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Environmental Impacts of Wind Energy Applications: "Myth or Reality?"

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Summary

Wind energy is the fastest growing energy sector for electricity production in various European countries. A substantial wind power penetration is also expected in the Greek energy market. This significant number of new wind turbines provokes serious reaction of local people, pretending important environmental impacts. For this purpose, an introductory survey is carried out to validate the real size of the wind energy applications’ impact on human societies and local ecosystems. During the present investigation, several important parameters, like visual impact, noise emissions, avian mortality, land usage and energy payback period-materials’ requirements are taken into account. On the other hand, the wind energy contribution to air pollution reduction is also considered.

Keywords:
Wind Energy; Environmental Impact; Noise Emissions; Avian Mortality; Visual Impact; Air Pollution; Climate Change.

Introduction

Aeolus, the ancient Greek god of winds, used to push sail-ships and move windmills for ages. Nowadays, wind energy has been the galloping energy sector for electricity production in various European countries. Three European countries - Germany, Spain and Denmark - are among the world’s leading nations in the field of wind energy applications [1]. During the last five years, the development rate of installed capacity in individual countries varies between 15% and 75% per year (Figure 1). Thus, the original E.U. target for 4,000MW of wind power by 2000 has been almost doubled, while the new EWEA (European Wind Energy Association) target attains 40,000MW by 2010 and 100,000MW by 2020.

According to extensive wind potential studies all over Europe, the best wind resources are located in the upland regions of Ireland, Britain and Greece, where average wind speeds (at hub height) may overpass the 8+11m/s. More precisely, in the Aegean Archipelago - a remote Hellenic area at the east side of the mainland- there exist several islands, which, along with the mainland coasts, possess high wind potential [2].
On the other hand, during the last two decades, the electricity demand in Greece increases by 4% per annum. This continuous electrical energy consumption acceleration has hitherto been primarily covered by either imported oil or locally extracted lignite (Figure 2), thus strongly contributing to environmental deterioration [3]. At the same time, the electricity production cost for the majority of the remote Greek islands is extremely high [4], approaching the value of 0.25 Euro/kWh, while the fuel cost is responsible for almost 50% of the above-mentioned value. Additionally, Greek dependency on imported fuel (≈70% of its domestic energy consumption is imported) leads to a considerable exchange loss, especially with countries outside the E.U. [5].

Finally, in March 1997, the European Commission undertook the obligation to reduce total E.U. emissions of greenhouse gases (in comparison with 1990) by 8% before the year 2012. Wind energy provides one of the cheapest renewable energy opportunities, reducing CO$_2$ emissions caused by electricity generation [6].

**POSITION OF THE PROBLEM**

For all the above-mentioned reasons, the Greek State is strongly subsidizing private investments in the area of wind energy applications [7], either via the 2601/98-development law or the "Energy Operation Program" of the Ministry of Development. As a result, several requests for new wind parks of more than 10,000MW exist in the Ministry of Development, in an attempt to take advantage of the total costsubsidy of 40% for the project. Hence, during the last two years, a substantial increase (of more than 100%) of the existing wind power has been encountered, suddenly pushing the installed wind power of the country over the 250MW (Figure 3).

A supplementary characteristic concerning the new wind parks installed has been their strict concentration in two geographical regions (i.e. East Crete and S. Euboea), while considerable new installations are being planned for the area of Peloponnesos (Greek Regulatory Authority for Energy [8]). This significant number of remarkably sized (500kW to 1MW) contemporary wind turbines, suddenly installed in those relatively restricted geographical areas, provoke serious local population reactions [9], which in some cases may even lead to cancellation of the complete wind power project, claiming important environmental impacts.

In this socio-techno-economic context, the RAE (Greek Regulatory Authority for Energy) decides -via international tenders- which companies have the ability to develop power stations, on a pure fiscal criteria basis. In view of this significantly scheduled wind power penetration (more than 1200MW have been accepted by RAE) in the local energy market and despite the expanded negative attitude of local societies encountered [10], an introductory investigation of the principal environmental impacts on the local societies-ecosystems is carried out, along with the techno-economic analysis regularly presented in similar cases [7]. The results obtained may be useful in any decision taken in the area of the European and local energy planning [11].

Generally speaking, public opinion surveys on both sides of the Atlantic are in strong support of the wind ener-
Energy development [12]. Typically, two-thirds to three-fourths of those polled encourage wind development even in areas with existing wind turbines. Several states of USA, including California, Colorado, Michigan and Texas, defend the so-called "Green Power" program, concerning the electricity produced by a renewable (green-clean) energy source, see also [13]. Additionally, "Green marketing" is the practice where an electric utility (municipal or private) offers blocks of "Green Power" to customers to support the development of renewable resources. Customers arrange to purchase a certain amount of "Green Power" (actually energy in kilowatt-hours) per month, for which they commonly pay a small premium to completely or partly offset any higher cost of renewable power sources.

On the other hand, there also exist other groups that find wind turbines "huge and noisy industrial machines damaging local amenity". Besides, "visual intrusion" is one of the major factors determining opposition to wind energy (Figure 4). Many researchers believe [14-15] that people unconsciously realize that opposition on aesthetic grounds is subjective and is, therefore, often dismissed by public officials. They, then, rationalize their opposition by rising concerns such as noise, shadow flicker and birds, which can be objectively evaluated.

![FIGURE 3 - Installed wind power capacity in Greece.](image3.png)

![FIGURE 4 - Public opinion for various power production alternatives [14].](image4.png)
To be objective, depending on the landscape characteristics, modern wind turbines -with a hub height of 60-100 meters and a blade length of 30-50 meters- form a visual impact on the scenery. However, in any case that man places structures in a terrain, its character immediately changes. Besides, it is a matter of taste -to a large extent-how people perceive that wind turbines fit into the landscape. Numerous studies [9, 12] in many European countries revealed that people who live near wind turbines are generally more favorable towards them than city dwellers.

Another important aspect of wind turbines operation is the noise emission. From the human perception point of view, most people find it pleasant to listen to the sound of the waves at the seashore, called "white noise" (random emissions). On the contrary, a neighbor's radio produces some systematic content, which one's brain cannot avoid discerning and analyzing. If one generally dislikes his neighbor, he will no doubt be even more annoyed with that noise. That's why sound experts define "noise" as "unwanted sound". According to this example, it is easy to conclude that the annoyance by wind turbine noise emissions is also a highly psychological phenomenon.

Therefore, in an attempt to obtain an unambiguous picture concerning the size and importance of the main environmental impacts of wind energy installations, the following topics are examined.

**VISUAL IMPACT**

Water and windmills have been in operation, during the last 800 years, all over Europe. Recently, wind turbines revived the matter of landscape aesthetics. They have been subject to hard criticism because they are "a new element" and because they are located in highly visible places in order to exploit wind conditions. The reaction to the sight of a wind farm is highly subjective. Many people see them as a welcome symbol of clean energy, whereas some find them unwelcome additions to the landscape. Thus, although a wind plant is clearly a man-made structure, what it represents "may be seen either as a positive or as a negative addition" to the landscape.

As already mentioned, the attitude towards wind energy is usually positive [9, 12]. However, the knowledge that a wind turbine will actually exist within a five-miles distance from their home seems to make people slightly less positive, i.e. the "NIMBY" (Not In My Back Yard) phenomenon [16]. According to various researchers [10, 12] a negative view of wind turbines on the landscape is the major factor determining opposition to wind energy applications.

Taking the above-described piece of information seriously into account, the industry has devoted considerable effort to carefully integrate the development of new wind-parks into the landscape. Computer-generated photomontages, animations and even fly-through, together with mapped zones of visual influence, provide objective predictions of appearance, e.g. [17-18].

One of the most significant methods to improve public acceptance has been visual uniformity; i.e. the rotor, nacelle and tower of each machine look similar. They don’t need to be identical. Additionally, it is equally important all towers to be of consistent height, while steel towers are found more aesthetically pleasing than the lattice ones, more widely used in the U.S.A. Professional designers have been employed by several wind turbine manufacturers to enhance the appearance of their machines. Finally, if turbines are faulty, the public may perceive a wind farm to be unjustified -a waste of visual resources. Thus, when turbines do not operate or are perceived as often broken, the public is far less likely to tolerate the turbines intrusion on the landscape.

Finally, a more objective case of visual impact is the effects of the periodic reflections (glinting) or interruption (shadow flicker) of sunlight from the rotor blades [19]. Wind turbines, like other tall structures, will cast a shadow (or a reflection) on the neighboring area when the sun is visible. This is a problem only when turbines are sited very close to workplace or dwellings and occur during periods of direct sunlight. These effects may be easily predicted and avoided by carefully considering the machine-site and the surface finish of the blades. A common guideline used in N. Europe is a minimum distance of 6-8 rotor diameters between the wind turbine and the closest neighbour. A house, 300 meters from a contemporary 600kW machine with a rotor diameter of 40 meters, will be exposed to moving shadows approximately 17-18 hours (out of 8760h) annually.

**NOISE**

Sound emissions from wind turbines may have two different origins, i.e. mechanical noise and aerodynamic noise. Additional analysis reveals [20] that for most turbines with rotor diameters up to 20m the mechanical component is the dominant one, whereas for larger rotors the aerodynamic component is the significant one. More precisely, mechanical noise may originate in the gearbox, in the drive train (the shafts) and in the electrical generator of the wind turbine. It is true that machines constructed during the early 80s or earlier do emit some mechanical noise, which in most cases may be heard even up to a 200m distance from the turbine. Nowadays, no manufacturer considers mechanical noise as a problem any longer, since within five years mechanical noise emissions had dropped to half their previous level due to better engineering practices.

On the other hand, three main categories of aerodynamic noise sources [21-23] may be distinguished:

- Discrete low frequency noise at the blade passing frequency and its harmonics.
Self induced noise due to direct radiation by the attached boundary layer on the rotor blade, due to flow field separation at the blade trailing edge and finally due to trailing edge instabilities involving quasi-discrete frequencies.

- Broadband noise due to interaction between the inflow turbulence and the rotor.

For almost all-existing commercial wind turbines operating under normal conditions, the most significant noise source is the self–induced noise of the blades. However, for very large wind turbines the interaction of the atmospheric turbulence with the rotor can become predominant under certain conditions.

Generally speaking, no landscape is ever completely quiet, since birds, animals and human activities create sound. Thus, when the wind hits different objects at a certain speed, it will start making a sound. From a technical point of view, as wind speed approaches the 6-7m/sec, the noise from the wind in leaves, shrubs, trees, masts etc. (background noise), will gradually mask any potential sound from wind turbines, (Figure 5). Of course, sound reflection or absorption from terrain and building surfaces may change the sound picture in different locations. The wind rose is, therefore, important to chart the potential dispersion of sound in different directions.

The dB(A) scale, used by public authorities around the world, measures the sound intensity over the whole range of different audible frequencies. As a matter of fact, it uses a weighting scheme, which accounts for the fact that the human ear has a different sensitivity (better at medium -speech range- frequencies) to each different sound frequency. Besides, the dB-scale is a logarithmic one. This means, that as the sound pressure (or the energy in the sound) is doubled the dB index increases by approximately three points (e.g. from 97dB(A) to 100dB(A)).

Other parameters being equal, sound pressure will increase with the fifth (4th to 6th) power of the speed of the blade relative to the surrounding area [20]. That is why modern wind turbines with large rotor diameters have very low rotational speed (Figure 6).

On top of that, the energy in sound waves (and thus the sound intensity) will drop with the square of the distance from the sound source (Figure 7).

According to this fact, at one rotor diameter distance (~40m) from the base of a wind turbine emitting 100dB(A) one will generally have a sound level of 60dB(A), corresponding to a European clothes dryer, while four rotor diameters (170m) away one will have 44dB(A), corresponding to a quiet living room in a house.

Of course, in cases of two or more wind turbines located at the same distance from one’s ears, the sound energy will double, increasing thus the sound level by 3dB(A). One will actually need ten wind turbines placed at the same distance from the measurement point, in order to perceive that the subjective loudness has doubled. Finally, the fact that the human ear (and mind) discerns pure tones more easily than (random) white noise must be taken into account when doing sound estimates.

**FIGURE 5** - Background noise and turbine noise vs. wind speed [24].
Summarizing, sound pressure predicted or measured is typically around 96-101dB(A) (Figure 6) for commercial wind turbines. Thus, the sound pressure level at a distance of 40m from a typical machine is 50-60dB(A), about the same level as a conventional speech. A farm of ten wind turbines, with the nearest at a distance of 500m would create a sound level of about 42dB(A) under the same conditions, equivalent to the sound inside a quiet office.

Ten years ago, wind turbines were louder than they are today (Figure 8). Serious effort has been devoted for the creation of the present generation of quiet machines, paying detailed attention to both the design of the blades [26-27] to avoid boundary layer separation [28] and to mechanical parts of the machine. As a result, noise is a minor problem for modern carefully sited wind turbines.

FIGURE 6
Noise emission level by contemporary wind turbines, market data.

FIGURE 7
Noise emission changes vs. the distance from the wind turbine.

FIGURE 8
Wind turbine technology amelioration impact on noise emission [25].
**IMPACT ON BIRDS**

Birds often collide with structures that they cannot easily detect, like high voltage overhead lines, masts, poles and windows of buildings. More than a few are also killed by moving vehicles. Accordingly, the impact of wind turbines on birds can be divided into:

- Direct impact, including risk of collision and effect on the breeding success.
- Indirect impact, including effects caused by disturbance from the wind turbines (noise and visual disturbance).

Studies in Germany, the Netherlands, Denmark and the UK conclude [29] that wind turbines do not pose any substantial threat to birds, since bird mortality due to wind turbines is only a small fraction of background mortality.

In Figure 9, the estimated number of annual bird deaths in the Netherlands from various man-made causes is presented [19]. According to the results given, more than **three hundred times** as many birds die from collisions with moving vehicles than with wind turbines and **seventy times** as many are killed by hunters. A parallel study in Denmark has estimated the maximum level of birds’ collision with wind turbines to be in the range of 6-7 birds/turbine/year. Equivalently, 25,000 to 30,000 birds annually die from collision with wind turbines that produce enough electricity for 600,000 families. For comparison purposes, in fact, over one million birds are annually killed by traffic in Denmark.

Isolated examples have been reported concerning significant damages on specific species, like geese and waders as well as golden eagles. For example, approximately three thousand cumulative bird deaths are related to the 625MW of installed wind power capacity at Altamont Pass each year, including 39 golden eagles [30]. However, in this area a "wind wall" of turbines on lattice towers is literally closing off the pass, while during the early development stages of wind farms practically no measures are taken to avoid this problem.

Another negative example [31] is referred to the Spanish wind farm of Tarifa, near the Strait of Gibraltar, which is a major bird migration route. This problem could have been avoided if the special circumstances in this area had been properly taken into account during the planning process of the wind farm.

On the other hand, for the majority of wind power installations one can say that the birds get accustomed to wind turbines rather quickly and there are several examples of falcons nesting in cages mounted on wind turbine towers. Radar studies (during day and night) show [19] that birds tend to change their flight route some 100-200m upwind of the turbine and pass above or around it at a safe distance.

Summarizing, we can say that the "avian mortality" is a real problem for commercial wind power plants (e.g. one bird for 100MWh of electricity-consumption of 25 families) and every death is regretted. However, results should not be concluded by a few extreme cases of increased bird mortality. Besides, wind power industry and wind farm developers have taken into account this issue seriously, and normally exclude new installations from bird-sensitive locations.
LAND USE

The Achilles’ heel of wind energy has always been the charge that it is too land-intensive. It is true that wind energy is diffuse (~500W/m²), while collecting energy from wind requires turbines to be spread over a wide area. More precisely, turbines should be separated by at least five to ten rotor diameters, in order the wind strength to be reformed and the air turbulence created by one rotor not to harm another machine downwind.

Therefore, the amount of land needed varies from as little as 0.05km²/MW for California’s densely packed arrays of small old-fashioned wind turbines to the 0.15km²/MW found in the openly spaced wind plants of northern Europe. As a rule of thumb, wind farms require 0.08 to 0.13km²/MW or wind farm arrays occupy 50m² of land for every m² swept by the wind turbine’s rotor [32]. Onshore wind farms have the advantage of dual land use, since the 99% of the area occupied by a wind plant can be used for agriculture or remain as natural habitat. Furthermore, part of the installations can be made offshore.

As stated above, less than 5% of the wind park area would be physically occupied by wind turbines, electrical equipment and access roads. Wind turbine foundations, though about 50m in diameter, are normally completely buried, permitting any existing agricultural activity to extend right up to the tower base.

There is no evidence that wind farms interfere to any greater extent than this with arable or livestock farming. Modern wind plants use no more land than other means of energy generation, see also Table 1. For direct comparison between wind energy and fossil fuels, the total fuel cycle in each case must be taken into account. For example, a wind plant in a moderately strong wind regime will use far less land than a coal mine and a conventional power plant, producing the same amount of electricity during a 20-year period.

Effects on other terrestrial ecosystem primarily result from construction activity, land take and hydrological disruption. The scale of these effects will depend on the type of ecosystem, drainage, construction techniques & timing and restoration practice. On typical flat on-shore sites, installation does not to any significant level affect vegetation or fauna. In almost all E.U. countries wind power developers are obliged to minimize any disturbance of vegetation under construction of wind farms in combination with road works etc., on sensitive sites as mountainous sites and offshore.

ENERGY BALANCE AND MATERIALS REQUIREMENTS

Though wind turbines do use energy-intensive materials, such as steel, glass reinforced polyester (fiberglass), and concrete (for foundations), according to three separate European studies [19] they quickly repay the energy consumed in their construction. More precisely, modern wind turbines rapidly recover all the energy spent in manufacturing, installing, maintaining, and finally scrapping them. A typical wind farm reimburses its energy debt in 3 to 4 months, in contrast to photovoltaics that present an amortization time of almost seven years.

As expected, most of the energy used to manufacture the turbine is contained in the rotor and nacelle. But more than one-third of the total energy consumed by the wind turbine is contained in the concrete foundation and the tower of the machine. A detailed life-cycle analysis [19] of wind turbines is done by D.W.T.M.A., estimating the energy content in all components of a wind turbine, and the global energy content in all links of the production chain. The resulting estimated energy requirements of a typical Danish 600kW wind turbine during its 20-year lifetime are shown in Table 2.

Manufacturing a state of the art 600kW wind turbine takes 3.2TJ, taking into account everything, from producing raw material to installing a ready to operate machine, including 20 years of operation & maintenance and decommissioning. In suitable locations, the wind turbine will generate 1.1 to 1.4GWh per year in its projected 20-year useful life.

<table>
<thead>
<tr>
<th>Production Technology</th>
<th>Maximum Land Required</th>
<th>Minimum Land Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wind Energy Installation</td>
<td>V=6m/s</td>
<td>V=9.5m/s</td>
</tr>
<tr>
<td></td>
<td>1300m²</td>
<td>750m²</td>
</tr>
<tr>
<td></td>
<td>North Greece</td>
<td>Crete</td>
</tr>
<tr>
<td>Photovoltaic-Solar Station</td>
<td>(1400kWh/m²)</td>
<td>(1650kWh/m²)</td>
</tr>
<tr>
<td></td>
<td>2900m²</td>
<td>2200m²</td>
</tr>
<tr>
<td></td>
<td>Low Quality</td>
<td>Medium Quality</td>
</tr>
<tr>
<td>Lignite-Fired Thermal Power Station</td>
<td>Megalopolis</td>
<td>Ptolemaida</td>
</tr>
<tr>
<td></td>
<td>9500m²</td>
<td>6800m²</td>
</tr>
</tbody>
</table>

TABLE 1
Land required per GWh of electrical energy for a 20-year period in Greece.
TABLE 2
Specific energy demand during the operational life of a wind turbine.

<table>
<thead>
<tr>
<th>Process</th>
<th>Specific Energy (MWh/kW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacture</td>
<td>0.880</td>
</tr>
<tr>
<td>Installation</td>
<td>0.228</td>
</tr>
<tr>
<td>Operation &amp; Maintenance</td>
<td>0.358</td>
</tr>
<tr>
<td>Scrapping (Total)</td>
<td>-0.098</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1.368</strong></td>
</tr>
</tbody>
</table>

According to the results obtained, at good sites, wind turbines pay for the energy in their materials within the first three to four months. Even at poor sites, energy payback occurs in less than one year.

A more extensive study was carried out in Germany examining wind turbines from 10kW to 3MW in size [33]. The analysis shows that even small wind turbines of 10-30kW took only a year to recover the energy spent in manufacturing, installing and decommissioning them, while turbines of 55kW took some six months to recover the corresponding energy spent.

A recent detailed study [34] concerning the material inputs of a wind farm is carried out for the Baix-Ebre wind farm in Spain, based on a life-cycle environmental impact assessment (LCA). Baix-Ebre wind farm comprises 27x150kW turbines on a high mountain ridge of Catalonia. While caution must be exercised with regard to this approach, as materials inputs may not be strictly proportional to installed capacity, the weights per MW give useful approximate generalized estimates, which are more widely applicable.

