in cucumber plant temperature should be at least 15 °C and maximum 25 °C. In the third week of October and in the first two weeks of November, temperatures in the province of Siirt have decreased from 12 °C to 6 °C over a period of four weeks, and temperatures continue to decline in the coming period. Harvest time in autumn cultivation lasts 8 weeks and is carried out between the third week of November and the first three weeks of January. The increase in the temperature values in the greenhouse during the fruit ripening and presentation of the market leads to an increase in the cost. Therefore, measures such as the use of heat screens and shortening of ventilation times in greenhouses can be effective in reducing these costs. In terms of spring cultivation, during January and August, the temperature of the pollination and flowering period should be between 19-21 °C [21]. January refers to the harvest for the autumn cultivation, while it refers to the planting and flowering period for spring cultivation. The heating demand calculated in autumn cultivation is also valid for this period. However, it is known that the plants are tolerant to lower temperatures than the seedling period. There are currently greenhouses in the central and Kurtalan districts of Siirt. In order to reduce the amount of heating load of the greenhouse, it is concluded that it can be installed in places close to the geothermal areas or that it can convert the solar energy into electrical energy (Photovoltaic) and that could reduce the heating costs. In addition, heat curtains in the greenhouse will be able to affect the heat loss. Due to the Siirt province’s farming calendar Turkey's highest greenhouse production history for unlike autumn productivity from Antalya has a production area received two weeks early date, in terms of spring production of the receipt date passed two weeks, greenhouse domestic heating to reduce financial-feed their It is thought that it can have a positive effect. The descriptive statistics of the data obtained from the experiment are given in Table 1. The minimum temperature value measured during the day as internal temperature is taken into consideration here.

The climatic characteristics of the table show significant differences with respect to months (p <0.01). Thus, although there was no significant difference between the minimum temperatures in May and the last 4 days of October, there was no significant difference in terms of minimum temperature for other months except in December. The same commentary could be done for the outside temperature. However, while there was no significant difference between the minimum temperatures in February and December, there was a significant difference between February and March, April and November.

In the input layer used for training with ANN has 9 neurons, there are 5 neurons in the hidden layer and 1 neuron in the output layer and the feed-forward multi-layer neuron network is used. (Figure 5).

![Model scheme of ANN](image)


Since the determination of the number of neurons in the hidden layer is also a problem, it is recommended to evaluate the success by increasing this number starting from a small number of neurons (2 or 3) in applications [24].

The Levenberg-Marquardt algorithm was used as the training algorithm. Matlab Neural Network Toolbox interface is used for training and testing in ANN.

All input values for use in ANN are subject to normalization. The normalization process was carried out with the formula (4).

$$||x|| = \frac{x_i - x_{\text{min}}}{x_{\text{max}} - x_{\text{min}}}$$

A total of 8053 data obtained during the breeding months were randomly divided into 70% training, 15% validation and 15% test. Initially, the training data was trained in the ANN and then run with the system test data. The estimation values found at the end of the test were compared with the values we have (Figure 6). In Figure 6, the scattering graph between the projected internal temperature values and the observed internal temperature (Target) data was plotted and the correlation coefficient (R) was calculated.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Month</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>95% Confidence Interval for Mean</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoor Temperature**</td>
<td>February</td>
<td>18</td>
<td>6.78</td>
<td>b</td>
<td>3.06</td>
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<td>b</td>
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<td>2.80</td>
<td>13.8</td>
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<td>May</td>
<td>27</td>
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<td>a</td>
<td>2.77</td>
<td>7.16</td>
<td>17.3</td>
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<td></td>
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<td>4</td>
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<td>a</td>
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<td>9.16</td>
<td>12.9</td>
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<td></td>
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<td>30</td>
<td>8.13</td>
<td>b</td>
<td>3.28</td>
<td>5.93</td>
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<td></td>
<td>December</td>
<td>31</td>
<td>4.13</td>
<td>c</td>
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<td>5.39</td>
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**p<0.0
In the training and testing phases, transactions were performed so that no unused data was left. By changing the number of neurons in the hidden layer, the best ANN model was determined. Model performance results for a different number of hidden neurons was given in Table 2.

As a result, 25 neuron ANN model with the lowest mean square error value was chosen as the best estimating model.

