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founded jointly by F. Korte and F. Coulston

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PHYTOPLANKTON DYNAMICS AND PHYSICAL-CHEMICAL FEATURES OF A SHALLOW LAKE (LAKE PAMVOTIS, GREECE).

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SUMMARY

The annual distribution of nutrients, Chlorophyll-a and the dynamics of the phytoplankton assemblages were studied during 1998-99 in Lake Pamvotis. High concentrations of nutrients were recorded, while the range of Chlorophyll-a values varied between 13-44 $\mu\text{g/l}$, reflecting the eutrophic character of the lake. Cyanophytes were the most important group during the warm period. The phytoplankton seasonal sequence is mainly correlated with environmental factors.

KEYWORDS: Chlorophyll-a, phytoplankton, seasonal variation, nutrients, Lake-Pamvotis.

INTRODUCTION

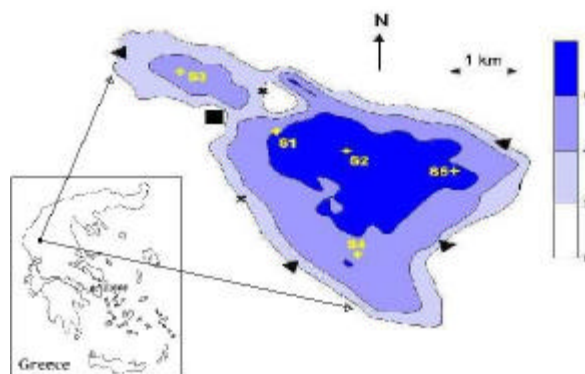
Data on the phytoplankton and the physico-chemical features of lake Pamvotis in Greece are lacking completely. The only published paper to date is restricted to phytoplankton taxonomy.¹ The study on lake Pamvotis was started in order to determine some physical-chemical features of the water as well as the phytoplankton dynamics.

Several studies indicate the effects of environmental parameters on phytoplankton dynamics^{2,3,4,5,6,7,8,9}. The influence of various factors on the seasonal appearance of phytoplankton differs significantly, with physical factors (temperature, water mixing underwater, light, climate) being the most important and chemical (nutrient level) as well as biotic factors (grazing and parasitism) being of lesser importance.¹⁰ Moreover, because of the fact that our knowledge is limited, the behaviour of algal population can sometimes be unpredictable.¹¹

Based on the above, it is clear that research on shallow lakes concerning the behaviour of abiotic and biotic factors is of particular importance. This kind of knowledge may be a useful management and restoration tool.

The present paper describes the seasonal fluctuations of three algal taxonomic groups (cyanophytes, chlorophytes, diatoms) in lake Pamvotis in relation to the environmental conditions.

FIGURE 1 - Bathymetry of lake Pamvotis (Greece) with the 5 sampling-stations, shown as +. Locations of inflows and outflows given by ? and city of Ioannina demarcated by ?



STUDY SITE AND METHODS

Lake Pamvotis is a shallow lake (maximum depth 8m), located 470 m above M.S.L in Northwestern Greece (Fig.1). The catchment area of Pamvotis consists of two morphological units, i.e. a western mountainous unit and an eastern flat one. According to geological data, lake Pamvotis is a karstic lake, which was formed during the late miocene to the pliocene period.¹² It receives substantial inputs of underground water, while there are no naturally occurring surface outflows. Drainage from the basin occurs through a system of sink holes that drain to the rivers Arachtos, Louros and Kalamas. The lake is an important ecosystem in the region supporting local agriculture, tourism and fisheries. During the past thirty years the ecosystem of Pamvotis sustained many activities such as irrigation, sediment deposits, input of agricultural and domestic sewages causing a serious problem in its trophic state.

Water samples were collected bi-weekly during April 1998–March 1999, in five sampling–stations (Fig. 1). The samples were taken at three depths (surface, mid-depth, bottom) for physicochemical analysis and intergrated samples were used (surface to bottom) for phytoplankton determination. Temperature and pH were measured using a thermometer and pH meter, WTW type. Water transparency was estimated using a Secchi disk. The following analytical procedures were used for the chemical analysis, according to APHA¹³. Dissolved oxygen was measured by Winkler's method, while nitrate-nitrogen was analysed according Cadmium Reduction method. Ammonium-nitrogen was determined using the Phenate method based on indophenol blue formation, SRP using the colorimetric method based on molybdenum blue formation and Silica according the Molybdosilicate method. For Chlorophyll-a determination, after filtering samples through GF/C glass fiber filters, Chl-a was extracted using 90% acetone sol. and concentration was calculated from the absorbance at 664nm. No corrections were made for pheophytin content. Algae samples after their pretreatment were counted according to Utermohl's¹⁴ sedimentation method and grouped into larger taxonomic groups. The determination of phytoplankton biomass (wet weight) was based on the estimation of the volume of each species, assuming a specific gravity of one.¹⁵

RESULTS AND DISCUSSION

Environmental Conditions

The response of lakes to meteorological forces has been considered a main factor for their dynamic.¹⁶ In Lake Pamvotis the diel variability of wind speeds was significantly greater during the summer (May–July) and major wind events (≈ 4 m/s as hourly average) occurred 2–3 times a month through the winter.¹⁶

During the study, no rainfall occurred from mid-June to mid-August. Precipitation occurred throughout the remainder of the year with high rainfall periods occurring the autumn and during February.

The surface water temperatures varied from 6.0 °C (in January) to 27.1 °C (in July) and bottom temperatures followed the same variation (i.e from 5.89 °C in January to 26.8 °C in July, Table 1). A weak thermal stratification was noticed from April through August while the water mixing began early in September. The lake's mixing regime is polymictic.

Dissolved oxygen (D.O.) in surface waters fluctuates between 5.3 mg/l and 11.9 mg/l, appearing the minimum values through the warm period (Table 1). Diurnal stratification was measured throughout the spring and summer, while the water column was isothermal in winter (Table 1). The maximum concentration, 11.9 mg/l, was recorded in January during the isothermal conditions. Decomposition process and sediment oxygen demand were sufficient to cause lower D.O. values near-bottom from June to October relative to other depths.

The pH fluctuated between 7.5 and 8.7 in surface waters, lower values appearing in June and July (Table 1). pH values above the sediment ranged between 7.5 and 8.4. Similar to temperature and dissolved oxygen, stratification of pH occurred during the warm period.

Secchi-disk transparency varied between 0.5 and 0.9 m (Table 1). The highest values (in Nov., Dec., Jan.) may be related to the low phytoplankton biomass, while there was low seasonal variability throughout the year. Factors which favor the turbid water state in lake Pamvotis are the algal biomass and the stirring-up of the sediment after wind events and heavy rainfalls.

TABLE 1 - Physical-Chemical data of lake Pamvotis(a:surface,b:mid-depth,c:bottom).

Month		A	M	J	J	A	S	O	N	D	J	F	M
Temp.°C	a	14.1	18.3	23.9	27.1	26.1	23.4	18.6	11.2	7.9	6	6.4	11.8
	b	13.8	17.6	22.3	26.8	26	23.2	18.5	11.1	7.7	5.8	6.1	10.6
	c	12.9	17.3	21.2	24.9	25.9	23.1	18.4	11	7.7	5.6	5.8	9.5
pH	a	7.7	8.2	7.8	7.5	8.7	8.5	8.4	8.2	8	8.4	8.5	8.2
	b	7.9	8.2	7.8	7.5	8.7	8.5	8.4	8.2	8	8.4	.5	8.1
	c	7.5	8	7.1	7.1	7.1	8.4	8	8.2	7.9	8.3	8.4	7.9
D.O. (mg/l)	a	10	8	7.8	8	6.6	5.3	6.8	7.5	8.7	11.9	10.6	9.6
	b	9.6	7.8	6.2	7.6	6	4.3	6.4	7.4	8.5	12	10.7	9.4
	c	8.2	7	3.6	4.1	4.9	3.8	5	7.3	8.5	11.5	10.3	8.3
Secchi d.(m)		0.7	0.7	0.6	0.6	0.5	0.5	0.6	0.8	0.8	0.9	0.8	0.7

FIGURE 2 - Seasonal distributions of nutrients in the three depths.

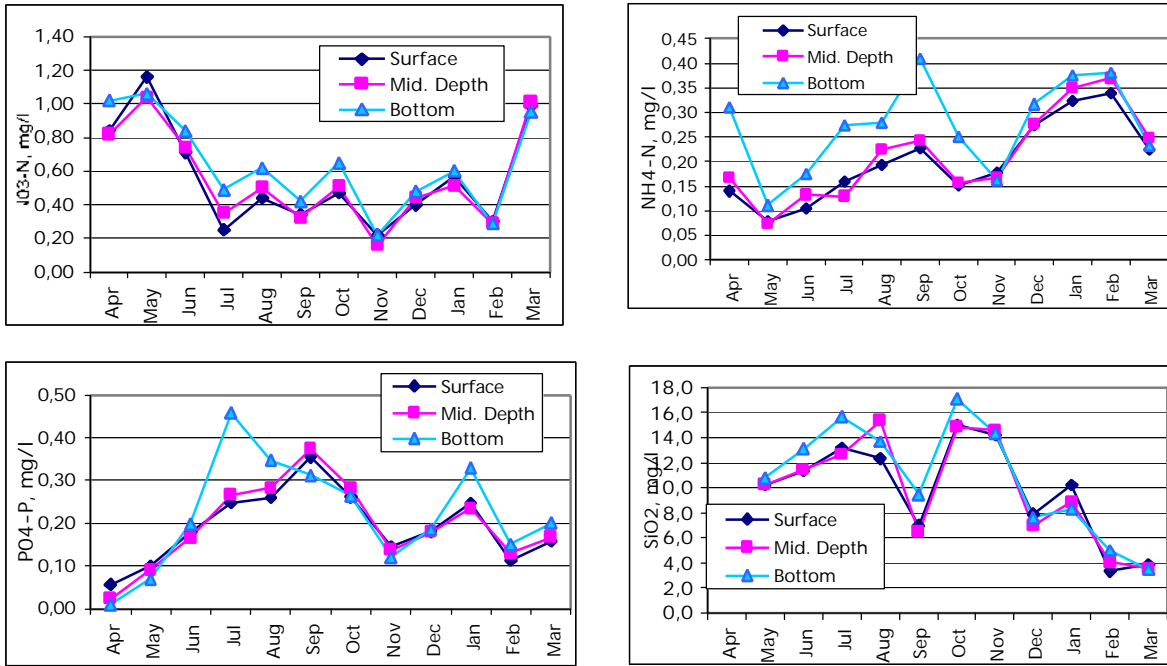
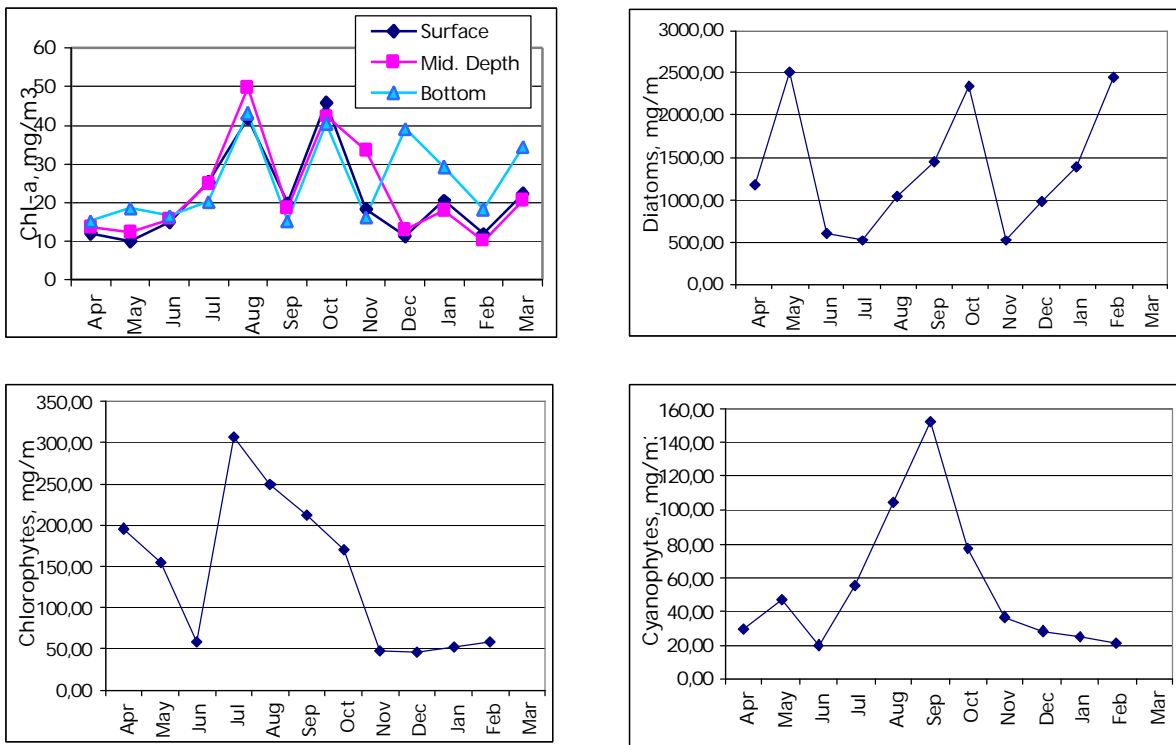


FIGURE 3 - Monthly fluctuations of Chla and algal biomass.