From the data gathered, it is clear that the material inputs required for a wind farm are dominated by the concrete (reinforced) for the turbine foundations and by the steel from which the turbine towers are fabricated. It is conceivable that a wind farm could, on reaching the end of its operating life, be refurbished by installing new nacelles and rotors on top of the existing towers and foundations. This would reduce the material inputs required for the "second generation" wind farm by well over 80%.

Lastly, if there is sufficient demand for the secondary raw materials, wind turbines can be regarded as being mainly composed of recyclable materials. The principal unresolved issue from an environmental perspective is the recycling of rotor blades [35].

Water use is another significant issue in energy production, particularly in areas where water is scarce. Conventional power plants use large amounts of water for the condensing portion of their thermodynamic cycle. Small amounts of water are used to clean wind turbine rotor blades in arid climates, to eliminate dust and insect build up, which otherwise deforms the shape of the airfoil and degrades performance [36]. According to calculation results, wind power plants use less than 1/600 as much water per unit of electricity produced as the nuclear does and approximately 1/500 as much as coal [37].

Finally, decommissioning will include the removal of all above ground elements of the development as a minimum, as well as the restoration of the original site. In most cases, the decommissioning costs can be recovered from the scrap value of the turbines and copper wiring from the project. Indeed, another significant environmental benefit of wind energy is that wind turbines can easily be decommissioned, in comparison with other generating technologies.

WIND ENERGY IMPACT ON THE DIMINUTION OF AIR POLLUTION

Air pollutants are primarily emitted from the various energy transformation processes based on fossil fuels. Today SO$_2$, NO$_x$, CO and volatile organic compounds (VOCs) are considered as the basic air pollutants, along with the CO$_2$, which is the result of using carbon as a fuel. These major pollutants may cause detriment at very different concentration levels, according to their toxicity factors [3]. Figure 10 presents the time varying contribution of the electricity production sector on the national annual production of the above pollutants. As it is obvious from the data of Figure 10 electricity production is responsible for about 48% of the national CO$_2$ emissions, along with 68% of SO$_2$ and 20% of NO$_x$.

More specifically, according to recent research and official data [3,38], every MWh of electricity consumed in Greece is considered to be responsible for almost 18kgr of CO, 4.3kgr of NO$_x$, 6.4kgr of SO$_2$ and 1054kg of CO$_2$. This significant environmental surcharge is directly connected to the continuous fossil fuel consumption in order to meet the amplified energy requirements of Greek society. Similar results [19] are also valid (Table 3) for almost all E.U. country members.
El e ct r i ci t y S e ct o r C o n t r i b u t i o n t o A i r P o l l u t i o n i n Gr e e c e

![Electricity Sector Contribution to Air Pollution in Greece](image)

**FIGURE 10** - Electricity sector contribution to air pollution in Greece.

**TABLE 3** - Specific emissions (kg/MWh) from fossil-fuelled electricity plants vs. wind parks.

<table>
<thead>
<tr>
<th>Air Pollutant</th>
<th>Netherlands</th>
<th>UK</th>
<th>Denmark</th>
<th>Greece</th>
<th>Wind Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$</td>
<td>872</td>
<td>936-1079</td>
<td>850</td>
<td>1054</td>
<td>7</td>
</tr>
<tr>
<td>SO$_2$</td>
<td>0.38</td>
<td>14.0-16.4</td>
<td>2.9</td>
<td>6.4</td>
<td>0.087</td>
</tr>
<tr>
<td>NO$_x$</td>
<td>0.89</td>
<td>2.5-5.3</td>
<td>2.6</td>
<td>4.3</td>
<td>0.036</td>
</tr>
</tbody>
</table>

**TABLE 4** - CO$_2$ Emissions (kg/MWh) from various electricity production technologies [19].

<table>
<thead>
<tr>
<th>Technology</th>
<th>Fuel Extraction</th>
<th>Construction</th>
<th>Operation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coal-fired</td>
<td>1</td>
<td>1</td>
<td>962</td>
<td>964</td>
</tr>
<tr>
<td>Oil-fired</td>
<td>-</td>
<td>-</td>
<td>726</td>
<td>726</td>
</tr>
<tr>
<td>Gas-fired</td>
<td>-</td>
<td>-</td>
<td>484</td>
<td>484</td>
</tr>
<tr>
<td>Nuclear</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Wind</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Small Hydro</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>10</td>
</tr>
</tbody>
</table>

Global warming due to anthropogenic emissions (e.g., CO$_2$ and CH$_4$) is now generally accepted as a fact; hence the IPCC (Intergovernmental Panel on Climate Change) scientists expect major ecological changes. In the EU, approximately one third of CO$_2$ emissions come from electrical power generation; thus for every 1% of conventional generation capacity displaced by renewables, a 0.3% reduction of total CO$_2$ emissions is being achieved.

Recapitulating, in Table 4 one may compare [19] CO$_2$ emissions from a large variety of electricity generation technologies. Thus far, neither satisfactory nor commercially viable means of abating CO$_2$ emissions from fossil fuelled plants have been devised. Among the most commercially competitive technologies, wind energy and hydro power stations are assumed to be responsible for only 5-10kg CO$_2$ per MWh produced. On the other side, coal-fired stations produce more than 950kg CO$_2$/MWh, while almost 730kgr CO$_2$/MWh is attributed to oil-fired installations.

Finally, SO$_2$ and NO$_x$ are mainly responsible for acidification agents. The most important quantified effects of acid deposition are upon human health, building materi-
als, historical monuments and commercial forestry. Furthermore, there are major impacts upon ecosystems, both terrestrial and aquatic. According to damage costs derived using previous estimates of acidification [39], an optimistic value is approximately 6000 Euro per tonne of either SO$_2$ or NO$_x$. Besides, impacts are non-localized, as they may be experienced hundreds or even thousands of kilometres from the initial emission point. Comparing for example the SO$_2$ and NO$_x$ emissions from fossil-fuelled generating plants (Table 3) with those produced by wind parks (i.e. 0.087kgSO$_2$/MWh and 0.036kgNO$_x$/MWh) on a wind turbine life cycle basis, one may state that the specific emissions of wind energy production plants are only a very small percentage, respectively, to those from fossil-fuelled plants.

**CONCLUSIONS**

It is the author’s articulated opinion that wind energy is a sufficient, mature, cost-effective and widely applicable technology, especially for the Greek socio-economic situation. However, in some exceptional occasions, remarkable negative environmental events are encountered. In order to explain and validate the real impact of wind energy applications on the environment, an introductory investigation is carried out, including visual impact, noise emissions, avian mortality, land use etc. Subsequently, the energy amortization period and the material requirements of a typical wind converter are estimated.

- The main conclusions drawn from the above-presented study are that the wind energy applications, especially during their first steps, impose -in a degree- unnecessary annoyance on human societies and local ecosystems. These sparse accidents at no case characterize the contemporary wind energy technology. Besides, one should seriously take into consideration the undeniable contribution of wind energy to the air pollution prevention.

- In this context, wind energy developers and turbine manufacturers have realized a lot during the twenty-years of their participation in the wind potential exploitation all over the world. Modern wind turbines are more quiet, safer, respect the landscape aesthetics, while special attention is paid during new project planning.

For all the above-mentioned reasons, the society -if properly informed- eagerly supports the efforts of wind power sector to fulfill the electricity demand with clean energy. It is common belief that wind turbines are not inherently dangerous. Therefore, every aspect of a wind plant should convey the sense that wind energy is more benign than other forms of energy. Of course, wind industry should continue placing the same effort on being a good neighbor as on being aerodynamic efficient, in order not only to maintain but also to increase the public acceptance of wind energy applications, all over the world. Recapitulating, the increase of wind energy penetration in the local fuel-mix is going to ameliorate the existing environmental situation without invoking the long and short-term hazards of thermal and nuclear power stations.

**REFERENCES**


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THE INFLUENCE OF SEWAGE AND SLUDGE TREATMENT PROCESSES ON CONCENTRATIONS OF POLYCYCLIC AROMATIC HYDROCARBONS

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SUMMARY

The analysis of PAH concentration changes was carried out in samples of sewage flowing into an urban sewage-treatment plant in Częstochowa, Poland, after each stage of purification, and in samples of sludge obtained in each (unit process) of the treatment process. Average total concentration of PAHs analyzed in sewage was 5.2 µg/dm³ in crude stage, 89.1 µg/dm³ after the sand trap, 48.2 µg/dm³ after the primary settling tank, and 7.8 µg/dm³ after a secondary settling tank. Further, 4280 µg/dm³ of dried sludge mass (d.s.m.) was observed in crude sludge, 12800 µg/dm³ of d.s.m. after the separated sludge digestion chambers (SSDC), 6160 µg/dm³ of d.s.m. after the open sludge digestion chamber (OSDC), and 259 µg/dm³ of d.s.m. in dewatering sludge (pyrene only). Biological sewage treatment with secondary sedimentation decreased PAHs' concentration by 83%. Mostly such a decrease was the result of absorption of these compounds on particulates and their decomposition by microorganisms of activated sludge. Highest average total concentration of PAHs was found in the digested sludge, which may reflect the adsorption of PAHs onto particulate matter. After stabilization and filtration of sludge the PAHs content decreased, respectively, by ca 50% and 95%, which may be caused by volatilization, oxidation, and photooxidation processes of these compounds or their desorption to water from the sludge. This is consistent with the enhanced amount of PAHs in sewage after a sand trap.

KEYWORDS: polycyclic aromatic hydrocarbons, sludge, sewage, sewage treatment.

INTRODUCTION

The pollution of natural environment by polycyclic aromatic hydrocarbons (PAHs), compounds that possess carcinogenic and mutagenic properties [1-4], is widespread. Therefore, knowledge of environmental sources by PAHs and the transitions of these compounds in environment will form the basis of the eventual elimination of sources of pollution and diminishing their concentrations in environment. One source of PAHs in the environment is sewage and sludge. The management of sewage is an important problem. Proper management should enable the utilization of nutrient substances and micronutrients contained in it. On the other hand, it should protect the secondary contamination of environment by toxic substances, among them PAHs, present in sludge [2]. Main sources of PAHs in sludge are liquid industrial wastes and, to a less degree, domestic sewage. Concentrations of PAHs in domestic sewage [2] are about 1 µg/dm³.

Liquid industrial wastes can contain much higher amounts of these compounds [3] - even up to 40 mg/dm³. Among liquid industrial wastes, the highest concentrations of PAHs are in coke, refinery wastes, and coal processing plant and steel plant liquid wastes. Rain can influence the PAHs present in sludge, in that the rainwater may contain components absorbed from air as well as abrasion products of car tires and pavement [4,5]. The concentration of PAHs in sludge of an urban sewage-treatment plant depends on liquid industrial waste fraction and kind of sewage system (combined sewage system, separate sewage system) [6].

Moreover, PAHs can be synthesized by microorganisms in purification processes and in biotransformation of organic materials [7]. Therefore, contents of these com-
pounds in sludge found by different laboratories were determined in range 0.2-295 mg/kg of dry matter [7 - 9]. These values were obtained using varying analytical methods and differing recovery protocols [10] for a wide variety of analytes. Given this, we decided to analyze the PAH content changes in samples of sewage flowing into urban sewage-treatment plant in Częstochowa, in sewage after each stage of purification, and in samples of sludge obtained in each (unit process) sewage treatment process (Scheme).

SCHEME - Urban sewage-treatment plant in Częstochowa.
MATERIALS AND METHODS

Methodology of investigation

Sewage and sludge from an urban sewage-treatment plant in Częstochowa were selected for investigation of PAH contents (Scheme). This plant is a classical mechanical-biological treatment plant, consisting of activated sludge technology with additional chemical treatment for the removal of phosphorus compounds. We estimated that percentage of liquid industrial wastes flowing into the plant at 40% of total flow. The process of sludge treatment is carried out in separated sludge digestion chambers, and then in an open sludge digestion chamber. Primary sludge and excess sludge after mechanical thickening with flocculent addition is directed to the fermentation process. Digested sludge is dewatered on a filter-press also with flocculent addition.

The following sewage samples were collected: crude sewage, before sand trap; sewage after sand trap, after settling tanks after primary sedimentation, and after secondary settling tanks after biological treatment and secondary sedimentation. The following sludge samples were collected: fresh sludge (primary) from primary settling tanks, digested sludge from separated sludge digestion chambers (SSDC), digested sludge from SSDC, additionally stabilized in open sludge digestion chamber (OSDC), dewatered sludge after a filter-press.

Analytical methodology

All solvents were purchased from PPH (POCh - Gliwice) and were of chemical purity, solvents used for chromatography were of chromatographic purity and purchased from Aldrich. From sewage and sludge samples, PAHs were extracted with cyclohexane using an ultrasonic method. Extract was decanted and used for chromatographic analysis. For qualitative and quantitative determination of PAHs, a gas chromatograph equipped with flame-ionization detector (GC-FID) was used. Sixteen PAHs were analyzed: acenaphthene, acenaphthylene, anthracene, benzo(a)-anthracene benzo(a)pyrene, benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(ghi)perylene, chrysene, dibenzo(ah) anthracene, phenanthrene, fluoranthene, fluorene, indeno [1,2,3-cd]pyrene, naphthalene, pyrene [1, 11]. Comparison was made to analytical standards, A PAH calibration mix (Catalog No 4-7940 = U, Supelco, Bellefonte, PA, containing 10 µg/mL of each PAH).

RESULTS AND DISCUSSION

Only six of sixteen investigated PAHs were found in the samples of sewage analyzed and the concentrations of these compounds varied considerably (Table 1). The lowest content of aromatic hydrocarbons determined was found in crude sewage flowing into sewage-treatment plant. In this sewage the presence of only two compounds, i.e., naphthalene (2.0 µg/dm³) and pyrene (3.2 µg/dm³) were found. The highest concentration of PAHs was found in the sewage after a sand trap. The sum of six PAHs determined was 89.1 µg/dm³, and 68% of this amount was pyrene. A small amount (0.9 µg/dm³) of benzo(a)anthracene, that is considered as carcinogenic, was also found.

In the sewage after primary sedimentation the concentration of the compounds studied was lower than after a sand trap, and was 48.2 µg/dm³. In this sewage sample the following four compounds were found: anthracene, fluoranthene, naphthalene and pyrene. The highest concentration found was of pyrene (41.5 µg/dm³), which was about 86% of a sum of four PAHs determined.

Analyzing a process of sedimentation in a primary settling tank, the total content of PAHs in sewage discharged from a settling tank was lower than in sewage supplied to this installation by 45% (content of PAHs decreased from 89.1 µg/dm³, to 48.2 µg/dm³).

In sewage after a secondary settling tank the presence of only naphthalene and pyrene in concentrations of 3.7 µg/dm³ and 4.1 µg/dm³, respectively, were found. Therefore, it can be stated that during biological treatment and secondary sedimentation, there was a concentration decrease of the PAHs analyzed in sewage by 83% (from 48.2 µg/dm³ to 7.8 µg/dm³).

Based on the results obtained, no single sludge sample contained all sixteen PAHs. Concentrations of the compounds determined in these samples were diverse (Table 2). In fresh sludge from primary settling tanks seven PAHs, anthracene, benzo(a)anthracene, phenanthrene, fluoranthene, fluorene, naphthalene, pyrene, were found. The highest concentration found was of naphthalene, i.e., 964.1 µg/kg of dried sludge mass (d.s.m.). In the sludge the presence of only one cancer suspect agent, i.e., benzo(a)anthracene, was found. It was present in an amount of 472.2 µg/kg of d.s.m. The highest concentration of PAHs was present in the digested sludge from the separated sludge digestion chambers. Here the amount was 12800 µg/kg of d.s.m. The most prominent among the five of compounds (anthracene, benzo(g,h,i) perylene, phenanthrene, fluoranthene, naphthalene) determined was naphthalene. Its concentration was 7649 µg/kg of d.s.m., which was 60% of the sum of five PAHs. In this sludge sample was found the most carcinogenic compound, i.e., benzo(g,h,i) perylene, in amount of 139.3 µg/kg of d.s.m. Comparing a kind and amounts of PAHs in the digested sludge from the separated digestion chambers with fresh sludge, it can be stated that the concentration of fluoranthene and naphthalene increased by 657% and 693%, respectively, while the concentrations of anthracene and phenanthrene decreased in this sludge by 87% and 81%, respectively. In the samples of this digested sludge benzo(g,h,i) perylene was found, which was absent in
TABLE 1 - Contents of PAHs in sewage.

<table>
<thead>
<tr>
<th>No.</th>
<th>Determined hydrocarbon</th>
<th>Contents of PAHs, µg/dm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude sewage</td>
</tr>
<tr>
<td>1</td>
<td>Anthracene</td>
<td>n.f.</td>
</tr>
<tr>
<td>2</td>
<td>Benzo(a)anthracene</td>
<td>n.f.</td>
</tr>
<tr>
<td>3</td>
<td>Phenanthrene</td>
<td>n.f.</td>
</tr>
<tr>
<td>4</td>
<td>Fluoranthene</td>
<td>n.f.</td>
</tr>
<tr>
<td>5</td>
<td>Naphthalene</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>Pyrene</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Σ PAH</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>Σ cancigenic PAH</td>
<td>n.f.</td>
</tr>
</tbody>
</table>

n.f. - not found

TABLE 2 - Contents of PAHs in sewage sludge.

<table>
<thead>
<tr>
<th>No.</th>
<th>Determined hydrocarbon</th>
<th>Contents of PAHs, µg/kg of d.s.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude sludge</td>
</tr>
<tr>
<td>1</td>
<td>Anthracene</td>
<td>768.9</td>
</tr>
<tr>
<td>2</td>
<td>Benzo(a)anthracene</td>
<td>472.2</td>
</tr>
<tr>
<td>3</td>
<td>Benzo(g,h,i)perylene</td>
<td>n.f.</td>
</tr>
<tr>
<td>4</td>
<td>Phenanthrene</td>
<td>620.7</td>
</tr>
<tr>
<td>5</td>
<td>Fluoranthene</td>
<td>639.1</td>
</tr>
<tr>
<td>6</td>
<td>Fluorene</td>
<td>385.6</td>
</tr>
<tr>
<td>7</td>
<td>Indeno[1,2,3-c,d]pyrene</td>
<td>n.f.</td>
</tr>
<tr>
<td>8</td>
<td>Naphthalene</td>
<td>964.1</td>
</tr>
<tr>
<td>9</td>
<td>Pyrene</td>
<td>427.7</td>
</tr>
<tr>
<td></td>
<td>Σ PAH</td>
<td>4280</td>
</tr>
<tr>
<td></td>
<td>Σ cancigenic PAH</td>
<td>472.2</td>
</tr>
</tbody>
</table>

n.f. - not found

the fresh sludge. These large increases in PAHs concentrations in the digested sludge may be caused by biotransformation of these compounds by microorganisms during the digestion process. Because of sludge stabilization in a separated digestion chamber, the total concentration of the five PAHs decreased from 12800 to 6160 µg/kg of d.s.m., i.e., by ca 50% (Table 2). Such a loss of total PAHs contents is attributed to the decrease of anthracene, benzo(g,h,i)perylene and phenanthrene contents to the detection limit of the method used for fluoranthene and naphthalene by 84 and 82%, respectively. Despite the considerable decrease of the total PAHs contents the presence of indeno[1,2,3-c,d]pyrene and benzo(a)-anthracene, not detected previously, was found in the sludge samples after the open digestion chamber. In sludge after dewatering, among the sixteen PAHs investigated, only one was found. It was pyrene in amount of 259 µg/kg of d.s.m., which amount in relation to the contents in sludge after OSDC decreased by over 95%. Based on the results obtained, the mass balance possibility of the compounds determined was considered. It should be emphasized, that the system of sewage and sludge treatment is a continuous system, and the sewage and sludge exhibit a variable chemical composition. This arises from the variable proportion between domestic sewage, liquid industrial wastes and rain water, flowing into a treatment plant, all of which may influence the PAHs present in the sewage and sludge. Therefore, preparation of such balance requires instrumentation and broad analysis of both the technological lines (Scheme). Also the possibility of PAHs volatilizing to air should be considered and validated in each technological process, and the determination of PAHs contents in the material used for aiding the sewage and sludge treatment processes.

CONCLUSIONS

Basing on the results obtained in this study it can be said, that polycyclic aromatic hydrocarbons were present in the investigated sewage and sludge. Their average total amounts in the sewage were 5.2 µg/dm$^3$ in the crude
stage, 89.1 µg/dm$^3$ after sand trap, 48.2 µg/dm$^3$ after the primary settling tank and 7.8 µg/dm$^3$ after a secondary settling tank. Biological sewage treatment with secondary sedimentation decreased PAHs concentration by 83%. That was caused probably by absorption of these compounds on particulates and their decomposition by microorganisms of activated sludge. In sludge samples, among the sixteen PAHs investigated, only nine were found. Total concentrations of PAHs were 4280 µg/kg of d.s.m. in fresh sludge, 12800 µg/kg of d.s.m. in sludge after SSDC, 6160 µg/kg of d.s.m. in sludge after OSDC and in the dewatering sludge only pyrene in amount 259 µg/kg of d.s.m. was found. Highest average total concentration of PAHs was found in digested sludge, which may be caused by biotransformation of organic compounds by microorganisms. After sludge stabilization in a separated sludge digestion chamber the PAHs content decreased by ca 50%, which may be caused by volatilization, oxidation or photooxidation processes of these compounds. After a filter-press the contents of PAHs decreased by over 95%, that may be caused by desorption of carbohydrates from particulates to water over sludge and change of these compounds’ solubility in the presence of flocculent added for dewatering. This can be confirmed indirectly by the enhanced amount of PAHs in the sewage after the sand trap in relation to PAHs in crude sewage (water over sludge after filtration is recovering before a sand trap).

