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ORIGINAL PAPERS

TOXICITY ASSESSMENT OF SEDIMENTS AND SOIL FROM RIVERS AND FLOODPLAINS IN CENTRAL POLAND USING A BATTERY OF MICROBIOTESTS – A CASE STUDY

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SUMMARY

In Poland, programmes of river-monitoring are based on traditional methods which examine the physico-chemical and biological parameters of water quality. However, these programmes do not include sediments and soil toxicity testing for environmental quality assessment. Rivers` sediments and soil from floodplain areas tend to accumulate various contaminants. Changes in physical and chemical characteristics in rivers may make contaminants bioavailable and hence capable of exerting their toxicity. Soils of floodplains serve as a natural remediation system by reducing the loads of pollutants. The aim of this study was to indicate application of a battery of microbiotests as tools for assessing the quality of sediments and/or soil from rivers and floodplains. Four rivers of Central Poland were selected for the study. Each of these rivers has been subjected to anthropogenic influences for several years. Samples were collected in spring and autumn 2005. The following micro-biotests were used to evaluate the toxicity: Microtox®SPT, Spirotox-SPT, Ostracodtoxkit F™ and Phytotoxkit™. The physico-chemical characteristics and respiration of soil and sediment samples have also been performed. To summarize, for the studied samples it was found that sediments exhibited higher toxicity than the soils. Furthermore, seasonal changes of toxicity and respiration of sediments' and soils' samples were observed. This project will be continued in 2006 and 2007.

KEYWORDS: battery of microbiotests, sediment, soil from floodplain, sensitivity of species.

INTRODUCTION

Development of agriculture, urbanization and industry contribute to decrease of surface water quality [1]. Rivers are open systems characterized by a high potential to self-purification. On the other hand, they can be easily changed by anthropogenic pressure, as aquatic organisms are very sensitive to changes in the quality of water [2]. Actually, the status of rivers in Poland is assessed mainly through the hydrological, physical and chemical monitoring program, which is included in the surface water monitoring according to legislation [3]. As a result, in Poland the complete ecological quality of rivers is unknown. On the other hand, sound methods for the assessment of the ecological status of surface water are required by the Water Framework Directive [4]. Traditional diversity, ecophysiological indices have been widely used to assess changes in biota caused by environmental disturbances in the river catchment. However, appropriate and standardized methods to assess the ecological status are still lacking. Against this background, ecotoxicological bioassays have been developed for screening the toxicity of river systems and assessing their ecological status [5, 6]. To asses the quality of rivers, the analyses of water, sediments and soil samples from floodplains are necessary.

The compositions of river's sediments depend on the physical and chemical agents. These factors influence the nutrient leaching and pollutants scavenging from sediments, which act as a sink for pollutants in water-bodies [5, 7-11]. Presently in Poland, the river deposits are monitored at 301 places of the different rivers. The study contains chemical and physical parameters [12].

During periods of flooding, sediments are deposited on floodplains. These areas serve as natural remediation system, reducing loads of suspended matter, nutrients and pollutants in rivers. That makes them excellent tools for the protection of freshwater ecosystems against eutrophication

and pollution. On the other hand, accumulated substances can be secondary effluents of river pollution [5, 13].

To determine the impact of chemicals on the structure and function of biological communities in environment, the battery of tests consisting of species belonging to different taxonomic groups and different trophic levels should be used [14-16]. For recent years, the traditional aquatic toxicity tests with water extracts from soil or sediment were applied to evaluate the toxicity of both [17, 18]. In these methods, the same organisms, used to evaluate the toxicity of water, were applied. However, complete toxicological information is possible only by direct-contact tests [7, 18-20]. Here, the organisms are incubated in the samples, making possible to evaluate the influence of all potential toxicity substances, present in samples. At this time, many standardized test procedures with microbiotests (direct contact tests), have been published [7, 19, 21]. Using them, the synergistic and antagonistic interaction between toxic substances in water, sediments and soil can be determined. For better sensitivity, repeatability, user-friendliness and reduction in costs, the standard microbiotest Toxkits are recommended [22].

The aim of the study was to evaluate the potential of the battery of microbiotests in a monitoring of the river's sediments and soil from floodplains of four rivers in Central Poland.

Study site description

The study area was located in two districts: Lodz and Warsaw. The samples were collected from four rivers located in Central Poland – Pilica (P), Bzura (B), Ner (N) and Utrata (U), and taken according to the scheme: sediments and soils "a" - "c" from the middle river stretch, and samples "d" from the estuary.

The Pilica River watershed in Central Poland (catchment area: 9273 km²) is the longest (342 km) left side tributary of Vistula River. It flows through both investigated regions – Łódzkie and Mazowieckie. Water from the study site on the Pilica River (P) has been classified to the 3rd purity class for rivers.

The Bzura River watershed (catchment area: 7787.5 km^2) is located in both of the research districts: Lodz and Warsaw. It is a 166.2 km long left side tributary of the Vistula River. Water from the study site on the Bzura River has been classified to the 4th (Bb) and 5th purity class (Ba and Bd) for rivers.

The Ner River (catchment area: 1865.5 km²) is 125.9 km long and the right-side tributary of the Warta River. It flows only through the district Łódzkie. At all its length, the water samples from Ner River have been classified to the 4th (Na) and 5th (Nb – Nd) purity class for rivers.

The Utrata River (catchment area: 792 km^2 km²) is a 68 km long right-side tributary of the Bzura River. It flows only through the district Mazowieckie. Water from the study sites Ua have been classified into the 4th, and those from other sampling places to the 5th purity class for rivers.

MATERIALS AND METHODS

Sampling

The samples were collected in April and September/ October 2005. In spring, the samples were taken at 9 sites. In autumn, there were 3 additional sampling points (12 sampling points altogether). At each sampling site, the river's sediment and soil from floodplain area were taken. Sediment samples at each location were collected with grab samplers (sampling depth approx. 20 cm) from pools. Soil samples (1 kg each) were taken from depths of 0–25 cm. All samples were stored at 4 °C until analysis.

Physical and chemical analyses

At the sampling sites, all soil and sediment samples were characterized for general parameters, such as organic matter content, pH and metals content. Soil sample pH (H_2O) was measured according to Myślińska and coworkers [23]. Prior to analysis, the subsamples were mixed, homogenized and sieved through a mesh-width of 500 μm to remove larger debris. Sediment pH was determined with a WTW 320 pH-meter. The content of organic matter was assessed by heating the samples in a stove at 550 °C [24]. The metal concentrations in the mixture (Zn, Ni, Pb, Cd, Cu, Fe, Co, Cr) were determined by atomic absorption spectrometry (AAS).

Soil and sediments respiration

The microorganism activity of sediment and soil samples was examined using the OxiTop®-Control system (WTW). The samples were cleaned from plants, put into measurement container OxiTop®, and incubated in temperature-controlled conditions (20 °C). The results were expressed as the amount of oxygen taken by soil or sediment samples [mg O_2/kg d.w.] during 12 hours.

Ecotoxicity tests

A battery of four microbiotests was used for this study. The battery was composed of test species representative of different trophic levels of food chine: primary producers (higher plant seeds), primary consumers (crustaceans), and decomposers (bacteria, protozoa) (Table 1).

Phytotoxkit™

The three-days germination and root growth inhibition test with seeds of three higher plant seeds (monocotyl *Sorghum saccharatum, and* dicotyls *Lepidium sativum* and *Sinapis alba*) was performed according to the standard operational procedure of the Phytotoxkit™ [25], which follows ISO standard 11269-1. The toxic effect was measured in comparison to the control soils. During the study, two controls were used, a reference soil provided by the Toxkit and a river sand. The sand was water-washed and sieved to eliminate contaminations and debris, and then

air-dried at 105 °C. The meaningful toxicity was observed for the reference soil, therefore, the results were not included in this paper. Estimation of root growth inhibition

TABLE 1 - Characteristic of the battery used for toxicity assessment of sediments and soil from floodplains.

Trophic level	Organisms	Test name	Endpoint	Test duration
Producers	**Higher plants**			
	Sorghum saccharatum *Lepidium sativum* *Sinapis alba*	Phytotoxkit™	growth inhibition	3 day
Consumers	**Crustaceans**			
	Heterocypris incongruens	Ostracodtoxkit F™	mortality/growth inhibition	6 days
Decomposers	**Bacteria**			
	Vibrio fischeri	Microtox®SPT	luminescence inhibition	20 minutes
	Protozoa			
	Spirostomum ambiguum	Spirotox-SPT	mortality (morphological deformations)	6 days

was made using a Nikon® digital camera connected with the view analysis PC system ImageTool®.

Spirotox-SPT

The six-days lethality test with *Spirostomum ambiguum* was based on the Spirotox-SPT procedure [21]. The test was carried out in disposable polystyrene multiplates (12 wells). The organisms were incubated in the Tyrod solution, and samples in concentration 200 g L^{-1}. As a control, rinsed marine sand was used.

Ostracodtoxkit F™

The six-days mortality and growth inhibition test with *Heterocypris incongruens* was performed according to the standard operational procedure manual of the Ostracodtoxkit F™ [26]. The organisms were incubated in EPA me-dium-hard water, and samples in concentration 200 g L^{-1}. As a control, rinsed marine sand was used.

Microtox®Solid Phase Test (SPT)

The solid phase luminescence inhibition test with *Vibrio fischeri* was performed according to the standard operational procedure of the producer (SDI). The samples were suspended in a diluent (3.5% NaCl), and serial dilutions were prepared. Bacteria were incubated with the samples for 20 min.

Toxicity assessment system

A toxicity assessment in a non-diluted sample is expressed as the percentage effect (EP). The effect depended on criteria of the respective assay: lethality of *S. ambiguum* and *H. incongruens*, inhibition of *H. incongruens* growth, inhibition of root growth of *S. alba*, *L. sativum*, and *S. saccharatum*. Only in the case of Microtox®SPT test, toxic units (TU) [g L^{-1}], according to the formula: $TU = 100/EC_{50}$, were calculated and presented. The percentage effect of the test is compared with the control sample. The sample was classified to be toxic, when the effects for Phytotoxkit™, Spirotox-SPT, Ostracodtoxkit F™ were above 20%, and that for Microtox®SPT TU was above 10.

RESULTS

Physical and chemical analysis

The pH values of the analyzed spring sediments and soil samples indicated their more alkaline reaction with regard to autumn samples, with the exception of two soil samples from Utrata River: Ua and Uc (Table 2). The pH values of the sediment samples were higher than those of the soils samples, except two autumnal samples: Na and Uc.

During the study, the seasonal changes of metal contents were not observed. The analyses of metal contents focused on the values, which exceeded the environmental background concentrations in sediment or the maximum tolerable levels in soils, according to Bojakowska and Gliwicz [12] and IUNG Puławy [27]. In sediment samples, the natural concentration was transgressed for Pb and Cu in the same spring samples from Utrata River: Ub (Table 2). In the majority of soil samples, the tolerable limits of metals were exceeded for Zn (with the exceptions of autum samples from P and Nc, as well as spring samples from Bb, Bd, and Ua). A high (near the threshold value) concentration of Cd was found in two soil samples: P – in autumn, and Bd – in spring (Table 2). The analyses showed that the concentrations of metals were higher in soil than sediment samples. Different relationship was observed only for 5% of the analyzed samples.