Concentrations of soluble reactive phosphorous (SRP) had a seasonal range of 0.06 ~ 0.35 mg P/l in surface water and exhibited higher values during the warm-dry period. Maximum value was recorded in September, while high values were also observed during Dec. – Jan. The near-bottom SRP profile shows a quite similar seasonal pattern, the highest values appearing during Aug. - Sept. (Fig.2). SRP concentrations are probably influenced by inflows, algal dynamics and internal loading. During the periods of high water level because of the inflows (Dec. to Jan.) large amounts of phosphorous enter the lake. During warm period D.O. and pH conditions (see Table 1) in the sediment-water interface enhance the internal loading process, enriching the water column with phosphorus from the sediment.^{17,18} This fact, in relation to the low water level, results in high concentrations of SRP.

Nitrate-Nitrogen had a number of maxima and minima through the monitoring year exhibiting higher concentrations during spring and winter (Fig.2). The higher values in the wet period varied between 0.22 and 1.16 mg/l, and may be attributed to the Nitrogen inputs from the inflows. During summer, lower concentrations, ranging from 0.34 to 0.71 mg/l, may be the result of biological utilization. Vertical differences were marked, especially during the warm period, higher concentrations appearing in the hypolimnion. This may be attributed to the nutrient release from the sediment and subsequent enrichment of the water column.

Elevated ammonia-nitrogen concentrations, caused by the inflows were observed during the wet period resulting in a maximum value of 0.34 mg/l in February (Fig.2). At near-bottom the highest value was recorded in August (0.41mg/l) probably caused by the decomposition of organic matter in the sediment-water interface.

Remarkably increased concentrations were found in the hypolimnion during the summer period. This increase coinciding with that of other nutrients (NO₃-N,SRP,SiO₂) suggests that lake's sediment is a significant source of nutrients, especially in the warm-dry period. Concentrations of silica ranged between 3.33 and 14.99 mg/l in the whole water column, the lower values appearing during early spring (3.33-5.0 mg/l) and late summer (6.5-9.0 mg/l) probably due to the increase of diatoms. Lower values have been observed in epilimnion during the thermal stratification period (Fig.2).

Seasonal patterns of chlorophyll-a and phytoplankton

The seasonal pattern of Chl-a is present in Fig.3. The average monthly concentrations of Chl-a ranged between 13 ~ 44 µg/l, reflecting the eutrophic state of the lake.¹⁹ Higher concentrations were observed during July-August (26.4~58.3 µg/l), Sep.-Oct. (10.7~64.2µg/l) and Jan. (5.1~30.6µg/l).

Looking at the phytoplankton biomass seasonal variations (Fig.3) it is considered that the Chl-a maxima occurring in July was made up of chlorophytes (maximum biomass, 306.3mg/m³). The high concentrations observed in late summer and autumn coincided with increased cyanophytes and diatoms biomass.

Vertical differences in Chl-a concentration were marked during the limited stratification period. During the winter-early spring period, the near-bottom Chl-a was generally greater than at the surface and this may be attributed to the sedimentation of the large size diatoms, which dominated during these periods.

The seasonal succession of algae in lake Pamvotis was: chlorophytes and diatoms in the spring, chlorophytes in early-summer, cyanophytes in late-summer and diatoms in autumn and winter. Generally, it is a complicated succession, as it happens in many shallow, productive lakes.²⁰ This sequence differs from the pattern of other eutrophic Greek lakes^{5,6,7} and may be attributed to the shallowness of the lake and to the weakness of thermal stratification.

Cyanophytes in lake Pamvotis start their seasonal growth in response to the nitrogen depletion, and simultaneous availability of phosphorus. In the spring-winter period, when high concentrations of nitrogen are present, cyanophytes, as nitrogen-fixing algae, had no advantage over other algal species and their abundance remained low. According to Tilman²¹ many cyanophyte species are good competitors for nitrogen, but poor competitors for phosphorus. Also, there was found a significant correlation between cyanophytes and total P in six temperate lakes.²

The high biomass of cyanophytes during the warm period coincided with the DIN (nitrate+ammonia nitrogen): SRP ratio (the ratio ranged between 1-9 with a mean value of 3), which suggests a competitive advantage to algae with nitrogen-fixing capabilities.

Among physical factors, which may supplement an advantage to cyanophytes, the low light available during the warm season in the lake seems to enhance their growth.¹¹ During this period Secchi depth is fluctuated between 0.5-0.6 m.

The annual cycle of diatoms has been characterized by quite stable trends with two major peaks: one in early spring and the second one in autumn. Populations of diatoms declined rapidly in May. During the warm, stratified period their population remained low. The autumn peak is coincided with low silica concentrations at the same time when phosphorus and nitrogen are available (Fig. 2), indicating that silica could have been the limiting factor for their growth²². Heavy rainfalls and the turbulence effects enhance the distribution of diatoms during their

peak appearance (Feb. Oct), since their decline is also due to the sedimentation, when these phenomena are absent. Grazing could also influence diatoms abundance, since the rotifers population during summer period was increased, ranging between 600-800 ind./l (Kagalou, unpublished data), after the development of diatom peak.

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BACTERIOCIDAL ACTIVITY AND PARTIAL CHARACTERISATION OF AN INHIBITORY COMPOUND FROM *Serratia marcescens* BN10 ISOLATED FROM *Balaninus nucum* L.

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SUMMARY

Utilising an “agar well diffusion assay”, we determined production of an inhibitory compound (bacteriocin) from *Serratia marcescens* Bn10 strain isolated from hazelnut beetle, *Balaninus nucum* L. (Coleoptera: Curculionidae). It was active against *Bacillus subtilis*, *Corynebacterium xerosis*, *Enterobacter cloacea*, *Escherichia coli*, *Proteus vulgaris*, *Klepsiella pneumonia*, *Neisseria flavescens*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Serratia marcescens*, but not active against *Citrobacter freundii*, *Staphylococcus aureus* and *Streptococcus faecalis*. Partial characterisation indicated that bacteriocin was heat stable, for activity remained after heating at 121 °C for 15 min. It was inactivated by trypsin, proteinase K and protease; however, it was not inactivated by lysozyme. The agent was also stable at pH values from 2,0 to 10,0. Since *S. marcescens* Bn10 did not contain plasmid, the gene encoding for the bacteriocin is presumably located on the chromosome.

KEYWORDS: Inhibitory compound, *Serratia marcescens* Bn10, *Balaninus nucum*.

INTRODUCTION

Bacteriocins are high-molecular-weight antibiotics synthesized by a variety of gram-positive and gram-negative bacterial strains. They are extracellular substances which often remain bound to the cell surface of the producer strain (1).

Bacteriocins exhibit a remarkable degree of specificity. Susceptibility to a given bacteriocin is usually restricted to bacterial species, which carry a specific receptor on the cell envelope. Different bacteriocins may attach to the same receptor but differ in their mode of action. Similarly, bacteriocins with similar mode of action may attach to different receptors. Thus, each bacteriocin has a specificity of attachment and a specific mode of action, and these specificities are independent (2, 3).

Certain bacteriocins, such as pyocin R (4), have very high molecular weights. Other bacteriocins including colicins E1 (5), Ia, Ib (6), K (7) and U (8) have been shown to be simple proteins with molecular weights in the range of 55,000 to 80,000 da.

Bacteriocin production in the genus *Serratia* was first described by Hamon and Peron in 1961 who found that 86% of the *Serratia* strains tested produced at least one bacteriocin. Up to now, a number of bacteriocins have been isolated and characterized from *S. marcescens* strains (2, 9, 10, 11). Bacteriocins produced by *Serratia* are of two kinds (12): (a) a trypsin-resistant, acid-sensitive (pH 2) structure called “group A bacteriocin” (13, 14) and (b) a trypsin-sensitive, acid-resistant protein called “group B bacteriocin” (13, 14).

In this study, inhibitory compound (bacteriocin) produced by *S. marcescens* Bn10 isolated from *Balaninus nucum* L. (Coleoptera: Curculionidae) was determined and partially characterized.

MATERIALS AND METHODS

Bacterial strains and media

Serratia marcescens Bn10 was isolated from *Balaninus nucum* L. (Coleoptera: Curculionidae) (15). The following bacteria were used as indicator organisms, *Bacillus subtilis* ATCC 6633, *Citrobacter freundii* ATCC 8090, *Corynebacterium xerosis* ATCC 7711, *Enterobacter cloacea* ATCC 13047, *Escherichia coli* ATCC 25922, *Klepsiella pneumonia* ATCC 13883, *Neisseria flavescens* ATCC 13120, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhimurium* RSKK 42, *Serratia marcescens* ATCC 8101, *Staphylococcus aureus* RSKK 250 and *Streptococcus faecalis* ATCC 19433. All bacteria were grown on nutrient broth and nutrient agar.

Activity assay

An agar well diffusion assay was used to detect the bactericidal activity of *S. marcescens* Bn10 (16, 17). A nutrient agar plate was inoculated with *S. marcescens* Bn10 and incubated at 30 °C for 18 h. After incubation, agar discs (3 mm in diameter) of *S. marcescens* Bn10 were cut out from the agar culture. On the other hand, 7 ml of soft agar containing 4% inoculum of culture of the indicator strain was overlaid onto nutrient agar and wells were cut. Either agar discs of the *S. marcescens* Bn10 or 300 µl of cell-free culture supernatant fluid were placed into each well.

To obtain the above-mentioned cell-free culture supernatant fluid, a *S. marcescens* Bn10 culture was centrifuged at 6,000 rpm for 10 min, filtered using a 0.2 µm pore size filter (Whatman, Maidstone England), neutralized to pH 7.0, and treated with catalase (5mg/ml) to break down the inhibitory activity which is eventually due to hydrogen peroxide. The plates were incubated at 30 °C for 24 h and subsequently examined for zones of inhibition.

Sensitivity to heat, pH and hydrolytic enzymes

Cell-free culture supernatants of *S. marcescens* Bn10 were exposed to heat treatments at 100 °C for 5, 10 and 20 min, respectively, and at 121 °C for 15 min. The samples were then tested for bactericidal activity against indicator organisms, using agar well diffusion assay. In a separate experiment, samples of supernatant were adjusted to pH values ranging from 2.0 to 10.0 using HCl and NaOH, incubated at 30 °C for 30 min, and tested for bactericidal activity. Resistance of bacteriocin to hydro-

lytic enzymes was determined by incubation of the bacteriocin samples in the presence of lysozyme, protease, proteinase K and trypsin at a concentration of 0.1 mg/ml. Samples with and without enzymes were held at 37 °C for 1 h. After incubation, the enzymes were heat-inactivated (5 min at 100 °C) and tested for bactericidal activity using agar well diffusion assay.

Plasmid isolation

Screening for plasmids was performed according to the method described by Birnboim and Doly (18). Plasmid DNA was separated by electrophoresis in 0.7% agarose gel. *Hind*III-digested lambda phage DNA was used as a molecular weight marker.

RESULTS AND DISCUSSION

In this study, the culture supernatant fluid and agar discs of *S. marcescens* Bn10 were examined for inhibition of the indicator strains. In both the culture supernatant fluid and the agar discs without induction, the inhibitory activity was determined. Foulds and Shemin (9) determined that bacteriocin L from *S. marcescens* has no inhibitory activity without induction. So, the inhibitory compound from *S. marcescens* Bn10 is different from the reported bacteriocin L.

The inhibitory compound produced by *S. marcescens* Bn10 had an extensive activity spectrum. Among the tested species, it inhibited *B. subtilis*, *C. xerosis*, *E. cloacea*, *E. coli*, *K. pneumonia*, *N. flavescens*, *P. vulgaris*, *P. aeruginosa*, *S. typhimurium* and *S. marcescens* (Table 1).

TABLE 1 - Bacteriocidal activity of *S. marcescens* Bn10 on some indicator bacteria.

Organisms	Strains	Inhibition
<i>Bacillus subtilis</i>	ATCC 6633	+
<i>Corynebacterium xerosis</i>	ATCC 7711	+
<i>Enterobacter cloacae</i>	ATCC 13047	+
<i>Escherichia coli</i>	ATCC 25922	+
<i>Klebsiella pneumonia</i>	ATCC 13883	+
<i>Neisseria flavescens</i>	ATCC 13120	+
<i>Proteus vulgaris</i>	ATCC 13315	+
<i>Pseudomonas aeruginosa</i>	ATCC 10145	+
<i>Salmonella typhimurium</i>	RSKK 42	+
<i>Serratia marcescens</i>	ATCC 8101	+
<i>Citrobacter freundii</i>	ATCC 8090	-
<i>Staphylococcus aureus</i>	RSKK 250	-
<i>Streptococcus faecalis</i>	ATCC 19433	-

+: inhibition, -: no inhibition; * inhibition was scored positive if the width of the zone around the wells was 1 mm or larger

TABLE 2

Comparison of bacteriocidal activity of agar disc and cell-free supernatant fluid of *S. marcescens* Bn10 on some indicator bacteria.