We also found that in this biological sewage treatment plant the PAH concentrations diminished after each step of purification. The exception was the sewage after the sand trap, where PAH concentrations increased 28 times. A possible interpretation is that during filtration considerable amounts of PAHs caused contamination of the sand trap. This phenomenon is probably connected with fractionation of sewage and will be the aim of further investigations at this and other sewage treatment plants.

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TREATMENT OF WASTEWATER FROM CAR WASHES BY ULTRAFILTRATION

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SUMMARY

This study evaluates the effectiveness of ultrafiltration (UF) process for the treatment of wastewater from car washes. The tubular membranes used in UF pilot plant were made from polyvinylidene fluoride (PVDF), poly(vinyl chloride) (PVC) and polyacrylonitrile (PAN) with MWCO of 100 kDa, 70 kDa and 50 kDa, respectively. The retention of the different chemicals varied greatly. These membranes exhibit 100% retention of suspended solids and produce the permeate with the content of oil and grease of less than 5 ppm. The flux and COD retention, when treating the wastewater from car washes with PVDF membrane, were 50-60 L/m²h and 60 %, respectively. The UF permeates have Silt Density Index (SDI) below 3, that renders them suitable for further treatment by nano-filtration (NF) using the spiral-wound membranes.

KEYWORDS: ultrafiltration, oily wastewater, tubular membranes, Silt Density Index.

INTRODUCTION

The discharge of water containing dispersed oil is of environmental concern in a number of industries, including: produced water from oil recovery, the discharge of bilge water from ships, metal working cutting fluids, and a range of food industry effluents [1]. Due to the presence of petroleum hydrocarbons, this wastewater is considered as a hazardous industrial waste and requires further treatment prior to discharge into the municipal sewers. The same concern is also about the wastewater from car washing stations, where an average of 600 dm³ of wastewater is generated per car [2].

Apart from the chemicals used in the washing process the wastewater from car washes contains: free oil, oil/water emulsion, grease and particles such as dust, carbon, asphalt and salt, which are washed off from the surface of the car. The wastewater may also contain hydraulic fluid and motor oil that have leaked out from the breaking system and engine. Therefore, the composition of wastewater is complex. In most cases the wastewater is collected in a separation tank, where the oil is separated from the water phase. The water phase is commonly sent to a municipal sewage treatment plant prior to final discharge. The reduction in gravimetrical separation efficiency of mineral oil in the separation tank creates a problem when the traditionally used petroleum solutions are replaced by micro-emulsion. The Polish environmental authorities demand that the content of mineral oil in the discharged effluent does not exceed 10 ppm, that is sometimes difficult to achieve with emulsions stabilized by degreasing agents.

To solve the problems with treatment of such a complex wastewater from car washes various separation techniques such as flocculation, biological treatment and the use of hydrocyclones have been studied [3].

The high cost of conventional effluent treatment plants makes membrane filtration a viable approach for the treatment of wastewater from car washes, where large volumes of water are used and where the recovery of water or by-products is possible.

Membranes are used to separate a liquid into two streams the concentrate and the permeate. Membrane filtration plants use a semi permeable material which acts

wash, an emulsifier (degreasing agent) solution is sprayed over the car body. The degreasing chemical desorbs the dirt covering the surface of the car body and solubilizes the dirt to remove it from the surface. In the last stage, the car is rinsed with water and hydrophobic chemical is added. The purpose of the hydrophobic chemical is to facilitate water drain-off. This stage often includes the addition of wax, as a final stage in the washing of the car.
as a barrier to particles of a particular size. By applying a pressure across this barrier, particles are driven from one side to the other. The material left behind in the filtration system is known as the retentate, or concentrate, while the liquid which flows on the other side of the membrane barrier is referred to as the permeate, or filtrate. Where wastewater is being cleaned, the membrane is used to remove molecules of unwanted chemicals. These are subsequently collected prior to disposal. If purification or recovery of material is desired, the same process can be applied. However, this time, the material of value is collected and recovered. Product recovery can take place from either the permeate or the retentate depending upon the application.

Membrane filtration can be divided into four broad groups, each determined by the size of particle which can be retained by the membrane material [4]. These range from reverse osmosis (RO) which provides the finest level of filtration, through nanofiltration (NF) and ultrafiltration (UF) which uses the coarsest of membranes. Ultrafiltration, which can separate particles up to a few tenths of a micron in diameter of different molecular weights, is widely used in the industry. Membrane filtration offers a number of benefits in the treatment of wastewater from car washes. Benefits offered by membrane filtration include volume reduction of aqueous wastes, production of high quality water which could be recycled for three washing stages, and recovery of degreasing chemicals used in the washing process.

Companies, including PCI, have developed a wide range of membrane materials and geometries. This means that a plant can be built to match the process flow of wastewater of car washes exactly. Tubular membranes for example are particularly robust. They are generally able to withstand aggressive chemicals and solvent cleaners and can handle suspended solids without blocking. This means that high throughput and performance can be achieved.

The objective of the investigation presented in this paper was to study the flux and retention of different types of chemical products used in car washes during the UF process. Three tubular UF membranes with different molecular weight cut-off (MWCO) were used in this investigation.

### MATERIALS AND METHODS

**Wastewater characteristics**

Wastewater was collected from car washing station in Szczecin. The composition of wastewater from car washes is very complex and includes a mixture of free oil, oil/water emulsion, and various inorganic and organic components. The parameters of wastewater from car washing are listed in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspended solids, mg/L</td>
<td>73</td>
</tr>
<tr>
<td>TOC, mg/L</td>
<td>970</td>
</tr>
<tr>
<td>Oil and grease, ppm</td>
<td>113</td>
</tr>
<tr>
<td>COD, mg/L</td>
<td>2230</td>
</tr>
<tr>
<td>Conductivity, µS/cm</td>
<td>740</td>
</tr>
<tr>
<td>TDS, ppm</td>
<td>538</td>
</tr>
<tr>
<td>pH</td>
<td>8.38</td>
</tr>
</tbody>
</table>

**Characterization of ultrafiltration membranes**

Three different tubular membranes made from PVDF, PVC and PAN were used for the determination of the water permeability and retention characteristics for dextran solutions. PVDF tubular membranes type FP 100 were purchased from PCI Membrane Systems. The membranes with an internal diameter of 0.0125 m and length of 1.2 m were mounted in B1 module (membrane area 0.9 m²). Tubular membranes from PVC and PAN with an internal diameter of 0.025 m and a length of 2 m (membrane area 0.15 m²) were prepared in our laboratory by the phase inversion method [5]. All ultrafiltration experiments were performed in the UF unit. A schematic diagram of UF pilot plant is illustrated in Fig.1. The ultrafiltration system consists of a 200L stainless steel feed tank. The feed temperature was controlled by heat exchanger. The feed flow rate was adjusted with the use of by-pass valve and the throttle valves located downstream of the UF modules.

The water permeability measurements were performed with RO permeate (χ=10µS/cm) as the feed. The flux across the membrane was calculated from \( F = \frac{V}{A \cdot T} \) where \( F \) is the rate of liquid flow across the membrane (L/m²h), \( A \) is the membrane surface area in contact with the liquid (m²), \( T \) is the run time of the experiment (h), and \( V \) is the volume of the permeate collected during time \( T \) (L). The transmembrane pressures in the range from 0.5 to 4.0 bars were used. All measurements were performed at 25°C.

The retention characteristic of ultrafiltration membranes is usually presented in the form of a molecular weight cut-off. MWCO is identical with the molecular weight of a tested substance that has a predefined retention, e.g. 90%. During the characterization tests it is necessary to choose appropriate operating conditions in order to minimize the polarization phenomena. The best operating conditions are low pressure, high recirculation rate (high turbulence), and very low solute concentrations. The choice of a solute with good chemical and conformational stability, without any physical or chemical interaction with the membrane, is another important factor. However, the membrane manufacturers use a wide range of test substances (dextrans, polyethylene glycols, proteins, etc.), as well as different test conditions [6].
FIGURE 1 - Schematic diagram of UF pilot plant.
1-feed tank, 2-pump, 3-by-pass valve, 4-module, 5-throttle valve, 6-pressure gauge, 7-rotameter, 8-flowmeter, 9-heat exchanger, 10-temperature regulator, 11-permeate tank.

Detailed information regarding test substances, test conditions or definition of MWCO is not found in manufacturer specification sheets. These missing or differing specifications make a reliable membrane comparison on the basis of manufacturer information impossible.

The test solutions used in our laboratory for the determination of MWCO had the following composition: distilled water 98.8 wt. %, dextran 1.0 wt. %, and sodium azide 0.2 wt. %, added to the solution to prevent bacterial growth. Industrial grade dextrans (Polfa, Poland) with molecular weights in the range of 5-100 kDa were employed without further purification. The measurements were performed at transmembrane pressure of 1 bar, the other conditions were the same as for the permeability tests.

The ultrafiltration of a testing solution with the lowest molecular weight dextran was run for 4 h in zero recovery (the retentate and the permeate were recirculated), then the feed and permeate samples were collected for analysis. After accomplishing this test, the UF installation was flushed with distilled water, and a subsequent solution of dextran with a higher molecular weight was tested in the same manner. The testing procedure was repeated for the consecutive dextran solutions.

The retention of different components was calculated by comparing the concentration of the substance in the permeate and in the feed, as follows:

\[ R = \left(1 - \frac{C_P}{C_F}\right) \times 100 \]  

where \( R \) is the retention (%), \( C_P \) is the concentration in the permeate, and \( C_F \) is the concentration in the feed.

Treatment of wastewater by UF system

The wastewater from car washing station was treated by UF process using the pilot plant shown in Fig. 1. The performance of UF membranes during the treatment of wastewater were studied in terms of flux, removal efficiency in relation to the membrane characteristics, and the values of Silt Density Index achieved for the permeates. Two modes of wastewater treatment with ultrafiltration were evaluated in the presented studies. Tests for the determination of flux stability during 50 h of wastewater treatment were carried out at the transmembrane pressure of 2 bar with constant feed concentration (the streams of both retentate and permeate were returned to the feed tank (Fig. 1, broken line). The other test was performed with permeate further purified by nanofiltration. The NF process was run on the pilot unit (PCI Membrane Systems) designed for pilot scale work. The permeate flux from PVDF membrane (FP 100) during the UF pretreatment was at the same level as NF permeate flux (at 20 bar), hence, these two processes were run in a continuous mode. During these tests, the permeate flux for each type of membrane was measured using a rotameter, and the samples of the permeate and feed were collected for the analysis. The pollution parameter reductions were calculated from eq. 1.

Methods of analysis

The wastewater from car washing station as well as the resulting permeates and retentates were analysed for their ionic content (conductivity, total dissolved solids (TDS), pH (Ultrameter 6P MYRON L), total organic carbon (TOC) (Analyzer multi N/K, Analytic Jena), oil
and lubricant (OCMA 310 Horiba analyser), chemical oxygen demand (COD) and suspended solids by the procedure outlined in standard methods [7]. The measurement of the SDI has been carried out with a filtration cell, which was connected to a pressure vessel such that the cell can be filled during filtration by keeping the pressure at 2 bars. Membrane filters used were 0.45 µm Millipore filters (0.047 m in diameter) of type HAWPO4700. For each measurement the pressure vessel and the filtration cell were filled with the feed water to be investigated. Both were sealed, and the pressure of 2 bars was applied and continuously adjusted to maintain for the total filtration time. The feed water flows through the membrane filter and the initial time \( T_i \) in seconds required to collect the 0.5L sample was measured. The filtration was then continued at 2 bars for 15 min (total elapsed test time \( T_t \)) and the time \( T_f \) in seconds required to collect the second 0.5L sample after test time \( T_t \) was measured. The SDI is calculated by using the equation below:

\[
SDI = \frac{1 - T_i/T_f}{T_t} \times 100
\]

(2)

**RESULTS AND DISCUSSION**

Separation and transport properties of PVDF, PVC and PAN membranes

The characterization of the ultrafiltration membranes made from PVDF, PVC and PAN was performed by the measurements of the retention of dextrans with different molecular weights. The resulting retention curves for dextran solutions are presented in Fig 2. The slope of the curves for both PVC and PAN membranes indicates that these membranes possess a diffusive cut-off corresponding to a wide pore size distribution. However, the commercial membrane FP 100 exhibits a rather narrow pore size distribution, which is reflected by a low retention of dextran 10 kDa and a high retention of dextran 40 kDa. MWCO determined from the retention curves amounts 50, 70 and 100 kDa for PAN, PVC and PVDF membranes, respectively. Moreover, the higher the MWCO the larger is the permeate flux of the respective membrane.

The water permeability results obtained for UF membranes as a function of the transmembrane pressure are shown in Fig. 3. The linear relationship between water flux and the transmembrane pressures was obtained for PAN and PVDF membrane. PVC membrane exhibits a deviation from linearity at higher pressures, namely, the increase of pressure from 3 to 4 bars results in the increase of permeate flux amounting half of that for the same gradient of the pressure but from a lower pressure region. This decline of the water permeability for PVC membranes can be explained by the compression of larger pores that have the major contribution to the membrane permeability at pressures exceeding 3 bars.
FIGURE 4 - Permeate flux stability during UF treatment of wastewater from car washes (oil content 113 ppm).

FIGURE 5 - Average Silt Density Index of UF permeates from PVDF, PAN and PVC membranes.

FIGURE 6 - Reduction of respective parameters achieved by PVDF membrane in UF treatment of wastewater from car washing station (t=25°C, p=2 bar).
Effectiveness of UF treatment of wastewater from car washing station.

The ultrafiltration process of wastewater obtained from car washing station was carried out within the range of process parameters recommended for oil emulsions. All UF experiments were performed using the tubular membranes. One of the major benefits of tubular membrane design is its ability to cope with high levels of suspended solids without the need for any prefiltration. Therefore, UF treatment of wastewater from car washing station should effectively reduce such parameters as the content of oil and grease, COD, TOC, and result in the complete rejection of the suspended solids. However, the retention of TDS should be very low. The UF pretreatment should allow to obtain the permeate at a level of the SDI required for spiral-wound elements. The performance of UF process in the treatment of wastewater is illustrated in Figs 4-6.

The UF process with properly selected membranes (FP 100) is very effective for the removal of oil and grease (>98%) and suspended solids (100%) from wastewater obtained from a car washing station. The study demonstrated that UF does not reduce the COD in the permeate to a level required by environmental authorities. The retention of TDS by UF membranes was below 5% over the entire pressure range used in UF process. The use of membranes with lower MWCO (50 and 70 kDa) does not result in a significantly lower value of SDI (Fig. 5) which is probably caused by a rather wide pore size distribution for these membranes. There was no significant difference in the retention between the membranes although the difference in cut-off was rather large. The flux for wastewater collected at the car washing station was 50-60 dm³/m²/h during the treatment by UF process. The studies demonstrate that contaminants such as oil, grease and suspended solids, which are retained by the UF membranes, can be removed from wastewater from car washing stations. Water and chemical compounds that pass through the UF membranes can be reused.

CONCLUSION

The application of UF membranes with MWCO in the range of 50-100 kDa results in the complete reduction of suspended solids and high retention of oil and lubricant (98-99%). The UF membranes demonstrate good flux stability during the treatment of wastewater from car washing station. The UF membranes do not reduce the COD in the permeate to the level demanded by environmental authorities.

The treatment of wastewater from car washing station by UF membranes allow to obtain the SDI value at a level recommended by manufacturers of spiral-wound elements for nanofiltration. The obtained UF permeates are suitable for further purification in NF process with spiral-wound elements. An outstanding advantage of the proposed combination of the membrane processes: UF and NF is the complete removal of oil and greases, TOC, and the reduction of TDS and COD by more than 70%, which allow to reuse the permeate obtained in the car washing process.

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CHROMIUM EFFECTS ON

*Anguilla anguilla* LIVER ORGAN CULTURE

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SUMMARY

One *Anguilla anguilla* L. weighing 500 g was injected intraperitoneally (i.p.) with 4 mg/Kg β-naphthoflavaone (BNF). The liver was excised after 24 hours and cultured in Minimal Essential Medium (MEM) for 24 hours in four different conditions: serum free (MEM-S); serum free with potassium dichromate 1 mM (MEM-S+Cr); serum free with potassium dichromate 1 mM (MEM+S+Cr); serum and potassium dichromate 1 mM (MEM+S). Liver ethoxyresorufin-O-de-ethylase (EROD) activity, cytochrome P450 (P450) content and glutathione-S-transferase (GST) activity were determined. Liver EROD activity significantly increases after 24 hours in organ culture either in MEM-S or MEM+S, compared to 0 hour level. However, tissue cultured in media with chromium, presents a highly significant EROD activity decrease compared to 0 hour. Nevertheless, serum addition to the culture media significantly prevents liver EROD activity decrease caused by chromium, when compared to MEM-S+Cr. The previous results demonstrate serum’s protective effect against chromium EROD inhibition in liver organ culture. Liver P450 content was not significantly changed under different experimental conditions. GST activity in MEM+S was significantly higher than in MEM-S. Liver GST activity significantly increased in MEM-S+Cr compared to MEM-S.

**KEYWORDS:** Anguilla, Cytochrome P450, EROD, GST, Organ Culture, Chromium.

INTRODUCTION

Among heavy metals, chromium VI (Cr(VI)) is a widespread toxic chemical, present in industrial effluents and wastes. Cr(VI) is isostuctural with sulphate and phosphate [1] allowing it to cross membranes and enter cells readily reacting with intracellular reductants originating unstable radical species which can induce lipid peroxidation as well as a number of genotoxic and carcinogen effects [2].

Fish liver is generally the organ where many absorbed aquatic xenobiotics go through a critical metabolic process of activation (oxidation), mediated by cytochrome P450-associated monoxygenases, and inactivation mediated by conjugases, allowing xenobiotics excretion and elimination. According to Santos and Pacheco (2000) in *vivo* *Anguilla anguilla* L. liver cultures are good ecotoxicological models for biotransformation studies since in *vivo* liver EROD induction is maintained and increased from 0 up to 24 hours, after previous *in vivo* BNF induction [3]. Organ cultures involve "the maintenance or growth of tissues, organ primordia or the whole or parts of an organ *in vitro* for a period of 24 hours or longer, in a way which may allow differentiation and/or preservation of architecture and/or function" [4]. These *in vitro* systems, used for toxicological research, have advantages such as the utilisation of a small number of animals, controlled experimental conditions, genetic heterogeneity removal, small quantities of test chemicals and amount of toxic wastes [3]. Despite the successful use of organ culture, concerning biochemical and toxicological research, fish organ culture has not been extensively used.

The present research work was designed to study, the Cr(VI) effects on eel liver organ cultures (24 hours) cytochrome P450 (P450) content, EROD and GST activities after 24 hours *in vivo* i.p. injection with BNF (4 mg/kg).

**MATERIALS AND METHODS**

**Animals:** One adult eel (500 g) caught at the Aveiro Lagoon and acclimated to laboratory conditions in 70 L aquarium containing aerated and filtered freshwater at 20 °C during 7 days. The fish was neither fed during recovery nor during the experimental period.

**Chemicals:** Culture Media - Minimum Essential Medium (MEM), Foetal Calf Serum (S), HEPES, β-naphthoflavone (BNF), 7-ethoxyresorufin, resorufin, potassium dichromate (K₂Cr₂O₇), NADPH, Nistatin and Streptomy-
cin-Penicillin were all purchased at Sigma Co (St. Louis, MO), E. Merck-Darmstadt (Germany), Boeringer Mannheim (Germany) and Gibco (Scotland).

**Experimental Conditions:** One eel was i.p. injected with 4 mg/kg β-Naphthoflavone (BNF) and killed 24 hours later. The liver was removed, cut into small cubes (2x2 mm) and cultured during 24 hours in the following way: 1) MEM–Serum (MEM-S); 2) MEM+S; 3) MEM–S+K2Cr2O7 1 mM (MEM-S+Cr); 4) MEM+S+Cr. Immediately after liver sampling and before organ culture initiation, a small number of liver pieces were frozen in liquid nitrogen. Anguilla anguilla L. liver organ cultures were stopped 24 hours later and the tissues were collected, frozen in liquid nitrogen and stored at −80 ºC until homogenization.

**Biochemical Analyses:**

- **EROD assay - Liver microsomes** were obtained according to the methods of Lange and co-workers [5] and Monod and Vindimian [6] as adapted by Pacheco and Santos [7]. Liver EROD activity was measured as described by Burke and Mayer [8]. EROD activity is expressed as picomoles per minute per milligram of microsomal protein.