The analyses of organic matter show low differences between spring and autumnal samples. During the study, considerable differences between soil and sediment samples were not observed (Table 2). The content of organic matter in sediment samples was between 1.6% (Ud) – 16.2% (Nc) and 1.6% (Ud) – 10% (Ua), in spring and autumn, respectively. In soil samples, the content of organic matter ranged between 2.9% (Ud) – 12% (Ba) and 0.8% (P) – 21.5% (Nd), in spring and autumn, respectively.

Respiration

During the study, higher sediments' and soils' respiration in spring than in autumn (with the exception of the sediment sample from the Pilica River) was observed (Fig. 1).

TABLE 2 - Characteristics of sampling sites with chemical characteristics of sediments and soil samples in compare with environmental background concentrations in sediment or maximum tolerable levels of toxic elements in soil. sed. - sediments; env.bac.con. - environmental background concentrations.

sample	date	organic matter [%]		pH		Zn [mg kg^{-1}]		Ni [mg kg^{-1}]		Pb [mg kg^{-1}]		Cd [mg kg^{-1}]		Cu [mg kg^{-1}]		Fe [mg kg^{-1}]		Co [mg kg^{-1}]		Cr [mg kg^{-1}]	
		sed.	soil	sed.	soil	sed.	soil	sed.	soil	sed.	soil	sed.	soil	sed.	soil	sed.	soil	sed.	soil	sed.	soil
P	spring	2.00	4.89	7.29	6.31	2.70	45.60	1.30	6.80	2.40	13.80	0.41	0.66	1.30	9.90	41.60	62.40	0.24	0.96	1.30	7.20
	autumn	6.98	0.80	6.89	6.16	9.70	42.30	2.83	6.30	3.70	11.20	0.25	0.95	0.80	3.40	56.80	79.90	0.51	1.25	2.00	4.10
Ba	spring	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
	autumn	4.30	15.57	6.45	6.05	3.54	69.30	2.42	2.90	3.82	20.30	0.23	0.14	0.95	15.30	58.60	82.40	0.37	1.26	1.86	1.90
Bb	spring	4.03	11.94	7.46	6.65	3.01	12.31	2.23	2,70	3.71	3.88	0.23	0.27	1.73	2.07	51.30	55.40	0.38	0.46	2.24	2.12
	autumn	1.92	4.50	6.72	6.26	12.31	66.80	2.70	5.12	3.88	18.60	0.27	0.15	2.07	14.10	55.40	79.90	0.46	0,86	2.12	2.80
Bd	spring	12.88	6.26	7.62	7.31	3.54	42.30	2.42	6.30	3.82	11.20	0.23	0.95	0.95	3.40	58.60	79.90	0.37	1.25	1.86	4.10
	autumn	2.16	2.52	6.97	6.45	3.01	68.20	2.23	3.50	3.71	19.40	0.23	0.12	1.73	14.70	51.30	86.20	0.38	0.77	2.24	3.40
Na	spring	11.53	10.78	7.27	7.08	4.45	56,10	2.67	6.70	4.13	12.30	0.26	0.57	3.12	7.20	61.70	88.40	0.71	1.09	2.14	7.30
	autumn	3.27	4.48	6.46	6.57	4.45	56,10	2.67	6.70	4.13	12.30	0.26	0.57	3.12	7.20	61.70	88.40	0.71	1.09	2.14	7.30
Nb	spring	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
	autumn	6.84	10.03	7.04	6.09	6.40	63.80	2.78	7.70	3.88	14.30	0.27	0.68	3.40	9.80	53.60	77.30	0.33	0.86	1.78	5.90
Nc	spring	16,21	3.95	7.32	6.64	6.40	63.80	2.78	7.70	3.88	14.30	0.27	0.68	3.40	9.80	53.60	77.30	0.33	0.86	1.78	5.90
	autumn	9.44	4.82	6.83	6.25	15.03	44.90	2.18	7.90	3.67	12.10	0.29	0.58	3.23	9.80	55.70	69.40	0.64	1.11	2.44	6.70
Nd	spring	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
	autumn	1.65	21.54	7.13	6.55	21.02	65.30	0.89	2.80	6,70	17.50	0.06	0.10	4.49	14.40	61.30	72.90	0.31	0.92	0.81	1.20
Ua	spring	2.50	3.34	7.44	5.89	15.03	44.90	2.18	7.90	3.67	12.10	0.29	0.58	3.23	9.80	55.70	69.40	0.64	1.11	2.44	6.70
	autumn	10.02	2.87	6.62	6.58	18.60	66.40	1.29	5.20	6.70	16.80	0.05	0.21	4.30	12.80	57.10	81.60	0.37	1.32	0.80	1.60
Ub	spring	6.40	6.10	7.37	6.77	65.30	65.30	2.80	2.80	17.50	17.50	0.10	0.10	14.40	14.40	72.90	72.90	0.92	0.92	1.20	1.20
	autumn	2.54	8.16	6.53	5.07	19. 80	63.40	1.34	4.85	5.90	17.80	0.06	0.18	5.10	13.10	53.20	83.40	0.40	1.12	0.90	2.40
Uc	spring	13.26	9.79	7.50	7.05	21.70	68.20	1.35	3.50	6.56	19.40	0.07	0.12	4.54	14.70	54.60	86.20	0.41	0.77	1.36	3.40
	autumn	2.28	2.88	6.78	7.09	21.70	62.90	1.35	3.92	6.56	18.60	0.07	0.16	4.54	14.20	54.60	85.70	0.41	0.98	1.36	1.90
Ud	spring	1.61	2.85	7.47	6.90	22.74	69.30	1.25	2.90	6.86	20.30	0.04	0.14	5.31	15.30	56.40	82.40	0.66	1.26	0.92	1.90
	autumn	1.62	1.49	7.17	6.79	22.74	64.50	1.25	4.65	6.86	16.90	0.04	0.14	5.31	13.80	56.40	84.90	0.66	1.23	0.92	3.20
env.bac.con./ tolerance limit		-	-	-	-	73.00	3-50	6	2-50	11	10-70	<0.5	0.1-1	7	2-60	-	10000-30000	-	20	6	15-70

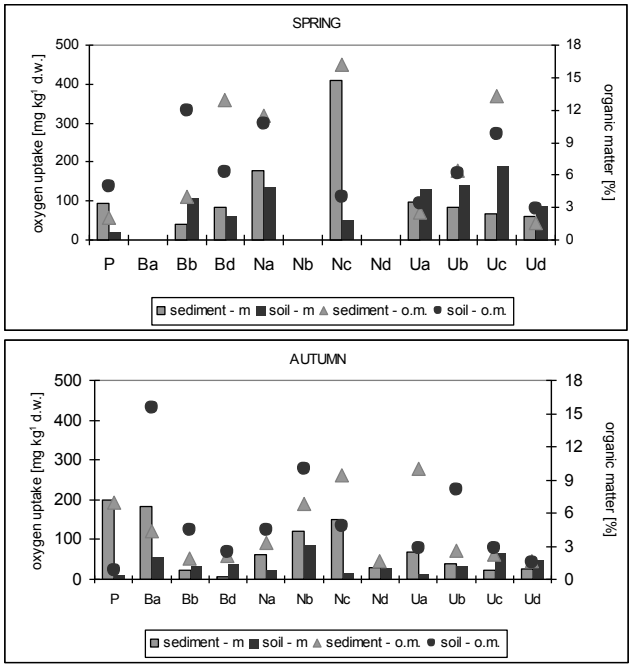

FIGURE 1 - Seasonal changes of soil and sediment respiration in relation with organic matter content. soil – m: soil respiration; sediment – m: sediments respiration; sediment – o.m.: organic matter content in sediments; soil – o.m.: organic matter content in soil.

In case of sediments, the highest activity of microorganisms in spring was noted for the samples from Ner River (Nc and Na sites: 408 mg O_2 kg^{-1} d.w. and 179 mg O_2 kg^{-1} d.w., respectively) (Fig. 1). During the second research season, the values of sediments ranged from 7 mg O_2 kg^{-1} d.w. (Bd sampling site) to 201 mg O_2 kg^{-1} d.w. (P sampling site).

The analyses of spring samples of soils show that those taken from Utrata River (Ua – 133 mg O_2 kg^{-1} d.w., Ub – 140 mg O_2 kg^{-1} d.w., Uc – 189 mg O_2 kg^{-1} d.w.) and Ner River (Na – 135 mg O_2 kg^{-1} d.w.) had high activity. During the autumn, the differences between soil samples were lower. The values of oxygen uptake were between 9 mg O_2 kg^{-1} d.w. (P) – 85 mg O_2 kg^{-1} d.w. (Nb) (Fig. 1). The analyses showed lack of differences between soils' and sediments' metabolisms.

The results indicated some relationship between the amount of oxygen uptake and organic matter content in the analyzed samples. The correlation coefficients for spring samples between respiration and organic matter in the same samples were 0.61 and 0.47 for sediment and soil samples, respectively. On the other hand, the correlation coefficient between respiration and organic matter in the same autumn samples were 0.65 and 0.22 for sediment and soil samples, respectively (Fig. 1).

Toxicity tests

The toxicity analyses of spring sediment samples showed that almost half of them react with over 20% effect. High lethality for *H. incongruens* in 4 samples was observed, from down stretch of Bzura (Bd), from middle stretch of Ner (Na) and Utrata (Ua), and from down stretch of Utrata (Ud) (Table 3). Two of the above samples (Bd and Ud), but also Uc, were toxic for *S. ambigum*. High toxicity was observed in Microtox®SPT for *V. fischeri* in Na, Nc and Ua samples. In case of Phytotoxkit™, inhibition of root growth higher than 20% was observed for *S. alba* in all samples (except that from Uc), and for *L. sativum* in P and Ua sediment samples.

Generally, no toxic effect for consumers and decomposers was observed for spring samples from middle stretch of Pilica, Bzura (Bb) and Utrata (Ub) Rivers. On the other hand, in the same sediment samples, toxicity effects above 20% for *S. alba* and *L. sativum* were observed (except Bb, Ub for *L. sativum*) (Table 3).

During the sediments sample analyses in spring, the *H. incongruens* in Ostracodtoxkit F™ (lethality test) and *S. alba* in Phytotoxkit™ test were the most sensitive species. PE values over 20% were observed in four and eight of the samples, respectively (Fig. 2).

In autum sampling, lower numbers of toxic sediment samples for *S. ambiguum* and *H. incongruens* were found (Table 3). On the other hand, in 42% of the analyzed samples (Bd, Nb, Nc, Nd, Ua) high luminescence inhibition of *V. fischeri* was noted. In the Phytotoxkit™ test, for each seed different reaction was observed. For *L. sativum*, all samples were toxic. The similar situation was observed for *S. saccharatum* (no toxic effects in Ba and Bb samples only). The lowest number of toxic samples was found for *S. alba.*

No hazards in sediment samples P, Na, Ub, and Uc, tested with Ostracodtoxkit F™, Spirotox SPT and Microtox®SPT, were observed in autumn. The most toxic samples were that from down stretch of Bzura River – Bd. On the other hand, toxicity effects were observed in each sample by Phytotoxkit™ tests (Table 3).

During sediment sample analyses in autumn, the most toxic effects (PE over 20%) were observed for 5 Microtox®SPT and 12 Phytotoxkit™ tests with *L. sativum*. The toxicities of the spring samples are summarized in Fig. 2.