Indicator Organisms	Disc	Fluid
<i>Bacillus subtilis</i>	5.5*	3.5
<i>Corynebacterium xerosis</i>	2.5	1.5
<i>Enterobacter cloacae</i>	5	3.5
<i>Escherichia coli</i>	3	1.5
<i>Klebsiella pneumonia</i>	4	2
<i>Neisseria flavescens</i>	3.5	2.3
<i>Proteus vulgaris</i>	4	1.8
<i>Pseudomonas aeruginosa</i>	4	2.5
<i>Salmonella typhimurium</i>	4	2
<i>Serratia marcescens</i>	3	1.5

* The values are in radius, mm

TABLE 3

Effect of heat, pH and some hydrolytic enzymes on the activity of inhibitory compound produced by *S. marcescens* Bn10.

	Activity
Heat: 5 min at 100 °C	+
10 min at 100 °C	+
20 min at 100 °C	+
15 min at 121 °C	+
pH: 2.0 – 7.0	+
8.0 – 10.0	W+
Enzymes: Protease	-
Proteinase K	-
Trypsin	-
Lysozyme	+
Control	+

W+: weak positive; * Inhibition was scored positive if the width of the zone around the wells was 1 mm or larger.

However, *C. freundii*, *S. aureus* and *S. faecalis* were not inhibited. The inhibitory compound had the highest effect on *B. subtilis* among the tested species. It is known that an inhibitory compound (bacteriocin) may exhibit bacteriocidal activity on different genus or species (16, 19, 20). Inhibition scores produced by the inhibitory compound of *S. marcescens* Bn10 on different species are shown in Table 2. Agar discs demonstrated more bacteriocidal activity than culture supernatant fluid, suggesting that the active compound produced by *S. marcescens* Bn10 was bound to the surface of the producer cell.

The results of sensitivity to heat, pH and hydrolytic enzymes are shown in Table 3. The inhibitory activity of the compound was not lost following heating for 5, 10 and 20 min at 100 °C or 15 min at 121 °C. The inhibitory compound was considered to be heat stable, as activity remained after heating for 15 min 121 °C. Foulds and

Shemin (9) determined that the activity of bacteriocin from *S. marcescens* P & S decreased 25% at 39 °C and 100% at 41 °C. However, it was determined that the final titers of bacteriocin from *S. marcescens* JF246 were <10 and 10,000 at 60 and 50 °C, respectively (2). This information demonstrates that the inhibitory compound produced by *S. marcescens* Bn10 was heat stable.

The inhibitory compound was active at pHs ranging from 2,0 to 10,0, but activity decreased at pH 8,0 or above (Table 3). Franz et al. (21) similarly determined that the activity of plantaricin decreased at pH 8,0 or above.

The inhibitory compound was inactivated by the trypsin, proteinase K and protease, but not lysozyme, indicating a proteinaceous nature, other bacteriocins isolated by Foulds (2), and Foulds and Shemin (9) also were sensitive to trypsin like *S. marcescens* Bn10 inhibitory compound.

Since most bacteriocins are plasmid encoded, the plasmid profile of *S. marcescens* Bn10 was studied. However, we did not detect any plasmid (data not shown). This suggests that the *S. marcescens* Bn10 inhibitory compound could be chromosomally encoded. Viejo et al. (10) cloned and sequenced a bacteriocin gene of *S. marcescens* encoded by chromosome.

These results show that the inhibitory compound produced by *S. marcescens* Bn10 is in the group B bacteriocin (12) as trypsin-sensitive, acid-resistant. Our data also suggest, it is heat stable, membrane bound and chromosomally encoded. Further experiments are required to determine the mechanism of activity and molecular characterization of the respective gene of this bacteriocin.

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TREATABILITY OF VEGETABLE OIL INDUSTRY EFFLUENTS THROUGH PHYSICAL-CHEMICAL METHODS

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ABSTRACT

In this study, a vegetable oil factory (Karadeniz Birlik Dogan Erdil) was selected as representative of this sector and its production method evaluated and its effluent investigated. During the first part of the test program, quality of the raw effluent was determined. Alternatives of possible physical-chemical treatment were examined by using pilot scale and laboratory models. The most efficient treatment combination has been determined under the objective of this study. The results showed that 17.65 - 69.12 % of COD and 28.50 - 76.32 % oil-grease were removed by using Dissolved Air Flotation (DAF), 63.19 - 80.66 % COD and 76.95 - 83.38 % of oil-grease removed in the acid cracking and coagulation with lime, 88.14 - 92.99 % of COD and 69.89 - 92.11 % oil-grease removed by using alum, and 94.66 - 96.25 % of COD and 93.81 - 96.16 % of oil-grease removed by using ferric chloride for treatment of the same wastewater. The treatment efficiency of coagulation by alum process was raised up to the range of 94.66 - 95.37 % of COD and 96.88 - 97.78 % of oil-grease by using alum-clay process.

KEYWORDS: Vegetable Oil Industry, Dissolved Air Flotation (DAF), Coagulation, Acid cracking.

INTRODUCTION

In recent time, the vegetable oil industry develops rapidly in Turkey with the cultivation of appropriate agricultural crops such as, especially, sunflower, cotton and maize. In Turkey, the pip of sunflower, amounting to 1000,000 tones was produced on an average in one year, and on an average, raw oil of 300,000 tons and edible oil of 110,000 tons were obtained from this production.

Wastewaters containing oil can be treated either in chemical or biological units. The basic process of chemical treatment is coagulation (Basu, 1967; Chin et al, 1987). The efficiency of treatment depends on the ratio of free oil to emulsified oil. The free oil can be easily removed from wastewater by physical processes using coagulating agents, e.g. alum and ferric chloride for phase separation. In order to remove emulsified oil from waste

water, before phase separation the acid cracking process (pH reduction to 2.0) is used for de-emulsification. The conventional treatment of vegetable oil industry wastewater consists of mechanical dewatering of sludge produced with physical, chemical and biological processes. Oily materials in low levels can be removed with biological systems. However, the COD removal above 50 % can be effected only by chemical treatment. Settling with lime and activated sludge systems is a very effective but cost- and labour-intensive combination for the treatment of oily wastewater. The oil-grease concentrations in domestic wastewater range from 30 to 50 mg/l, are higher than 1,000 mg/l in wastewaters of oil industry (Tsugita and Ellis, 1981) and sometimes reach up to 2000 mg/l (Uslu and Turkman, 1987).

In this study, a 2-hour composite sample of wastewater from Karadeniz Dogan Erdil Oil Factory was characterized and its treatability by physico-chemical methods investigated.

MATERIALS AND METHODS

The experiments were carried out on the laboratory scale by taking the samples of composite wastewater for 2 hours from Karadeniz Dogan Erdil Oil Factory. The flow rate of wastewater was approximately 315.88 m³/day. In the factory the wastewater is passed through an oil trap. To determine its efficiency, the samples were taken from inlet and outlet of the oil trap. The samples in the other stages of this study were taken only from its outlet.

Dissolved Air Flotation (DAF) system consisted of a 10 liter feeding tank, an air pump, a 3 liter aeration tank (pressure tank) and a 121.5 liter flotation tank. The pressure tank was fed with air (pressure 4 bar) by a peristaltic pump. Then the wastewater/air mixing was fed into the flotation tank. The dimension of the tank equipped with two parallel skimmers were 90 x 45 x 30 cm. The wastewater was passed from bottom of the baffles and discharged from near the upper end of the tank. The free oil was collected by the skimmers at the wastewater surface in the flotation tank (Fig. 1). The average hydraulic accumulation/ detention time was kept to 24 h.

The above-described acid cracking was simulated in the laboratory with concentrated HCl and H₂SO₄ solutions. The samples were filled into 1 liter flasks, magnetically stirred, pH was measured and adjusted to 2.0 by admixing mineral acids. Then the free oils accumulating at the surface were removed and used for analysis of process efficiency.

The chemical treatment studies were conducted with a jar test apparatus. The acidic wastewater samples separated from the free oil were subjected to coagulation process with lime. Additionally, alum [Al₂(SO₄)₃.18H₂O] and ferric chloride (FeCl₃.6H₂O) were tried as coagulants. The jars filled with acidic wastewater were rotated for rapid mixing (100 rpm for 4 min), slow mixing 20 rpm for 30 min) before sedimentation.

The amount, temperature and pH of wastewater were determined *in situ*. The samples were analysed for chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total phosphorous, total solids (TS), suspended solids (SS), volatile suspended solids (VSS) and oil-grease (O&G) according to the corresponding Standard Methods (APHA, AWWA, WPCF, 1985).

The main clay compounds that aided as coagulants (gypsum > anorthite > montmorillonite > quartz; see Fig. 2) were determined by routine x-ray diffraction analysis (Cu-α test tube, chart speed 1cm min⁻¹; working conditions: FWHM = 1°2 θ/min, counting of 100 cps).

FIGURE 1 - Schematic representation of the Dissolved Air Flotation (DAF) Unit .

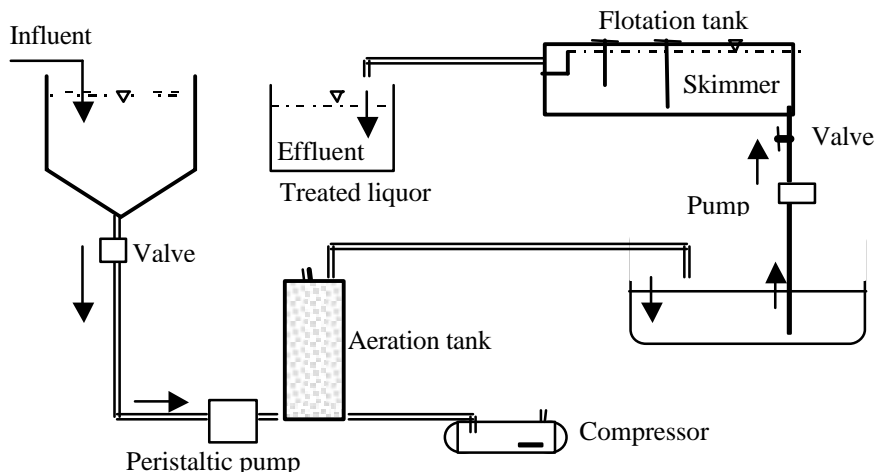


FIGURE 2 - XRD analysis of clay samples used.

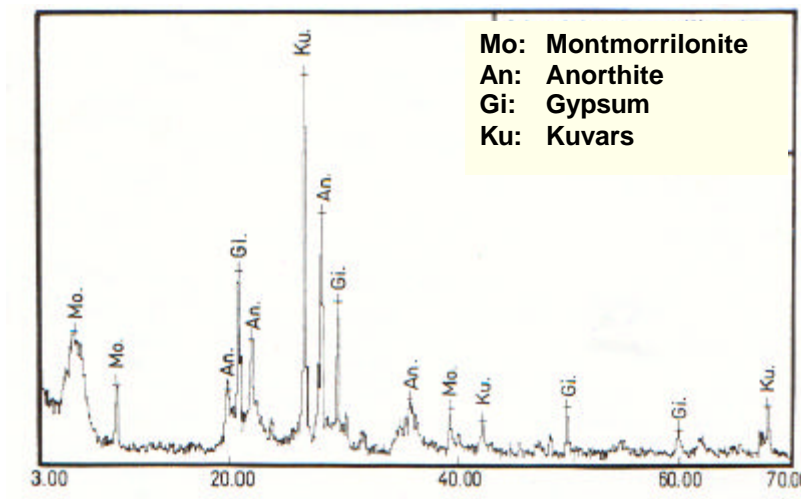


TABLE 1 - The Characteristics of Wastewater from Sunflower Oil Factory.

Parameter	Minimum	Maximum	Average
Temperature, °C	46.45	65.26	49.56
pH	7.43	9.56	8.52
Alkalinity, mg CaCO ₃ /L	800	1990	1680
Oil-grease, mg/l	540	7640	3030
COD, mg/l	5600	15300	9350
BOD ₅ , mg/l	1050	10300	5260
Sulphate, mg/l	160	1080	620
Total phosphorus, mg/l	216	556.20	378.56
TKN, mg/l	19.80	125.40	76.92
TSS, mg/l	3700	12800	7830
SS, mg/l	410	3240	1650
VSS, mg/l	4300	9680	5820
TDS, mg/l	4590	10200	6180
Flow rate, m ³ /day	308.60	321.80	315.88

TKN = total Kjeldahl nitrogen; TSS = total suspended solids;

SS = suspended solids; VSS = volatile suspended solids; TDS = total dissolved solids.

RESULTS AND DISCUSSION

The characteristics of wastewater

During the study, 32 samples collected from the oil trap were analysed in order to determine the wastewater characteristics. The results are given in Table 1.

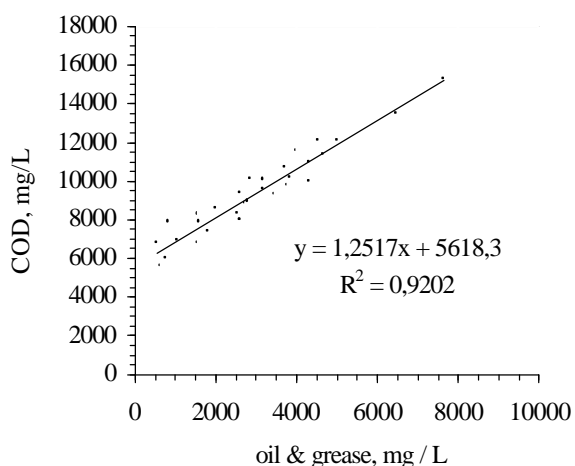
In order to determine the relationship between oil & grease and COD, as shown in Fig. 3, the following correlation was applied to the results:

$$y = 1.2517x + 5618.3 \quad (1)$$

where y is COD and x is oil & grease.