- **Liver Cytochrome P450 Content - Liver P450 content** was quantified by measuring the 490- to 450-nm absorbance spectrum as described by Hermens and co-workers [9]. P450 content was expressed as nanomoles per milligram of microsomal protein.

- **Liver GST determination - GST activity** in the liver cytosol was determined with CDNB as substrate according to Habig and co-workers [10] at 25 ºC. GST activity was expressed as nanomoles per minute per milligram cytosolic protein.

Measurements of protein concentration - Microsomal and cytosolic protein contents were determined according to the Biuret method [11] using bovine serum albumin as standard.

**Statistical Analysis:** The results were expressed as mean ± standard error (SE) and statistical analysis was performed using a two-tailed Student t test [12].

**RESULTS**

*Anguilla anguilla* L. liver organ cultures significantly increased (P<0.001) their EROD activity from 0 up to 24 hours (Fig. 1) either in MEM-S or MEM+S. Culture media with or without serum, containing Cr(VI) 1 mM significantly inhibited EROD activity (P<0.001) in the eel liver organ cultures (approximately 93 % and 98%). Liver EROD activity in MEM+S+Cr is significantly higher (P<0.01) than in MEM-S+Cr.

However, *Anguilla anguilla* L. P450 content remained constant in all the liver organ culture conditions studied (Fig. 2).

Furthermore, GST activity decreased in MEM-S compared with 0 hours-control tissue (P<0.05) (Fig. 3), despite its significant increase (P<0.01) in MEM–S+Cr liver organ culture compared to 0 hours-control tissue.

**FIGURE 1** – Liver EROD activity after *in vivo* i.p. exposure to 4 mg/Kg BNF at 0 hours *in vitro* sample/control; liver organ culture in MEM-S, MEM+S, MEM–S+Cr and MEM+S+Cr. Values represent mean ± SE. **** P<0.001 difference from 0 hours/Control; ♂ ♂ ♂ ♂ P<0.001 difference from MEM-S; ♠ ♠ ♠ ♠ ♠ P<0.001 difference from MEM + S; ♦ ♦ ♦ P<0.01 difference from MEM–S+Cr 1 mM.
DISCUSSION AND CONCLUSION

Liver Anguilla anguilla L organ cultures either in MEM-S or MEM+S significantly increased their EROD activity from 0 up to 24 hours. Our experimental results agree with Santos and Pacheco (2000) previous findings where in vivo i.p. injection or water diluted BNF significantly increased liver EROD activity induction in vitro, from 0 up to 0.5, 4 and 24 hours, when compared to their controls. Liver exposed to Cr 1 mM in organ culture media, significantly inhibited liver EROD activity either with serum (93 %) or without serum (98 %). MEM serum proteins seem to prevent the cell uptake and protect it from Cr (VI) inhibitory effect since liver EROD activity in liver organ cultures is significantly higher in MEM+S+Cr than in MEM-S+Cr. The effect of chemicals on cellular processes is dependent on cells external environment composition and their capability to enter the cell. However, Anguilla anguilla L. liver P450 content remained constant in all the organ culture conditions studied. According to George’s (1989) studies, cadmium injection in European plaice showed a reduced EROD activity and a decrease in enzymatic protein rather than a catalytic inhibition [13]. Liver organ culture EROD inhibition by chromium is not associated with a cytochrome P450 decrease. Viarengo and co-workers’ (1997) studies with copper and mercury have shown that these metals may inhibit liver EROD in fish by direct binding to exposed sulphydryl groups [14].

GST activity decreased in MEM-S compared with 0-hour control tissue. However, in MEM-S+Cr liver organ culture a significant increase in GST activity was observed compared to MEM-S and 0-hour control tissue, suggesting Cr(VI) GST induction. Glutathione S-transferases play a
major role in protecting against the toxic effects of electrophilic and genotoxic compounds, as well as reactive intermediate metabolites produced during cellular oxidative processes [15].

Biotransforming enzymes in aquatic species are usually good indicators of environmental contamination by PAHs. Therefore, several studies have successfully used MFO activity in the liver [16] as well as pollutant levels in water and their metabolites in the gallbladder as biomarkers with a good correlation between them. However, the presence of heavy metals may inhibit EROD activity [14].

Anguilla anguilla L. Liver organ culture proved to be an important alternative to in vivo biotransformation studies. The above experiment demonstrates that liver organ culture biotransformation enzymes such as EROD activity is well preserved and increases either with or without serum, whereas GST seems to decrease in the absence of serum. However, culture medium composition (-S or +S) is very important and may interfere with the cellular uptake of contaminants such as Cr(VI). Serum demonstrated to be very important as a liver organ culture EROD activity protector against Cr (VI) present in MEM. Therefore, water contamination by high levels of chromium associated with organic compounds may cause low levels of liver EROD activity induction. Thus, since heavy metals (and other compounds) may inhibit EROD activity, great care should be taken when using liver EROD activity as PAH biomarker in environmental monitoring.

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CONCENTRATIONS OF HEAVY METALS IN EUROPEAN BATS (MICROCHIROPTERA)

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SUMMARY

Kidney samples of 150 bats belonging to 16 European species, which were found dead or moribund in Styria (Austria) were analysed for residues of 5 heavy metals (Pb, Cd, Hg, Cu, Zn). Tissue concentrations of the essential elements Cu and Zn were relatively high compared with the non-essential and potentially toxic elements Pb, Cd and Hg. The concentrations varied between species (except Cu), age, year of collection and collection site, but not between sex of the animals. Juvenile bats showed higher tissue concentrations for Pb and Zn than the adult bats.

KEYWORDS:
Bats, Microchiroptera, heavy metals, kidney, accumulation.

INTRODUCTION

Heavy metals exist ubiquitously, naturally or being released into the environment by anthropogenic sources. Some of the elements, especially lead, cadmium or (organ)mercury, are known to accumulate along the food chain and to exert toxic effects on higher trophic levels when critical tissue concentration is attained. The accumulation of heavy metals in the tissue of wild vertebrates is related to the availability of these elements in the foraging habitat. The contamination by environmental pollutants is often discussed as one of the reasons for the remarkable decline of bats during the past decades. Insects constitute to be the only dietary source of bat species occurring in Austria. Therefore, the daily consumption of insects feeding on other invertebrates and/or plants allows the passage and accumulation of environmental contaminants in bats. The presence of metal contaminants in the inner organs of bats is relatively poorly documented. Only the papers by Streit & Nagel [1, 2] give data about European bats on that topic. An overview on the tissue concentrations of heavy metal residues was given for North American bats [3-6] and for Japanese bats [7]. The reason for the few references available on the effects of heavy metals in bats, might be seen in the fact that due to the small body size of these animals the detection limits of the former analytical techniques were too high. Therefore, many authors used either pooled organ samples or whole body analyses. Both methods were not able to yield an overview of the individual tissue concentrations of selected organs. The development of flameless AAS as well as the mercury-hydride technique lowered the detection limits continuously and allow the analysis of very small samples like the individual organs of the bats. However, as a consequence of the decline of bats, it becomes difficult to obtain samples. In this study we choose to investigate the individual kidney concentrations of five heavy metals in bats. The kidney was selected, because it is known to be an organ that accumulates the heavy metals like cadmium in its tissue.

MATERIALS AND METHODS

Organ samples of 150 animals were obtained during necropsies of bats found dead or having been euthanised due to serious injuries in the Bat Rescue and Nursing Station in Styria. The bats belonged to 16 species, all of them members of the family Vespertilionidae with the exception of Rhinolophus hipposideros (Rhinolophidae). All bats are endangered species and protected by law in Austria. Table 1 gives an overview on the species and numbers of individuals analysed.

The sampling period was from November 1996 to August 2000. The samples were frozen immediately and kept at –20 °C until analyses for lead, cadmium, mercury, copper and zinc at the Research Institute for Wildlife Ecology at the University of Veterinary Medicine Vienna.

Bats were classified as juvenile, if the low degree of ossification of the epiphyses allowed to conclude that the animal was at the maximum one year old. Also the conditions of the teeth and the patagium were considered, as juvenile animals should show low wear in both cases.
**TABLE 1 - Species, sex and age of the bats.**

<table>
<thead>
<tr>
<th>Species (scientific name)</th>
<th>male, adult</th>
<th>female, adult</th>
<th>male, juvenile</th>
<th>female, juvenile</th>
<th>Sample size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbastella barbastellus</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eptesicus nilssonii</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eptesicus serotinus</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Hypsugo savii</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Myotis daubentonii</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myotis emarginatus</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Myotis mystacinus</td>
<td>6</td>
<td>11</td>
<td>4</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Myotis nattereri</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Nyctalus noctula</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Pipistrellus kuhlii</td>
<td>9</td>
<td>13</td>
<td>1</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Pipistrellus pipistrellus</td>
<td>12</td>
<td>16</td>
<td>4</td>
<td>11</td>
<td>43</td>
</tr>
<tr>
<td>Plecotus auritus</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Plecotus austriacus</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Rhinolophus hipposideros</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Vespertilio murinus</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>58</td>
<td>18</td>
<td>22</td>
<td>150</td>
</tr>
</tbody>
</table>

For chemical analysis the kidney samples were thawed and individually weighed in glass test tubes for digestion in 5 ml nitric acid (about 30%) for 2 hours on a 110°C-hot plate. Deionized water was added to bring the final volume to 10 ml. The solution was transferred to PE tubes for storage until analysis. Lead, cadmium and copper were determined by flameless AAS (Perkin-Elmer 4100 ZL with autosampler AS 71), mercury by hydride-technique (Perkin-Elmer AAS 5000 with MHS-1) and zinc with flame atomic absorption spectrophotometer (Perkin-Elmer AAS 3030 B). Each sample was measured at least two times.

**RESULTS AND DISCUSSION**

**Lead**

Lead could be detected in all kidney samples, the concentrations ranged from 0.131 to 15.85 ppm (Table 3). Species including juvenile bats with relatively small kidney masses had larger concentration ranges than species including only adult bats. Adult bats showed only half of the lead tissue concentrations of juvenile bats, but there was no significant difference between the sex of the bats. One explanation might be that bats seem to have a serious problem with the supply of enough calcium for the offspring [8]: During a quite short period bats need a relatively high amount of calcium for dentification and skeleton development. This demand in calcium can only be met with the amount contained in the milk. However, the lactating bats cannot cover their need in calcium, neither with drinking water nor with their food, as calcium concentrations in insects are low. Therefore, it is assumed that lactating females cover the demand of calcium of the offspring by liberating calcium from their own skeleton, as some lactating females showed signs of osteoporosis in jaw and wing bones. Lead behaves similar to calcium in the bones and can be stored there. Under certain circumstances like stress, lactation or even diseases, lead contained in the bones is mobilized together with the calcium. Thus young bats seem to be more susceptible to lead contamination due to this mechanism, especially during suckling.

**STATISTICAL ANALYSIS**

All measurements were highly skewed to the right. Due to this nonnormal distribution we used exclusively nonparametric statistics, therefore, median and limit ranges of the interquartile area are given instead of arithmetic mean and standard deviation. Nonparametric Kruskal-Wallis analysis of variance by ranks and Mann-Whitney two-sample t-test were applied to data for significant differences at the 0.05 level. All data are given in ppm (mg/kg) on a wet weight basis. Values below the detection limit were considered as zero for statistical processing. For comparison with references found in the literature, the arithmetic mean is also listed in tables (Tables 2-7) indicating the heavy metal concentrations.
TABLE 2 - Concentrations of heavy metals in the kidneys of bats.

<table>
<thead>
<tr>
<th>Metal</th>
<th>n</th>
<th>Median</th>
<th>25 Percentile</th>
<th>75 Percentile</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>150</td>
<td>0.859</td>
<td>0.496</td>
<td>1.312</td>
<td>0.131</td>
<td>15.854</td>
<td>1.256</td>
</tr>
<tr>
<td>Cd</td>
<td>150</td>
<td>0.175</td>
<td>0.063</td>
<td>0.626</td>
<td>n.d.</td>
<td>6.052</td>
<td>0.530</td>
</tr>
<tr>
<td>Hg</td>
<td>150</td>
<td>0.329</td>
<td>0.153</td>
<td>0.694</td>
<td>n.d.</td>
<td>5.308</td>
<td>0.580</td>
</tr>
<tr>
<td>Cu</td>
<td>149</td>
<td>7.60</td>
<td>5.41</td>
<td>11.32</td>
<td>2.29</td>
<td>118.51</td>
<td>10.01</td>
</tr>
<tr>
<td>Zn</td>
<td>149</td>
<td>53.2</td>
<td>32.5</td>
<td>127.7</td>
<td>13.0</td>
<td>820.9</td>
<td>104.3</td>
</tr>
</tbody>
</table>

* below detection limit

TABLE 3 - Lead concentrations in kidney tissue of the different bat species.

<table>
<thead>
<tr>
<th>Bat species</th>
<th>n</th>
<th>Median</th>
<th>25 Percentile</th>
<th>75 Percentile</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eptesicus serotinus</td>
<td>10</td>
<td>0.373</td>
<td>0.267</td>
<td>0.762</td>
<td>0.174</td>
<td>0.962</td>
<td>0.461</td>
</tr>
<tr>
<td>Myotis emarginatus</td>
<td>11</td>
<td>1.209</td>
<td>0.615</td>
<td>2.138</td>
<td>0.457</td>
<td>3.500</td>
<td>1.407</td>
</tr>
<tr>
<td>Myotis mystacinus</td>
<td>26</td>
<td>1.167</td>
<td>0.712</td>
<td>2.161</td>
<td>0.131</td>
<td>13.810</td>
<td>1.937</td>
</tr>
<tr>
<td>Nyctalus noctula</td>
<td>5</td>
<td>0.406</td>
<td>0.356</td>
<td>0.528</td>
<td>0.312</td>
<td>0.879</td>
<td>0.482</td>
</tr>
<tr>
<td>Pipistrellus kuhlii</td>
<td>23</td>
<td>0.864</td>
<td>0.676</td>
<td>1.106</td>
<td>0.416</td>
<td>2.550</td>
<td>0.994</td>
</tr>
<tr>
<td>Pipistrellus pipistrellus</td>
<td>43</td>
<td>0.957</td>
<td>0.672</td>
<td>1.524</td>
<td>0.385</td>
<td>15.854</td>
<td>1.517</td>
</tr>
<tr>
<td>Rhinolophus hipposideros</td>
<td>5</td>
<td>0.847</td>
<td>0.430</td>
<td>1.371</td>
<td>0.374</td>
<td>2.318</td>
<td>1.016</td>
</tr>
<tr>
<td>Vespertilio murinus</td>
<td>6</td>
<td>0.676</td>
<td>0.414</td>
<td>1.092</td>
<td>0.335</td>
<td>1.415</td>
<td>0.772</td>
</tr>
</tbody>
</table>

* in the Tables 3-7 the results for species with less than 5 specimens are not listed.

TABLE 4 - Cadmium concentrations in kidney tissue of the different bat species.

<table>
<thead>
<tr>
<th>Bat species</th>
<th>n</th>
<th>Median</th>
<th>25 Percentile</th>
<th>75 Percentile</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eptesicus serotinus</td>
<td>10</td>
<td>0.108</td>
<td>0.056</td>
<td>0.343</td>
<td>n.d.</td>
<td>1.974</td>
<td>0.354</td>
</tr>
<tr>
<td>Myotis emarginatus</td>
<td>11</td>
<td>0.035</td>
<td>0.005</td>
<td>0.051</td>
<td>0.001</td>
<td>2.914</td>
<td>0.293</td>
</tr>
<tr>
<td>Myotis mystacinus</td>
<td>26</td>
<td>0.304</td>
<td>0.040</td>
<td>0.825</td>
<td>n.d.</td>
<td>6.052</td>
<td>0.689</td>
</tr>
<tr>
<td>Nyctalus noctula</td>
<td>5</td>
<td>0.192</td>
<td>0.100</td>
<td>0.825</td>
<td>0.077</td>
<td>1.290</td>
<td>0.408</td>
</tr>
<tr>
<td>Pipistrellus kuhlii</td>
<td>23</td>
<td>0.202</td>
<td>0.099</td>
<td>0.361</td>
<td>0.007</td>
<td>4.000</td>
<td>0.447</td>
</tr>
<tr>
<td>Pipistrellus pipistrellus</td>
<td>43</td>
<td>0.147</td>
<td>0.078</td>
<td>0.740</td>
<td>0.014</td>
<td>2.817</td>
<td>0.537</td>
</tr>
<tr>
<td>Rhinolophus hipposideros</td>
<td>5</td>
<td>0.103</td>
<td>0.030</td>
<td>0.279</td>
<td>0.003</td>
<td>0.388</td>
<td>0.144</td>
</tr>
<tr>
<td>Vespertilio murinus</td>
<td>6</td>
<td>0.363</td>
<td>0.217</td>
<td>0.725</td>
<td>0.215</td>
<td>1.307</td>
<td>0.500</td>
</tr>
</tbody>
</table>

* below detection limit

Cadmium

Mammals accumulate cadmium in the liver and, especially, in kidney tissue, during their life. As cadmium does not cross the placenta, the organs of newborns normally do not contain detectable amounts of this element. Cadmium tissue levels rise therefore usually with the individual age. Bats’ kidney cadmium levels demonstrated a number of significant differences between species (Table 4) and age groups.

The highest cadmium concentrations (medians) were found in bats belonging to the species of *Vespertilio murinus* (Parti-coloured bat) and *Myotis mystacinus* (Whiskered bat). The Parti-coloured bats analysed were all adult individuals, while the Whiskered bat group included about 50% juveniles. One should expect higher tissue concentrations in the case of adult animals, especially when considering that some bats like *M. mystacinus* are relatively...
long living animals in relation to their body size, as they can live up to 23 years. Juvenile bats had concentrations about a tenth of those of the adults. Therefore, the group of *Myotis emarginatus* (Geoffroy’s bat) which contained up to 90% of juvenile individuals showed one of the lowest cadmium levels among the analysed species.

No statistically significant differences in kidney tissue concentrations could be found regarding the sex and the collection site of the bats.

**Mercury**

Mercury kidney tissue levels ranged from below detection limit (17 samples) to 3.2 ppm (Table 5). No statistically significant differences could be observed considering age and sex of the bats. Median tissue concentrations were the highest for *Myotis mystacinus*. The median tissue concentrations of this species was more than twice the median tissue concentration of the other bats. Maximum kidney tissue concentrations in European bats were higher than those found in the carcasses of American *Eptesicus fuscus* (Big brown bat) by O’Shea et al. [9] but considering toxic mercury kidney concentrations given by Wren et al. [10], there is no indication of a potentially harmful situation for bats foraging in Styria. The tissue concentrations found might be partially due to the former use of mercury containing seed dressings, which were commonly applied in agriculture. A few years ago this kind of chemical seed protection treatment was banned in Austria because of the ecotoxicological effects.

**Copper**

As an element occurring in different enzymes copper is an essential trace element whose concentration in the body is normally under metabolic control. Therefore copper was found in all specimens. The kidney tissue levels ranged from 2.62 ppm to 118.5 ppm (Table 6). There was no significant statistical difference between the species, the age and the sex of adult bats. Among juvenile bats the females had significantly higher copper tissue levels than males.

The highest kidney tissue level of 118.5 ppm belonged to an adult female *Myotis emarginatus*, which also showed a high cadmium level. In that specific case an intoxication with cadmium and/or copper cannot be excluded, as this bat showed signs of enteritis during necropsy. Cadmium occurs as soiling of copper ore, and both elements are known to cause intestinal troubles as symptoms of an intoxication. Nevertheless, the high tissue level may also be the result of a disorder in this bat’s copper metabolism. A significant positive correlation between the tissue concentrations of cadmium and copper has been found for all bats examined.

With the exception of the adult female *Myotis emarginatus* the kidney tissue levels of the other European bats were only slightly higher, compared to those found by Streit & Nagel [1] in their study on different European bat species, but there is no indication of any harmful effect of this element to bats in general at the moment in Styria. The higher copper concentrations compared to other literature data might also be explained as a result of the use of copper containing pesticides in viniculture, as there are some vineyards in the south-eastern region of Styria.

**Zinc**

Like copper, zinc is an essential trace element. Zinc showed the highest level among all examined five heavy metals. The kidney tissue concentrations ranged from about 14 to 821 ppm (Table 7). The median concentrations varied among the bat species and age. Young animals had higher renal concentrations than the adults. Adult bats showed a zinc tissue concentration about three times lower than those of the juvenile bats. This fact may be the result of the activity of the alkaline phosphatase, a zinc metalloenzyme, which regulates the bone metabolism. In juvenile bats this enzyme has a greater activity, as it is necessary for the development and the growth of the skeleton. Thus in juvenile bats the concentration of this enzyme and, therefore, of zinc is remarkably higher than in adult bats when skeleton growth is completed.