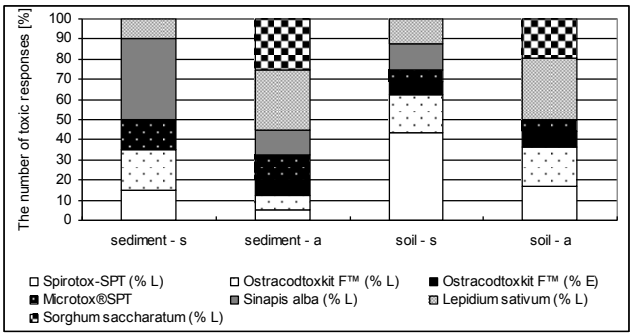

FIGURE 2 - The most sensitive species from the test battery. L – lethal; E – growth inhibition.

TABLE 3 - Results of toxicity tests performed in spring and in autumn 2005. L – lethal; E – growth inhibition; NP – not performed;

sample	date	Spirotox-SPT (% L)		Ostracodtoxkit F™ (% L)		Ostracodtoxkit F™ (% E)		Microtox®SPT (TU)		*Sinapis alba* (% E)		*Lepidium sativum* (% E)		*Sorghum saccharatum* (% E)	
		sediment	soil	sediment	soil	sediment	soil	sediment	soil	sediment	soil	sediment	soil	sediment	soil
P	spring	10	10	0	5	0	0	2.92	1.18	40	-16	50	25	11	-92
	autumn	0	0	13	5	-31	43	5.26	1.51	18	-26.6	61	56	54	-5
Ba	spring	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
	autumn	15	10	53	100	37	NP	8.55	3.81	-22.3	-44.9	42	59	15	58
Bb	spring	12	32	12	15	1	14	3.7	9.23	26	1	1	7	-125	-147
	autumn	15	85	35	90	34	NP	1.33	11.9	20.1	-62.1	49	18	12	34
Bd	spring	30	55	90	5	NP	22	3.8	3.27	55	7	8	-33	-65	-205
	autumn	5	75	23	63	30	NP	14.4	6.79	-7.4	-37.1	46	34	35	36
Na	spring	15	15	55	10	NP	8	111	3	42	-6	-21	1	-89	-107
	autumn	5	65	13	35	0	50	5.81	3.54	39.6	-41.5	51	42	52	18
Nb	spring	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
	autumn	45	30	20	73	17	NP	36.9	2.69	75.3	-15.9	67	32	59	73
Nc	spring	17	37	5	5	0	0,0	11.1	1.4	63	6	-19	-10	-103	-24
	autumn	2	20	20	30	1	42,0	38.8	2.51	-20.6	-49.3	54	45	29	31
Nd	spring	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
	autumn	0	20	-5	75	-2	NP	31.1	9.6	28.0	-39.7	62	37	21	47
Ua	spring	5	45	52	70	NP	NP	13.9	1.17	49	20	68	-4	-2	-75
	autumn	0	15	3	13	0	18	26.3	3.63	-26	-67.2	49	33	51	-1
Ub	spring	20	82	20	55	8	NP	3.9	1.7	48	29	-7	38	-41	-20
	autumn	0	0	8	0	0	2	9.6	3.95	59.3	-36.5	56	34	44	12
Uc	spring	42	80	12	10	8	10	4	3.24	16	23	16	0	-56	-64
	autumn	0	30	5	8	4	9	9.2	30	6.9	-2.9	57	33	36	-43
Ud	spring	32	50	22	37	13	14	1.7	0.51	24	17	6	-14	-129	-96
	autumn	25	30	5	0	9	4	0.7	6.3	-50.4	-55.8	50	52	60	63

In spring, the Spirotox was sensitive to all soil samples from Utrata and Bzura Rivers, whereas Ostracodtoxkit F™ mortality test was sensitive only to Ua, Ub and Ud, and Ostracodtoxkit F™ inhibition of growth was examined for Bd sample. Microtox®SPT was also sensitive to the sample from Bzura River – Bb. But in case of Phytotoxkit™ test, toxic effect was observed only in two samples, Ub and Uc, for *S. alba*, and P and Ub, for *L. sativum* (Table 3).

All the samples were toxic to at least one test from the battery of bacterial, protozoan and crustacean tests (except the samples from P and Na) (Table 3).

In spring, *S. ambiguum* in Spirotox SPT test was the most sensitive species in soil concerning tests with primary consumers and decomposers (Fig. 2). In 78% of the samples, mortality rates above 20% were analyzed. However, the Phytotoxkit™ showed this 20% effect only in 3 samples, P, Ub, and Uc (Table 3). *S. alba* and *L. sativum* were the most sensitivity species (Fig. 2).

During the autumn's bioanalyses of soil samples, the toxicity effect above 20% was noted in the samples Bb, Bd, Na, Nb, Uc, and Ud for *S. ambiguum*, in the samples from Bzura and Ner Rivers for *H. incongruens* in mortality test, as well as in Na and Nc samples from Pilica for *H. incongruens* in inhibition of growth-test, and, finally, in Bb and Uc for *V. fisheri*. An inhibition of root growth below 20% of the studied plants was observed for *L. sativum* only in Bb, for *S. saccharatum* in the samples P, Na, Ua, Ub, and Uc, but for *S. alba* in each sample. The bioanalyses with decomposers and primary consumers indicated that the most toxic soil sample was that from middle stretch of Bzura River – Bb (Table 3).

The obtained results suggested that the most sensitive species for pollutions in soil samples taken in autumn were *H. incongruens* and *L. sativum* (Fig. 2).

DISCUSSION

The rivers are located in the lower parts of landscapes and, therefore, collect and transport pollutants downstream from catchment [29]. The sediments accumulate pollutants in water-bodies and can provide a long-term record of toxic discharge. Chemicals bound to the sediments, and may persist long after the actual discharge has been stopped [10]. Moreover, during periodic floods, deposition of sediments is observed. Soil pollution has to be analyzed in a broad context due to the potential for interactions among soil, surface water and air [29]. Pilica, Bzura, Ner and Utrata rivers have been changed by humans to a different degree. The diversity is a consequence of: unsustainable development of agriculture, deforestation and urbanization. Rivers' sediments and soil from their floodplain contain a broad spectrum of different types of discharges, which can cause diversified toxic effects on test species. The physico-chemical analyses provided reliable information about actual sediment and soil pollution. Moreover, the content of organic matter and pH values create the environmental background for the interpretation of biological, physical and chemical processes in the sediments and soil samples.

The obtained results indicated that concentrations of metals were higher in soil samples than in the sediments, in spite of more acidic reactions of soil samples, suggesting that metals accumulate in soils. Lower concentrations of metals in river deposit arise from washing-out of metals from sediments during river discharge.

The mobility and bioavailability of metals bound to sediments and soils depend on multiple factors, for example reaction, organic matter content, oxidation state, biological activity [30-33].

According to Polish classification, the soil samples with pH lower than 6.8 belong to acid soils, with 6.9–7.2 to neutral ones, and above 7.2 to alkaline-reacting ones [34]. On this basis, the majority of the tested soil samples were acidic, 4 indicated neutral reaction (spring: Na, Uc, Ud; autumn: Uc), and only one was alkaline (spring: Bd). The standardized methods to assess the reaction of sediment samples are still lacking. Prokop and coworkers [30] showed that the rate of leaching from such sediments probably strongly increases at pH < 4.5. This could not be observed in the analyzed soil and sediment samples from the presented rivers. On the other hand, the above-mentioned authors observed that pH values ranging between 4.4–6, favour the bioavailability of metals to be high. The pH values in the analyzed samples of this study were all above 6.

Organic matter content is the parameter, which may strongly influence the mobility and bioavailability of metals and different pollutants [10, 33, 35]. Karuppiah and coworkers [35] showed that higher organic matter and clay content favour adsorption and retention of pollutants. Authors studied the toxicity of river and wetland sediments for the bacterium *Vibrio fischeri*. They observed that samples with identical metolachlor level, and higher amounts of organic matter (10 times) and clay (3.5 times) than the river sediment, were not toxic. But the chemical analyses of Prokop et al. [30] showed that not more than 10% of total metal concentration was adsorbed to solid organic matter. According to the same authors, 4–40% of total Cd and Zn were bound to dissolved organic carbon in the sediment samples. During our investigation, differences between organic matter in soil and sediment samples was not observed. Simultaneously, standard chemical analyses do not include determination of dissolved organic matter.

Prokop et al. [30] analysed the mobility and toxicity in sandy river deposits, and showed correlations between the rate of leaching and concentration of metal, pH values and organic matter, and the value of toxicity to plants in the sediment and to invertebrates in the eluate. They found that mobility and leaching of Cd and Zn in sediments increased with decreasing pH and decreasing content of organic matter. During the ecotoxicological study, these authors observed higher toxicity of the eluates, and, on the

other hand, lower toxicity of the sediments during high-flow of water through the sediments. So, the results suggested that the water flow can reduce the actual toxicity of the upper layer of river deposits. Dubova and Zariņa [36] elucidated in their toxicological analyses that more acidic conditions increase the sample`s toxicity.

The biological classification of rivers was performed by a lot of authors. The usage of microbiotests in biomonitoring of surface water was performed by Blinova [6]. Their results indicated that rivers belonging to the same class of biological quality (according to the classification of rivers based on macroinvertebrate taxonomical composition), forced the tested species (*Selenastrum capricornutum*, *Thamnocephalus platyurus*, *Daphnia magna*) to show different toxicity of water. The necessity of using ecotoxicological analyses of river water and sediments was underlined by Latif and Licek [5]. Their investigation of the toxicity of water and sediment samples showed that the water samples were toxic to any of the species of the test battery, but the bioassay of the sediment samples showed more or less pronounced impacts on several tests of the biota. Furthermore, it was necessary to use the direct contact sediment bioassay in this test battery for assessing the toxic hazard of poorly or non-soluble contaminants. They observed higher mortality of several *H. incongruens* samples used in the direct contact tests of the studied river sediments, compared to two crustaceans (*Daphnia magna* and *Thamnocephalus platyurus*) used for pore water toxicity test. The same result was obtained by Fernández and coworkers [33]. They suggested that using of extracts is less effective than that of whole soil samples, because the ionic strength in extraction is lower than in the pore water of the soil. Samples, which were non-toxic to *Hyalella azteca* in traditional contact test, gave the same effect signals as the presented direct contact microbiotests with *H. incongruens* [7].

A lot of studies [15, 16, 36, 37] suggested using a test battery composed of bacterial assay, protozoan test, microalgal test, jointly with one of the following bioassays: higher plants, rotifers or crustaceans.

The same was confirmed by Montvydienė and Marčiulioninė [38], who suggested that the response of plants and animals on toxic substances can generally be different. They published that Zn is the most toxic metal to animals, and, simultaneously, one of the least toxic to plants. On the other hand, Cr is one of the least toxic to animals, but rather toxic to plants. Moreover, the results indicate that the difference in the sensitivity of tested organisms to heavy metal mixture solutions might depend on the different en-vironmental origins of the tested plants. The toxic reaction of two plants was measured by these authors, and *Spirodela polyrrhiza* (freshwater plant) was more sensitive to heavy metals than the seeds of *Lepidium sativum* (terrestrial plant).

In the present study, the test battery was composed of microbiotests with Ostracodtoxkit F™, Spirotox-SPT, Microtox®SPT and Phytotoxkit™. The results demonstrated that the tested organisms react differently in the same sediments and soils (Table 3). In Ub sediment samples from Utrata and Pilica, the lowest toxicities were observed for decomposers and primary consumers. In the same samples, the root growth inhibition of plants was considerable. Similar results were noted in the soil samples. The most toxic sample (Bb) for Ostracodtoxkit F™, Spirotox-SPT, Microtox®SPT characterized low toxicity for plants, simultaneously.