The correlation coefficient R^2 was found to be 0.92.

FIGURE 3 - The relationship between COD and oil & grease in wastewater from the Sunflower Oil Factory



The analyses of the composite samples collected at the outlet of the oil trap indicate that the wastewater contained COD, sulphate and oil & grease in high levels. However, the temperature of the wastewater was very high (50-65 °C). pH values of the wastewaters vary according to acidic or alkaline operation type. COD values correlate with oil & grease on a large scale (correlation coefficient R^2 0.959). This leads to an opinion that a decrease in oil & grease concentration may highly reduce the COD values. The ratios of COD / oil & grease, which are accepted as one of the criteria for the method selection in wastewater treatment studies, were found to be 2.05-3.08 which are in agreement with that of Dart 1974 (2-3.2) but different from those of Eroglu et al., 1990 (5), and Sengul et al., 1992 (2-6.7).

Treatability Studies

The samples from inlet and outlet of the oil trap taken at various dates were analysed. Fig. 4 represents the treatment efficiencies determined in oil trap effluent at different pH values in the DAF unit. The removal of COD and oil & grease by means of the oil trap varied depending on pH. The removal of COD and oil & grease were approximately 25.34 and 21.43 % at pH 6.96, respectively. These values decreased to approximately 16.27 and 12.08 % at pH 8.46 because only a minor portion of emulsified oil was converted to free oil rising up to the surface.

According to the results obtained, the pH-dependent removal efficiency was increased in the DAF unit at lower pH values similar as in the oil trap. At pH 3.56, the removal of COD and oil & grease was 69.12 and 76.32 %

and at pH 7.48 only 17.65 % and 28.50 %. Keller and Cantrell (1975) reported that 33% BOD₅, 32 % SS and 62 % oil & grease were removed in the DAF unit. Ho and Tan (1989) observed a percentage removal of 79.2 for COD and 59.2 for SS, respectively.

The jar experiments with the combination acid cracking and coagulation with lime were carried out under laboratory conditions. 2 ml of 98% H₂SO₄ or 4.36 ml of 37 % HCl were used to reduce the pH of wastewater from 11.05 to 2. To prove the settling out capacity during increasing of pH from 2 to 10, the average dose of lime was 16 g/l.

FIGURE 4

Treatment efficiencies of oil trap effluent at different pH values in the DAF unit (O&G = oil & grease); TSS = total suspended solids).

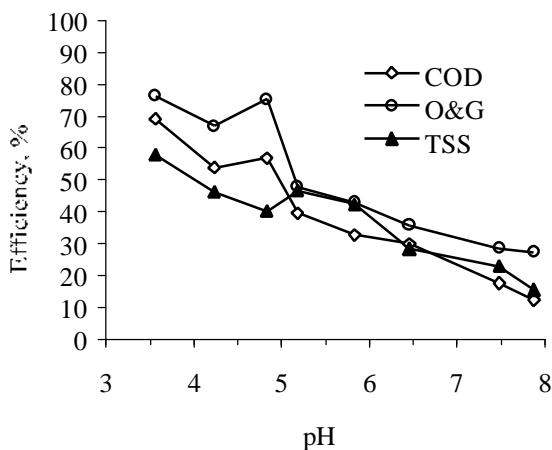
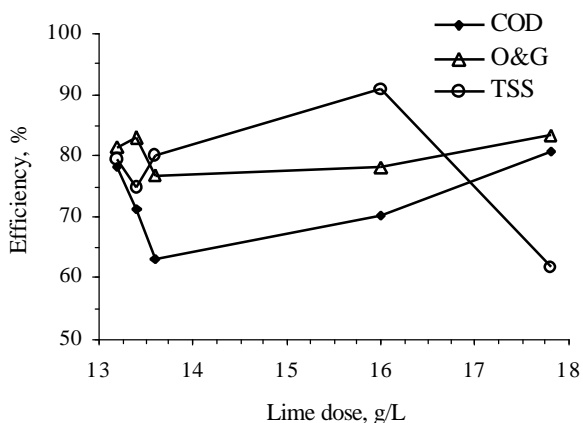


FIGURE 5 - Wastewater treatment efficiency of the combination acid-cracking + coagulation with lime (O&G = oil & grease, TSS = total suspended solids).



The percentage removals were 63.19- 80.66 % for COD, 76.95- 83.38 % for oil & grease and 6.10- 85.0 % for SS (Fig. 5). 1.88-2.52 g/l sludge was produced in the coagulation process with lime. However, to prove the “receiving media standard”, it was necessary to add 1-1.2 ml/l H₂SO₄ or 1.35-1.56 ml/l HCl solutions (pH 10).

The sulphate removal was found to be 24-27%. The acid cracking process combined with lime-coagulation has several disadvantages such as high acid consumption, high formation of sludge, and high sulphate concentration in the effluent.

FIGURE 6

Wastewater treatment efficiency using the coagulating agent alum (O&G = oil & grease, TSS = total suspended solids).

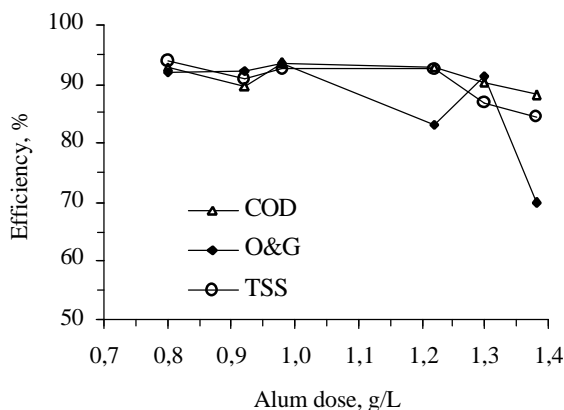
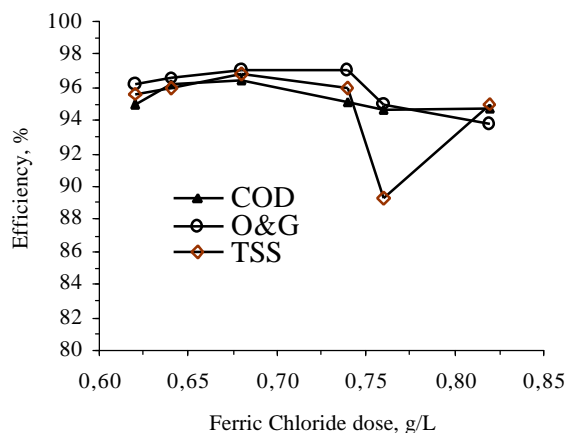


FIGURE 7

Wastewater treatment efficiency using ferric chloride as coagulating agent (O&G = oil & grease, TSS = total suspended solids).



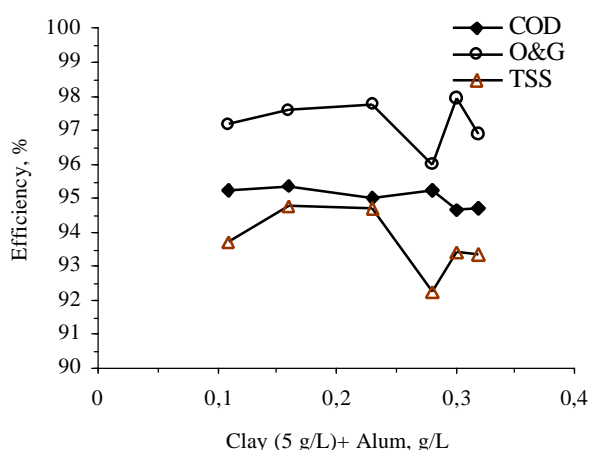
In the coagulation experiments using alum and ferric chloride, the optimal dose was found to be 0.80-1.8 g/l for alum and 0.62-0.82 g/l for ferric chloride. However, the optimal range of pH was 6.00-6.20 for alum and 6.80-7.05 for ferric chloride. When the treatment efficiencies for both coagulants were compared, as seen in Figs. 7 and 8, it could be shown that ferric chloride was more suitable than alum for the treatment of an oil factory effluent. Only 55-69% amounts of ferric chloride were necessary compared to alum to improve removal of COD, oil & grease, and TS.

In the coagulation experiments using mixtures of clay with alum or ferric chloride, it was shown that the use of clay/alum (5g clay + desired alum) was more efficient

than that of clay/ferric chloride mixing. The optimal dose for alum decreased from 0.80-1.38 g/l to 0.16-0.32 g/l with addition of clay. However, the removal percentage of COD and oil & grease increased from 88.14-92.99% to 94.66-95.37% and from 69.89-92.11 to 96.88-97.78%, respectively. The amount of sludge produced was only 58% compared to the lime experiments.

FIGURE 8

The wastewater treatment efficiency with 5 g/l clay/alum mixture added O&D = oil & grease, TSS = total suspended solids).



CONCLUSION

The analyses have shown that COD concentration of wastewater depends on its oil & grease content. This means that COD load could be importantly decreased with a decrease in oil & grease concentration. The COD/oil & grease ratio varied from 2.05 to 3.08. The efficiency of wastewater treatment increased depending on the reduction of pH using the DAF unit. There are two disadvantages such as exceeding use of chemical matter and exceeding formation of sludge using the combination acid cracking and coagulation with lime. Therefore, alum + 5 g clay were used, but the optimum dose decreased to 0.16-0.32 g/l. However, the amount of sludge produced was reduced to 58 % compared to lime treatment and it was shown that clay could be an alternative to lime.

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THE PERFORMANCE OF ZEISS GFAAS-5 INSTRUMENT ON THE DETERMINATION OF TRACE METALS IN WHOLE BLOOD SAMPLES OF SOUTHERN ELEPHANT SEALS (*MIROUNGA LEONINA*) FROM ANTARCTICA

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SUMMARY

The severe matrix interference on chromium determination in whole blood samples of Southern elephant seals from Antarctica by a GFAAS (ZEISS-5 model) at 357.9 nm is demonstrated. Such interference was due to overlapping absorption spectra of chromium and iron at 357.9 nm and appeared as a systematic constant error, which was not corrected with the application of the standard addition technique. Thus, the interference was eliminated using the second resonance wavelength (359.4 nm). No iron interference was observed in nickel and lead determinations. However, for cadmium a suppression of the signal (15 -20%) was observed.

KEYWORDS:

metals, elephant seal, Antarctica, blood, GFAAS.

INTRODUCTION

The effects of environmental degradation are now being felt on regional and global levels. However, there are "remote" places, like Antarctica, which might be still considered undisturbed by humankind's activities. Data obtained from such areas may be of special importance for comparative studies and for establishing background values in seawater, biota, etc.

It is known that organic life cannot develop and survive in the absence of metallic ions (1). Many of the heavy metals (Co, Cu, Fe, Zn, etc) are essential to metabolism at low concentrations. However, all of them can be toxic to estuarine and marine organisms when concentrations above the threshold level are reached. Some of them, such as Hg, Cd, and Pb, do not have known biological function and may greatly affect biotic communities (2).

For low-level analyses of metals various techniques are applied, including graphite furnace atomic absorption spectrometry (GFAAS), neutron activation, inductively coupled plasma-mass spectrometry (ICP-MS), and stripping voltammetry. GFAAS and ICP-MS are frequently used in clinical and hydrochemical laboratories and are capable of accurate and precise results. However, it has been demonstrated that for complex matrix samples like brines, geological, and biological samples, interference problems exist (3, 4, 5). The analysis of trace elements like Cr, Cd, Pb, and Ni in biological samples is wrought with difficulties due to low concentrations and interference problems. In such cases, great care should be taken throughout sample processing procedures to prevent contamination. In addition, applying a proper digestion procedure and diminishing the matrix interference are also extremely important. Several authors have reported interference on the determination of Cr, Cd, Pb, and Ni by GFAAS. Attempts to eliminate these interferences have been done by using matrix modifiers and optimising furnace conditions (3, 5, 6, 7). Bannon et al. used matrix standards for diminishing the interference on lead determination in blood samples (8). Quinaia and Nobrega report the using of a calibration curve prepared with Cr spiked urine correcting all potential matrix interference (9). Nevertheless, it is known that the matrix interference is not always avoided by using standard addition method, especially when systematic constant error is present (10).

In light of the above, our aim was to assess the background levels of Fe, Cu, Zn, Hg, Cd, Pb, and Cr in whole blood samples of Southern elephant seals. However, during analysis some troubles were observed especially with Cr when analysed using a ZEISS AAS-5 model. It is known that the absorption signal of Cr is greatly reduced by the presence of Fe when AAS in flame mode is used,

due to the formation of compounds, which are difficult to atomise (11). In whole blood samples of Southern elephant seals, the iron concentration found is about 700-800 µg/ mL. During Cd, Pb, Cr, and Ni determinations, in which levels are less than 15 ng/mL, iron is a likely interfering element when the samples are diluted 5 to 10 times. In this study is shown that during Cr determination by GFAAS, Fe brought about a significant overestimating of Cr concentration, demonstrating the presence of a different interference type compared with the interference encountered in flame AAS. Some data obtained and the difficulties faced are presented and discussed.