<table>
<thead>
<tr>
<th>Bat species</th>
<th>n</th>
<th>Median</th>
<th>25 Percentile</th>
<th>75 Percentile</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eptesicus serotinus</em></td>
<td>10</td>
<td>0.287</td>
<td>0.153</td>
<td>0.416</td>
<td>0.092</td>
<td>0.668</td>
<td>0.308</td>
</tr>
<tr>
<td><em>Myotis emarginatus</em></td>
<td>11</td>
<td>0.312</td>
<td>0.000</td>
<td>0.690</td>
<td>n.d.</td>
<td>1.087</td>
<td>0.350</td>
</tr>
<tr>
<td><em>Myotis mystacinus</em></td>
<td>26</td>
<td>0.853</td>
<td>0.297</td>
<td>1.617</td>
<td>n.d.</td>
<td>3.211</td>
<td>1.053</td>
</tr>
<tr>
<td><em>Nyctalus noctula</em></td>
<td>5</td>
<td>0.181</td>
<td>0.172</td>
<td>0.267</td>
<td>0.168</td>
<td>0.337</td>
<td>0.212</td>
</tr>
<tr>
<td><em>Pipistrellus kuhlii</em></td>
<td>23</td>
<td>0.245</td>
<td>0.112</td>
<td>0.645</td>
<td>n.d.</td>
<td>1.051</td>
<td>0.364</td>
</tr>
<tr>
<td><em>Pipistrellus pipistrellus</em></td>
<td>43</td>
<td>0.322</td>
<td>0.165</td>
<td>0.671</td>
<td>n.d.</td>
<td>3.200</td>
<td>0.451</td>
</tr>
<tr>
<td><em>Rhinolophus hipposideros</em></td>
<td>5</td>
<td>0.211</td>
<td>0.000</td>
<td>0.461</td>
<td>n.d.</td>
<td>0.649</td>
<td>0.226</td>
</tr>
<tr>
<td><em>Vespertilio murinus</em></td>
<td>6</td>
<td>0.251</td>
<td>0.171</td>
<td>0.495</td>
<td>0.152</td>
<td>0.695</td>
<td>0.326</td>
</tr>
</tbody>
</table>

* below detection limit
TABLE 6 - Copper concentrations in kidney tissue of the different bat species.

<table>
<thead>
<tr>
<th>Bat species</th>
<th>n</th>
<th>Median</th>
<th>25 Percentile</th>
<th>75 Percentile</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eptesicus serotinus</td>
<td>10</td>
<td>5.72</td>
<td>4.96</td>
<td>7.96</td>
<td>3.40</td>
<td>11.10</td>
<td>6.39</td>
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<td>11</td>
<td>5.15</td>
<td>3.97</td>
<td>10.74</td>
<td>2.75</td>
<td>118.51</td>
<td>17.16</td>
</tr>
<tr>
<td>Myotis mystacinus</td>
<td>26</td>
<td>8.11</td>
<td>5.72</td>
<td>12.92</td>
<td>2.29</td>
<td>38.05</td>
<td>10.22</td>
</tr>
<tr>
<td>Nyctalus noctula</td>
<td>5</td>
<td>8.57</td>
<td>4.88</td>
<td>12.01</td>
<td>4.59</td>
<td>14.54</td>
<td>8.47</td>
</tr>
<tr>
<td>Pipistrellus kuhlii</td>
<td>23</td>
<td>8.44</td>
<td>6.08</td>
<td>12.75</td>
<td>4.16</td>
<td>20.38</td>
<td>9.85</td>
</tr>
<tr>
<td>Pipistrellus pipistrellus</td>
<td>43</td>
<td>8.43</td>
<td>5.94</td>
<td>12.56</td>
<td>3.31</td>
<td>36.18</td>
<td>10.38</td>
</tr>
<tr>
<td>Rhinolophus hipposideros</td>
<td>5</td>
<td>7.34</td>
<td>5.08</td>
<td>13.26</td>
<td>4.38</td>
<td>17.81</td>
<td>8.80</td>
</tr>
<tr>
<td>Vespertilio murinus</td>
<td>6</td>
<td>5.53</td>
<td>3.50</td>
<td>7.49</td>
<td>2.62</td>
<td>8.10</td>
<td>5.48</td>
</tr>
</tbody>
</table>

TABLE 7 - Zinc concentrations in kidney tissue of the different bat species.

<table>
<thead>
<tr>
<th>Bat species</th>
<th>n</th>
<th>Median</th>
<th>25 Percentile</th>
<th>75 Percentile</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eptesicus serotinus</td>
<td>10</td>
<td>52.9</td>
<td>30.8</td>
<td>73.8</td>
<td>22.4</td>
<td>122.5</td>
<td>61.6</td>
</tr>
<tr>
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<td>11</td>
<td>118.1</td>
<td>88.5</td>
<td>189.7</td>
<td>34.5</td>
<td>597.8</td>
<td>171.8</td>
</tr>
<tr>
<td>Myotis mystacinus</td>
<td>26</td>
<td>76.5</td>
<td>51.9</td>
<td>221.5</td>
<td>28.2</td>
<td>820.9</td>
<td>161.6</td>
</tr>
<tr>
<td>Nyctalus noctula</td>
<td>5</td>
<td>25.2</td>
<td>21.1</td>
<td>30.4</td>
<td>13.6</td>
<td>33.8</td>
<td>25.1</td>
</tr>
<tr>
<td>Pipistrellus kuhlii</td>
<td>23</td>
<td>40.8</td>
<td>33.2</td>
<td>60.2</td>
<td>14.0</td>
<td>425.5</td>
<td>77.9</td>
</tr>
<tr>
<td>Pipistrellus pipistrellus</td>
<td>43</td>
<td>53.1</td>
<td>30.4</td>
<td>179.2</td>
<td>17.1</td>
<td>440.0</td>
<td>108.9</td>
</tr>
<tr>
<td>Rhinolophus hipposideros</td>
<td>5</td>
<td>163.5</td>
<td>73.5</td>
<td>267.0</td>
<td>25.3</td>
<td>389.6</td>
<td>178.8</td>
</tr>
<tr>
<td>Vespertilio murinus</td>
<td>6</td>
<td>39.5</td>
<td>30.3</td>
<td>68.6</td>
<td>26.9</td>
<td>89.4</td>
<td>49.0</td>
</tr>
</tbody>
</table>

Among the five elements examined in this study, only lead may constitute a potential problem to European bats. However, the elemental analysis results only document its presence and provide no further toxicity information on the reproductive success of these animals, even the young bats showing higher kidney tissue concentrations than adults.

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REFERENCES


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BIOMARKERS OF OIL-TYPE POLLUTANTS IN SURFACE SOIL

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3IChTM, Chemistry Centre, Belgrade; Yugoslavia
4Federal Institute for Geosciences and Natural Resources, Hannover; Germany

SUMMARY

In this paper, biomarkers were applied for identification of oil-type pollutants in surface soil and their microbial changes were investigated. Samples were taken at destroyed fuel storage facilities in Niš (Yugoslavia). Steranes and triterpanes were used for identification and differentiation of oil-type pollutants in surface soil. n-Alkanes were found to be affected by biodegradation and biosynthesis of “new” even-carbon numbered homologues was observed. Both processes showed differences in intensity, even if layer distances were as small as 25 cm.

KEYWORDS: Surface soil, oil-type pollutants, biodegradation, n-alkanes, steranes, triterpanes.

INTRODUCTION

Biomarker compounds have been already successfully applied for identification of oil-type pollutants in river sediments [1-3] and ground waters [4, 5]. Polycyclic alkanes of sterane and triterpane type were found to be more reliable for this purpose due to their lower susceptibility to biodegradation, compared to n-alkanes [5]. Namely, it was shown that microorganisms indirectly control distribution of n-alkanes in sediments [2] and ground waters [4], thus having important role in changing the composition of petroleum contaminants in these environments. In this paper, an attempt was made to apply biomarkers for identification of oil-type pollutants in surface soil, as well as to investigate their potential microbial changes in topsoil layers. For that purpose, surface soil samples from destroyed fuel storage facilities in Niš were used, collected 5–6 weeks after the release of significant amounts of oil into environment.

MATERIALS AND METHODS

The surface soil samples analyzed in this investigation were taken at the fuel storage facilities in Niš (Yugoslavia), bombed several times during the war activities in 1999, on the 5th, 8th and 11th of May. Although most of the reservoirs were empty, some of them had still been filled with petroleum, as the reservoir R-7 with the volume of 5000 m³. Sampling was performed on the 18th of June 1999 at two locations. The first sampling location was the crater in the immediate vicinity of petroleums’ reservoir R-7, wherefrom samples from the depths of 5 cm (sample Ia) and 30 cm (sample Ib) were taken. Sampling was also performed 100 meters away from the crater, from the depths of 5 and 30 cm (samples IIa and IIb), respectively. All the samples had strong odour, characteristic of most of the petroleums.

Organic substance was extracted by Soxhlet extraction of the samples with chloroform for 36 hours. The group composition was determined by the means of column chromatography, using the adsorbents Al₂O₃ and SiO₂. Saturated hydrocarbons were eluted with petroleum-ether and aromatic hydrocarbons with benzene. Content of NSO-compounds was calculated on the basis of difference. Analyses of the saturated hydrocarbon fractions included determination of n-alkane distribution and the isoprenoid hydrocarbons pristane and phytane. They were performed on a Varian 3300 gas chromatograph (FID) fitted with a 25 m × 0.25 mm capillary column coated with non-polar BP-1 using hydrogen as a carrier gas at 1 cm³/min flow rate.

After GC-analyses, polycyclic alkanes of the sterane and triterpane types were analyzed by gas chromatography-mass spectrometry (GC-MS) using the single ion monitoring (SIM) method. Steranes were identified from m/z 217 and triterpanes from m/z 191 fragmentograms. Analyses were performed on a Hewlett Packard 5890, Series II, gas chromatograph fitted with a capillary column with HP-5MS and helium as a carrier gas at 1 cm³/min flow rate. GC system was equipped with a HP 5972 mass-selective detector (MSD) operated at 70 eV.

RESULTS AND DISCUSSION

The amount and group composition of organic matter isolated from the investigated soil samples are presented in Table 1.
TABLE 1 - Content and group composition of the extracted organic matter.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Organic matter (%)</th>
<th>Alkanes (%)</th>
<th>Aromates (%)</th>
<th>NSO-compounds (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>9.98</td>
<td>69.54</td>
<td>18.21</td>
<td>12.25</td>
</tr>
<tr>
<td>Ib</td>
<td>3.3</td>
<td>67.28</td>
<td>11.76</td>
<td>20.96</td>
</tr>
<tr>
<td>IIa</td>
<td>0.41</td>
<td>64.76</td>
<td>14.09</td>
<td>21.15</td>
</tr>
<tr>
<td>IIb</td>
<td>0.17</td>
<td>38.48</td>
<td>15.15</td>
<td>46.37</td>
</tr>
</tbody>
</table>

The naturally occurring organic matter content in soil is very variable. Therefore, it is not reliable to determine the presence of oil-type pollutant in soil solely on the basis of the amount of the extracted organic substance. Nevertheless, it is evident that samples from the first sampling location (Ia and Ib) have been contaminated, having significantly larger organic matter content than samples IIa and IIb. Although spilt petroleum penetrated the topsoil contaminating the layer at the depth of 30 cm, the Ib sample contains much smaller amount of organic substance than its corresponding sample Ia. This is also noticeable for the samples taken at the second sampling location, however, their contamination is yet to be proven. The saturated hydrocarbon fraction, consisting of \( n \)-alkanes and branched and cyclic alkanes, dominates aromates and NSO-compounds in most of the samples. This is a typical characteristic of petroleum and its derivatives [6], and therefore corroborates aforementioned inferences of oil-type pollutants’ presence. The first sampling location shows a gradual decrease in alkane content with depth. For the second one, more conspicuous decline is observed (Table 1).

Both gradual and prominent reduction in the amount of saturated hydrocarbons with depth could be explained as a net effect of the abiotic and biotic processes. These processes comprise the “weathering” process, to which petroleum is subjected when spilt in the environment [7]. However, due to susceptibility of \( n \)-alkanes to microbial degradation, there is a strong possibility that the evident decrease in alkane content is a consequence of intensive biodegradation, over 5-6 weeks period, rather than of abiotic reactions. This presumption of \textit{in situ} biodegradation must be further supported with reliable parameters.

Gas chromatograms of alkane fractions of the investigated samples are shown in Fig.1. As one of the most investigated group of biomarkers, \( n \)-alkanes provide important information on weathering and biodegradation processes occurring when petroleum is spilt in the environment. For the purpose of monitoring these processes parameters are calculated from the abundance and distribution of \( n \)-alkanes and isoprenoid aliphatic alkanes pristane (Pr) and phytane (Phyt) and presented in Table 2.

The gas chromatogram of the sample Ia (Fig.1) is characterized by uniform distribution of \( n \)-alkanes with an even and odd number of carbon atoms (carbon preference index, CPI=0.99, Table 2), as well as by higher abundance of pristane and phytane relative to \( n \)-alkanes C\(_{17}\) and C\(_{18}\), respectively (Pr/\( n \)-C\(_{17}\) >1, Phyt/\( n \)-C\(_{18}\) >1, Table 2). The observed bimodal distribution of \( n \)-alkanes with CPI around 1 is typical for alkane fraction of crude oils [6,8]. The ratios Pr/\( n \)-C\(_{17}\) and Phyt/\( n \)-C\(_{18}\) are primarily maturation parameters, but are also affected by microbial degra-
ation [8]. Since n-alkanes are generally attacked by microorganisms prior to the isoprenoids, a higher abundance of pristane and phytane compared to n-alkanes, in most cases, is a result of commenced biodegradation. Therefore, the gas chromatogram of alkane fraction of the sample Ia confirms the anthropogenic contribution from thermally mature organic matter i.e. petroleum and reveals that biodegradation has already started.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Parameters calculated from the distribution of n-alkanes and isoprenoid aliphatic alkanes pristane and phytane.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Pr/n-C&lt;sub&gt;17&lt;/sub&gt;</td>
</tr>
<tr>
<td>Ia</td>
<td>1.28</td>
</tr>
<tr>
<td>Ib</td>
<td>ND</td>
</tr>
<tr>
<td>IIa</td>
<td>ND</td>
</tr>
<tr>
<td>IIb</td>
<td>ND</td>
</tr>
</tbody>
</table>

The sample Ib corroborates these conclusions, also exhibiting CPI value around 1 (Table 2) and indicating microbial degradation, although more intensive, resulting in the absence of n-C<sub>17</sub> and increase in Phyt/n-C<sub>18</sub> parameter due to the n-C<sub>18</sub> degradation (Fig.1, Table 2). Moreover, the more prominent microbial process is an explanation for a gradual decrease in alkane content with depth, observed for the two samples investigated (Table 1).

The IIA and IIb samples are rather different from the samples Ia and Ib. Their gas chromatograms (Fig.1) are characterized by a large, unresolved complex mixture (UCM or “hump”) that rises above the baseline. This is a well-known characteristic of more heavily biodegraded petroleums [8]. The UCM represents a complex mixture of branched and cyclic hydrocarbons resistant to weathering and biodegradation processes, indicating that substantial degradation of the oil has already occurred [9]. Compared to the samples from the first sampling location, there is a clear absence of n-C<sub>17</sub> and the values of Phyt/n-C<sub>18</sub> ratios are significantly lower (Table 2).

Furthermore, a distinct drop of this parameter in IIB sample is quite the opposite of the observed increase in the sample Ib. This could lead to a conclusion that for this location biodegradation is more prominent in the layer from the depth of 5 cm. Yet, a complete n-alkane distribution reveals a clear predominance of even-carbon numbered n-alkanes, especially conspicuous in IIB sample. As it is shown in Fig.1, there is a moderate increase in n-C<sub>16</sub>, but also n-C<sub>16</sub>, n-C<sub>20</sub> and other higher even-carbon numbered homologues. A pronounced domination of n-alkanes with an even-carbon number is reflected on CPI values of the two above-mentioned samples showing values of 0.76 and 0.53, respectively (Table 2). In the literature [10], the occurrence of “new” even-carbon numbered n-alkanes is described as direct biogenic contribution from metabolic products of some microorganisms such as Desulfovibrio desulfuricans, Corynebacterium sp., Escherichia coli, Penicillium sp. and Rhizopus stolonifer. It was shown earlier that these microorganisms could indirectly control the distribution of n-alkanes in river sediments [2] and that changes include degradation of “oil-type” n-alkanes, followed by the synthesis of new n-alkanes which are dominated by even-carbon numbered homologues with maxima at n-C<sub>16</sub> and n-C<sub>18</sub> [4]. In this study, results indicate that biosynthesis of even-carbon numbered n-alkanes can also occur in the soil environment, i.e. surface soil layers. Moreover, it seems that this process is more intensive in the layer at the depth of 30 cm.

Previous indications of the presence of two different contaminants in the samples from the two locations could be further investigated by using GC-MS analyses of polycyclic alkanes of the sterane and triterpene type. Additionally, they could be used for excluding the contribution from the background i.e. indigenous organic matter to all investigated samples. Since oil is the most mature form of lithosphere organic matter, it is assumed that indigenous organic matter in surface soil is of lower maturity than that comprising the oil-type pollutant [8]. The fragmentograms of steranes and triterpanes of the alkane fractions isolated from the samples Ib and IIb, chosen as representative for their sampling locations, are presented in Fig. 2. Namely, it was observed that their mass chromatograms are almost identical to those of corresponding samples from the depths of 5 cm (Ia and IIA, respectively), indicating that biodegradation of steranes and triterpanes still was not commenced. The sterane distributions (m/z 217 mass chromatograms, Fig. 2) are characterized by a predominance of biolipid C<sub>27</sub> αα (20R) steranes over C<sub>28</sub> and C<sub>29</sub> isomers (peaks b, c and d, Fig. 2) and by significant abundance of geolipid isomers as diasteranes, for both samples, proving unambiguously the presence of oil-type pollution in the surface soil at both sampling locations. However, there are clear dissimilarities in sterane fingerprints. The dominance of C<sub>27</sub> biolipid isomer is striking in Ib sample and only slight in the sample IIb. The difference between samples is further verified with triterpane fingerprints (m/z 191 mass chromatograms, Fig. 2). They are dominated by thermodynamically stable, geolipid C<sub>25</sub> αβ-norhopane and C<sub>30</sub> αβ-hopane (C and D, Fig. 2), with a smooth decrease in the abundance of C<sub>31</sub>–C<sub>33</sub> (C<sub>34</sub> for the sample IIB) αβ-homohopanes (E and subsequent doublets, Fig. 2). The triterpanes with αβ-configuration, in the range C<sub>27</sub>–C<sub>33</sub>, are characteristic of petroleum, as one of the most mature organic material [8]. Therefore, contribution from the background organic matter is excluded. The relative abundances of C<sub>27</sub>-hopanones, Ts and Tm (peaks A and B, Fig. 2), are considerably different. Their ratio (Ts/Tm) is often used for differentiation of oils, the Ts being a source parameter, not affected by maturity changes, whereas Tm is influenced by these reactions [11]. Moreover, there is a noticeable presence of oleanane in IIB sample and its absence in the sample Ib. Evidently, two different oil-type pollutants are present, most likely due to the mixing with petroleum derivatives from other destroyed reservoirs.
CONCLUSION

The results obtained indicate that, as for biomarker compounds, n-alkanes are the most affected by indigenous microorganisms in the surface soil. Microbial degradation of “oil-type” n-alkanes, as well as biodegradation followed by biosynthesis of “new” even-carbon numbered n-alkanes, exhibit significant differences in their intensity, even if layer distance is as small as 25 cm. Both processes are more intensive in the layers at the depth of 30 cm than in those at the depth of 5 cm, which might be contributed to the smaller amount of organic pollutant that is to be degraded. Steranes and triterpanes remained unaffected by biodegradation in the topsoil layers. Their distributions revealed the presence of different petroleum contaminants, proving their significance for identification and differentiation of oil-type pollutants in the surface soil.

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COMPARATIVE ACUTE TOXICITY OF THE COMMERCIAL HERBICIDES GLYPHOSATE TO NEOTROPICAL TADPOLES Scinax nasicus (ANURA: HYLIDAE)

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SUMMARY

We investigated the effect of glyphosate (GLY) to Scinax nasicus tadpoles and the influence of GLY degradations in the variation of LC₅₀ values. These results showed that 96-h LC₅₀ for S. nasicus tadpoles exposed to continuous applications of GLY in the renewal tests (RT) was 3.13 mg GLY/L, compared to an estimated LC₅₀ in static tests (ST) of 5.27 mg GLY/L. These data indicate large differences in toxicity between RT and ST, S. nasicus tadpoles did not die when the GLY exposure was not continuous.

KEYWORDS:
Glyphosate, amphibians, Scinax nasicus, tadpoles, toxicity.

INTRODUCTION

Glyphosate, (GLY) or N-phosphonomethyl glycine, is a systemic herbicide used for non-selective weed control on 'rights-of-way', forestry plantations, sites prepared for plantings, and as a foliage desiccant for selected crops [1, 2]. It is considered to have low toxicity to bees, fish, and other aquatic organisms [3, 4]. However, some of the surfactants used in agricultural formulations have been found to be significantly more toxic to fish, amphibians, and aquatic invertebrates than the herbicide itself [5-7].