The toxicity of soils and sediments was lower than that expected from analyses of historical changes in the presented rivers. Moreover, during the study, no relationship could be established between the contents of total metals and ecotoxicological findings. These results are in agreement with that obtained by several authors [5, 39, 40]. The sediment sample, in which higher concentrations of Cu and Pb were noted than in the environmental one (Ub – spring), was not toxic (except for *Sinapis alba*). To summarize, the obtained results suggested that in the river deposits toxic effects were more often observed (60 times, corresponding to 41% of all analysed sediment samples) than in the soil (52 times, only 35% of all analysed soil samples), in spite of lower contents of metals. This result is an effect of influences from different chemicals present in the samples, or more acidic reactions.

Samples collected in spring and autumn were investigated. Seasonal changes (spring and autumn bio-analyses) showed high differences in sediment and soil samples` toxicity. In sediment samples, toxicity effects could be observed in 32% during spring, but in 48% during autumn. Similar dependencies were noted for soils samples. In 25% of spring soil samples toxicity effects were observed. During autumn bio-analyses, this value increased to 43%. The seasonal change was assessed by Latif and Licek [5]. These authors showed higher loads of chemicals in winter than in summer. As consequence, they observed differences in the toxic impact of sediments on the tested organisms.

Microorganisms' activity was assessed as background of ecotoxicological tests. Microbial systems during exposition to organic and inorganic pollution responsed temporary decreases in species diversity, and changes in the metabolic functions. Consequently, microorganisms might be used as bioindicators for the natural and anthropogenic changes in soil. But the same microbial communities are unaffected until very large concentrations of pollutants are introduced [13]. Hence, many microorganisms demonstrate resistance to metals in water, soil and industrial wastes [41]. Perhaps, for this reason during analyses, the relationship between quantity of oxygen uptake and content of metals in sediment and soil samples was not observed. On the other hand, lower respiration was observed in autumn samples, simultaneously accompanied by higher toxicity for organisms. Moreover, the seasonal changes of reactions can be consequences of changes in the decomposition of organic matter rate. Higher microorganisms' activity is as-

sociated with faster transformations of organic matter at higher temperatures during spring. The obtained results showed higher relationship between oxygen uptake and content of organic matter in spring than in autumn (Fig. 1). The optimal pH of sediments and soils for microorganisms are between 5.5–7.2 [42], and the values obtained in the present study were in this range.

The aim of this preliminary study was to evaluate the potential of a microbiotests` battery as monitoring system for Polish rivers sediments and soils from the rivers` floodplains. The obtained results showed that the most sensitive tests were Phytotoxkit™ with *L. sativum* and *S. alba* for sediment samples, and Spirotox SPT and *L. sativum* for soil samples.

The study revealed that toxicity tests of sediments and soils from floodplains are necessary to indicate negative changes in river systems. Our research was part of a 3-years programme, and analyses will be continued in 2006 and 2007.

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EXTRACTION OF IRON(III), ANTIMONY(III) AND CADMIUM(II) FROM HYDROCHLORIC ACID BY TRIOCTYL AMINE AND TRIOCTYL PHOSPHINE OXIDE IN ISOAMYL ACETATE

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SUMMARY

The extraction of iron (III), antimony (III) and cadmium (II) from hydrochloric acid solutions has been investigated using high-molecular weight amine (trioctyl amine, TOA, R_3N) and a neutral organophosphorus compound (trioctyl phosphine oxide, TOPO) in isoamyl acetate (IAA). It was found that the efficiency of batch extraction method for the studied elements is almost similar for trioctyl phosphine oxide and trioctyl amine, both at low and higher acidity. This method was applied to the analysis of the elements in wastewaters. In addition, the back-extractions for iron, antimony and cadmium were studied. The interfering effects of sodium, potassium, magnesium, calcium, lead and cobalt were investigated. Blank values were low and recoveries were 100%, 100% and 88.3% for iron, antimony and cadmium, respectively, at 95% confidence level. The relative standard deviations for the determinations were found to be 1.0-6.0%. Detection limits (3σ) were 0.5 μg L^{-1} for iron, 0.6 μg L^{-1} for antimony and 2.9 μg L^{-1} for cadmium.

KEYWORDS: Extraction, trioctyl phosphine oxide, trioctyl amine, isoamyl acetate, batch method, atomic absorption spectrometry.

INTRODUCTION

The extraction technique in conjunction with atomic absorption spectrometry (AAS) has great advantages because it enables rapid and sensitive determination of micro-amounts of iron, antimony and cadmium, spraying the extract directly into the flame. Moreover, interferences from matrix elements are avoided by selective extraction [1-10].

It is well-known that extraction of ions from an aqueous solution is only possible, if the charge is neutralized by chelation, or by association with other species of opposite charge. A further requirement to aid the extraction is that, at least, one of the ions involved contains large hydrophobic groups.

Consequently, we thought that organophosphorous compounds like trioctyl amine (TOA) and trioctyl phosphine oxide (TOPO) could be suitable for those purposes. Their extraction capabilities with respect to various metals are unique [11-12]. TOA is a high-molecular weight amine and one of the most widely used extractants. Analytically, TOA is used for extraction of elements that yield anionic complexes, therefore, in many cases, chemical and electrostatic interactions are important. TOPO has also been used to extract several metals. Usually elements in their highest oxidation state yield most extractable TOPO complexes [3]. Isoamyl acetate (IAA) has suitable physicochemical properties for the extraction process; it is stable and active with respect to the extracted compounds.

In this paper, we investigated the extraction of iron, antimony and cadmium from hydrochloric acid solutions with TOA and TOPO in IAA. In addition, this method was applied to analyze the studied elements in wastewaters, because TOA and TOPO have been recommended for the removal of iron, antimony and cadmium from industrial wastewater. The proposed method is simple, rapid and sensitive, and should be applicable to other metals.

MATERIALS AND METHODS

Reagents

All reagents were of analytical grade and without further purification.

TOPO solution: Dilute 7 mL of TOPO (Alpha Aesar) to 133 mL with IAA.

TOA solution: Dilute 7 mL of TOA (Merck) to 133 mL with IAA.

Mixed standard solutions of iron (10 mg L^{-1}), antimony (40 mg L^{-1}) and cadmium (5 mg L^{-1}) were prepared by appropriate dilution of 1000 mg L^{-1} AAS-standard stock

solutions. All solutions were prepared with doubly distilled water.

Apparatus

A UNICAM 929 model flame atomic absorption spectrophotometer (FAAS) with a 100 mm long slot burner and hollow cathode lamps was used. The instrumental conditions for each element were taken from the manufacturer`s manual (Table 1).

TABLE 1 - Instrumental parameters of Fe, Sb and Cd in FAAS determination.

	Fe	Sb	Cd
Sensitivity	0.060 mg L^{-1}	0.370 mg L^{-1}	0.032 mg L^{-1}
Wavelength	248.3 nm	217.6 nm	228.8 nm
Band pass	0.2 nm	0.2 nm	0.5 nm
Lamp current	20 mA	15 mA	5 mA

Extraction and Analytical Procedures

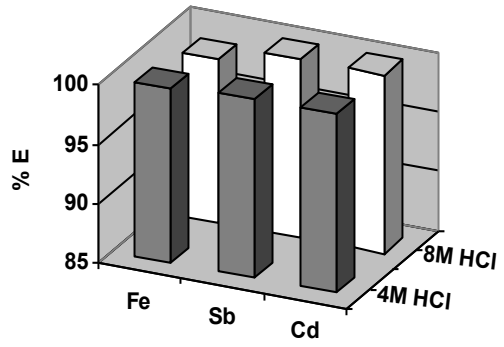
Equal volumes (15 mL each) of the aqueous and organic phases were placed in a 50-mL separatory funnel, which was shaken for 2 min, and then the content was allowed to stand for 1 h. The aqueous solution was separated from the organic layer, and the elements extracted into the organic phase were analyzed by FAAS, on the basis of the residual elements in the aqueous phase. Chloride complexes of iron, antimony and cadmium have been investigated with respect to their extractability by 5% solutions of neutral TOPO and TOA. The organic layers containing the extracted elements were back-extracted with aqueous solutions of 0.5 M HCl, 1 M HNO_3 and 0.5 M H_2O_2 + 0.5 M HNO_3. To the separatory funnel containing the organic phase, 15 mL of 0.5 M HCl, 1 M HNO_3 and 0.5 M H_2O_2 + 0.5 M HNO_3 solutions were added and mixed for 2 min. After phases were allowed to stand for 1 h, the aqueous solution was separated and used for determination of the re-extracted elements.

RESULTS AND DISCUSSION

Extraction with TOA and TOPO

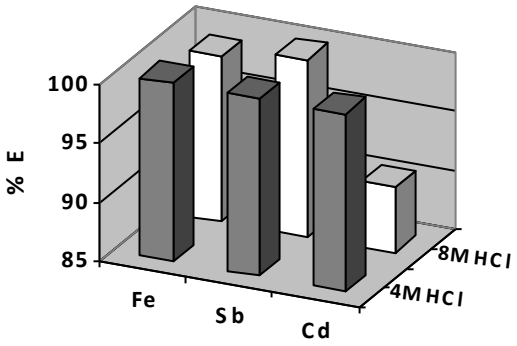
The TOA-in-IAA extraction results of iron, antimony and cadmium from hydrochloric acid solutions are illustrated in Figs. 1 and 2. The extraction efficiency for iron resembles both at low acidity and higher acidity, and follows the order TOPO > TOA. Similarly, the extraction efficiency for antimony is similar both at low acidity and higher acidity, but follows the order TOPO = TOA. Extraction of cadmium follows the order TOA>TOPO at high HCl acidity, and TOA = TOPO at low HCl acidity. They were found to be high with 4M and 8M HCl and TOA as well as TOPO in IAA. From the results shown in Figs. 1 and 2, TOPO and TOA appeared to be the most suitable extractants for the studied elements.

The extracted elements in the organic phase can be back-extracted with suitable reagents, and in this study 0.5 M HCl, 1 M HNO_3 and 0.5 M H_2O_2 + 0.5 M HNO_3 were used as stripping solutions. The studied elements can be partially or completely back-extracted to the aqueous phase, as presented in Tables 2 and 3.



	Fe	Sb	Cd
4M HCl	99,6	100	100
8M HCl	98,8	100	100

FIGURE 1 - The extraction of Fe(III), Sb(III) and Cd(II) with TOA in IAA.



	Fe	Sb	Cd
4M HCl	100	100	100
8M HCl	99	100	90,5

FIGURE 2 - The extraction of Fe(III), Sb(III) and Cd(II) with TOPO in IAA.

The stripping results for the IAA-TOPO extraction system are given in Table 2.

Iron was extracted by 1 M HNO_3 (100%), 0.5 M HCl (93%) and 0.5 M H_2O_2 + 0.5M HNO_3 (78%) at higher acidity. In the other cases, iron was only partially back-extracted (around 36-89%). Antimony was poorly stripped by 0.5 M HCl (only about 42%) and 1 M HNO_3, but it was completely back-extracted (100%) to the aqueous phase by 0.5 M HCl + H_2O_2 mixture from both media, 4M and 8M HCl solutions. Cadmium was partially back-extracted in all extraction cases studied (around 57-79%).

TABLE 2 - Effect of eluents on the back-extraction of the elements (IAA-TOPO system).