MATERIALS AND METHODS

All reagents used were Suprapur™ from Merck. The glassware was subjected to careful cleaning procedure (12). Fe, Zn, and Cu were determined by flame using a CG-AA 7000 model (Instrumentos Científicos C.G. Ltda., Brazil). For Cd, Cr, Pb, and Ni, a ZEISS GFAAS-5 (Carl Zeiss Jena GmbH, Germany) was used. GFAAS parameters for chromium are presented in Table 1. Mercury was determined using a constructed cold vapour device coupled with the CG-AA 7000 instrument. Deuterium background correction was used in all measurements.

Southern elephant seals (*Mirounga leonina*) were captured at the Elephant Island during the XVII Brazilian Antarctic Operation (1998-1999) and blood samples were collected using plastic syringes containing heparin. After collection, whole blood samples were kept deep-frozen (-20 °C) during transportation and storage until analysis.

For sample digestion, about 2-3 g of whole blood was thawed and treated with 5-6 mL, HNO₃ cc in Teflon vials. The vials were tightly closed and heated on a hot plate at 100 °C. The temperature was then increased to 140 °C until a yellow transparent liquid was obtained. All manipulations were carried out in a laminar flow hood. Magnesium nitrate was used as matrix modifier to permit somewhat higher temperatures on the pyrolyses step, particularly for Cd and Pb, but also for Cr (3). For Hg determination, an aliquot of the digested sample was treated with 0.5 mL BrC1 solution.

Quality control included the use of blanks and analysis of a tuna fish-350 reference material (IAEA, Monaco). No reference blood sample was available in our laboratory. Blanks with Milli-Q water and heparin were prepared in the sampling site. Respective blanks were also prepared during digestion procedure. Results obtained for the reference sample were in agreement (± 7 %) with the certified values. The field and laboratory digestion blanks showed concentration values below the detection limit.

TABLE 1 - GFAAS parameters employed for Cr analyses.

Step	Temp (°C)	Ramp °C/sec)	Hold (sec)	Gas flow (argon)
1	105	5	25	Max
2	300	20	15	Max
3	1100	20	15	Max
4	1100	0	6	Stop
5	2600	Max	3	Stop
6	2700	1500	3	Max

TABLE 2 - Chromium concentration (µg/g) in the whole blood of adult male Southern elephant seals (*Mirounga leonina*) measured at 357.9 and 359.4 nm wavelengths.

Sample	Wavelength (nm)	
	357.9	359.4
Blood		
1	26.4	6.58
2	24.3	6.48
3	25.5	6.74
4	25.5	4.71
5	35.6	8.76
6	37.2	9.74
Mean ± SD	29.08 ± 5.73	7.17 ± 1.80
Reference Material		
Observed	0.62	0.59
Reported	0.64	0.64

FIGURE 1
Absorption spectra of Cr in a whole blood sample of Southern elephant seal (*Mirounga leonina*) at 357.9 nm (A) and 359.4 nm (B). The small peaks correspond to the background signal.

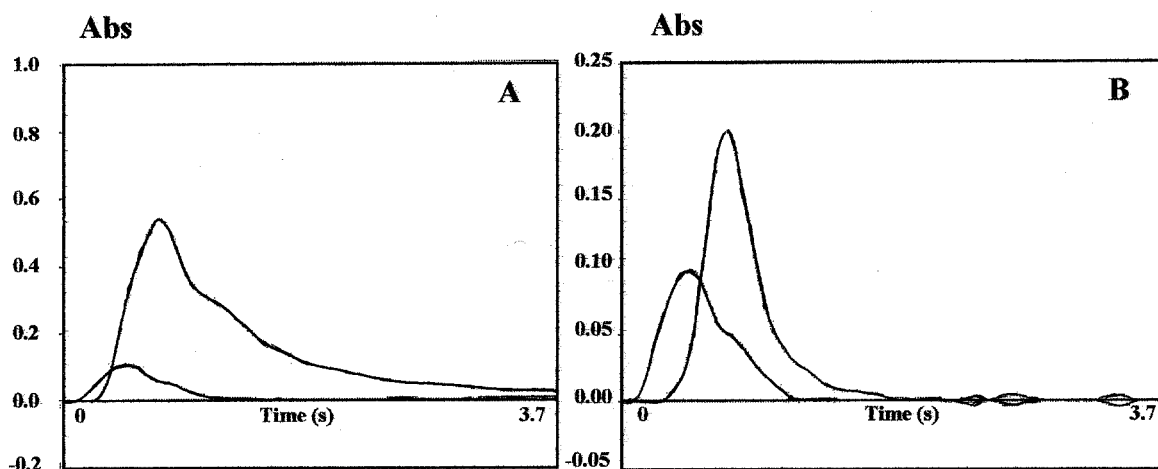


TABLE 3
Metal concentrations in whole blood samples of adult male Southern elephant seals (*Mirounga leonina*) from Antarctica and reference material (tuna fish-350). Data are means \pm SD (n=6). Hg, Pb, Cd and Cr in ng/g; Fe, Cu and Zn in μ g/g.

Metal	Sample		
	Blood	observed	Tuna 350 reported
Hg	99.50 \pm 15.40	4.47	4.68
Pb	9.20 \pm 2.15	0.092	0.10
Cd	3.79 \pm 1.23	0.022	0.020
Cr	7.10 \pm 1.79	0.61	0.65
Fe	700.60 \pm 28.5	69.8	72.1
Zn	3.13 \pm 0.11	17.9	17.4
Cu	1.04 \pm 0.04	2.83	2.69

RESULTS AND DISCUSSION

Table 2 summarizes the measurement of Cr performed in some whole blood samples of Southern elephant seals and reference material (tuna fish-350), at two wavelengths (357.9 and 359.4 nm). It is known that the sensitivity obtained at 357.9 nm is slightly better than that at 359.4 nm. However, a significant interference at 357.9 nm seems to occur. At this wavelength, the highest peak that corresponds to the Cr is very big and asymmetric, due to a matrix effect (Fig. 1A). On the other hand, the peak is rather symmetric and the interference seems to have been removed at 359.4 nm (Fig. 1B). Furthermore, such significant difference was not observed for Cr when the reference material (tuna fish-350) was analyzed (Table 2).

These results indicate that the discrepancy appears only in the blood sample matrix. Considering that Fe concentration in tuna fish sample is relatively low, we believe that the discrepancy observed in blood samples

might be due to high iron concentration in the final digested blood solution, which was diluted no more than 5 times. Consequently, the Fe concentration was in the 100-200 ppm range. The observed interference is likely due to spectra overlapping due to the presence of Fe, which absorbs at 358.1 nm, a wavelength very close to 357.9 nm. Such spectral interference produced a systematic constant error on Cr assessment, which was confirmed by the ineffectiveness of the standard addition method.

For Fe interference assessment, synthetic samples were prepared containing 4 μ g/L Cd, Pb, Ni, and Cr, while Fe was added in concentrations similar to those found in diluted samples (100 to 200 mg/L) analysed. Unfortunately, the blank of Fe prepared from Fe stock solution showed relatively high Cr contamination (about 2 μ g/L Cr). In spite of this, an asymmetric and big peak, similar to that presented in Fig. 1A was obtained at 357.9 nm, whereas at 359.4 nm a similar peak response

was not observed. This result supports the idea that the high Fe concentration in the blood sample is causing interference in the Cr determinations. No interference of Fe was observed during Ni and Pb determination, while for Cd a suppression of the signal was observed (15-20 %). Some data of metal concentrations in the whole blood samples of Southern elephant seals from Antarctica are presented in Table 3.

In conclusion, our results suggest that the second resonance wavelength (359.4 nm) should be used for Cr determination in blood samples when a graphite furnace ZEISS AAS-5 instrument is used. They also indicate that the strong interference in Cr determinations at 357.9 nm is due to high Fe concentration in the blood sample

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ANAEROBIC CO-COMPOSTING OF SUGARBEET WASTE AND WINE FACTORY WASTES

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ABSTRACT

The present research deals with evaluation of the efficiency of an anaerobic batch composting system to produce a product safe to use from a hygienic standpoint. So, using bacteriological parameters as a guideline, faecal coliforms (FC) and total coliforms (TC) on raw waste, leachates and finished compost were determined. Measurements also include total solids (TS), volatile solids (VS), pH, conductivity, chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonium, total phosphorus (TP) and alkalinity in the leachate. Determinations on some metals, VS, cellulose, pH, conductivity, moisture, C, N, S, Ca, Mg, Na and K were carried out in the starting mixture and the compost obtained. The mixture of the composting materials was as follows: 70% wine factory wastes, 20% sugarbeet waste and 10% biological treatment sludge. By the end of the composting process (day 108), the TC and FC numbers in co-compost were reduced from $1609 \cdot 10^4$ and $918 \cdot 10^4$ MPN/g.ww to $0.33 \cdot 10^4$ and $0.16 \cdot 10^4$ MPN/g.ww, respectively. Although FC and TC numbers were not completely eliminated in both leachate and compost at the end of the composting, their levels were very low. The fact that pathogen die-off is faster than that of the indicator organisms suggests that low numbers of indicator organisms are sign of elimination of pathogens.

KEYWORDS:

Anaerobic co-composting, sugarbeet waste, wine factory wastes, biological treatment sludge, faecal coliforms, total coliforms.

INTRODUCTION

During the past decade, composting technology has received considerable attention in two distinct areas: microbial ecology of the composting process and potential uses of the end product of compost. The composting process is ideally a controlled microbial digestion utilizing indigenous bacteria, actinomycetes and fungi to decompose an organic substrate. Observed patterns

of microbial succession are influenced by the relative degree of decomposition of the organic matter within the system (1).

The compost microbiota determine the rate of composting, influence the quality of the product, and produce most of the physical and chemical changes in compost (2). The capacity of compost microflora to degrade a wide variety of organic contaminants has been clearly demonstrated (3, 4, 5).

An important aspect of compost quality is the absence of several types of pathogens (6). In cases where the compost is to be applied to agricultural soils and where public health aspects are of concern, the levels of pathogens and their elimination during the composting process are important criteria that must be evaluated (7). However, monitoring pathogens is a difficult and lengthy procedure unsuited to routine application. In view of the wide array of pathogens that can be present in raw waste, a thorough analysis of the entire compost output for its pathogen content would become an enormous problem (8). The use of indicator organisms, whose removal characteristics are similar to these pathogens, has also become popular, because it is a shorter and more convenient technique (9). The study of Tiquia et al. (8), as well as those of previous workers (9, 10, 11) examined the use of faecal coliforms and faecal streptococci as indicators of pathogens and showed that the two indicators are abundant in the initial compost materials. Faecal coliforms and faecal streptococci were both found to be more resistant than the pathogenic bacterium *Salmonella*, in the study of Tiquia et al. (8).

Leachate recycling was shown to be an important integral part of an anaerobic composting. The cost and simplicity of a leachate recycling system can be approximately the same as for an aeration system in a static compost pile. The recycled leachate continually redistributed moisture, bacteria, buffer and nutrients throughout the solid waste pile. The recycling of the leachate also provides a means for redistributing bacteria throughout the decomposing solid mass (12).

The co-composting process can be used in two ways: (i) by combining the suitable wastes because of economic and operating conditions for composting, (ii) by combining the waste with convenient wastes (13) if the waste does not contain nutrients/other elements in adequate quantities for optimum composting (inadequates of stated elements are compensated). In this study, the sugarbeet wastes with high nitrogen content and wine factory wastes with low nitrogen content were co-composted, characteristics of leachate were monitored and various properties of the compost obtained were investigated.

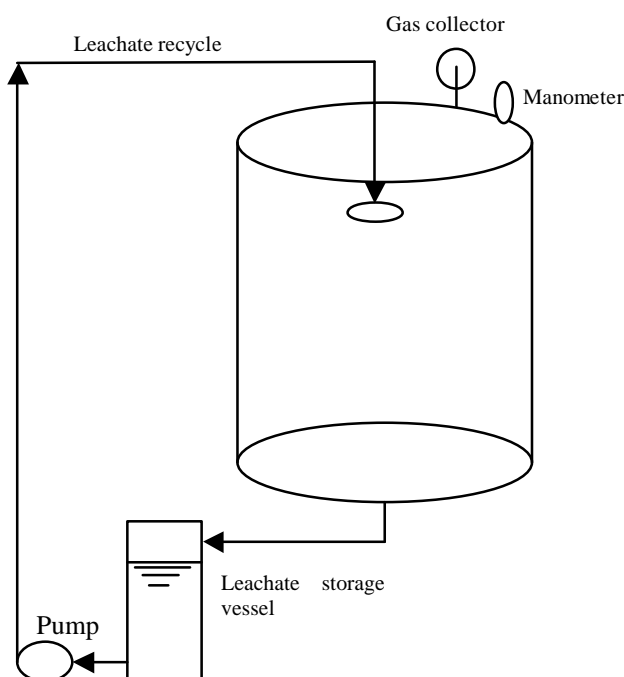
MATERIALS AND METHODS

Sugarbeet waste and biological treatment sludge showed a high organic matter content. Biological treatment sludge supplemented very well the sugarbeet waste; adding biomass and the moisture needed. The wine factory waste was obtained from a wine factory in Elazig (Turkey). The sugarbeet wastes were supplied by a farm in Elazig (Turkey). Aerobic treatment sludge was obtained from a treatment plant located in Elazig (Turkey). The materials were mixed together to form a 73.5 kg wet load. An anaerobic batch reactor of type depicted schematically in Figure 1 was filled with wastes consisting of: 70% wine factory wastes, 20% sugarbeet waste and 10% biological treatment sludge. The reactor was seeded with six kilograms of sludge from an anaerobic treatment plant of a wine factory.