The pesticide degradation phenomenon implies the possibility to decrease toxic concentrations of xenobiotics in the environment. In water, GLY is rapidly dissipated, and has under laboratory conditions a half-life for degradation ranging from a few days to approximately 20 days [2]. Moreover, it has been found to dissipate from the sediment of a farm pond with a half-life of 120 days, and still be present in pond sediment at a concentration of 0.1 ppm one year later [8]. The presence of GLY in surface water is most likely to occur as a result of heavy rainfall after recent application on neighboring land, with subsequent rapid displacement into stream sediment [2]. The presence of an environmental contaminant can decrease larval amphibian activity [9, 10] and alter swimming and feeding behavior [11]. Alternatively, it is possible for contaminants to increase the anuran larval activity and, therefore, predation rates [12]. The objective of this study was to evaluate and compare the acute toxicity of glyphosate formulations (GLY-F) on Scinax nasicus tadpoles. Furthermore, the behavioral responses are described.

Scinax nasicus was selected for this study because of its wide Neotropical distribution (i.e. present in Argentina, Paraguay, Uruguay, Bolivia, and Brazil) [13]. This anuran is common in forests, wetland, riparian areas, urban, and agricultural lands [14]. In addition, another study indicated that natural S. nasicus populations could be affected by herbicide application [15].

MATERIALS AND METHODS

Two hundred and fifty S. nasicus tadpole individuals (Gosner Stage 25-26) [16] were collected from an unpolluted temporary pond in the floodplain of the Paraná River (31° 42´S; 60° 34´O, Santa Fe, Argentina) and maintained under laboratory conditions. The tadpoles were acclimatized over 7 days to a 12 h light/12 h dark cycles in glass tanks with artificial pond water (APW) (pH: 8.2 ± 0.3, conductivity: 237 ± 25 µmhos/cm¹, dissolved oxygen: 8.4 ± 0.1 mg/L, and hardness: 56 ± 12 mg CaCO₃/l, at 22 ± 2 ºC).

96-h acute toxicity test was conducted according to ASTM Standard Methods [17], with 26-27 Gosner Stage larvae. Glass tanks (35 cm diameter and 60 cm high), with 4 L of APW and 10 tadpoles (average weight: 0.025 ± SE
0.006 g) per tank, were used in the experiments. The tested product was Glyfos®®, a commercial herbicide containing 48% GLY, that was measured by high performance liquid chromatography (HPLC). In a renewal test (RT), solutions were renewed at 24-h intervals during the test period and tadpoles that had been taken out were transferred into the new solution. Simultaneously, a static test (ST) was conducted, in which solutions were not renewed during exposure. In both acute toxicity tests, the concentrations used were: 0, 3.07, 3.84, 4.8, 6.0, and 7.5 mg of GLY-F/L. Tests were conducted at 22 ± 2 °C and 12-h /12-h light-dark. The mortality was recorded every 24-h and the dead animals were removed at each observation time. The LC₅₀ with confidence limits (P ≤ 0.05) were estimated using the probit analysis [18]. The criterion of non-overlapping 95% confidence intervals was used to determine the significant differences (P ≤ 0.05) between LC₅₀ values in RT and ST. Moreover, bobbing (swimming to the surface for air) rates were measured as well as the number of times that larvae broke the water surface with the anterior part of their body in a 10 minute interval was recorded.

GLY was identified and quantified by Water 600 HPLC system with IC-Pack ion-exclusion 50 Å-7μm (7.8 x 150 mm) column, mobile phase phosphoric acid: 0.05% at 55 °C. Water postcolumn derivatization system at 38 °C, fluorescence spectrophotometer detector at 339-nm excitation and 345-nm emission wavelengths by Milenium manager data processor. This method was adapted from Winfield [20].

RESULTS AND DISCUSSION

The 96-h LC₅₀ for S. nasicus exposed to GLY-F in the RT was 3.13 mg GLY-F/L, compared to an estimated higher LC₅₀ in ST (where solutions were not renewed during exposure) of 5.27 mg GLY-F/L. Although at 24-h LC₅₀ the RT and ST did not differ statistically (P > 0.05), differences was observed at 48, 72, and 96-h (P < 0.05) (Figure 1). In RT, we observed a decrease in LC₅₀ values at all time during the bioassay (Figure 1 A). In ST, mortality stabilized at 24-h, which was related to the GLY degradation. Nevertheless, we found that the half-life of GLY was approximately 96-h (Figure 1B).

After 24-h of exposure in RT, cephalic edemas and bent tail occurred in 3.84 mg/L of GLY and there were mouth deformities at 4.8 mg/L. These manifestations were increased with the time and intestinal vacuity was detected at 48-h exposure, although these tadpoles showed different behaviors at differing GLY concentrations with the control. The rates of the tadpoles coming to the surface to gulp air is lower without herbicides, Chi-square statistic (with Yates correction) = 26.34; P < 0.01. In contrast, in the ST test, the alterations were not so marked and they were only poorly observed at the highest concentration, 7.5 mg/L. The specific behavioral differences may also be important, many temporary aquatic environments are highly eutrophic and have low levels of dissolved oxygen. Clearly, the ability of larvae to meet their respiratory requirements, unduly favors their exposure to predation risk. To what extent amphibians are presently limited in their range by the contamination of potential breeding sites is unknown.

Mann and Bidwell [21] found, in test solutions renewed after 24-h with freshly made solutions, several LC₅₀ values for tadpoles of Australian frogs (Crinia insignifera, Heleioporus eyrei, Limnodynastes dorcas, and Litoria moorei) (48-LC₅₀: 8.1-32.2 mg GLY/L). An Australian study confirmed that Roundup® herbicides are toxic to frogs, especially to tadpoles, because of the effect of inert ingredients on tadpole gills [22]. The herbicide Glyphos® contains polyethoxylated tallowamine (POEA) [23], a surfactant bound to suspended material that can have toxic effects in water systems. The same surfactant is
included in Roundup® formulations because it aids to counteract the surface tension on leaf surfaces, but it may also interfere with gill respiration in tadpoles [24].

In this context, transgenic soya (resistant to Roundup-Ready) is the most extensively cultivated, compared to others crops, in Argentina agricultural lands and during 2000-2001 it had occupied 19 million hectares [25]. Moreover, the GLY-F use in Argentina increased from 1 to 58 million liters from 1991 to 2000 [26]. The validity of extrapolating laboratory based acute toxicity data to the field situation is contentious [21]. However, our results indicated a variability of toxicity of GLY-F according to the type of exposure. Specifically, we found that non-target tadpoles did not die when the GLY exposure was not continuous. Moreover, we argue that the GLY-F pulse can affect native populations of neotropical tadpoles. Also, we suggest that prevention of possible contamination of lentic, or ephemeral water bodies where most of the anuran breed, should be considered. On the other hand, the impacts and the costs of the required alternatives to the GLY formulations should be minimized for uses away from aquatic situations.

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EXPLORATION OF BIOLOGICAL RICHNESS AND WATER QUALITY OF STREAM KELKIT, TOKAT-TURKEY

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SUMMARY

This study examines the biological richness combined with measurement of physicochemical parameters and some biotic indices, in order to determine the water quality at ten different sites along the Niksar part of the stream Kelkit between March 2000 and February 2001. Standard methods have been used to determine the physicochemical properties of water samples. The metal ions were determined by atomic absorption spectrometry after liquid-liquid extraction. Metal accumulation factors were calculated for some macroinvertebrates and fish species. Biological and chemical results are in good agreement with respect to the water quality in the stream Kelkit. In particular, macroinvertebrate and phytoplankton communities responded quite variably probably because of substantial differences in flow regimes. We concluded that a rich biota population in the stream Kelkit will be achievable.

KEYWORDS:
Stream Kelkit, biological richness, water quality, physicochemical analysis, atomic absorption spectrometry.

INTRODUCTION

The biological richness of freshwater in many regions of Anatolia has not been discovered yet. Macrozoobenthos have been rarely used to determine quality of running water in Turkey and biotic indices have not been used by governmental institutions yet. However, in the meanwhile ecological recovery and rehabilitation of aquatic ecosystems have become objectives of many governmental institutions [1]. In order to define the fauna structure in the region biological parameters have to be used. Afterwards, the methods are changed to be appropriate to the members of fauna and a regional index is prepared according to these changes. In our study, the biological richness was defined as accurate as possible and macroinvertebrates and physicochemical parameters were used to determine the water quality.

The main constituent of water are living organisms. The weight percentage of water in invertebrates is about 70% [2]. For instance, typical insects have water contents of 65-75% of fresh weight, although the levels range from only 17% to more than 90% in different life stages of different species [3].

Benthic macroinvertebrates are the group of organisms most widely used for assessment of water resources [4-9]. Water quality determines the number of species, continuity and present densities with the use of biotic indices [10]. However, various researches have been performed for monitoring water quality [11,12].

The stream Kelkit is the longest tributary of the river Yeşilirmak which is one of the longest in Turkey (1144.5 km², 210 km in length, \(Q_{\text{max}}=978.5 \text{ m}^3/\text{s}, \ Q_{\text{min}}=1.85 \text{ m}^3/\text{s}\) with an average discharge of 3224 km³/s per year. The stream is situated in Northern Turkey, east of the city of Tokat. This part of the stream runs fast in all seasons and receives mainly agricultural runoff, but also urban sewage and little industrial waste.

The major aim of this project was to determine the spatial and temporal pattern in the community structure of biota richness with particular emphasis on the relationships between structure of community and the physical and chemical environment. Furthermore, if macroinvertebrate community structure could be predicted from physicochemical characteristics of the stream, this information might be useful to assess the status of adjacent stream systems.
MATERIAL AND METHODS

Sampling

Ten sites were sampled for benthic invertebrates monthly from March 2000 to February 2001. Five stations, south of Niksar county, were the first sampling stations and other five, west of Niksar, were the second ones (Fig. 1). The first stations receive mainly agricultural runoff and no industrial wastes (except the waste from one limestone mine). Generally, the bottom of the stream has various sizes of rocks, but gravel is found in the parts nearest to the county centre. The second stations have mostly gravel and in some areas, sand and silt. This part of the stream receives mainly urban sewage, agricultural runoff and little industrial wastewater. Samples were taken six times with a Surber sampler covering an substrate area of 0.1 m². The net (mesh size 475µm) was 50 cm long with a straining surface area of 0.77 m². Riffle areas were the major habitat of this fast running stream, with a substratum consisting predominantly of gravel and some rocks. When sampling was restricted in some areas, the procedure followed was that outlined by Surber [13]. Larger stones were first picked out and washed into the net to remove pupae and other attached invertebrates. The latter were washed in the field through a sieve and immediately stored in 4% formalin. Invertebrates were then transferred into 70% ethyl alcohol and later identification of macroinvertebrates was carried out to determine the lowest taxa possible (in most cases genus or species). Samples of zooplankton were taken by a plankton net (55 µm) and the phytoplankton using the methods of Sladeckova [14]. Formalin (4%) was used to preserve them until identification by inverted microscopical analysis.

Temperature, dissolved oxygen, conductivity and pH were measured in the field using portable instruments. The samples were filtered through a 0.45 µm Millipore membrane, immediately acidified to pH ≤ 2 using high purity HNO₃, and kept in the refrigerator at 4 ºC until analysis [15].

Reagents

All reagents were of analytical grade unless otherwise stated. Double-deionised water (Milli-Q Millipore 18.2 MΩcm⁻¹ resistivity) was used for all dilutions. All the plastics and glassware were cleaned by soaking in dilute HNO₃ (1+9) and rinsed with distilled water prior to use. The stock solutions of metals (1000 mg/l) were obtained by dissolving appropriate salts or the corresponding metals (E. Merck) and further diluted prior to use. APDC was dissolved in a water/ethanol (75/25, v/v) mixture.
FAAS analysis

A Varian SpectrAA-220 flame atomic absorption spectrometer was used in this study. All measurements were carried out in an air/acetylene flame. The operating parameters for working elements were set as recommended by the manufacturer. The wavelengths (nm) used for the determination of the elements were as follows: Fe: 248.3, Cu: 324.8, Mn: 279.5, Zn: 213.9, Pb: 283.3, Cd: 228.8. Trace metal concentrations were measured by flame atomic absorption spectrometry (FAAS) after preconcentration by liquid-liquid extraction (APDC-MIBK) [16]. Ammonia nitrogen, nitrate, nitrite, sulphate, phosphate, chloride, hardness, organic matter, total dissolved solids and boron in the samples were determined according to APHA, AWWA, and WPCF [17]. The digestion of biological samples were made according to [18] and concentrations of Pb, Cd and Fe, Cu, Mn and Zn were determined using graphite furnace and flame AAAS, respectively.

RESULTS AND DISCUSSION

Forty five species of algae, 4 species of amoebae and 11 of Rotifera were determined up to the genus level (Table 1). The dominant algal groups found were Bacillariophyta: Achnanthes (3), Asterionella (1), Cocconeis (1), Cymbella (4), Fragilaria (2), Synedra (3), Tabellari (1), Mastogloia (1), Navicula (2), Pinnularia (4), Navicula (2), Pinnularia (4), Nitzschia (4) and Cymbatopleura (3). Additionally, algal flora that is part of Chlorophyta and Cynophyta has been identified. It has been observed that, although the assorted species belonging to Cynophyta are few, the density of these organisms is high. On the other hand, it is found that the number of species belonging to Chlorophyta is high, but their density is low. Stochastic events and circumstances contribute to the variability, but certain species-association may occur preferentially in certain kinds of rivers. For instance, Navicula and Cymbella are frequently encountered in limestone stream beds. Zygnema, Pinnularia and Nitzschia occurred in less alkaline granites, sand and limestone [19]. Consequently, these observations fit well with the findings in our study. The benthic component is augmented by habitually free-living species common in the stream. According to these results obtained, it was observed that epilith, epipel and epiphyte components mingle each other. This may be partly related to the rates of dislodgement and the flushing out by the flow.

The composition of macroinvertebrate fauna identified as Platyhelminthes (1), Mollusca (4), Annelida (2), Crustacea (2) and insecta (31) is shown in Table 2. According to the results, the first stations of the stream Kelkit were dominated by Plecoptera, Ephemeroptera and Tricoptera and found to be “first class” by diversity. The sum results for the first stations were calculated using ecocological condition categories and found to be 75 by Chandler Score, 8.1 by BMWP (Biological Monitoring Working Party) Score and 11.2 by Expanded Trent Biotic Index (ETBI). The scores calculated for second stations were 50.9 by Chandler Score, 6.2 by BMWP Score and 11.2 by Expanded Trent Biotic Index. Among a great variety of schemes, we selected three for the class determination. It has been claimed to distinguish well for small changes in water quality. The BMWP and ETBI were chosen because they are easy to use, and ETBI has been used previously in many studies. The mean density of macroinvertebrates differed in the stations (F= 4.175, p<.001) and in seasons, as well (F= 15.238, p<.001). The highest mean density in individual organisms was recorded in summer and the lowest in winter. From the seasonal records of stream temperature, pH, dissolved oxygen, hardness, organic matter, chloride, sulphate, phosphate, ammonia nitrogen, nitrate, nitrite and biotic indices a correlation matrix was calculated. Significant correlations were found between temperature and BMWP (r=0.960 p<0.04), temperature and Chandler score (r=0.993 p<0.007), temperature and ETBI (r=0.985 p<0.015), BMWP and Chandler score (r=0.981 p<0.019), ammonia nitrogen and nitrite (r=0.982 p<0.018), ammonia nitrogen and nitrate (r=0.957 p<0.043), organic matter and nitrate (r=0.998 p<0.002), organic matter and nitrite (r=0.955 p<0.011), organic matter and hardness (r=0.944 p<0.05), nitrite and nitrate (r=0.982 p<0.018), nitrite and phosphate (r=0.964 p<0.036), phosphate and sulphate (r=0.951 p<0.049), hardness and chloride (r=0.945 p<0.001) and a negative correlation between temperature and dissolved oxygen (r = -0.993 p<0.018). BMWP, Chandler score, ETBI and temperature intercorrelate. Each is tied into a seasonal cycle with the same timing (e. g. higher temperatures and higher biotic scores in summer).

Classification of composite samples for the first and second stations showed differences between catchments, based on the presence-absence of taxa. Heptagania, Capnia, Perla, Dinocras, Elmis, Paraleptophlebia and Hydropsyche were major indicator species of the first stations, and the physicochemical results were mostly class I for the first stations. Gammarus and Baetis were major indicator species of the second stations. This will confirm that Gammarus and Baetis species (except B. alpinus) are often dominant and frequent in weakly polluted water. This was confirmed by the chemical results.

Fish richness shows that this part of the stream is not polluted yet (Table 3). Species of Alburnus and Leuciscus are sensitive to pollution. Species of Salmonidae are not found in this study, but the water quality and stream-bed are suitable for Salmonidae farming.

Macroinvertebrate species as bioindicators of chemical pollution were determined to the species level and evaluated with the physicochemical parameters used in biomonitoring. The results of physicochemical values were classified by Turkish Standards [20]. The values of the physicochemical parameters and their classes of water quality are given in Table 4.
TABLE 1 - The species of phylum of algae, Sarcodina and Rotifera found in Kelkkit stream.

<table>
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<th>FAMILY</th>
<th>GENUS/SPECIES</th>
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<td>Cymbella a fli ns. Kütz.</td>
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<td>Cymbella lata. Grun.</td>
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<td>Cymbella turgida. Gregory.</td>
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<td>Pennales</td>
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<td>Frag illaria sp.</td>
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<td>Frag illaria intermedia. Grun.</td>
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<td>Synedra sp.</td>
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<td>Synedra acus. Kütz.</td>
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<td>Synedra ulna. Nitzsch</td>
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<td>Tabellaria sp.</td>
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<td>Mastogloia sp.</td>
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<td>Navicula sp.</td>
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<td>Navicula cryptocoephal. Kütz.</td>
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<td>Pinnularia sp.</td>
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<td>Pinnularia biceps. Greg.</td>
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<td>Pinnularia fasciata. Lagersted.</td>
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<td>Pinnularia microstauron Ehr.</td>
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<td>Nitzchia sp.</td>
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<tr>
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<td>Nitzchia acicularis. W. Smith</td>
</tr>
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<td>Nitzchia dicephala. W. Smith</td>
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<td>Nitzchia falaisiensis. Grun.</td>
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<td>Cymatopleura sp.</td>
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<td>Cymatopleura angulata</td>
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<td>Cymatopleura elliptica. Brebisson</td>
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<tr>
<td>Sarcomastigophora</td>
<td>Superclass: Rhizopoda</td>
<td>Amoebae</td>
<td>Hyalodiscidae</td>
<td>Amoeba proteus. Pallas</td>
</tr>
<tr>
<td>Subfilum: Sarcodina</td>
<td>Lobosea</td>
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<td>Astramoeba radiosa. Ehrenberger</td>
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<td>Dinamoeba sp.</td>
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<td>Dinamoeba horrida. Schaeffer</td>
</tr>
<tr>
<td>Rotifera</td>
<td>Bdelloidea (Digononta)</td>
<td>Bdelloidea</td>
<td>Habrotrochidae</td>
<td>Habrotrocha sp.</td>
</tr>
<tr>
<td></td>
<td>Monogononta</td>
<td>Ploima</td>
<td>Dicranophorida</td>
<td>Cephanodella sp.</td>
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<td>Cephanodella auriculata</td>
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<tr>
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<td>Cephanodella euderby</td>
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<td>Cephanodella exigua</td>
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<td></td>
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<td>Cephanodella megalocoephalas</td>
</tr>
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<td></td>
<td></td>
<td>Lecane ohiensis</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>Lecane elasma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Monostyla hulla</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Elosa woralli var spinifera Wisen.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Elosa woralli Lord.</td>
</tr>
</tbody>
</table>
TABLE 2 - Systematic list of taxa of macroinvertebrates found in Niksar stream.

<table>
<thead>
<tr>
<th>FHYLUM</th>
<th>CLASS</th>
<th>ORDO</th>
<th>FAMILY</th>
<th>GENUS/SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platyhelminthes</td>
<td>Turbellaria</td>
<td>Tricladida</td>
<td>Tricladida</td>
<td>Polycelis sp.</td>
</tr>
<tr>
<td>Mollusca</td>
<td>Bivalvia</td>
<td>Unionoida</td>
<td>Unionoida</td>
<td>Unio pitorum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sphaeriida</td>
<td>Sphaeriida</td>
<td>Sphaerium spp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Superorder)</td>
<td>Basommatophora</td>
<td>P(Coretus) vorneiss</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P(Tropidiscus) planorbis</td>
</tr>
<tr>
<td>Annelida</td>
<td>Clitellata</td>
<td>Hirudina</td>
<td>Glossiphoniida</td>
<td>Hellobdella stagnalis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oligochaeta</td>
<td>L. variegatus</td>
</tr>
<tr>
<td>Crustacea</td>
<td>Malacostraca</td>
<td></td>
<td></td>
<td>Gammarida sp.</td>
</tr>
</tbody>
</table>

TABLE 3 - Systematic list of taxa of fish found in Niksar stream.