Organic Phase	Eluent	Back-extraction (%)[a]		
		Fe(III)	Sb(III)	Cd(II)
IAA-TOPO (4 M HCl)	0.5M HCl	89.5±3	42.0±3	77.0±2
	1M HNO_3	78.4±3	43.0±4	79.6.±2
	0.5M H_2O_2+HNO_3	36.0±2	100±1	75.5±3
IAA-TOPO (8 M HCl)	0.5M HCl	93.4±4	40.0±6	57.0±5
	1M HNO_3	100±2	36.0±6	72.2±3
	0.5M H_2O_2+HNO_3	78.0±1	100±2	72.4±3

[a] Average of three determinations with 95% confidence level.

The stripping results for IAA-TOA extraction system are given in Table 3.

Iron was again completely back-extracted by 0.5 M HCl + H_2O_2 and 1M HNO_3 as eluents from both media, 4M and 8M HCl solutions (100%), but poorly returned to the aqueous solution by 0.5 M HCl. Antimony was partially back-extracted by 0.5 M HCl + H_2O_2 (around 81%), but poorly returned to the aqueous solution in other eluents. Cadmium was only back-extracted by 1 M HNO_3 from both 4M and 8M HCl solutions (around 71-88%).

TABLE 3 - Effect of eluents on the back-extraction of the elements (IAA-TOA system).

Organic Phase	Eluent	Back extraction (%)[a]		
		Fe(III)	Sb(III)	Cd(II)
IAA-TOA (4 M HCl)	0.5M HCl	24.4±4	18.2±4	23.1±3
	1M HNO_3	100±1	46.7±6	88.3±3
	0.5M H_2O_2+HNO_3	100±2	80.6±3	48.0±3
IAA-TOA (8 M HCl)	0.5M HCl	42.0±4	27.4±5	8.9±5
	1M HNO_3	100±1	53.3±2	71.0±2
	0.5M H_2O_2+HNO_3	100±2	81.4±1	52.2±3

[a] Average of three determinations with 95% confidence level.

Iron and antimony was completely back-extracted (100%) in some eluting solutions by both TOPO and TOA in IAA. The best extraction efficiency for cadmium was obtained by 1M HNO_3 from 4M HCl solution, and it seems that it is very strongly bonded to TOPO and TOA, and, therefore, the used eluents are not effective in this case.

The detection limits, based on three times the standard deviation of the blanks (n=10), were 0.5 μg L^{-1}, 0.6 μg L^{-1} and 2.9 μg L^{-1} for Fe, Sb and Cd, respectively, and relative standard deviations ranged between 1.0-6.0%. The relative standard deviations of the recovered values, and accuracy and precision of the above findings suggest that the proposed method could be used for the determination of trace metals in environmental water samples.

Finally, extraction procedures are ideally suited for the isolation of trace quantities of iron, antimony and cadmium.

Effect of the Diverse Ions

A sample solution of 1 L containing 10 mg iron, 40 mg antimony and 5 mg cadmium, metal ions (sodium, potassium, magnesium, calcium, lead and cobalt; 50 mg L^{-1} each) was prepared. The extraction of iron, antimony and cadmium was carried out according to the general procedure described above. It was observed that metal ions did not interfere with the adsorption of iron, antimony and cadmium.

Application

From the studied systems, IAA-TOPO extraction system was applied for the determination of iron, antimony and cadmium in wastewater. Wastewater sample was obtained from a factory near Edirne. An aliquot (300-500 ml) of the water sample was filtered to remove suspended material, adjusted to the appropriate acidity with HCl, and then subjected to the used procedure. The analytical results are shown in Table 4. Extraction of metal ions by this system showed that it can be reliably applied for their determination in wastewater, as well as in other samples. Since the applied system has no affinity for alkaline and earth alkaline metals, it is useful for water sample analyses of environmental concern.

TABLE 4 - Determination of Fe, Sb and Cd in wastewater sample using IAA-TOPO system (volume of IAA-TOPO 10 mL; sample volume 10 mL; eluents volume 10 mL; eluent 1M HNO_3 for Fe and Cd, 0.5 M H_2O_2+ HNO_3 for Sb).

Element	Wastewater (mg L^{-1})	Added (mg L^{-1})	Wastewater Found (mg L^{-1})
Fe	$0.572±2.1x10^{-2}$	2.00	$2.567±1.6x10^{-2}$
Sb	$0.063±2.9x10^{-3}$	2.00	$2.060±3.2x10^{-3}$
Cd	$0.019±2.6x10^{-3}$	2.00	$2.010±2.1x10^{-2}$

[a] Average of three determinations with 95% confidence level.

CONCLUSION

A rapid and sensitive method has been developed for the determination of iron, antimony and cadmium metals. Optimal conditions have been established for the extraction and determination of the studied elements. Iron, antimony and cadmium are extracted from hydrochloric acid by trioctyl amine and trioctyl phosphine oxide in isoamyl acetate, and determined by flame atomic absorption spectrometry. The proposed extraction systems were successfully applied for the determination of micro-amounts of iron, antimony and cadmium from various aqueous mixtures. The proposed organic extractants exhibit good chemical stability, good analytical reliability, and fast rates of equilibrium. Both, the uptake of the metals from the aqueous phase and stripping of these metal ions, are fairly rapid. The interfering effects of other metal ions were also investigated, and it was observed that metal ions do not interfere in the adsorption of iron, antimony and cadmium. The proposed method is rapid and sensitive, and considered to be satisfactory.

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ORGANOCHLORINE RESIDUES IN SOME SERBIAN AGRICULTURAL PRODUCTS

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SUMMARY

The contents of organochlorine pesticides (OCPs) were determined in wheat grain, bran, and sunflower oil, obtained from Vojvodina Province (Serbia). Samples were prepared by the official method proposed for isolation of OCPs. Qualitative and quantitative analyses were done by GC/ECD. The total concentration of OCP residues ranged from <0.01 to 4.93 ng/g for wheat and bran, and 0.71 to 0.95 ng/g for edible oils. Contents of determined OCPs in the investigated samples were compared with the regulated maximum levels according to Serbian legislation, and with that from literature.

KEYWORDS:
Organochlorine pesticides, wheat grain, bran, sunflower oil.

INTRODUCTION

Organochlorine pesticides (OCPs) have been particularly effective in the control of pests and diseases, but their resistance to degradation has resulted in universal contamination of water, soil, and also foods by them. High toxicity of these compounds has made their use very restrictive reaching the point of being forbidden in most countries.

According to literature data, endosulfan is still in use in Spain [1], Korea [2] and both endosulfan and lindane are also used in Serbia and USA [3], for these two tend to be much less persistent than those falling under the proposed treaty. The use of lindane is in the process of being banned in Europe [4]. In China, technical HCH was replaced by the purified active isomer γ-HCH (lindane) in 1991; while chlordane as a broad-spectrum contact pesticide is still being extensively used against termites [5]. Endosulfan is one of the remaining OCPs registered in Argentina for control of a large spectrum of insect pests on fruits and vegetables [6, 7]. In Canada, lindane is one of the 10 top insecticides used for protection of livestock, crop-seed and poultry [8].

OCPs are compounds with a very low solubility in water and because of their lipid character they cannot be metabolised by the organism, but accumulate in fats. These compounds are known of inducing or aggravating certain health problems in humans, such as cancer, immune systems and the disruption of hormonal functions [9].

Due to their hydrophobicity, OCPs are persistent to degradation in the environment, and they are ubiquitously found contaminants. Therefore, they have become a major issue of environmental research in order to investigate levels of their occurrence, biochemical and toxic effects, human exposure and health risk assessment, and have been subjected to national and international regulations and controls. The general population is mainly exposed to residues of OCPs through the ingestion of contaminated food. The control of their presence in foodstuffs is important, i.e. they should be monitored in order to ensure that public health is not endangered by residue concentrations that are in excess of the official tolerance levels.

The aim of the present study was to investigate the contents of organochlorine compound residues in composite wheat grain samples collected from three important wheat growing regions of Vojvodina Province (northern part of Serbia) in 2002; in composite wheat bran sample representative for the northern part of Vojvodina (North Bačka) collected in 2003; and composite sunflower oil samples representative for a chosen factory located at West Bačka (Vojvodina) in 2003. This study was of a limited and local scale, but to date no survey exists in Vojvodina concerning the levels of these compounds in some crops and their products.

MATERIALS AND METHODS

Sampling

Grain samples of wheat were collected in the frame of the yearly monitoring of wheat quality performed by the National Center for Cereal Technology-Novi Sad, Vojvodina Province, Serbia.

For the purpose of this study, three important wheat growing regions of Vojvodina (North Bačka, South Bačka and South Banat) were investigated, and regions were subdivided into provinces. Within each province, samples of grains were collected during the harvest 2002 in different representative fields located in the whole provincial territory. Individual samples coming from the same province - each weighing 1 kg - were pooled together (35, 39 and 34 samples from North Bačka, South Bačka and South Banat, respectively) to obtain the test samples. Test samples coming from each region (5 from North Bačka, 6 from South Bačka, and 5 from South Banat) were also pooled together to obtain the composite sample, representative for each selected region. In this way, three representative wheat samples were prepared.

During 30 days in 2003, fifteen individually taken samples of wheat bran produced in flour milling industry located in North Bačka were collected to obtain the representative sample for this region.

For 40 days during the edible oil production in 2003, sixteen individually taken samples of sunflower oil (refined and unrefined) were pooled together to obtain the composite sample representative for a chosen factory located in West Bačka. This factory accounted for about 32% of sunflower oil production in Serbia.

Composite samples (~1 kg) of each item were stored in a closed glass beaker at about 4 ^{o}C before analysis.

Chemicals

Organic solvents, sodium chloride and sodium sulphate were purchased from Merck (Hohenbrunn, Germany); Florisil from Fluka (Buchs, Switzerland); and EPA 608 Pesticides Calibration Mix from Supelco (Bellefonte, PA, USA). Distilled water used in extraction procedure was previously extracted with dichloromethane. The laboratory glassware was washed with detergent, rinsed with distilled water and acetone and then heated to 130 ^{o}C overnight prior to use.

Analysis

OCPs in the samples were analysed according to the official method AOAC 970.52 [10]. Twenty grams of ground wheat and bran samples were extracted with distilled water/ acetonitrile mixture and filtered through Whatman paper No. 1. Filtrate was treated with petroleum ether, saturated sodium chloride solution and distilled water. After removing the aqueous phase, anhydrous sodium sulphate was added, and the solution was concentrated to ca 5 mL in a rotary evaporator. Clean-up was done by adsorption chromatography using Florisil packed in a glass column, as well as 6% and 15% ethyl ether in petroleum ether as eluting solvents, respectively.

In the case of edible oil, thirty grams of oil was extracted with petroleum ether and acetonitrile. The acetonitrile layer was drained and treated with petroleum ether, saturated sodium chloride and water. Petroleum ether solution was separated and treated with additional portion of acetonitrile. Both acetonitrile solutions were combined. After discarding the aqueous layer, organic layer was passed through anhydrous sodium sulphate in a glass column, and then it was concentrated in rotary evaporator. Clean-up procedure was the same as for the above mentioned samples.

Sample analysis was carried out using an HP 6890 gas chromatograph equipped with HP-5 fused silica column (60 m x 0.32 mm i.d., film thickness 0.25 μm) and ECD. Temperature program was as follows: initial temperature 120 ^{o}C, held for 2 min, then 3 ^{o}C/min to 295 ^{o}C, kept for 3 min. Helium was used as carrier gas with nominal initial flow of 2.1 mL/min through the column. The injector and detector temperatures were maintained at 290 and 310 ^{o}C, respectively. A 1 μL aliquot of extracts was injected (splitless mode) with a purge time of 0.75 min. OCPs identification was performed by comparison of their retention times with those of authentic standards. The quantitative analysis was done by the external standard method.