A constant temperature room was used to maintain the temperature of the reactor at 35-40 °C. The leachates were collected in the leachate storage vessel of the reactor. Leachate from leachate storage vessel (13 l) to reactor was recycled and was distributed uniformly over reactor.

Leachates were sampled during composting and pH, conductivity, TS, VS, COD, TKN, NH_4^+ , TP, alkalinity and microbiological analyses were performed. Compost samples were analyzed for VS, cellulose, pH, conductivity, moisture, C, N, S, Ca, Mg, Na, K, Cu, Mn, Zn, Fe, Cd, Pb, Cr, Ni and microbiological contents at the beginning and end of the composting. Immediately after sampling, the samples were transported to the laboratory and homogenised in a food blender. The dried compost samples were sieved and prepared to analyze with a size of 0.2 mm. FC and TC densities on raw waste, leachates and finished compost were demonstrated by using direct most probable number (MPN) technique according to Standard Methods (14). Alkalinity, moisture, TS, VS, COD, TKN, NH_4^+ , TP were made as described in Standard Methods (14). Cellulose were performed according to AOAC Methods (15). Cu, Mn, Zn, Fe, Cd, Pb, Cr, Ni were measured by atomic absorption spectrophotometry (ATI Unicam 929). Ca, Mg, Na and K were measured by atomic emission spectrophotometry. The compost samples were analyzed for pH (1:10 w/v sample:water extract) using a pH probe (WTW pH 330), conductivity (1:10 w/v sample:water extract) using a conductivity probe (WTW LF 330), C, N, S using a CHNS analyzer (Leco CHNS analyzer).

FIGURE 1 - Scheme of the anaerobic composting process.

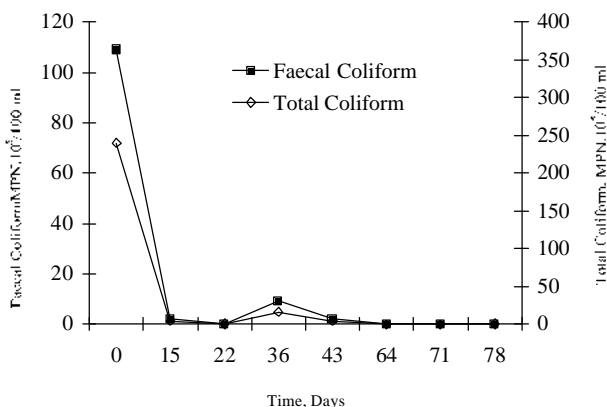


RESULTS AND DISCUSSION

The variations of the microbiological and chemical characteristics in leachate

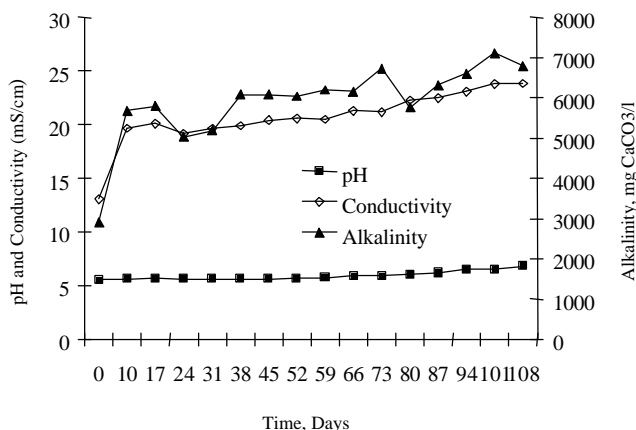
Both populations of FC and TC decreased with time, as seen in Fig.2. Towards the end of the decomposition process, the numbers of coliform bacteria declined noticeably. Thus enumeration of bacteria in leachate could be useful as another indicator of decomposition process progress.

FIGURE 2
Population changes of faecal and total coliforms with time.



The pH of the leachate increased during composting (Fig. 3). The pH was 5.56 at the beginning of the process in leachate. It is known that composting is possible in the pH range 3-11 (16). The pH increased to 6.81 at the end of the process in leachate. Increases in pH values may have been caused by the formation of $(NH_4)_2CO_3$ during anaerobic decomposition (17). An initial fall in pH was observed, due to the production of organic acids by microorganism. Low pH values were the sign of rapid degradation and acidogenic phase.

FIGURE 3 - Changes in the values of pH, conductivity and alkalinity during composting period.



In leachate, alkalinity as $CaCO_3$ was increased from 2920 to 6800 mg/l at day 108 due to the NaOH addition. Optimum alkalinity as $CaCO_3$ is 1000-4000 mg/l for anaerobic microorganisms (18). There was enough alkalinity for anaerobic microorganisms in our study (Fig.3). Conductivity value increased from 13.06 to 23.85 mS/cm in leachate (Fig. 3), at the end of the composting, which could be due, in part, to the effect of salt concentration as a consequence of the degradation of the organic matter fraction (19).

FIGURE 4 - Changes in the values of total phosphorus and COD during composting period.

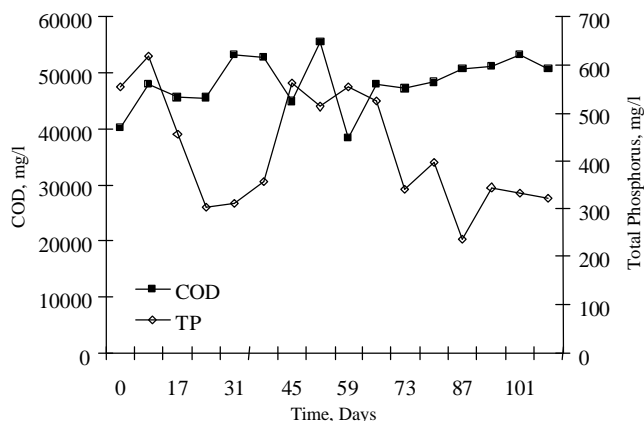
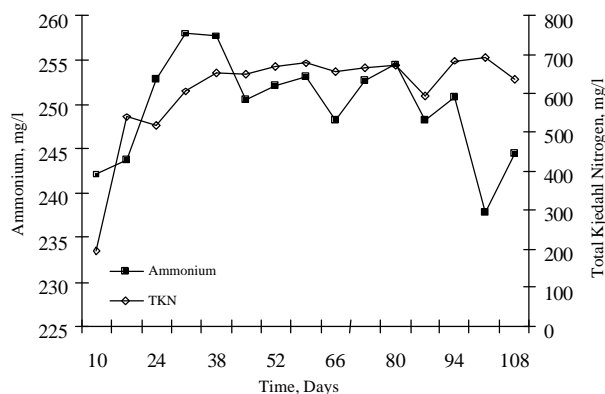


FIGURE 5 - Changes in the values of ammonium and total Kjeldahl nitrogen with time.

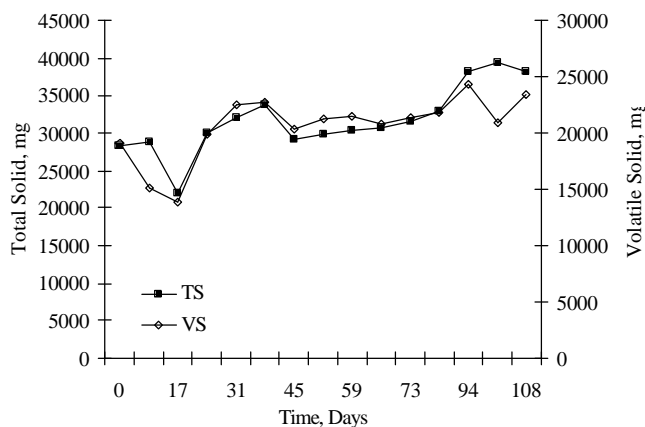


The total phosphorus showed a decrease in the leachate, at the end of the composting (Fig. 4). The COD values in the leachate were stable during composting process (Fig. 4).

The changes in ammonium and TKN are given in Fig. 5. The changes in ammonium may be due to a combination of factors, such as biodegradation of organic nitrogen, volatilization of part of ammonium in the recirculation of the leachates (20). TKN values did not show high differences with time. Cecchi et al. (21) and Pera et al. (22) reported that TKN was not changed with time. These results are in agreement with our results.

Decreases in the TS in the leachate as the study progresses are due in part to VS destruction (12). VS and TS values with time is given in Fig. 6.

FIGURE 6 - The variation of total and volatile solids with time.



The compost quality

Table 1 illustrates the measured parameters, which quantify some of the physical, chemical and microbiological properties of the anaerobic compost obtained in the study.

With regard to the hygienic aspects, the indicator microorganisms in the initial co-compost were drastically reduced at the end of the process. The fact that pathogen die-off is faster than that of the indicator organisms suggests that low numbers of indicator organisms are sign of elimination of pathogens. Coliform organisms are more resistant to inactivation than *Salmonella* sp. and thus are good indicator organisms (23). Faecal coliform was found to be more resistant than the pathogenic bacterium *Salmonella*, in the study of Tiquia et al. (8). In the study of Pera et al. (22), *Salmonella* was not found when total and faecal coliforms were 460.10^4 and 27.10^4 cells/g dw, respectively. In the light of the literature, it can be said that the final co-compost of our study is safe to use from a hygienic standpoint.

There are some mechanisms that influenced pathogen destruction or suppression occurred in the reactors. Bertoldi et al. (11) reported that bacteria and pathogenic organisms can generally metabolize readily assimilable organic matter, such as alcohols and sugars whereas they can not multiply on complex compounds, such as cellulose, lignin and humic substances. During composting, the raw composting material is mineralized and humified to more complex organic matter. The resulting limitation in available organic matter during the later stage of composting might explain why TC and FC are drastically reduced in the compost material in our study. By the end of the composting process (day 108), the TC and FC

numbers in compost were reduced from 1609.10^4 and 918.10^4 MPN/g.ww to $0.33.10^4$ and $0.16.10^4$ MPN/g ww in the reactor, respectively. Pera et al. (22) mixed domestic solid waste with leachate of thermophilic digester. They reported that TC numbers were decreased from 460.10^4 to $0.62.10^4$ cell/g. dry weight at the end of the anaerobic composting process. These results are in agreement with our data.

TABLE 1 - The physical, chemical and microbiological properties of the anaerobic compost.

Parameters	Start	Finish
Moisture(%)	75	77
pH	4.55	6.04
Conductivity(mS/cm)	4.44	6.56
C(%)	49.68	45.00
N(%)	2.597	2.602
C/N(%)	19.13	17.29
S(%)	0.226	0.165
Cellulose(%)	21.89	19.12
VS(%)	91	88
Cu(ppm)	52	74
Mn(ppm)	3400	64
Zn(ppm)	73	155
Fe(ppm)	658	964
Mg(ppm)	2166	2180
Ca (ppm)	20170	18914
Na(ppm)	1533	8534
K(ppm)	11889	10841
Cd(ppm)	*	*
Pb (ppm)	*	*
Cr(ppm)	*	*
Ni (ppm)	*	*
TC(MPN/g ww)	1609.10^4	$0.33.10^4$
FC(MPN/g ww)	918.10^4	$0.16.10^4$

* below detection limit

The nitrogen and carbon content of the composting mixture at the end of the process were 2.6 % and 45 %, respectively. Taking into account the weight loss at the end of the process, it can be said that nitrogen losses were very reduced in reactor (24). As regards organic carbon, a decrease was observed over process due to CO_2 loss by microbial respiration. During the composting process, CO_2 is emitted from the composting mass as a metabolic end product. Thus, the total carbon content of the composting mass decreases as composting proceeds (25). These carbon decreases in conjunction with the nitrogen evolution observed, produced a C/N ratio decrease over the process in mixture (24). The C/N ratio was decreased from 19.13 to 17.29, at the end of the composting. It has been stated that when the C/N ratio is less than 20, the compost is mature and can be used for agricultural purposes without any restrictions (26, 27).

During composting the moisture content of the composting mixture increased from 75 % to 77 % within 108 days because of water-holding capacity of the materials. The pH increased during composting. The pH was 4.55 at the beginning of the process. The pH increased to 6.56 at the end of the process. A high quality compost would have a pH between 6 and 7 (28).

Cellulose content was decreased from 21.9 % to 19.1 % in final co-compost. This resulting low cellulose degradation is in agreement with that of Madejon et al. (29). They co-composted concentrated depectified beet vinasse and grape marc. Cellulose content was decreased from 205 to 196 g/kg at day 150, in their study. Two theories can explain the lack of degradation of cellulose: (i) grape skins and seed contain high levels of tannins that reduce the rate of cellulose decomposition by inhibiting cellulosic enzymes (30), (ii) the high lignin content of grape marc can inhibit decomposition of cellulose. This inhibition is thought to be strictly physical, because the presence of lignin between the cellulose fibrils decreases the surface area accessible to microorganisms (31).

A decrease of potassium during the composting was observed. The K value in the co-compost changed from 11889 to 10841 ppm. The Na and Mg contents were increased in co-compost at the end of the process. Increase in the value of Na may be due to addition of NaOH.

Metal concentrations were increased at the end of the process because of the loss of mass. Heavy metals and other metal ions are often a concern in any waste treatment process. Heavy metal toxicity has been cited as a cause of toxic inhibition in anaerobic treatment (12). Heavy metal concentrations of the final co-compost were below the limits established by several legislations, e.g. Spanish legislation (32). Cd, Pb, Cr and Ni contents were below detection limit.