<table>
<thead>
<tr>
<th>PHYLUM</th>
<th>CLASS</th>
<th>ORDO</th>
<th>FAMILY</th>
<th>GENUS/SPECIES</th>
<th>LOCAL NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chordota</td>
<td>Teleostei</td>
<td>Cypriniformes</td>
<td>Cyprinida</td>
<td>Cyprinus carpio</td>
<td>Pullu Sazan, Çıplak Sazan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chalcobars hemicordis</td>
<td>Tatlisu Kefali</td>
</tr>
<tr>
<td>Siluriformes</td>
<td>Siluridae</td>
<td>Silurus</td>
<td>Silurus glanis</td>
<td>Yayan Balığı</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lentiscus cephalus</td>
<td>Tatlisu Kefali</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Capoeta tinca</td>
<td>Karabalık veya Siraz Balığı</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hydrolagus piceus</td>
<td></td>
</tr>
<tr>
<td>Perciformes</td>
<td>Percidae</td>
<td></td>
<td></td>
<td>Elmis aenea</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 4 - Seasonal mean parameters and the classes of the water quality in the first five and the second five stations of the stream Kelkit.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>16-15 (I-I)</td>
<td>9-10 (I-I)</td>
<td>15-14 (I-I)</td>
<td>22-22 (I-I)</td>
</tr>
<tr>
<td>pH</td>
<td>8.34-8.45 (I-I)</td>
<td>7.76-7.90 (I-I)</td>
<td>8.23-8.32 (I-I)</td>
<td>8.27-8.22 (I-I)</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>8.38-9.40 (I-I)</td>
<td>8.96-9.90 (I-I)</td>
<td>9.24-9.44 (I-I)</td>
<td>7.68-7.89 (I-I)</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>510-504 (I-I)</td>
<td>480-498 (I-I)</td>
<td>540-572 (I-I)</td>
<td>590-569 (I-I)</td>
</tr>
<tr>
<td>Total dissolved solids (mg/L)</td>
<td>726±58-689±40 (I-I)</td>
<td>655±44-670±55 (I-I)</td>
<td>686±39-705±85 (I-I)</td>
<td>842±60-760±50 (I-I)</td>
</tr>
<tr>
<td>Hardness (mg CaCO₃/L)</td>
<td>160±15-154±20 (I-I)</td>
<td>156±19-170±25 (I-I)</td>
<td>193±10-184±16 (I-I)</td>
<td>240±21-225±14 (I-I)</td>
</tr>
<tr>
<td>Organic matter (mg/L)</td>
<td>2.30±0.16-3.18±0.25 (I-I)</td>
<td>2.76±0.24-3.12±0.41 (I-I)</td>
<td>2.92±0.40-3.41±0.37 (I-I)</td>
<td>3.28±0.56-3.70±0.42 (I-I)</td>
</tr>
<tr>
<td>Chloride (mg/L)</td>
<td>89.9±7.5-74.3±6.4 (I-I)</td>
<td>109.6±11.8-94.5±8.1 (I-I)</td>
<td>117.5±9.3-102.5±11.7 (I-I)</td>
<td>191.1±16.5-205.8±19.3 (I-I)</td>
</tr>
<tr>
<td>Sulphate (mg/L)</td>
<td>70.8±6.4-90.5±10.3 (I-I)</td>
<td>70.6±5.7-86.4±7.8 (I-I)</td>
<td>80.1±9.3-94.3±10.7 (I-I)</td>
<td>63.3±4.2-76.9±8.5 (I-I)</td>
</tr>
<tr>
<td>Phosphate (mg/L)</td>
<td>0.96±0.20-0.84±0.32 (IV-IV)</td>
<td>0.22±0.05-0.19±0.08 (II-II)</td>
<td>3.65±0.54-3.86±0.48 (IV-IV)</td>
<td>1.86±0.78-1.98±0.34 (IV-IV)</td>
</tr>
<tr>
<td>Ammonia nitrogen (mg/L)</td>
<td>0.77±0.15-0.90±0.23 (I-I)</td>
<td>1.11±0.24-1.25±0.13 (II-II)</td>
<td>1.34±0.35-1.43±0.22 (II-II)</td>
<td>1.50±0.40-1.32±0.19 (II-II)</td>
</tr>
<tr>
<td>Nitrate (mg/L)</td>
<td>10.24±1.1-9.15±1.29 (II-II)</td>
<td>10.45±1.86-12.6±1.4 (II-II)</td>
<td>13.57±0.93-15.8±1.21 (II-II)</td>
<td>11.86±1.79-12.7±1.33 (II-II)</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>0.40±0.07-0.38±0.04 (IV-IV)</td>
<td>0.35±0.05-0.36±0.03 (IV-IV)</td>
<td>0.55±0.06-0.56±0.05 (IV-IV)</td>
<td>0.60±0.09-0.68±0.06 (IV-IV)</td>
</tr>
<tr>
<td>Lead (µg/L)</td>
<td>4.62±0.28-5.79±0.21 (I-I)</td>
<td>6.27±0.25-5.84±0.30 (I-I)</td>
<td>4.18±0.13-4.82±0.23 (I-I)</td>
<td>3.94±0.10-4.32±0.26 (I-I)</td>
</tr>
<tr>
<td>Cadmium (µg/L)</td>
<td>1.38±0.17-1.50±0.11 (I-I)</td>
<td>2.85±0.20-2.40±0.32 (I-I)</td>
<td>2.47±0.18-2.12±0.13 (I-I)</td>
<td>2.62±0.25-2.45±0.20 (I-I)</td>
</tr>
<tr>
<td>Iron (µg/L)</td>
<td>50.5±4.23-65.74±5.4 (I-I)</td>
<td>42.39±3.9-37.6±2.51 (I-I)</td>
<td>85.65±3.07-97.32±4.63 (I-I)</td>
<td>93.45±5.87-99.16±8.38 (I-I)</td>
</tr>
<tr>
<td>Copper (µg/L)</td>
<td>5.82±0.32-8.43±0.50 (I-I)</td>
<td>7.65±0.24-6.36±0.48 (I-I)</td>
<td>6.41±0.30-7.73±0.41 (I-I)</td>
<td>6.82±0.38-8.11±0.49 (I-I)</td>
</tr>
<tr>
<td>Manganese (µg/L)</td>
<td>14.89±1.2-19.56±2.0 (I-I)</td>
<td>19.7±1.84-17.32±0.9 (I-I)</td>
<td>22.60±1.46-29.88±3.20 (I-I)</td>
<td>29.83±3.26-34.46±3.18 (I-I)</td>
</tr>
<tr>
<td>Zinc (µg/L)</td>
<td>0.36±0.05-0.54±0.04 (I-I)</td>
<td>0.72±0.07-0.66±0.05 (I-I)</td>
<td>0.53±0.03-0.68±0.06 (I-I)</td>
<td>0.48±0.04-0.63±0.07 (I-I)</td>
</tr>
<tr>
<td>Boron (µg/L)</td>
<td>0.20±0.03-0.34±0.05 (I-I)</td>
<td>0.32±0.02-0.25±0.03 (I-I)</td>
<td>0.75±0.04-0.83±0.06 (I-I)</td>
<td>0.86±0.05-0.77±0.04 (I-I)</td>
</tr>
</tbody>
</table>

I: High quality water, II: weakly polluted water, III: Polluted water, IV: High polluted water
The first values are given referring to the first five station and second values for the second five stations.
and accumulation factors calculated. The results are given in some macroinvertebrates, fish and residues samples of Yesilirmak river [16].

All physicochemical parameters determined in Kelkit stations and were found to be class I except for ammonia nitrogen, nitrate, nitrite and phosphate. Ammonia nitrogen was class II except in autumn, nitrate class II and nitrite class IV in all seasons. The use of fertilizers in agriculture and urban sewage is believed to increase the nitrate and nitrite concentration. In addition, the absence of freshwater plants might cause an increase in nitrogen ion concentrations in the stream. Especially, excess nitrite concentration restricts the life of living organisms. Phosphate was found to be as class II in winter and class IV during the other seasons. The exceeding amounts of phosphate concentration are thought to be mainly a result of the use of dung in agriculture and detergents including phosphate.

Trace metal concentrations were determined to be class I. All physicochemical parameters determined in Kelkit stream are in agreement with those earlier reported for Yesilirmak river [16].

The concentrations of trace metals were determined in some macroinvertebrates, fish and residues samples and accumulation factors calculated. The results are given in Table 5. The accumulation of metals changed depending on the species. For example, some species (Gammarus sp., Baetis sp. and Chironomus sp.) accumulated the metals at high ratios. The minimum and maximum accumulation factors were 3 and 43 for Pb in Elmis aenea and Gammarus sp., 4 and 79 for Cd in Heptagenia sp. and Baetis sp., 67 and 544 for Fe in Chalcalburnus chalcoides and Baetis sp., 83 and 467 for Cu in Sphaerium spp. and Baetis sp., 24 and 88 for Mn in Hydropsyche sp. and Gammarus sp., 200 and 1200 for Zn in Heptagenia sp. and Alburnus alburnus, respectively.

Various metal accumulations in biological samples have been made and the values found are in agreement with those reported in the literature [21, 22]. The concentrations of metals in residues (agricultural runoff, domestic sewage and industrial waste) were found to be high, but their dilution in Kelkit stream decreased metal concentrations. Recoveries were nearly quantitative for all elements studied (≥ 95%) with relative standard deviations less than 10%.

TABLE 5
Concentrations of metals determined in some macroinvertebrates, fish and residue samples.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pb</th>
<th>Cd</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroinvertebrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphaerium spp.</td>
<td>40±5</td>
<td>53±6</td>
<td>12±4</td>
<td>0.5±0.1</td>
<td>0.8±0.1</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Gammarus sp.</td>
<td>216±30</td>
<td>184±21</td>
<td>49±5</td>
<td>2.1±0.2</td>
<td>2.2±0.2</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Baetis sp.</td>
<td>170±24</td>
<td>198±35</td>
<td>56±7</td>
<td>2.8±0.1</td>
<td>1.8±0.1</td>
<td>0.4±0.05</td>
</tr>
<tr>
<td>Heptagenia sp.</td>
<td>23±4</td>
<td>10±1</td>
<td>8±2</td>
<td>1.1±0.2</td>
<td>-</td>
<td>0.1±0.01</td>
</tr>
<tr>
<td>Paraleptophlebia sp.</td>
<td>57±6</td>
<td>36±2</td>
<td>7±0.6</td>
<td>-</td>
<td>1.1±0.2</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Capnia sp.</td>
<td>32±2</td>
<td>42±5</td>
<td>10±2</td>
<td>1.8±0.1</td>
<td>0.9±0.1</td>
<td>-</td>
</tr>
<tr>
<td>Perla bipunctata</td>
<td>18±2</td>
<td>11±2</td>
<td>9±1</td>
<td>2.0±0.4</td>
<td>0.7±0.1</td>
<td>0.3±0.02</td>
</tr>
<tr>
<td>Dinocras cephalotes</td>
<td>65±10</td>
<td>45±6</td>
<td>16±3</td>
<td>-</td>
<td>1.2±0.2</td>
<td>-</td>
</tr>
<tr>
<td>Hydropsyche sp.</td>
<td>86±14</td>
<td>70±6</td>
<td>-</td>
<td>1.3±0.2</td>
<td>0.6±0.1</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Simulium sp.</td>
<td>38±5</td>
<td>49±8</td>
<td>15±2</td>
<td>0.8±0.3</td>
<td>1.4±0.2</td>
<td>-</td>
</tr>
<tr>
<td>Chironomus sp.</td>
<td>204±28</td>
<td>143±32</td>
<td>23±4</td>
<td>2.5±0.2</td>
<td>1.2±0.1</td>
<td>0.5±0.05</td>
</tr>
<tr>
<td>Elmis aenea</td>
<td>15±3</td>
<td>28±4</td>
<td>7±0.4</td>
<td>0.9±0.1</td>
<td>-</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyprinus carpio</td>
<td>66±8</td>
<td>34±5</td>
<td>11±2</td>
<td>1.4±0.4</td>
<td>1.0±0.1</td>
<td>0.2±0.04</td>
</tr>
<tr>
<td>Chalcalburnus chalcoides</td>
<td>45±6</td>
<td>29±3</td>
<td>6±0.3</td>
<td>1.9±0.5</td>
<td>1.5±0.2</td>
<td>0.5±0.08</td>
</tr>
<tr>
<td>Alburnus alburnus</td>
<td>84±12</td>
<td>57±9</td>
<td>8±0.5</td>
<td>1.0±0.4</td>
<td>1.9±0.1</td>
<td>0.6±0.05</td>
</tr>
<tr>
<td>Leuciscus cephalus</td>
<td>37±5</td>
<td>82±10</td>
<td>18±2</td>
<td>1.2±0.2</td>
<td>0.8±0.1</td>
<td>-</td>
</tr>
<tr>
<td>Capoeta tinca</td>
<td>72±9</td>
<td>60±8</td>
<td>14±4</td>
<td>1.6±0.3</td>
<td>1.6±0.2</td>
<td>0.3±0.04</td>
</tr>
<tr>
<td>Silurus glanis</td>
<td>25±4</td>
<td>14±3</td>
<td>12±2</td>
<td>0.9±0.1</td>
<td>0.7±0.1</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Perca fluviatilis</td>
<td>30±6</td>
<td>19±5</td>
<td>9±0.8</td>
<td>1.3±0.2</td>
<td>1.2±0.2</td>
<td>0.4±0.03</td>
</tr>
<tr>
<td>Residues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Agricultural runoff</td>
<td>134±26</td>
<td>120±18</td>
<td>24±3</td>
<td>15±3</td>
<td>9±1</td>
<td>6±0.5</td>
</tr>
<tr>
<td>Domestic sewage</td>
<td>105±18</td>
<td>85±12</td>
<td>10±2</td>
<td>12±5</td>
<td>5±0.8</td>
<td>8±0.7</td>
</tr>
<tr>
<td>Industrial waste</td>
<td>275±52</td>
<td>200±30</td>
<td>36±4</td>
<td>23±4</td>
<td>8±0.6</td>
<td>10±1</td>
</tr>
</tbody>
</table>

*: not detected

concentrations of Pb and Cd (µg/kg), all others (mg/kg)
ACKNOWLEDGEMENT

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THE REMOVAL OF CHROMIUM(VI) FROM SYNTHETIC WASTEWATER BY *Ulothrix zonata*

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**SUMMARY**

The removal of Cr\(^{6+}\) ions with *Ulothrix zonata*, a species of green algae, was studied at a fixed temperature and mixing rate. Initial metal ion concentration, initial pH, biomass concentration and the effect of contact time were investigated and their suitability to adsorption isotherms was examined. It was observed that the results were in conformity with the Freundlich adsorption isotherm. The highest uptake was achieved at 30 min. and pH=1.0. The removal ratio of Cr\(^{6+}\) respectively founded 96%, 47%, 44%, 34%, 30% at 5, 25, 50, 100, 150 mg/L metal concentration.

**KEYWORDS:** Adsorption isotherms, Algae, Biosorption, Chromium ion removal.

**INTRODUCTION**

The release of heavy metals into our environment is still large [1]. Heavy metals such as copper, lead, chromium, cadmium, etc., in waste water are hazardous to the environment. Because of their toxicity, their pollution effect on our ecosystem presents a possible human health risk [2].

The maximum levels permitted in waste water are 5 mg/L and 0.05 mg/L, respectively Cr\(^{3+}\) and Cr\(^{6+}\). They exist as low levels in the environment. Cr\(^{3+}\) apparently plays an essential role in plant and animal metabolism, while Cr\(^{6+}\) is directly toxic to bacteria, plants and animals [3].

The main sources of chromium pollution are mining, leather tanning and cement industries, electro plating, production of steel and other metal alloys, photographic material and corrosive paints. The chromium compounds most severely responsible for environmental pollution are the trivalent compounds, chromium oxide and chromium sulfate and the hexavalent compounds, chromium trioxide, chromic acid and dichromates [4].

Conventional methods for removing Cr\(^{6+}\) from waste water include chemical reduction, electro chemical treatment, ion exchange and evaporative recovery. The search for new and innovative treatment technologies has focused attention on the metal binding capacities of various microorganisms. Many yeasts, algae, bacteria and various aquatic flora are known to be capable of concentrating metal species from dilute aqueous solutions and accumulating them within the structure of the microorganism [5].

The adsorption of heavy metal ions is also a very beneficial property of most algae. Because of this property more economic, practical and efficient techniques are being developed for the treatment of industrial waste waters and algae have been successfully used as adsorption agent for heavy metals. The uptake of metal ions by microorganisms in batch systems has been suggested to occur in two stages: an initial rapid uptake (passive uptake), followed by a much slower process (active uptake). The first stage is thought to be physical adsorption or ion exchange at the cell surface. The adsorption equilibrium occurs within 5-10 min at the end of rapid physical adsorption. Dead cells accumulate heavy metal ions to the same or greater extent than living cells; this kind of adsorption is called ‘biosorption’ [6].

Kinetics of biosorption by algal cells involves two stages. The first phase is very rapid, occurring immediately after initial contact with the cell and usually lasting for less than 30 minutes. This initial phase is thought to be passive, involving physical sorption or ion exchange at cell surfaces. The second phase is slow and extended, and has been observed to continue for more than one month. The slow phase appears to be active and related to metabolic activities of the cell. The relative importance of these two stages depends on the organism involved [7].

Many types of biomass in non-living form have been studied for their heavy metal uptake capacities and suitability to be used as bases for biosorbent development. These include bacteria [8-10], fungi [11], fresh water algae [12, 13], marine algae [14-17]. In general, the heavy metal uptake capacities have varied significantly for different types of biomass studies.
In this study, the effects of some parameters on the removal of Cr\textsuperscript{6+} from synthetic wastewater by *Ulothrix zonata* were investigated. These parameters are initial metal concentration, algal biomass concentration and pH, which mainly affect the waste water treatment by the biosorption technique.

**MATERIALS AND METHODS**

**Preparation of algae and Cr\textsuperscript{6+} solutions for biosorption**

*Ulothrix zonata*, a species of green algae collected from the fresh water channels of the fish farm at Atatürk University, Erzurum, Turkey was used as adsorbent in this study. For the biosorption studies, the collected living algal filaments were rinsed with distilled water and then inactivated in an oven at 100 °C for 5-6 hours. A given amount of inactivated dried *U. Zonata* was then suspended in double distilled water homogenizing for 45 min in a waring blender. Algal suspension was prepared as 10 g alg/L solution and used by dilution. Specific BET (N\textsubscript{2}) surface area of algae is 1.1 m\textsuperscript{2}/g.

Feed Cr\textsuperscript{6+} solution was prepared by dilution of stock 1.0 g/L solution which was obtained by dissolving the exact quantity of potassium dichromate in 1 liter of double distilled water. Before mixing with the algal suspension, the pH of metal solution was adjusted to 1.0 with 1M H\textsubscript{2}SO\textsubscript{4}.

Cr\textsuperscript{6+} concentration was then determined by using an indirect UV-Visible spectrophotometric method based on the reaction of Cr\textsuperscript{6+} and diphenylcarbazide, which forms a red-violet colored complex. The absorbance of the colored complex was measured in a double beam spectrophotometer at 540 nm wavelength, and Cr\textsuperscript{6+} concentration was determined by comparing absorbance to a calibration curve prepared by using standardized concentrations of Cr\textsuperscript{6+} between 0.005 mg/L and 2 mg/L.

**Biosorption Studies**

For biosorption studies, a weighed amount of dry cells was suspended in distilled water and homogenized in a mixer to disperse cell aggregates. The adsorption of Cr\textsuperscript{6+} by *Ulothrix zonata* was investigated in batch experiments. All biosorption experiments were conducted in flasks on a rotary shaker. The temperature of the system during the experiment was kept constant at 20 °C.

Equal and known quantities of suspended homogenized algal solution were added to flasks containing Cr\textsuperscript{6+} solutions with known concentration. The initial pH was adjusted with dilute solutions of HNO\textsubscript{3} and NaOH. The flasks were shaken at 150 rpm on a thermostated shaker for 2 hrs which is enough time to reach equilibrium of adsorption at room temperature. Samples were filtered using Whatman GF/A retained. The concentrations of unadsorbed Cr\textsuperscript{6+} ions in the remaining solution were determined.

**RESULTS AND DISCUSSION**

In this study, adsorption capacities of dried algae are investigated considering several factors which are important in the biosorption of Cr\textsuperscript{6+}. These factors include pH, time, initial metal ion concentration, dried algal concentration and specific surface properties of the alga.

**Effect of contact time**

Figure 1 shows the variation of percent removal of Cr\textsuperscript{6+} with contact times. As contact time increases, percent removal also increases initially, but then gradually approaches a constant value.

![FIGURE 1 - Effect of contact time on the removal of Cr\textsuperscript{6+} (adsorbent concentration=0.1 g/L, temperature=20 °C, agitating rate=150 rpm, pH=1.0).](image-url)
The rate of removal of Cr\(^{VI}\) is high in the first 30 min, but thereafter the rate significantly decreases and eventually approaches zero, finally the equilibrium point has been attained. These changes in the rate of removal may be due to the fact that, initially all adsorbent sites are vacant and the solute concentration gradient is high. The decrease in rate with time also indicates monolayer adsorption of Cr\(^{VI}\) on biosorbent surface and the presence of diffusion from solution to pores of biosorbent.