Quality control consisted of analysis of blank and spiked samples. The detection limit of the applied method for individual components was 0.01 ng/g. The fortified samples were prepared by adding a known volume of mixed OCP standard solutions. They were stored (for equilibrium), and then analysed as previously described. The 69-90% recovery rates of analytes were acceptable [11].

Obtained results were blank-corrected and presented as mean value of duplicates. They have not been corrected for the recovery percentage.

RESULTS AND DISCUSSION

The concentrations of OCP residues in analyzed samples (wheat grain, wheat bran, and sunflower oil) obtained from the Vojvodina Province are given in Tables 1-2. Total sum values were given using limits of detection (LODs) equal to zero, when the content was below the detection limit.

The total content of OCPs in cereals ranged from <0.01 to 4.93 ng/g fresh weight. The following order in wheat from North Bačka was found: HCHs>DDTs>endosulfan; HCHs were below the detection limit for grain collected at South Bačka region, while OCPs were not detected above detection limit in the sample from South Banat (Table 1). The discrepancy of OCP profiles of the analysed samples representative for the chosen regions could be explained with different past and present usage of agrochemicals. The contents of α-HCH and β-HCH in the wheat sample from North Bačka indicated that lindane was completely decomposed or bio-transformed into its isomers. The levels of p,p'-DDT in wheat samples from South Bačka and North Bačka, and wheat bran from North Bačka showed that this pesticide was recently used, although its

TABLE 1 - The mean contents of OCP residues (ng/g wet weight) in wheat grain and bran from the Vojvodina Province, and literature data for cereals, mean value and range (ng/g wet weight).

Compounds	Wheat grain, South Bačka	Wheat grain, North Bačka	Wheat grain, South Banat	Wheat bran, North Bačka	Cereals from India [12]	Cereals from Australia [13]	Serbian legislation for cereals [14]
α-HCH	<0.01	2.65	<0.01	<0.01	**ΣHCH=35 (27-39)**	**ΣHCH=0.44 (0.03-1.8)**	**sum of α+β+δ HCH** 20
β-HCH	<0.01	1.25	<0.01	<0.01			
δ-HCH	<0.01	<0.01	<0.01	<0.01			
γ-HCH	<0.01	<0.01	<0.01	<0.01	4.8 (3.9-5.8)	0.09 (<0.01-0.36)	100
p,p'-DDE	<0.01	<0.01	<0.01	0.44	ΣDDT = 3.5 (1.7-5.4)	ΣDDT = 0.82 (0.02-2.3)	DDT and its metabolites 50
p,p'-DDT	0.15	0.54	<0.01	0.26			
p,p'-DDD	<0.01	<0.01	<0.01	<0.01			
Endosulfan I	<0.01	<0.01	<0.01	<0.01	ND	ND	sum of endosulfan I+ endosulfan II+ endosulfan sulfate 100
Endosulfan II	0.16	0.49	<0.01	<0.01	ND	ND	
Endosulfan sulfate	<0.01	<0.01	<0.01	0.35	ND	ND	
Endrin	<0.01	<0.01	<0.01	0.09	ND	ND	10
Aldrin	<0.01	<0.01	<0.01	<0.01	1.3 (0.017-2.3)	0.03 (<0.01-0.36)	sum of aldrin+dieldrin 10
Dieldrin	<0.01	<0.01	<0.01	<0.01	0.75 (0.28-1.4)	1.6 (0.2-3.5)	
Heptachlor	<0.01	<0.01	<0.01	<0.01	0.08 (0.04-0.11)	0.07 (0.02-0.12)	sum of heptachlor + heptachlor epoxide 10
Heptachlor epoxide	<0.01	<0.01	<0.01	<0.01	ND	0.16 (<0.01-0.4)	

ND – not determined

application has been banned. Maybe it was used in formulation of solutions for protection against mosqitous. This practice, unfortunately, is still in use in Serbia. The presence of p,p'-DDE in the wheat bran could be attributed to a previous use of DDT.

The contents of p,p'-DDT in wheat bran and wheat grain were of the same order, although the level of this compound would be expected to be higher in the bran, due to fact that organochlorine compounds deposit on the outer layer of the grain because of precipitation from atmosphere.

It could be easily seen that the levels of OCP residues in wheat grain were below the allowed maximum concentration given by Serbian Legislation [14]. Levels of OCPs in wheat bran are not regulated by Serbian Legislation, but it can be said that their contents in bran were very low in comparison with the official tolerable limits for cereals.

Mukherjee and Gopal [15] obtained in the wheat grain from different parts of Delhi the contents of p,p'-DDE, p,p'-DDT, α-HCH, and β-HCH as 34, 1, 3, and 16 ng/g, respectively. However, Bakore et al. [16] showed that the amount of pesticides detected in wheat flour from Jaipur, India was high, i.e. the average contents of 10 OCPs were 13.83, 6.32, and 9.39 μg/g during summer, winter and rainy seasons, respectively, and the following order was found: heptachlor and heptachlor epoxide>HCHs>DDTs. The explanation of this was that maximum use of heptachlor was being done on the standing crop just before harvest to prevent pest attacks. Contamination of wheat flour with isomers of HCH suggested the practice of mixing HCH in wheat during storage.

Kannan et al. [12] investigated contamination of Indian food (purchased from grocery stores) by organochlorine residues and found the following order in cereals: HCHs> DDTs>aldrin and dieldrin>heptachlor (Table 1). As can be seen, HCHs were the predominantly noticed compounds in the mentioned foods, because HCH was preferentially used for the control of agricultural pests, while DDT levels were apparently high, but mainly due to mosquito control in urban areas.

Concentrations of OCPs in cereal products (rice, wheat flour, corn flour) collected from different metropolitan locations in Australia followed the order of aldrin and dieldrin> DDTs> HCHs> heptachlor and heptachlor epoxide (Table 1) [13]. Although the use of OCPs was banned in Australia, the observed residues were derived from their past use against termites and external parasites.

The total content of OCPs in refined and unrefined sunflower oil from West Bačka accounted for 32% of total sunflower oil production in Serbia ranged from 0.71-0.95 ng/g wet weight.

The concentrations of OCPs decreased for unrefined oils as follows: DDTs>heptachlor>HCHs, but for refined ones: DDTs>HCHs~heptachlor (Table 2). The content of these compounds in the oil samples was the result of their presence in raw material (sunflower seeds), probably due to atmospheric deposition on the crops. Concerning the levels of pesticide residues in unrefined and refined oil, it could be concluded that oil refining generally did not influence the levels of the identified OCPs, only the content of heptachlor was slightly reduced. This conclusion coincides with finding of Smith et al. [19], who showed that neither the solvent extraction nor the bleaching affected the pesticide levels in vegetable oils.

TABLE 2 - The mean contents of OCP residues (ng/g wet weight) in sunflower oil from West Bačka, and literature data for oils, mean value and range (ng/g wet weight).

Compounds	Unrefined oil (this work)	Refined oil (this work)	Oils from India [12]	Oils from Australia [13]	Oils from Serbia [17]	Oils from the Middle East and the Mediterranean countries[18]	Serbian legislation for vegetable oil [14]
α-HCH	0.12	0.13	ΣHCH=220 (6.9-480)	ΣHCH=0.89 (0.44-1.5)	<0.01	<0.1-1.6	sum of α+β+δ HCH 200
β-HCH	<0.01	<0.01			<0.01	<0.1-0.3	
δ-HCH	<0.01	<0.01			<0.01	ND	
γ-HCH	<0.01	<0.01	35 (1.2-100)	0.31 (0.05-0.76)	<0.01-0.29	<0.1-1.9	not defined
p,p'-DDE	0.22	0.23	ΣDDT =21 (1.8-57)	ΣDDT =2.1 (0.36-5.3)	<0.01	<0.1-7.0	DDT and its metabolites 100
p,p'-DDT	0.34	0.23			<0.01	<0.1-4.5	
p,p'-DDD	<0.01	<0.01			<0.01-0.09	<0.1-7.0	
Aldrin	<0.01	<0.01	19 (<0.1-47)	0.15 (<0.1-0.69)	<0.01	ND	not defined
Dieldrin	<0.01	<0.01	24 (<0.1-47)	4.2 (0.86-15)	<0.01	ND	not defined
Heptachlor	0.27	0.12	0.45 (0.08-1.6)	0.20 (<0.01-0.43)	<0.01	ND	not defined
Heptachlor epoxide	<0.01	<0.01	ND	1.6 (0.15-5.0)	<0.01	ND	not defined

ND – not determined

It could be easily seen that the levels of OCP residues in edible sunflower oil were below the allowed maximum concentration given by Serbian Legislation [14].

Vukša et al. [17] investigated contents of OCPs in sunflower oils taken from the market. Levels of lindane and p,p'-DDD in domestic oils were <0.01-0.29 ng/g and <0.01-0.09 ng/g, respectively. Concentrations of lindane and p,p'-DDT in oils from abroad were <0.01-0.26 and <0.01-0.02 ng/g, respectively.

Kannan et al. [12] investigated contamination in the oils (sunflower oil, groundnut oil, gingelly oil, coconut oil, palm oil) from India, and found the following order: HCHs> aldrin and dieldrin>DDTs>heptachlor (Table 2). Concentrations of OCPs in oils (soybean oil, peanut oil, sunflower oil, safflower oil and vegetable oil) collected from different metropolitan locations in Australia followed the order of aldrin and dieldrin>DDTs> heptachlor and heptachlor epoxide>HCHs (Table 2) [13].

Jacobs and Covaci [18] investigated OCPs in olive and vegetable (sunflower and corn) oil from the Middle East and some Mediterranean countries, and detected them in olive and corn oil, but not in sunflower oil samples (Table 2).

OCP contamination in investigated groups of foodstuffs from India and Australia can be rated as follows: oils>cereals. The observed trend is expected due to lipophilicity of OCPs [12, 13]. This trend was not observed in the samples from Vojvodina Province, the levels of OCPs were in the same order of magnitude. It could be explained with the similar effects of atmospheric deposition influencing OCP burden in different crops.

CONCLUSION

The contents of OCP residues in wheat grain, and edible oil taken from different localities of the Vojvodina Province were well below the allowed maximum concentrations given by Serbian Legislation. According to literature data in developing countries like India, consumption of organochlorine is primarily due to their low costs and versatility in action against various pests. Therefore, the contents of ΣHCH and ΣDDT in cereals of India were 9 and 6.5 times higher than the levels of these pesticide residues in wheat of North Bačka (Vojvodina Province). In developed countries (e.g. Australia), using of OCPs is increasingly restricted or banned, and so the level of ΣDDT in cereals was 9 times lower than in wheat from North Bačka, while the content of ΣHCH was of the same order of magnitude. The OCP levels in edible oil were significantly lower in samples of West Bačka than in Indian samples, but in the range of concentrations for the Australian samples.

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OCCURRENCE AND FATE OF POLYCYCLIC AROMATIC HYDROCARBONS IN SEWAGE TREATMENT PLANTS USING MICROWAVE-ASSISTED EXTRACTION FOLLOWED BY LIQUID CHROMATOGRAPHY COUPLED WITH FLUORESCENCE DETECTOR

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SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) are highly persistent compounds in the environment, recalcitrant to biodegradation and highly lipophilic, while some of them cause acute carcinogenic and mutagenic toxicity. Due to the risk of soil contamination whenever sewage sludge is disposed to agricultural land, the investigation of their presence in sewage sludge is a priority need.