**CONCLUSION**

Estimating the indoor temperature values of greenhouses can prevent economic losses for producers. Especially in the period of seedling plants have temperature sensitivity. According to the results of the study; alternative energy (geothermal) resources can be benefited from cultivation in places where it is considered appropriate. In order to produce the autumn period for greenhouses in Siirt, heating systems should be operated in greenhouses from the third week of November until the second week of December and January. In this period, it is very important to keep the temperature values in the greenhouse in constant degrees, so the costs of the heating load will increase the cost of production. In the spring season, the active and passive heating systems are required to be used in full performance for 8 weeks in February and March, as they include February and March flowering and fruit set.

In this period, the neglect of heating with economic concern may cause damage to the plant and
humidity estimates should be increased. In addition, the accuracy of indoor relative temperature estimation models, the ANN model improvement over other similar methods. The presented advantage of the model is the quick calculation and very good predictions with missing data. Another wall ratio, etc. data.

It does not require assumptions and can make very good predictions with missing data. Another advantage of the model is the quick calculation and continuous learning from real data. The presented model was compared with other similar methods. Future potential studies include further reduction of the forecast interval and expansion of the forecast horizon. In addition, the accuracy of indoor relative humidity estimates should be increased.

REFERENCES


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CHARACTERISTICS OF AIR POLLUTION IN THE CITY OF ERZURUM

presented in the 19th International MESAEP Symposium, Rome-Italy from October 04-06, 2017

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Department of Landscape Architecture, Faculty of Architecture and Design, Atatürk University, 25240 Erzurum /Turkey

ABSTRACT

It is known that air pollution known to have negative effects on environment and human health is an important factor on urban life. It is of vital importance in metropoles to determine emissions causing air pollution and know the levels of air quality generated by these emissions.

The present study was conducted in the city of Erzurum, the second most elevated city of Turkey. In order to determine changes in air pollution in the study area, measurements of (SO\(_2\)), (NO\(_2\)), (O\(_3\)), (H\(_2\)S), (HF), (HCl), (BENZEN), (TOLUEN) and (KSILEN) concentrations were carried out in winter, spring, summer and autumn 2013 simultaneously at 18 points for 15±day periods. The values obtained from these measurements were analysed spatially and distribution maps were produced using Geographical Information System (GIS) based on a data base. From the results, urban air pollution distribution maps were also drawn.

As for the results of the study, it was found from temporal and spatial distribution maps, representing air pollutant concentrations that there are significant differences between (SO\(_2\)) and (NO\(_2\)) concentrations depending on the local sources. It was also seen that one of the concentrations of the most important pollutant in the city, SO\(_2\), exceeded limit values in winter and combustion periods, in the attraction centres of the city in and around Havuzbaşi and Yenişehir Bridge. Furthermore, it was detected that an area so called Dağ Mahallesi, where dense use of fossil fuels with low calorie and high sulphur rate and lower amount of natural gas are observed, is among the zones where pollution concentration is dense.

KEYWORDS:
Air pollution, Erzurum, GIS, SO\(_2\), Urbanization

INTRODUCTION

Rapid unplanned urbanization, high influx of population, ever-increasing number of vehicles and industrialization amidst urban areas are among the major sources of air pollution. Smaller size particulate matter is recognised to cause adverse health effects (REF). Concentrations of air pollutants can build up to levels significant to cause adverse effects on plant, animal and human health [1]. As a result of developments on urbanization and especially increase in the city populations, air pollution is the leading environmental problem affecting human health and decreasing quality of life in especially cities.

Besides all other environmental problems, air pollution due to transportation is one of the most important problem in Erzurum, and this has affected the planning and developments. Transportation is a major contributor of principal greenhouse gas (carbon dioxide) and the atmosphere of Erzurum is polluted with carbon monoxide, hydrocarbons, oxides of nitrogen, lead, sulphur oxides and particulate matter. These pollutants have negative effects on environment and health [2, 3, 4, 5, 6, 7]. The sources of air pollution in Turkey, are industrial activities, fossil fuels, and exhaust gases. However, the main reasons triggering these sources are the uncontrolled population increase and consequently, urbanization leads to reduction or elimination of green spaces [8].

The main objective of this paper is to determine the character of air pollution in Erzurum city. For this purpose, (SO\(_2\)), (NO\(_2\)), (O\(_3\)), (H\(_2\)S), (HF), (HCl), (BENZEN), (TOLUEN) and (KSILEN) values were used and air pollution maps were created by Geographical Information System (GIS).

It is seen that air pollution known to have negative effects on environment and human health is an important factor on quality of urban life. It is of vital importance in metropoles to determine emissions causing air pollutions and know the levels of air quality generated by these emissions.