Effect of pH on Cr\(^{VI}\) uptake

The equilibrium Cr\(^{VI}\) uptakes at various pH values are presented in Figure 2. The effect of pH was studied by varying the suspension pH from 1.0 to 5.0. The uptake of Cr\(^{VI}\) increases with the decreasing suspension of pH.

Figure 2 shows that the lowest biosorption occurred at pH 5.0 and the greatest biosorption occurred at pH 1.0. It has been known that Cr\(_2\)O\(_7^{2-}\) ions precipitate at pH’s above 7. Hence all sorption experiments were conducted at the acidic pH range (pH: 1-5). The high percent removal observed at pH 1.0 can be attributed to the positive surface charge gained depending on the adsorption of H\(^+\) ions on the algal surface. Similarly results have been found by Aksu et al. [2] a strain of Cladophora crispata collected from irrigation water channels.

![Figure 2](image_url)

**Figure 2** - Cr\(^{VI}\) uptake by *U. zonata* as a function of solution pH (initial metal concentration=50 mg/L, adsorbent concentration= 0.1 g/L, temperature=20° C, agitation rate=150 rpm, contact time=120 min).

![Figure 3](image_url)

**Figure 3** - Effect of algae concentration on the removal of Cr\(^{VI}\) (temperature=20° C, agitation rate=150 rpm, initial Cr\(^{VI}\) concentration=150 mg/L, pH=1.0)
Effect of algae or sorbate concentration

The dependence of Cr(VI) sorption on *U.zonata* concentration was studied at room temperature and optimum pH value by varying the sorbent amount from 0.1 to 1 g/L. The results are shown in Figure 3.

It is apparent that the percent removal of Cr(VI) increases rapidly with increasing concentration of the alga, due to the greater availability of the exchangeable sites or surface area at higher concentration of the sorbent.

The results are presented in Figure 3. It can be seen from this figure that Cr(VI) uptake is high for higher dosages. This may be due to the fact that the higher doses of the adsorbent, the more sorbent surface and pore volume will be available for the adsorption.

Effect of initial metal concentration

The effect of initial metal concentration on the capacity of biosorption is shown in Figure 4 where the data obtained at the end of the experiment are given. Percent removal of Cr(VI) determined at given contact times for five different initial Cr(VI) concentrations at certain conditions.

Figure 4 shows that the percentage removal of the metal ions decreases with the increasing of the initial concentration of metal ion. This indicates both the presence of equilibrium and the formation of monolayer on the algal sorbent surface for adsorption of Cr(VI) ions. Hence, it can be said that the sorption process occurs through electrostatic interactions.

Adsorption Isotherms

The variation of amount adsorbed with equilibrium Cr(VI) concentration were investigated. Obtained results are graphed in Figure 5. This shows that the amount adsorbed of Cr(VI) increases with increasing of equilibrium concentration of Cr(VI). The Freundlich adsorption model was used to describe the adsorption process. To obtain adsorption isotherm, experiments were performed at the concentration of adsorbent of 0.1 g/L, the several metal ion concentration and pH 1.0. The Freundlich model has been widely adopted to characterize the adsorption experiments.

\[
q_e = \frac{X}{M} = K_f C_e^{1/n} \quad (1)
\]

\[
\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (2)
\]

\[
X = C_o - C_e \quad (3)
\]
The adsorbent dosage, $M$, required to reduce the initial concentration, $C_o$, so the desired final concentration, $C_e$, is calculated from Eq 3. The value $X/M$ at log $C_e = 0$ can be determined from intercept of log $q_e$ graph versus log $C_e$. This is helpful for large-scale applications of batch systems. A logarithmic plot linearizes the equation, enabling the exponent $n$ and the constant $K_f$ to be determined: where $q_e$ is the amount of Cr^{6+} adsorbed by unit mass of adsorbent, $K_f$ the sorption capacity, indicator of sorption intensity, and $C_e$ is the equilibrium concentration.

$$\log q_e = \log K_f + \frac{1}{n} \log C_e$$  \hspace{1cm} (4)

The values of constants $K_f$ and 1/n have been determined from Figure 6. They are 1.79 and 0.38, respectively. The correlation coefficient is 0.9863. The high value of correlation coefficient indicates that the experimental data fitted well to the Freundlich model. $n$, higher than 1.0 imply that Cr^{6+} adsorption is quite intense. In addition, the magnitude of $K_f$ and $n$ also show easy uptake of Cr^{6+} from wastewater and high adsorptive capacity of algal biomass.

![Freundlich Isotherm for Cr^{6+} Adsorption onto the Algal Biomass (20°C).](image)

**FIGURE 6**

CONCLUSION

Algae have a great potential to remove metal ions in very low concentrations and to accumulate large amounts of specific toxic elements. The obtained results showed that initial pH and ion concentration significantly affected the uptake capacity of the biosorbent. At the biosorption levels, a process using algal biomass for the removal and recovery of a heavy metal ion is potentially more sustainable than current process technology. The highest sorption of Cr^{6+} was observed at pH 1.0 and the lowest sorption was observed at pH 5.0. Since different metal ions have different properties with regard to acidity of the solution, optimum pH for various metal ions can change.

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DEGRADATION PROCESS OF PROCYMIDONE IN SOIL

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SUMMARY

In this work, the behaviour of an antibotrytic fungicide, Procymidone (PCM) has been evaluated in both soil without and with addition of different percentages of compost in order to verify the influence of this organic matter on its degradation. The results obtained showed that the degradation process of PCM in soil was faster than that obtained in soil with compost addition, while the metabolites formed were the same.

KEYWORDS:
Procymidone, metabolites, soil and compost.

INTRODUCTION

In the light of the new technical-commercial strategies being derived from the present environmental philosophy, the users are encouraged to concentrate their attention on the environmental fate of the pesticides employed. Pesticides can also represent an environmental risk when they accumulate in the soil or, when they give rise to toxic products caused by their degradation. These compounds can be transported into the groundwater by leaching or into surface bodies of water by runoff from the soil, or they can be absorbed by plants [1].

The addition of organic matter to the soil by means of compost is an ever more widespread agricultural practice. An increase in organic matter can lead to either the immobilisation of pesticides with a consequent reduction in bio-availability or an increase in the degradation rate due to the greater biomass activity [2, 3].

Dicarboximidic fungicides (Iprodione, Procymidone, Vinlozinol and Chlozolinate) are widely used on various crops against Botrytis cinerea, Helmintosporium, M. nilinia and Sclerotinia [4]. The correct application of antibotrytic fungicides does not generally produce a residual content greater than the legal limits in foods [5, 6]. Nevertheless, frequent applications mean that the phenomena of accumulation in soil cannot be excluded, not only for the active ingredient, but also for its metabolites.

In the present work, the degradation rates in soil of Procymidone (PCM), a widely used dicarboximidic fungicide, were studied, paying particular attention to the presence of specific metabolites already identified during the composting process [7], those being 3,5-dichloroaniline and 2-(3,5-dichlorophenylcarbamoil)-1,2-dimethylpropane carboxylic acid (metabolite I). The chemical structures of PCM and their metabolites are shown in Figure 1.

![Chemical structures of PCM and metabolites](image-url)
After the addition of PCM to the soil, the degradation process of the active ingredient was verified by using the HPLC-DAD-MS system, after an extraction procedure with ultra-sound technique carried out in the presence of acetonitrile [7,8]. These results were then compared with those obtained in the same soil with an increasing amount of compost added.

MATERIALS AND METHODS

The PCM [N-(3,5-dichlorophenyl)-1,2-dimethyleclopropane-1,2-dicarboximide] and 3,5-dichloroaniline were supplied by Dr. Ehrenstorfer GmbH. The commercial product Sialex 50 WDG (PCM) was supplied by SIAPA. The 2-(3,5-dichlorophenyl)carbamoyl)-1,2-dimethylpropane carboxylic acid (metabolite I) was obtained after abiotic hydrolysis of the active ingredient at pH=8.7 in phosphate buffer, as described in a previous piece of work [7]. The reagents used were: acetonitrile for HPLC (G-Chromasolv, super gradient grade, Riedel-de-Haën) and ultra pure water, supplied by Elgastat purification systems. The compost added, coming from the Regional Disposal Consortium, was made up of vegetable residues (barks, prunings) and selected organic wastes. Its maturation time was three months. The soil was collected from an horticultural field in Piedmont (North-West Italy), air dried to 15% water content (w/w), sieved to obtain a fraction below 2 mm, and stored at + 4° C for about 15 days. Two parts of the soil had 1% and 25%, respectively, of added compost, according to common applications in vineyards (1%) and in floriculture (25%).

The Italian Society of Soil Science methods [9] were used to determine the soil sample characteristics, some of these are reported in Table 1. Soil samples (100 g air-dried) were placed in closed incubation systems as described by Nègre et al. [10], adjusted to 50% of WHC and then spiked with 2 ml of a 625 µg/ml PCM (obtained by dissolving 250 mg of the technical product in 200 ml of distilled water). Incubation was performed in the dark at 25 °C. The degradation process was observed for five months. At different times, triplicate 10 g samples were withdrawn and extracted. The compounds were extracted in acetonitrile using a sonication disruption technique previously reported [7, 8] and subsequently analysed through the HPLC-DAD-MS system.

A Lichrosphere, (HP) RP-C18 column (250 mm x 2 mm, particle size 5 µm) at room temperature was used for the analysis at a flow rate of 0.3ml/min.

The solvent programming was as follows: initially 1min. isocratic with 40% acetonitrile, 48 min. linear gradient to 70% acetonitrile. An additional isocratic step of 30 min. with 100% acetonitrile was necessary to wash the column of non-polar substances. For the detection of compounds, two detectors were used: DAD (absorbance data was recorded from 190 to 400 nm and chromatograms were monitored at λ<sub>max</sub>= 210 nm during every analysis) and MS (source APCI or ESI both positive and negative polarity) according to the procedure optimised in a previous work [7]. Experimental data was fitted by the equation of first order exponential decay: C=C<sub>0</sub> e<sup>-kt</sup>

RESULTS AND DISCUSSION

The experimental kinetic curves of degradation of the active ingredient in the three cases pointed out pseudo first order kinetics as reported in Figure 2, where, with the linear regression of ln (C/C<sub>0</sub>) versus time (minutes), it was possible to estimate the value of k<sub>obs</sub>

Operative parameters and kinetic results regarding active ingredient degradation during the composting process and in soils are compared in Table 1. The degradation half-life (t<sub>1/2</sub>) of the active ingredient in the original soil was 42 days.

TABLE 1

Operative parameters and kinetic results in native soil, in soil+1% compost and in soil+25% compost and during the composting process.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Soil</th>
<th>Soil + 1% compost</th>
<th>Soil + 25% compost</th>
<th>Compost (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.8</td>
<td>6.6</td>
<td>6.5</td>
<td>5.5 + 8.7</td>
</tr>
<tr>
<td>Organic matter (％)</td>
<td>1.60</td>
<td>1.70</td>
<td>6.89</td>
<td>28.5</td>
</tr>
<tr>
<td>T,°C</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25 + 60</td>
</tr>
<tr>
<td>k&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2.9 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>2.7 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>1.9 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>3.2 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>k&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.2 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>3.8 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>1.4 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>4.1 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
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<tr>
<td>k&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.7 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>1.1 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>1.4 × 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>/</td>
</tr>
</tbody>
</table>
The addition of compost has slowed down the degradation process, but not in a directly proportionate way (see Table 1). It is well-known that the organic matter of the soil represents a highly active adsorption phase, when pesticides are concerned and that, moreover, it constitutes a substrate for the development of microorganisms that degrade them [2, 3]. PCM has a great affinity with the organic soil matter, as it can be seen from its high $K_{ow}$ (1380) reported by the Pesticide Manual [4]. Therefore, it is possible that the added compost reduced the quantity of the active ingredient available for the degradation. Nevertheless, it can be assumed that the greater quantity of compost increased the activity of microorganisms able to degrade this molecule and that its degradation favoured the sorption process.

Actually, the degradation of Procymidone in the compost (its organic matter content was 28.5%), was similar to that observed in the original soil.

The mass spectra of the breakdown products formed in all examined samples (spectra not showed), suggest the formation of the same metabolites, 3,5-dichloroaniline (3,5-DCA) and metabolite I (2-(3,5-dichlorophenylcarbamoil)-1,2-dimethylethylene carboxylic acid), already identified during the previous composting process investigations [7], but the metabolic pathway is different.

In fact, in the compost we observed only the formation of the two metabolites, whereas in the soil (with and without compost added), the additional transformation of metabolite I to 3,5-DCA occurs, as shown in Figure 3.

![Figure 3 - Metabolic pathway proposed for PCM in studied systems (→) and in the compost (→→).](image)

In the original soil and in the soil with added compost the degradation process seems to proceed through two parallel reactions ($k_1$ – $k_2$) (for values, see Table 1) producing two different metabolites simultaneously as in the composting process. Moreover, in this study the presence of an additional reaction step has been shown ($k_3$) (see Table 1), in which the intermediate product (metabolite I) can again contribute to the 3,5-DCA formation.

This last aspect underlines that the presence of 3,5-DCA in soil and in the compost added soil is supported not only by the PCM degradation, but also by its metabolite I degradation. The values of kinetic constants evaluated confirms the previous statement.

This metabolic pathway has been obtained by employing a pseudo-first order equation, through parallel-consecutive reactions [11]. The disappearance of PCM did not correspond to the formation of equal quantities of the known derivative 3,5-DCA. Nevertheless, this behaviour is in agreement with the results obtained for this fungicide in other natural matrices, as for example, in the must and in the wine [5]. Actually, in this case, metabolite formation does not reach the initial amount of the parent product. In the case of the soil, there is an increase in soil absorption during the experimental time (this is suggested by the PCM high $K_{ow}$ value) that could justify a non-quantitative metabolisation of the fungicide.

Taking into account the $t_{1/2}$ values reported in Table 1, the degradation rate in soil is similar to that during the composting process. Nevertheless, in the latter, degradation proceeds only through parallel reactions, while in the former a consecutive reaction also occurs.

Inversely, on the addition of compost to the soil (a matrix rich in organic matter) substantially limits the whole degradation process. It is likely that the organic matter reduces the PCM bioavailability - thus slowing down its degradation.

So, the employment of the compost could promote the bioaccumulation of pesticides in soil, hence, because of the toxicity of these substances, this aspect cannot be left out.

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Quality in Chemical Measurements –
Training Concepts and Teaching Materials

Bernd Neidhardt, Wolfhard Wegscheider (Eds.):

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ISBN 3-540-65994-3; Hardcover €51.00/US$49.95.

The concepts of “Analytical Quality Assurance
(AQA)” and “Analytical Quality Management (AQM)”
developed in the wake of the harmonization of the Euro-
pean market have now been formally established via the
appropriate directives and norms (ISO 25, EN 45001, and
recently ISO 17025). These developments have become
widely accepted as market-regulating elements by both
the chemical industry and independent laboratories for
routine chemical analysis and are practised extensively in
the form of accreditation. But this has taken place without
any perceptible participation on the part of the universi-
ties. But quality of chemical measurements must become
a sustaining element of modern research and teaching in
the chemistry departments of universities. The prerequi-
site is an improvement in the teaching and training in
Analytical Chemistry via changes in content, concepts
and organization of teaching in foundation, undergraduate
and graduate courses in Chemistry, the introduction of
Analytical Chemistry as a compulsory subject or intensive
support for the new generation of academics in Analytical
Chemistry. Academic freedom in teaching and research
involves a responsibility to adapt oneself to changed con-
ditions, to prepare students for new tasks, to face the
competition from other universities and to give priority to
fulfilling duties, if necessary, at the expense of one’s own
scientific interests.

The 2nd EURACHEM Workshop on Current Issues
in Teaching Quality in Chemical Measurements (27-29
Oct. 1998) was a meeting of 50 experts from 14 European
countries to fill the gap between theory and reality in this
field. The output is published in this textbook comprising
a collection of transparencies on a CD-ROM. This helpful
material will assist in reducing the activation barrier asso-
ciated with the preparation of lectures and seminars on
this topic.

Environmental Analysis Volume 3
included in the series Handbook of Analytical Sepa-
rations by series editor Roger M. Smith

Wolfgang Kleiböhmer (Editor)

344 pages, numerous tables and figures; Elsevier Science
Shannon – Tokyo, 2001; ISBN 0-444-50021-9; Hard-
bound €184.69.

In the series “Handbook of Analytical Separations”
Volume 1 (Separation Methods in Drug Synthesis and
Purification by K. Valkó) and Volume 2 (Forensic Sci-
ence by M. J. Bogusz) have already been published. Now
the series is continued with Volume 3 – “Environmental
Analysis”. This volume is not an addition to the long list
of already published excellent textbooks dealing with
analytical separation techniques, but an up-to-date review
on both new solutions for well-known and still lasting
problems as well as for new existing problems in envi-
ronmental analysis. Therefore, the editor and his co-
working experienced team of authors cover a compilation
of methods in the field of environmental analysis for the
determination of a wide range of environmental pollu-
tants, such as amines, polycyclic aromatic hydrocarbons
(PAHs), pesticides, phenols, PCBs, organometallic spe-
cies, polycyclic aromatic sulphur heterocycles, and me-
tabolites of PAHs.

The book can be strongly recommended to practition-
ers in environmental analysis. Primarily it gives up-to-
date information on sampling and sample pre-treatment,
extraction techniques, clean-up steps, pre-fractionation,
different types of chromatographic methods and quality
assurance. Additionally, information on actual or upcom-
ing analytical problems, such as determination of polycy-
clic aromatic sulphur heterocycles or metabolites of PAHs
are presented. In the last chapter of the book the important
role of analytical separation techniques in water quality
control is elucidated and explained.

The Diels-Alder Reaction -
Selected Practical Methods.

Francesco Fringuelli and Aldo Taticchi

340 pages; Wiley-VCH, Weinheim, Chichester, 2002;
ISBN 0—471-80343-X; Hardcover 219.00 EUR.

The new book of Fringuelli and Taticchi is an excellent
and state-of-the-art review on the Diels-Alder reaction.
This special [4 + 2] cycloaddition reaction, which allows
the thermal reaction of an alkene (philodiene) with a conju-
gated diene, was invented by Diels and Alder in 1928 and
is still very much in use in organic chemistry documented
by more than 17,000 publications since that time. It is the best-known organic reaction and has been studied extensively for synthetic and mechanistic purposes.

The book contains seven chapters. The first chapter gives a more general introduction into the field. By use of this reaction a great variety of six-membered rings can be constructed with up to four stereogenic centers. This is shown for simple molecules as well as for complex structures of natural products such as chlorotrichloride. The reaction can be coupled with other reactions in a consecutive fashion, e.g. as cascade and Domino-Diels-Alder reaction. Representative examples of dienophiles and philodienes are presented in several informative tables. Also some examples of Retro- and Homo-Diels-Alder reactions are outlined there. Besides of normal pericyclic reactions some examples for ionic and radical 4+2 cycloadditions are cited. Characteristic behaviour of Diels-Alder reactions like regio- and stereochemistry along with effects of substituents on reactivity, and regioselectivity is discussed in this chapter. These mechanistic results of Diels-Alder reactions have been used to apply FMO theory: in general the superfacial interaction of the HOMO of the diene with the LUMO of the philodienes can explain the behaviour in normal Diels-Alder reactions, in special cases inversely cycloaddition behavior is observed.

The next chapters present many special examples for thermal Diels-Alder reactions. A great variety of six-membered rings with carbon and heteroatoms can be prepared, e.g. vinylarenes and heteroarenes can be extended to higher homologues by addition to chinoidic systems as philodienes. [60]fullerene has been shown to react as philodiene with some activated dienes. Some seleno-substituted alkynes have been used as ketene equivalent. A special chapter is contributed to Lewis-acid catalysis in Diel-Alder reactions. Addition of aluminium chloride enhances reaction times in many cases and the facial selectivity will also be greatly improved by this Lewis acid catalyst. Recently Scandium triflate and Samarium iodide have been proven as very useful catalysts along with various chiral catalysts. Also here typical and new examples of various catalytic effects are well presented in a table. In a special chapter new results of biocatalyzed Diels-Alder reactions are presented. A special enzyme, Diels-Alderase, was isolated in 1995 from a fungus growing on tomato and potato plants (Alternaria solani). It catalyses the intramolecular 4+2 addition of the natural product, prosolana pyrone, to the toxic isomer under very mild in vivo conditions. It is the first example of a natural Diels-Alder reaction.

A special chapter is devoted to high pressure Diels-Alder reactions. Also very interesting is the chapter of unconventional reaction conditions. Breslow showed kinetically strong rate enhancements of simple Diels-Alder reactions in water compared to organic solvents. Other researchers like Engberts and his group worked intensively in this field. It was also shown in 1992 that lithium perchlorate diethyl ether is an excellent solvent for many pericyclic reactions. Furthermore, effects of microwaves, sonochemistry and performance in micro-emulsions are extensively discussed. The book is a very modern review on this topic “Diels-Alder reaction” with more than thousand citations, excellently written and a must for a well-assorted research library.
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