This work concerns the occurrence of five PAHs, selected from the priority list of the US Environmental Protection Agency (phenanthrene, fluoranthene, pyrene, benzo[b]flouranthene, and benzo[a]pyrene), in municipal sewage sludge and to assess their fate in a sewage treatment plant. An analytical method based on microwave-assisted extraction (MAE), followed by HPLC-UV coupled with a fluorescent detector (FD) was used for their measurement. The method efficiency was evaluated as to its linearity, repeatability, accuracy and sensitivity.

The contamination levels of the PAHs were determined in sludge from the sewage treatment plants of three main Greek cities: Athens, Patras and Heraklion. PAHs ranged from 0.2 to 4 mg kg^{-1} d.m for each PAH in mixtures of primary and secondary sludge, while they were significantly lower in secondary sludge (less than 0.1 mg kg^{-1} d.m).

KEYWORDS:
Microwave-assisted extraction, polycyclic aromatic hydrocarbons, sewage sludge, waste water, sewage treatment plant.

INTRODUCTION

Polycyclic aromatic compounds (PAHs) are mainly originated from the incomplete combustion, whether natural or anthropogenic [1], of almost any organic material. PAHs are hydrophobic compounds and their persistence in the environment is mainly due to their low aqueous solubility [2]. Some of the PAHs have also been shown to be potentially highly carcinogenic and mutagenic [3], and as a result, have been listed by regulatory agencies such as the U.S. E.P.A. as top priority pollutants.

Sewage sludge has been used for many years as an organic fertilizer in agriculture, thus it is important to study the contamination levels of municipal sewer systems by PAHs. The main sources for PAHs to sewers are municipal and industrial wastes, rainfall and runoff waters and street-sweeping waters that collect atmospheric dry deposition [4].

Usually, the nature and the amounts of organic contaminants in solid samples are determined by an exhaustive extraction into an appropriate solvent and subsequent analysis of the so-obtained extracts. This approach generally involves the application of extraction techniques, such as Soxhlet extraction [5], sonication, [6] microwave assisted extraction (MAE), pressurized liquid extraction (PLE) [5, 7] and supercritical fluid extraction (SFE). The need for extraction techniques that are low-cost and less solvent- and time-consuming, has generated significant research effort for the development of new techniques.

MAE has several advantages [8]. One important advantage is that small volumes of solvents are needed for the extraction. Minimization of the required extraction time is another advantage of MAE; as microwave energy heats the samples in a closed pressurized extraction vessel, the

temperature can be rapidly increased, allowing the extraction of samples in minutes, in contrast with traditional methods, for which hours are needed.

MAE has been used mainly for the determination of chloroacetanilide herbicides and pesticides in soils and plant matrices, whereas there are only a few articles reporting the use of MAE for the determination of surfactants in dried sewage sludge, soil and sediment [9-12]. To our knowledge, there are only two publications so far, referring to the use of MAE in the determination of PAHs in sludge [13, 14].

The aim of this work was to investigate the occurrence of five PAHs selected from the priority list of the US E.P.A. in municipal sewage sludge and their fate in a sewage treatment plant, by applying an analytical method based on MAE followed by HPLC-UV coupled with a fluorescent detector.

MATERIALS AND METHODS

Reagents

Individual analytical standards of five PAHs: phenanthrene, fluoranthene, pyrene, benzo[b]flouranthene, and benzo[a]pyrene were obtained from Sigma-Aldrich. The standards were of high purity (> 99 %).

All organic solvents were of analytical grade or HPLC grade from Merck. Water was prepared on Milli-Q purification system (Millipore).

Sample Preparation

10 ml of the sample were filtered. The aquatic phase was filtered again and the extract was loaded to a C18 SPE-cartridge which was pre-conditioned with 1 ml methanol and 1 ml water. The PAHs were removed from the cartridge by passing 2 ml of acetonitrile. 20 μl of that solution were used for the analysis to the HPLC-FD. The solid phase was extracted by microwave assisted extraction.

Microwave- Assisted Solvent Extraction

The filter was transferred to the extraction vessel with 20 ml of acetone/hexane: 1/1. The extraction was at 100 ºC and the extraction energy was set at 600 W for 15 min.

After the extraction, the vessels were allowed to cool to room temperature before being opened. The extract was evaporated to dryness using a rotary vacuum evaporator at 30 ºC and redissolved in 10 ml of acetonitrile. After filtration, 20 μl were used for the analysis in the HPLC-UV-FD without any further clean up.

Instrumentation

The microwave–assisted extraction was carried out using a Microwave Accelerated reaction System for Extraction; model MARS 5 from CEM (North Carolina, USA). This model is able to extract 6 samples simultaneously in PTFE-lined extraction vessels under the same conditions.

The HPLC system consisted of a Star 9010/9001 Solvent Delivery System from Varian. The chromatographic separation was done using a reversed-phase XTerra® RP-18 analytical column of 250 x 4.6 mm and 5 μm particle diameter preceded by a guard column (20 x 3.9 mm) of the same packing material, both from Waters. Detection was carried out using a Star 9050 Variable wavelength UV-VIS detector and a ProStar 363 Fluorescence detector both from Varian. The mobile phase consisted of 60% acetonitrile and 40% water. The analysis was run isocratically with a flow rate of 0.7 ml min^{-1}. The detection on the fluorescent detector was carried out at at λ_{ex}: 250 nm and at λ_{em}: 370 nm from 0 to 22 min and λ_{ex}: 234 nm and at λ_{em}: 420 nm from 22 to 58 min.

Environmental Samples

The sludge samples used for the development of the method were taken from the sewage treatment plant of the city of Patras, Greece. The concentrated secondary sludge that was used for the development of the method was completely characterised. Some of the most important characteristics were: Total Suspended Solids: 33,5 g L^{-1}, Volatile Suspended Solids: 25,6 g L^{-1}, dissolved-COD: 5,21 mg L^{-1} and Total-COD: 36,2 mg L^{-1}. Environmental samples (sludges and mixed liquor) were collected from the sewage treatment plants of three main cites of Greece: Athens, Patras and Heraklion. The STP of Athens (4.000.000 inhabitants) is a new biological treatment plant treating 750.000 m^3 of sewage daily from the greater Athens area and has a nominal daily capacity of 1.000.000 m^3. The STP in Patras, Western Greece District, serves a population of approximately 180.000. The annual treated volume is 5.1 Mm^3 with an average influent BOD_5 of about 300 mg O_2 L^{-1}. Finally, the STP of Heraklion, Crete, Southern Greece District, is a secondary treatment plant (including nitrogen removal) with chlorine disinfection of the effluent and anaerobic digestion of the sludge for the production of biogas. The STP of Heraklion has a daily capacity of 28.000 m^3 and treats mainly domestic discharges from the city of Heraklion (150.000 inhabitants).

The samples were conserved by immediate addition of 1 % of formaldehyde and, when not immediately analysed, were stored in the dark at 4 ºC.

RESULTS AND DISCUSSION

Method Validation

The performance of the method was evaluated through the estimation of the linearity, repeatability and sensitivity, according to Standard Methods [15].

For quantification, six-point calibration curves were constructed using least-square linear regression, from the HPLC-FD analysis of standard solutions of a mixture of the selected PAHs in acetonitrile, at concentrations ranging from 0.01 μg ml^{-1} to 0.50 μg ml^{-1}. The calibration curves

were linear with correlation coefficients (R^2) higher than 0.999 for all target compounds (Table 1).

The recoveries of the proposed method were relatively high for all five PAHs. The overall method repeatability, calculated as the relative standard deviation (RSD) of the replicate (n=7) analysis of secondary sludge spiked with a standard mixture of the analytes at 2.99 μg g^{-1} d.m, was satisfactory, with RSD values from 2.95 to 8.53 % (Table 1).

The limits of detection (LOD) and quantification (LOQ) of the method were experimentally estimated from the analysis of secondary sludge at the minimum concentration of each analyte, giving a signal to noise ratio of 3 and 8, respectively. As shown in Table 1, the LODs obtained were 0.27 μg g^{-1} d.w., 0.21 μg g^{-1} d.w., 0.17 μg g^{-1} d.w., 0.57 μg g^{-1} d.w. and 0.38 μg g^{-1} d.w., respectively, using an injection volume of 20 μl. In case a bigger loop (during this study we could inject up to 100 μl) was used, these concentrations could be much lower. The LOQs, which were approximately 2.7 times higher than the corresponding LODs, were determined 0.73 μg g^{-1} d.w., 0.57 μg g^{-1} d.w., 0.46 μg g^{-1} d.w., 1.54 μg g^{-1} d.w., and 1.03 μg g^{-1} d.w., respectively.

Environmental Samples

The analytical method developed was used to monitor the presence of PAHs in sludge samples collected from different sewage treatment plants and to evaluate the fate of these compounds at different stages in a particular sewage treatment plant, the one of the city of Patras, in order to evaluate the removal efficiency of the treatment applied.

Table 2 lists the concentrations of the selected PAHs obtained in this study at the different sewage treatment plants and the characteristics of each of the STPs. The highest concentrations were determined in the secondary sludge from the STP of Athens and the lowest in the secondary sludge at the STP of Patras during the second sampling period.

Comparison with data from literature on domestic sewage sludge indicated higher concentrations determined in Athens, than those determined in Catalonia (Spain) and in Porto (Portugal) with fluoranthrene ranging from 0.055 to 0.7 mg kg^{-1} [16]. In general, the monitoring data of PAHs found in the literature were between 0.1 and 100 mg kg^{-1}. In a study of 25 STPs in Germany, concentrations of the sum of PAHs ranged between 2 and 80 mg kg^{-1} [17], and in a later study in 12 German STPs from 1995 to 1998, the sum of PAHs was determined between 2-15 mg kg^{-1} [16]. In the present work the sum of the 3 compounds was determined (Σ 3: fluoranthrene, benzo(b)fluoranthene and benzo(a)pyrene) (Decision No. 2455/2001/CE, 2001, Official Journal L331) and the results are comparable with the concentrations reported in the articles mentioned above. Individually the concentrations of PAHs are far below the

TABLE 1 - Linearity of the Calibration curves (R^2), % recoveries, repeatability as relative standard deviations (RSD), and limits of detection (LOD) and quantification (LOQ) characterizing the MAE-HPLC-FD analysis of the target compounds in sludge.

	R^2	Recovery (%)	RSD (%)	LOD (μg g^{-1} d.m)	LOQ (μg g^{-1} d.m)
Phenanthrene	1	80.27	4.16	0.27	0.73
Fluoranthene	1	76.45	2.99	0.21	0.57
Pyrene	1	76.15	2.95	0.17	0.46
Benzo(b)fluoranthene	0.999	82.03	8.53	0.57	1.54
Benzo(a)pyrene	1	80.38	5.98	0.38	1.03

TABLE 2 - Characterization of sludges from different STPs and the levels of the PAHs determined on these samples.

	Athens (07/2004)	Heraklion (07/2004)	Patras (06/2003)	Patras (07/2004)
Type of sludge	Concentrated primary	primary	Concentrated secondary	Concentrated secondary
TS (g L^{-1})	55.80	29.98	44.33	46.55
VS (g L^{-1})	34.38	19.46	30.12	31
d-COD (mg L^{-1})	875.28	1290	980	1100
pH	6.32	6.68	6.8	6.83
PAHs				
Phenanthrene (mg kg^{-1})	4.01	0.86	1.04	0.10
Fluoranthene (mg kg^{-1})	2.06	0.63	0.99	0.10
Pyrene (mg kg^{-1})	1.96	1.51	0.50	0.05
Benzo(b)fluoranthene (mg kg^{-1})	1.92	2.40	n.a.	n.d.
Benzo(a)pyrene (mg kg^{-1})	0.21	0.22	n.a.	n.d.
Σ 3	4.18	3.25	0.99	0.10

(n.a.: not analysed, n.d.: not detected)

TABLE 3 - Weekly variation of PAHs in the influent of STP of Patras.