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KINETIC ANALYSIS OF COUPLED TRANSPORT OF NITRATE IONS THROUGH LIQUID MEMBRANES AT DIFFERENT TEMPERATURES

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SUMMARY

Non-steady state kinetics of coupled transport of nitrate (NO_3^-) ions through liquid membranes (n-hexane 85% + trichloromethane 15%) containing tetraoctyl ammonium chloride as a carrier was examined at temperature range of 273-303 °K. In this study, kinetics of nitrate ion transport has been analyzed in the formalism of two consecutive irreversible first order reactions. The influence of temperature on the kinetic parameters (k_{1d} , k_{2m} , k_{2a} , t_{\max} , R_m^{\max} , J_m^{\max} , J_a^{\max}) has been investigated. The membrane entrance (k_{1d}) and exit rates (k_{2m} , k_{2a}) have been increased with temperatures. For maximum membrane entrance (J_m^{\max}) and exit (J_a^{\max}) fluxes the activation energies were calculated from the slopes of the two linear relationships: $E_{ad}=6.99$ kcal/mol and $E_{aa}=6.97$ kcal/mol, respectively. The values of the calculated activation energy indicate that the process is diffusional controlled.

KEYWORDS: Nitrate Removal, Liquid Membranes, Wastewater Treatment, Coupled Transport.

INTRODUCTION

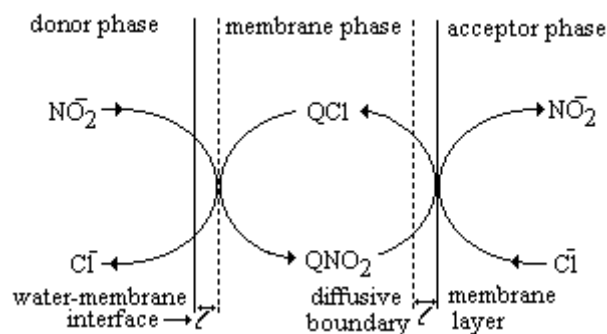
Liquid membranes play an important role in separation processes (1,2). In separation technology, the liquid membrane technique has been used for a wide range of different applications, such as toxic waste and metal removal from wastewaters (3,4) or gas separation (5).

Almost every research work on liquid membrane technique is done on system in which metal ions are being recovered, for economical reasons. Besides hundreds of cation separation system published in literature, only a few examples are known for separation of anions (6, 7). Especially in industrialized countries, it is no longer possible to use polluted surface waters for drinking water

supplies. Nitrate pollution in surface originates mainly from domestic and industrial wastewaters, and drainage and surface run off from agricultural areas (8).

For these purposes, the coupled transport kinetics of nitrate ions transport through liquid membranes (Fig.1) has been studied at different temperatures. The coupled transport kinetics of temperatures varied in the range of 293-308 °K.

FIGURE 1 - A schematic view of liquid membrane system.



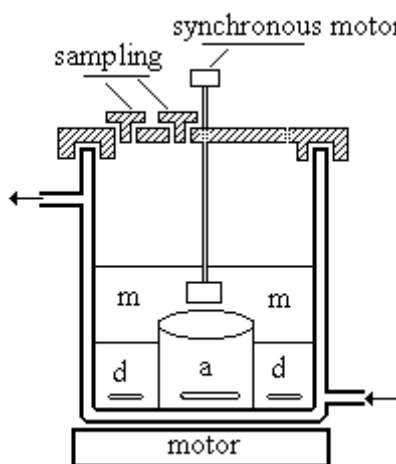
MATERIALS AND METHODS

Coupled transport experiments were conducted using the thermostated apparatus shown in Fig. 2. The initial composition of the phases: the donor phase (d, 75 ml) was an aqueous NaNO_3 solution (initial nitrate ion concentration, $C_{do}=50$ mg $\text{NO}_3^- \text{ l}^{-1}$), while the acceptor phase (a, 75 ml) was an aqueous 2 M HCl solution. The organic membrane phase was made up by dissolving the carrier tetraoctylammonium chloride in n-hexane (85 %) and trichloromethane (15%) mixtures ($C_{\text{carrier}}=10^{-3}$ M). The membrane phase (m, 200 ml) under the water phases was stirred magnetically. Stirring speeds for the donor, membrane and acceptor phases were 150, 200, and

150 rpm, respectively. The duration of a kinetic run was 500 min. The interface surfaces were as follows: $S_{d/m}=36.37 \text{ cm}^2$, $S_{a/m}=17.317 \text{ cm}^2$.

Samples (0.5 ml) were taken from both water phases (acceptor and donor phases) at regular time intervals and the nitrate ion concentration was analyzed by spectrophotometric method (9). Each reported experimental result is the arithmetic mean of two independent samples (error <1%).

FIGURE 2 - Apparatus used for coupled transport of nitrate ions through liquid membranes.



It was previously shown (10) that the kinetic behaviour of nitrate ion transport through liquid membranes described a general scheme of two consecutive irreversible first order reactions as given below:



It can be analyzed by full non-steady-state kinetics approximation; for non-steady state kinetics using reduced concentration.

$R_d = C_d / C_{d0}$, $R_m = C_m / C_{d0}$ and $R_a = C_a / C_{d0}$ (C_d , C_m and C_a are the nitrate concentrations in molar units in the donor, membrane and acceptor phases, respectively; C_{d0} being the initial nitrate ion concentration in the donor phase).

The following equations have been obtained (for more details see refs. 9):

$$R_d = \exp(-k_1 t) \quad (2)$$

$$R_m = \frac{k_1}{k_2 - k_1} [\exp(-k_1 t) - \exp(-k_2 t)] \quad (3)$$

$$R_a = 1 - \frac{1}{k_2 - k_1} [k_2 \exp(-k_1 t) - k_1 \exp(-k_2 t)] \quad (4)$$

where values k_1 (k_{1d}) and k_2 (k_{2m} , k_{2a}) are the apparent membrane entrance and exit rate constants, respectively.

Theoretical curves are calculated by eqns. (2), (3) and (4), respectively. First order time differentiation of eqns. (2)-(4) leads to the final forms of maximum value of flux equations:

$$\frac{dR_d}{dt} \max = -k_1 \left(\frac{k_1}{k_2} \right)^{-k_1/(k_1-k_2)} \equiv J_a^{\max} \quad (5)$$

$$\frac{dR_a}{dt} \max = k_2 \left(\frac{k_1}{k_2} \right)^{-k_2/(k_1-k_2)} \equiv J_a^{\max} \quad (6)$$

At $t = t_{\max} = t_{\text{ind}}$ the system is in steady state since the concentration of nitrate ions in the membrane (R_m) does not vary with time ($dR_m/dt_{\max}=0$) because the maximum value of penetration (J_d^{\max}) and exit (J_a^{\max}) fluxes are equal but of opposite sign (9):

$$-J_d^{\max} = +J_a^{\max} \quad (7)$$

The actual numerical analysis was carried out by non-linear curve fitting using a BASIC iteration program. The first rate constant, k_1 was obtained from eq. (2) using the donor phase data (k_{1d}), while the membrane exit rate constant, k_2 , may be obtained either directly from the acceptor phase kinetic data (k_{2a}) using eq. (4) or indirectly from the membrane phase data calculated on the basis of eq. (3) (k_{2m}). In both cases the k_{1d} value obtained from eq. (2) was used in the calculations.

RESULTS AND DISCUSSION

Time evolution of reduced concentrations of nitrate ion donor, membrane and acceptor phases are shown in Fig. 3 ($T = 298 \text{ }^\circ\text{K}$). It is apparent that R_d decreases mono-exponentially with time. On the other hand, the time variation of both R_m and R_a is bi-exponential. R_m increases at first, then decreases with time, i.e. it has a maximum when $dR_m/dt=0$.

The obtained kinetic parameters k_{1d} , k_{2m} , k_{2a} , J_d^{\max} , and J_a^{\max} are given in Table 1. For maximum membrane entrance and exit flux values, J_d^{\max} and J_a^{\max} , the activation energies, were calculated as $6.99 \text{ kcal.mol}^{-1}$ and $6.97 \text{ kcal.mol}^{-1}$, respectively. Activation energy (E_a) values were obtained from membrane exit rate [$\ln(J_a^{\max})$ versus $(1/T)$ plots] which is given in Fig. 4.

Those calculated activation energies indicate that temperature has an influence on the transportation rate constants of nitrate ion. The values of E_a obtained for a given process can serve as an indicator whether diffusion or chemical reaction is the rate-controlling step. For diffusion-controlled processes the value of the apparent activation energy is below 5 kcal.Mol^{-1} , whereas those for the intrinsic chemical reactions have been reported to be above 10 kcal.mol^{-1} (11).

FIGURE 3 - Time dependence of reduced concentrations of nitrate ions R_d , R_m and R_a phases in coupled transport through liquid membranes ($T= 298\text{ }^\circ\text{K}$); theoretical curves calculated from eqns. (2), (3) and (4), respectively).

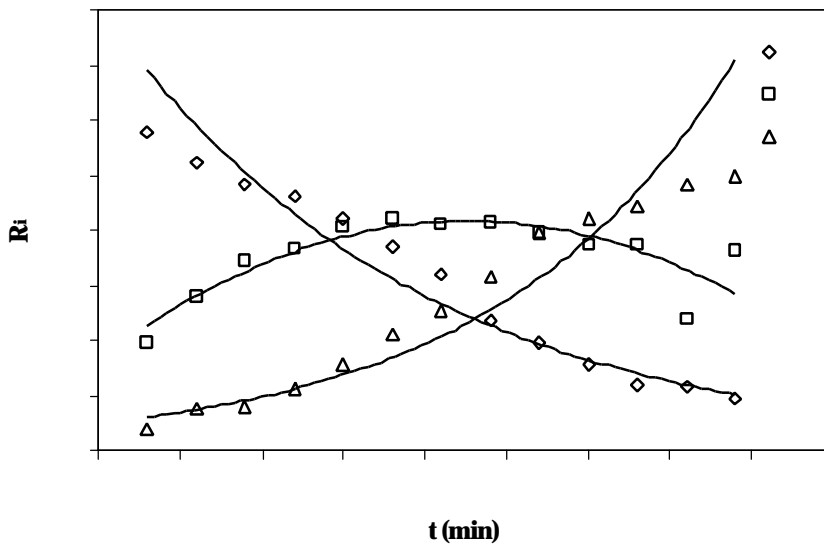
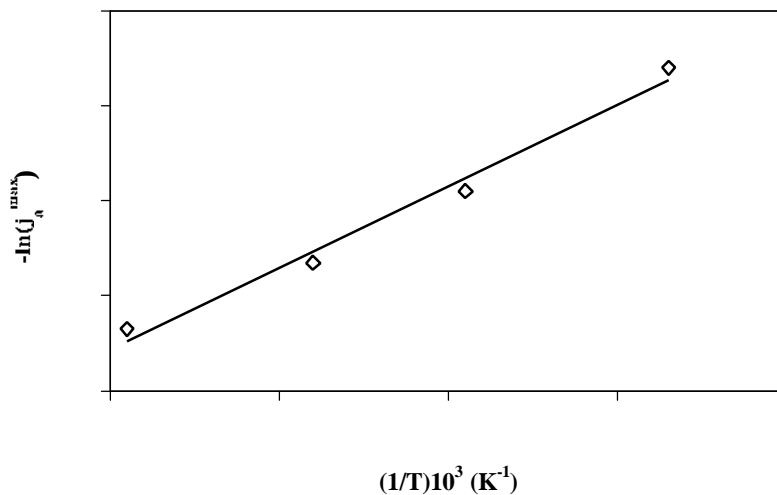


TABLE 1

Kinetic parameters for coupled transport of nitrate ions through liquid membranes at different temperatures.

T (°K)	$k_{1d} \cdot 10^{-2}$ (min^{-1})	$k_{2m} \cdot 10^{-2}$ (min^{-1})	$k_{2a} \cdot 10^{-2}$ (min^{-1})	J_d^{max} (min^{-1})	J_a^{max} (min^{-1})
293	4.125 ± 0.013	1.811 ± 0.017	1.778 ± 0.024	2.776	2.765
298	5.206 ± 0.039	2.198 ± 0.032	1.983 ± 0.046	3.594	3.566
303	6.323 ± 0.043	2.549 ± 0.017	2.331 ± 0.049	4.189	4.183
308	8.091 ± 0.016	2.654 ± 0.007	2.591 ± 0.017	4.845	4.845

FIGURE 4 - Arrhenius plot of nitrate ion transport, J_a^{max} .



CONCLUSIONS

In this study kinetics of nitrate ion transport has been analyzed in the formalism of two consecutive irreversible first order reactions. The membrane exit and entrance rates increased with temperatures. For membrane exit rate constants, k_{2m} and k_{2a} , the activation energies were found as $6.99 \text{ kcal.mol}^{-1}$ and $6.97 \text{ kcal.mol}^{-1}$, respectively. The values of the apparent activation energy indicate that the process is diffusionally controlled.