As the result of the developments witnessed in urbanization issues and the increase in the city populations in recent years, air pollution has become the leading environmental problem affecting human health and decreasing human quality of life in especially large cities.

When questioned the reasons of air pollution in Turkey, it is seen that this phenomenon results
from industrial facilities, fossil fuels, and exhaust gases. However, the main reasons triggering these processes may be uncontrolled population increase and concordantly urbanization increasing depending on the population and loses or harms in green spaces [8].

It is vitally important to monitor air quality in order to detect air pollutant sources and their spatial distribution, develop control strategies and follow their efficacy. Main air pollutants, which are possible to exist in the air outdoor in urban areas are carbon monoxide (CO), sulphur dioxide (SO\textsubscript{2}), particle materials (PM), ozone (O\textsubscript{3}), nitrogen oxides (NO\textsubscript{x}), and lead (Pb).

Air Quality Index (AQI) is used to report daily the air quality in the ambient air in which humans live. Such an index can inform people how clean/dirty the air is in their living environment and what type of health impacts they may face depending on the air quality. AQI may also give information about the health impacts of polluted air in hours or days when it is inhaled, based on a scale designed in a range from 0 to 500 intervals (Table 1). As the value increases pollution rate also increases and health problems are expected to be seen depending directly or indirectly on the index value [9]. AQI scale can be divided in 6 categories (Table 1).

<table>
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<tr>
<th>AQI Scale Value</th>
<th>Air Quality Index</th>
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<tr>
<td>1</td>
<td>0 - 50</td>
<td>Good</td>
</tr>
<tr>
<td>2</td>
<td>51 - 100</td>
<td>Moderate</td>
</tr>
<tr>
<td>3</td>
<td>101 - 150</td>
<td>Unhealthy for the sensitive groups</td>
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<td>6</td>
<td>301 - 500</td>
<td>Hazardous</td>
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</table>

AQI is calculated individually for each pollutant (ozone, particles, carbon monoxide, sulphur dioxide and nitrogen dioxide) in the mentioned location. The daily highest AQI value calculated is the AQI of the day in question [10].

The main objective of this paper is to determine the character of air pollution in Erzurum city. For this purpose, (SO\textsubscript{2}), (NO\textsubscript{2}), (O\textsubscript{3}), (H\textsubscript{2}S), (HF), (HCl), (BENZEN), (TOLUEN) and (KSILEN) values were used and air pollution maps were created by (GIS).

**TABLE 1**

<table>
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<th>Air Quality Index categories (WHO, 2005)</th>
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Erzurum is the largest and the coldest city in the northern temperate zone at the highest altitude. It has a surface area of 25.066 km\textsuperscript{2}, being the 4th largest province of Turkey in terms of surface area and covering 3.2% of the country’s whole area. Erzurum plain has bowl-shaped area which covers 825 km\textsuperscript{2} (Figure 2). It is surrounded by high mountain series with different heights, like Dumlu Mountain (3169 m.), Kargapazari Mountain (3045 m.) and Palandöken Mountain (3176 m.) in the south of the city. [11]. The city shelters nearly 800,000 people [12].

**FIGURE 1**

Location map of Erzurum city

Since the topography of the area, where the city is located, does not allow air movement at low rates to penetrate in the city, wind speed in especially winter season in the city centre is very weak in the periods out of frontal systems [13].

**FIGURE 2**

Topography of Erzurum city

**FIGURE 3**

Prevalent wind direction in Erzurum city
Wind seems to be an inefficient climate element to prevent air pollution since it is too weak to clear the polluted air prevalent in the city centre in winter season when the combustion activity is needed the most. Depending on the violent inversion formation in extremely cold winter density of air pollution increases (Figure 3).