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DETERMINATION OF TOTAL PETROLEUM HYDROCARBONS (TPH), ORGANIC CARBON AND HEAVY METALS IN SOILS ADJOINING THE NATIONAL ARTS THEATRE WATER FRONT AND ABEGEDE CREEK IJORA LAGOS

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ABSTRACT

This work attempts to assess the levels of total petroleum hydrocarbons (TPH), heavy metals and organic matter in soils collected randomly from the Abegede creek (A), and National Arts Theatre water front (B). Control samples were collected from an undeveloped area in Lagos State University Ojo. Atomic Absorption spectrophotometry was used in determining heavy metals, while TPH in the samples were estimated gravimetrically in accordance with standard methods.

KEYWORDS:

TPH, cadmium, copper, lead, pollution, organic matter.

INTRODUCTION

Soil quality is emerging as issue of vital importance in the use and management of land, water and air. Clearly, we must maintain soils in a clean state that is suitable for agriculture, that minimizes the pollution of water and air, and that allows for the safe and productive use of wastes and by-products as soil amendment. It is imperative to remediate many unclean soils that have been severely impacted by anthropogenic activities¹.

The analytical test procedures used to assess soil contamination by petroleum products are petroleum hydrocarbons and heavy metals determination²⁻⁶.

It has been documented that petroleum hydrocarbons and heavy metals can impact soil ecosystems sufficiently to results in significant losses in soil quality⁹⁻¹⁰. Their negative impact results from their toxicity to biological processes catalyzed by soil microorganisms.

Field studies of contaminated soils have demonstrated that elevated loading of these contaminants can result in diminished microbial biomass, reduced viable bacterial

population densities, inhibition of organic matter mineralization as well as decreased leaf litter¹¹⁻¹³.

Remarkable accumulation of petroleum hydrocarbons and heavy metals have been observed in organism found in contaminated soils¹⁴⁻¹⁸. These have their attendant toxicological and health implications as the pollutants find their way into the complex food chain^{16,19-23}. Bioremediation techniques using plants and microorganism have been widely applied in cleaning up petroleum hydrocarbons and heavy metals polluted soils, as most contaminants are usually transformed via natural processes to innocuous compounds^{3, 24-28}.

However, the degree of soil decontamination using bioremediation techniques depends largely on the nature and levels of heavy metals and petroleum hydrocarbons present in the soil^{3, 6, 10, 25}.

The areas adjoining the Abegede creek are among other populated by some major soil marketing companies. The activities of these companies inadvertently lead to the continual release of varying forms of refined petroleum products into the creek. In addition, large volumes of untreated septic domestic waste materials are indiscriminately emptied from commercial trucks into the wet lands adjoining the Abegede creek.

The objective of this work was to examine the effect of human activities on the quality of soils around Abegede creek near Lagos State water corporative headquarters Ijora and National Arts Theatre water front Iganmu.

The outcome of this study is expected to assist in the development of a remediation action plan (RAP) of the area, as well as to alert appropriate agencies on the need to formulate and enforce a comprehensive environmental plan for Nigeria's sprawling urban cities.

MATERIALS AND METHODS

Two sites were used – Lagos Water Corporation, Ijora and National Arts Theatre water front along the course of the Abegede creek. In addition control samples were collected from an undeveloped area in Lagos State University, Ojo.

Composite samples were collected randomly from each site within a depth of 15cm using a locally fabricated soil auger (screw down and pull)²⁶. The sampling was done thrice a week over a period of one and half months between January and February 2001.

Nature of sites

Auto- mechanical workshop is located around site A. There is also a pathway adjoining the site, which carries a high volume of pedestrian and vehicular traffic.

There is a continual discharge of effluent and refined petroleum product (petrol, diesel and lubricating oil etc) spills into the creek by the petroleum marketing companies adjoining the sites.

Apart from a service road that cut across site B, there is also a constant heavy volume of traffic on the Eko bridge traversing this site, along with the discharge of septic waste materials. The control site is an undeveloped area located within the Lagos State University, Ojo.

Chemicals

Analytical grade reagents, silica gel (kieselgel 60 F₂₅₄, 70 – 230 mesh) and metal standard stock solutions (1 mg dm⁻³) were purchased from Aldrich chemical company and Fluka AG.

Instrumentation

The determination of the heavy metals was performed by the use of a Buck Scientific 200A Atomic Absorption Spectrophotometer. The instrument's setting and operational conditions were done in accordance with the manufacturer's specifications. The instrument was calibrated with analytical grade metal standard stock solution (-1 mgdm⁻³) in replicate.

Physico-chemical analysis

The soil moisture and pH were determined according to the methods previously described²⁷. Soil organic carbon was measured by dichromate digestion, using standard methods²⁹. The heavy metals were extracted from the soil sample for analysis using 2N HNO₃ following the methods reported earlier^{2,17,26}. The total petroleum hydrocarbons in the samples were determined gravimetrically using 100 g air-dried soil samples, 3 g KOH with reflux for 2 ½ h in 100 ml n-hexane and cleaned up with a short silica gel column (kieselgel 60 F₂₅₄, 70 – 230 mesh) following the methods previously described^{2, 4-6, 27}.

Statistical analysis

ANOVAS were used to estimate statistically significant levels at 95% confidence level. Pearson's correlation coefficient (P <0.01) was used to determine relationships between organic matter and heavy metals.

RESULTS AND DISCUSSION

Soil pH

Mean soil pH values ranged between 3.98±0.34, 6.08±0.83, 4.96±0.56 (Table 1). The relatively high acidic values for the control site can be attributed to nearness of sulphuric acid plants located at Agbara, transportation of raw material for these acid plants through the adjoining Lagos – Badagry expressway may also be a contributive factor^{2, 26}.

Moisture content

The mean percentage moisture content shown in Table 1 reveals values ranging from 21.83±4.33% to 29±4.11%. These relatively high values are expected because the two sites are close to a water body, as well as the soil texture, which is predominantly loamy clay³⁰. The percentage of moisture for the control sample (Table 1) is 8.61±3.69 %, this was found to be lower than values obtained from the two sites.

Organic matter content

The mean percentage of organic matter content is generally low as shown on Table 1 and it ranges between 4.37±3.89% and 4.95±4.97%. This is presumably due to the presence of heavy metals which causes a stronger reduction in decomposition of organic matter in soils²⁸. The soil organic matter was positively connected (Pearson's correlation coefficient P <0.01) with Pb (r = 0.54 and 0.81), Cu (r = 0.52 and 0.88) Cd (r = 0.64 and 0.74) in sites A and B, respectively. A similar trend has been observed in the sediments of some environment⁴. The control being the lowest with mean value of 0.67±0.68%, because it is a well – drained site.

Total petroleum hydrocarbon

The results of the total petroleum hydrocarbon TPH are indicated in Table 2. The mean TPH control values of 20.00±18.00 µg/g are expected to be lower than the mean values of 255.00±94.00 µg/g and 157.00±127.00 µg/g, respectively, for sites A and B. A similar trend has been observed before^{22, 23}, which is an indication of petroleum hydrocarbons pollution. It should be noted, however, that the TPH value of 20.00 ± 18.00 µg/g obtained for the control site is higher than the range, 1.00 to 10.00 µg/g, earlier reported for unpolluted soils^{5,6,32}. The occasional resort to bush burning using some refined petroleum products as fuel may not be unconnected with the relatively higher values of TPH recorded for the control site samples.

TABLE 1 - Some characteristics of the sampled soil.

Parameter	Control	A	B
pH	3.98±0.34	6.08±0.83	4.96±0.56
Moisture content %	8.61±3.69	21.83±4.33	18.29±4.11
Organic Carbon %	0.40±0.40	2.53±2.2.5	2.86±3.05
Organic matter %	0.67±0.68	4.37±3.89	4.95±4.11
Texture	Loamy clay	Sandy loam	Loam

TABLE 2
Mean levels of TPH (mg/g) and heavy metals mg/g ± SD in the sampled soils.

Parameter	Control	A	B	F <0.05
TPH	20.00±18.00	2.55.00±94.00	157.00±127.00	14.88*
Lead	0.80±1.02	54.18±43.03	42.60±61.35	3.79*
Copper	2.76±3.54	17.22±16.16	11.93±15.23	2.86
Cadmium	ND	0.10±0.16	0.024±0.073	2.49

ND = not detected; * = significant at 95% confidence level.

Heavy metals

The mean lead, copper and cadmium values ($\mu\text{g/g}$) for the control sites are 0.80 ± 1.02 , 2.76 ± 3.54 , ND, respectively, whereas the values ($\mu\text{g/g}$) for sites A and B are 54.18 ± 43.03 , 43.60 ± 61.35 for lead; 17.22 ± 16.16 , 11.93 ± 15.23 for copper and 0.10 ± 0.16 , 0.024 ± 0.073 for cadmium, respectively (Table 2).

The burden of heavy metals (Table 2) in the soil samples collected from sites A and B are generally higher than those of samples from the control sites. This trend has been observed before in contaminated soils^{2,17,18,26,33-35} and may be taken as an indication of pollution of the sampled sites.

The ANOVAS (Table 2) for TPH and lead reveal statistically significant differences at 95% confidence level; whereas the distribution of copper and cadmium shows no significant difference at 95% confidence level.

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FEB PRESS RELEASES
8th FECS Conference on Chemistry and the Environment: Chemistry for a Sustaining World, Athens, Greece, 31 August to 4 September 2002

Conference website:

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Organized by the University of Athens, Department of Chemistry and under the auspices of:

1. The Federation of the European Chemical Societies, Division of Chemistry and the Environment (FECS-DCE)
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CONFERENCE AIMS

To fulfil its part in sustainable World development, chemistry is changing. This 'greening' of chemistry involves two main thrusts. **First**, production, use and disposal of hazardous chemicals is being reduced and where possible eliminated. This must, however, be achieved whilst maintaining or improving the quality of human life, the natural environment and industrial competitiveness. **Second**, the environmental impact of anthropogenic chemicals is being studied so that it may be better understood, monitored and controlled.

Research is continuing to support these goals. New synthetic pathways are being developed using renewable feedstocks, alternative solvents, catalysts and reaction to increase energy and atom efficiency and reduce waste. Simultaneously the toxicology, metabolism and biogeochemical cycling of environmental contaminants and pollutants are being elucidated.

Although sustainability in chemistry has become established in many parts of the industry, there is still a lack of general awareness amongst academics, industrialists, regulators and the media.

The aim of this conference is to bring together scientists from universities, industry and governments to discuss and promulgate the current state of knowledge, latest research findings and likely future developments in all aspects of chemistry in the environment to point the way to an integrated approach to chemistry for a sustainable and sustaining world in the twenty-first century.

The conference will include invited plenary lectures from world authorities, parallel sessions of oral presentations of submitted papers arranged to cater for both specialist and generalist participants, dedicated workshops and exhibitions of commercial laboratory and field equipment.

A special session will be devoted to the **2004 Olympic Games** and its potential impact on the city of Athens.

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CALL FOR ABSTRACTS

Deadline for abstracts is March 2002
(use the Instructions for the Preparation of Abstracts)

PRE-REGISTRATION

Can be done by visiting the website of the conference.

REGISTRATION FEE

Participants: 300 Euro

Graduate students: 100 Euro

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EPMR 2002:

**International Conference on the Environmental Problems of the Mediterranean Region
12-15 April 2002, Near East University, Cyprus**

This will be a third conference of its kind after the "Water Problems in the Mediterranean Countries 1997" with 300 participants from 43 countries and the "Earthquake Hazard and Risk in the Mediterranean Region, 1999" with 500 participants attending from 53 countries. At EPMR 2002 we expect to host over a thousand delegates from 62 different countries in Northern Cyprus.

OBJECTIVE

The objective of the conference is to develop an interdisciplinary holistic approach for the integrated and sustainable management of the Mediterranean ecosystem. A gathering of scientists, policy-makers and stakeholders of the Mediterranean belt throughout the world is sought for developing suitable resource management plans to save the future of the Mediterranean ecosystem by discussing new concepts for developing individual or collective projects on problems related to soil, water, minerals and tectonic movements of the Mediterranean environments.



TOPICS

- Land and Soil Degradation
- Water Quality Management – Contamination, Treatment and Monitoring
- Wastewater Recovery and Reuse
- Atmospheric, Meteorological and Hydrologic Impacts
- Coastal Environmental Management – Impacts of Tourism and other Land Use
- Fisheries and Marine Pollution
- Physiologic, Sociologic and Legislative Aspects of the Environmental Protection
- Innovative Technology
- Modelling, GIS Applications and Remote Sensing Systems for Environmental Evaluation
- Environmental Effects of Natural Disasters
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- Mediterranean Ecosystem
- Renewable Energy Sources
- Solid and Hazardous Waste Management
- Environmental Health Aspect
- Industrial Water and Waste Management
- Integrated Water Resources Management
- Biodiversity, Environment and Sustainable Development
- Groundwater Resources Management and Contamination
- Wastewater Management in Sensitive Coastal Areas
- Impact of The Environmental Changes on Natural Life
- Others

CALL FOR ABSTRACTS/ PAPERS AND POSTERS

Your abstract of a proposed paper should be relevant to the objectives and the topics outlined as above and be not more than 200 words. Papers will be presented orally or as poster sessions. For poster presentations, panels of 200 x 150 cm will be available. For further information, please see the format proceedings.

DEADLINES

Last date for sending in the abstracts/ papers and posters: **14 January 2002**

Announcement of Acceptance of abstracts/ papers and posters: **21 January 2002**

Last date for sending in the papers:
18 February 2002

Announcement of papers: **25 February 2002**

Last date for payment of fees: **18 March 2002**

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