In order to determine the spatial distribution of air pollution in the study area, (HCl), (BENZEN), (TOLUEN), (SO₂), (NO₂), (O₃), (H₂S), (HF), and (HF) were measured by Erzurum Clean Air Center (THM) in the body of Ministry of Environment and Urbanism Survey and Monitoring Department in winter, spring, summer and autumn periods in 2013 (Table 2). The data obtained were stored at the Erzurum Clean Air Centre, which also set up the inventory of sources of air pollutants in the province. The locations of monitoring stations are given in Figure 4.
GIS functions can be used to monitor, analyse, model and map air pollution. In the spatial distribution studies of air pollutants, spatial interpolation methods can be used to monitor the distribution of air pollutants in the urban atmosphere. The impact of industrial air pollutants and city topography on the urban atmosphere can be modelled using GIS [14, 15, 16]. In the “Ecological Corridor Scenario in Air Pollution” of the “Erzurum city” Project, (SO₂), (NO₂), (O₃), (H₂S), (HF), (HCl), (BENZEN) and (TOLUEN) measurements were obtained at 18 points by Erzurum Clean Air Center (THM) and distribution maps of pollution load, based on each pollutant, were prepared. For this purpose, Geographic Information Systems (GIS) was used as a tool. Location of monitoring sites were detected using ArcGIS 10.2 software (ESRI), based on the national coordinate system (ED-1950-UTM-Zone-37N). Interpolation - Inverse Distance Weighted (IDW) interpolation in Arc-Toolbox of ArcGIS 10.2 software was used to generate maps showing the most accurate distribution of Air Pollutant values in the field. IDW is an interpolation technique based on the principle that the closer locations play more important role in the estimation of a parameter. Spatial analyses were performed on the maps prepared based on this technique to determine the spatial distribution of (SO₂), (NO₂), (O₃), (H₂S), (HF), (HCl), (BENZEN), (TOLUEN).

RESULTS AND DISCUSSION

When the maps showing both temporal and spatial distribution of air pollution concentrations in Erzurum city are examined, significant differences are seen in (SO₂) and (NO₂) concentrations between monitoring sites. It was observed that (SO₂) concentrations, one of the most important air pollutants of the city, exceeded the limit value at Havuzbaşi and Yenişehir Bridge sites, which are the central business zones of the city, in winter combustion periods. It was also determined that Dağ Mahallesi site, which is one of the monitoring sites where low calorie and high sulphurcontent fuels are used densely and natural gas is used less, is among the places where the pollution rate is high (Figure 5-6). It was found in the study that due to the main transportation arteries without alternative, which is among the main problems of the city, (NO₂) concentration is the largest at Havuzbaşi ve Bosna Street sites (Figure 7). In addition, it was detected that (NO₂) concentration exceeds limit values on the main line connecting Yenişehir and Yıldızkent.
FIGURE 6
Spatial analysis map of (SO₂) concentrations for autumn 2013-2014

FIGURE 7
Spatial analysis map of (NO₂) concentrations for winter and autumn 2013-2014
FIGURE 8
Spatial analysis map of A: (O₃); B: (H₂S); C: (HF); D: (HCl); E: (BENZEN); F: (TOLUEN) concentrations for autumn 2013-2014
collective housing areas and Atatürk and Yavuz Sultan Selim boulevards. Concentrations of (O₃), (H₂S), (HF), (HCl), benzene and toluene were obtained using IDW location analysis method and the results were mapped to determine in which part of the city these concentrations are higher. Spatial distribution maps are presented in Figures 5-6-7-8 for the concentrations of (O₃), (H₂S), (HF), (HCl), benzene and toluene respectively.

CONCLUSION

In order to ensure the sustainability of habitats, pressures on earth’s natural resources like soil, water and air must be removed. Despite requirements of regulations on air quality, air pollutant concentrations are over limit values in Turkey caused mainly by the increase of emissions from industrial facilities that use low quality heating fuels as well as from vehicles exhaust gases. Rapid urbanization may be taken responsible for these pollutant sources and the reduction of green spaces, which are effective on the removal of their impacts.

In the present study, threshold values were exceeded at 3 monitoring sites Havuzbaşı, Yenisehir Bridge and Dağ Mahalleli, which are the central business zone, close to this zone and the point where low quality fuel is combusted for domestic heating. Elevations of the sites are very close to each other. Air pollution in the city does not result from the industrial production but combustion for domestic heating and traffic. Even in Erzurum, which is among the small size and unindustrialised city [13], air pollution may sometimes reach alarming levels. For that reason, in order to establish and develop cities by considering human health and comfort, all features of the areas including climatic and topographic structures, which are closely associated with the hazardous or risky phenomena like air pollution or air borne disasters.

Turkey has been in a rapid economic and urbanisation process over the last years. Such a process has some negative impacts on cities including the study area, where unplanned and distorted structuration is seen clearly. However, more environmentally friendly and climate sensitive spatial development should be considered by adopting the principles of city and landscape planning.

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Special Thanks to (give the name), Head of Measurement and Monitoring Department at the Ministry of Environment and Urbanism, for sharing the monitoring results of Erzurum city.

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