Influent of STP	Tuesday	Wednesday	Thursday
Phenanthrene (μg L^{-1})	3.19	3.34	2.10
Fluoranthene (μg L^{-1})	<	<	0.43
Pyrene (μg L^{-1})	<	<	0.44
Benzo(b)fluoranthene (μg L^{-1})	<	<	0.46
Benzo(a)pyrene (μg L^{-1})	<	<	0.47

(<: below detection limit)

TABLE 4 - The effect of the treatment on the presence of the PAHs, in the STP of Patras.

	Phenanthrene	Fluoranthene	Pyrene	Benzo(b) fluoranthene	Benzo(a) pyrene
Influent of STP (μg L^{-1})	2.87	0.14	0.14	<	<
Activated sludge (mg kg^{-1} d.m)	0.86	<	<	<	<
Concentrated activated sludge (mg kg^{-1} d.m)	0.104	0.099	0.050	<	<
Dehydrated sludge (mg kg^{-1} d.m)	<	<	<	<	<
Effluent of STP (μg L^{-1})	0.08	<	<	<	<

(<: below detection limit)

CEE standards (Directive 440, CEE 06/16/1975): 5 mg kg^{-1} for fluoranthrene, 2.5 mg kg^{-1} for benzo(b)fluoranthene and 2 mg kg^{-1} for benzo(a)pyrene [18], except for the city of Heraklion (2.4 mg kg^{-1}) which is close to the CEE standard.

The weekly variation of PAH concentrations was also monitored. Samples of the Patras STP influent were collected on three consecutive days (21, 22, 23 September 2004). Table 3 shows PAH concentrations for three days. The PAH concentration ranges in the influent were found to be: 2.1 μg L^{-1} to 3.34 μg L^{-1} for phenanthrene; 0 μg L^{-1} to 0.43 μg L^{-1} for fluoranthene; 0 μg L^{-1} to 0.44 μg L^{-1} for pyrene; 0 μg L^{-1} to 0.46 μg L^{-1} for benzo(b)fluoranthene; and 0 μg L^{-1} to 0.47 μg L^{-1} for benzo(a)pyrene. Blanchard et al. [19] found in wastewaters influents to the Paris treatment plant (Seine Aval STP), concentration values for fluoranthene, benzo(b)fluoranthene and benzo(a)pyrene ranging respectively from $0.2x10^{-3}$ μg L^{-1} to 0.40 μg L^{-1}, $2x10^{-2}$ μg L^{-1} to 0.10 μg L^{-1} and from $3x10^{-3}$ μg L^{-1} to 0.06 μg L^{-1}, values which are similar with our results.

In the sequel, the effect of the treatment on the presence of the PAHs in the Patras treatment plant was assessed (Table 4). Wastewaters from the influent of STP and the plant outlet were sampled. Sludge from the aeration basin, the secondary clarifier and dehydrated sludge were collected. A significant reduction of PAH concentrations in the effluent of the STP was observed. Evaporation from the different basins in relation with the high PAH vapour pressure and aerobic biodegradation processes [20] were believed to be the main reasons for the observed PAH concentration reduction. Similar reduction of the concentration of the PAHs have been reported elsewhere, [21] although the concentrations in both influent and effluent in our study were much higher.

Finally, daily PAH variation in the influent of STP of Patras is shown on Figure 1. It appears that there was no significant difference in the influent values. Although in the early morning (7:00 a.m) PAH concentrations were the lowest.

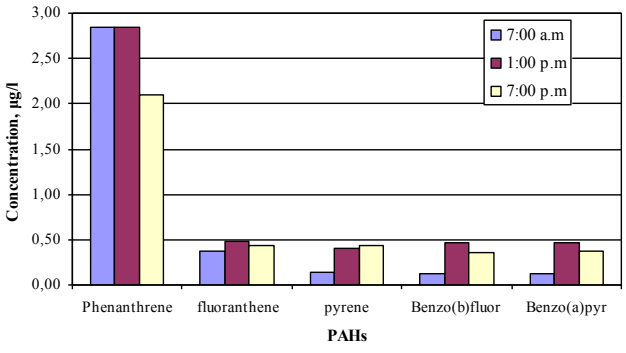

FIGURE 1
Daily variation of PAHs in the influent of STP of Patras.

Manoli and Samara [22] showed that in the raw sewage of the Thessaloniki treatment plant (Greece), only the season had a significant impact on the variability of PAHs (highest concentrations found during winter).

CONCLUSIONS

MAE extraction was developed for the determination of PAHs in sewage sludge. The developed method provides good performance in terms of precision, linearity, LODs and LOQs. The method was applied to real sewage sludge from different STPs in Greece. It was also used to study the fate of PAHs during the wastewater treatment in the STP of Patras. Daily variations were observed for PAHs with lower levels in the morning with the exception of phenanthrene. In the plant, a significant reduction in the amount of PAHs was observed both in the STP water effluent and the dehydrated sludge.

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UPTAKE OF TRACE METALS BY *Lycopersicum esculendum L.* AT A SITE ADJACENT TO THE MAIN ROAD ATHENS-THESSALONIKI, GREECE

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SUMMARY

This site-specific study tests the hypothesis that major roads may have an adverse impact on the (trace) elemental composition of *Lycopersicum esculentum* L. Surface and subsurface soil samples were also collected. All the samples were analysed for the elements lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), cobalt (Co), aluminium (Al), chromium (Cr) and manganese (Mn) using a digestion procedure followed by Atomic Absorption Spectrophotometry (AAS). Leaves and roots of *Lycopersicum esculentum* L. were collected upto distances of 0, 5, 10, 50 and 100 m from the major road. The highest concentrations of Al, Pb, Zn, Cu, Mn, Cr, and Co were found in the soils adjacent to the Lamias road, with regard to those collected further away from the road. In addition, the highest concentrations of metals were measured in both the road-soil interface and the sub-surface soils. The influence of the traffic on the soils was identified in both the leaves and roots of *Lycopersicum esculentum* L.

KEYWORDS:
Lycopersicum esculentum L., road pollution, metal ion, soil.

INTRODUCTION

Several studies have investigated the impact of heavy traffic on metal-ion (element) levels in road dust and adjacent soils, but there appears to be very little information on the metal-ion content of crops for human consumption grown near major roads.

Many studies throughout the world have linked the sources of contaminants in street-dust to traffic, and have recognized street dust as a significant pollution source itself [1].

Heavy metals in deposited street dusts have been studied in large and small urban areas in the United Kingdom: Birmingham and Coventry, West Midlands [2]. The levels of Cd, Cu, Fe, Mn, Pb and Zn in samples of soils from roads within North-West London were also measured [3]. The impact of a major rural highway in the United Kingdom on soil contamination has been examined by [4].

Dust samples from various roads situated in the Globetown Neighborhood of East London were studied [5]. Furthermore, heavy metal contents in the soils from more than sixty parks and public amenity areas in old urban districts, industrial areas and New Town of the territory in Hong Kong were determined [6]. The distributions of heavy metals in street dusts and soils of an industrial city in Northern Spain and in soils of Palermo city, Italy were also in-vestigated [7, 8].

The availability of several metals in urban soils of Seville suggesting that pollution with Cu, Pb and Zn could occur at some sampling sites was examined by Madrid et al. [9].

The fluxes of four metals (Cd, Cu, Pb and Zn) emitted from a major rural highway in West France have been evaluated, including wet and dry deposition into the roadside soils [10]. The data show that over 8.3% of lead and 2.7% of Cu were deposited in the local road environments. The budget calculation was not possible for cadmium and rather satisfactory for zinc: it seems that a large proportion of Pb may disperse in the atmosphere, whereas Cd sources may be ill-identified and probably overestimated.

Pb, Zn and Cd concentrations were evaluated in liquid and solid depositions, vegetation and in soil by [11]. Each site was equipped with 640-m transects centered on the motorway with 5 measuring points on each side of the road. By reason of Zn, middle rates (higher traffic) and roads equipment, the limit value is exceeded for Zn by upto 80 meters.

The concentrations of Pb and Cd in two plant species *Pittosporum sinensis* and *Nerium oleander* grown in soils at two major parks in Athens, Greece, were studied [12]. The concentrations of both metals were found to be considerably higher at the peripheral zones, and decreased towards the interior of the parks.

Several elements (Cd, Co, Cu, Mn, Ni, Pb, and Zn) in twelve different kinds of vegetables (tomatoes, eggplant, carrots, parsley, cucumber, marrow, potatoes, green pepper, lettuce, spinach, salad onions, leek, watercress and cabbages) from Saudi Arabia were investigated [13]. The results indicate that metal-ion concentration in each vegetable is dependent upon the selective uptake of the metal by the plant. The study reveals that the salad onions and watercress exhibited higher metal-ion concentrations than the other vegetables, and the metal-ion concentrations of the vegetables were within safety baseline levels for human consumption.

The levels of total Cr in *Lycopersicum esculentum* were determined to range between 0.008 – 0.026 μg/g (fresh weight of the edible fraction) [14]. These levels are similar to those obtained by [15, 16]. Cr levels tend to be higher in roots and tubers than in bulbs, whereas leaves and tender stalks have the lowest levels [17]. The excessive usage of fertilizers or the utilization of sewage as watering water are factors, which increase considerably the content of Cr in the vegetable products, but also industrial contaminations were found [18].

The concentrations of heavy metals of *Zea mays* cultivated nearest to the major road in Araxos area, Greece, in the different depths of soils as well as leaves and roots were studied [19]. Also two vegetable crops, *Lycopersicum esculentum* and *Solanum melongena,* cultivated in two different areas (Araxos and Lappas, Greece) nearest the edge of the major road traffic Patras-Pyrgos, Greece were studied, analyzing their soils, leaves and roots trace element levels [20].

Description of the study area

The area under investigation is a major agricultural area in Lamias district adjacent to a major road, which carries a significant volume of traffic. The road connects the capital of Greece, Athens, with the second-most populated city in Greece, Thessaloniki. The study area is roughly midway between Athens and Thessaloniki: an area of 325 ha is cultivated with vegetables, such as *Lycopersicum esculentum* tomato, and *Solanum melongena* during the summer period.

The total cultivated land area around Lamias amounts to 13244 ha, and is cropped with *Criticum aestivum* (3483 ha), *Zea mays* (150 ha), *Oryza sativa* (710 ha), *Ospria* (17 ha), *G. hirsutum* (4860 ha), and *Tabacum officinales* (369 ha). Further cultivation in the district includes areas of grassland (160 ha), other vegetables (325 ha), *Olea europaea* (common name) (2510 ha), other trees (370 ha), *Vitis vinifera* (20 ha) and *grasses* (300 ha).

It is noted that most producers have cultivated vegetables at almost the edge of the major road for a long time. Figure 1 shows the total number of vehicles travelling along the major road during 2002.

MATERIALS AND METHODS

Collection of field samples

One transect, at right angles away from the road, was selected for this initial study. Samples of leaves and roots were collected at 0, 5, 10, 20, 50 and 100 m distances from the road. Two soil samples were collected at each location at depths of 0-5 and 5-15 cm.

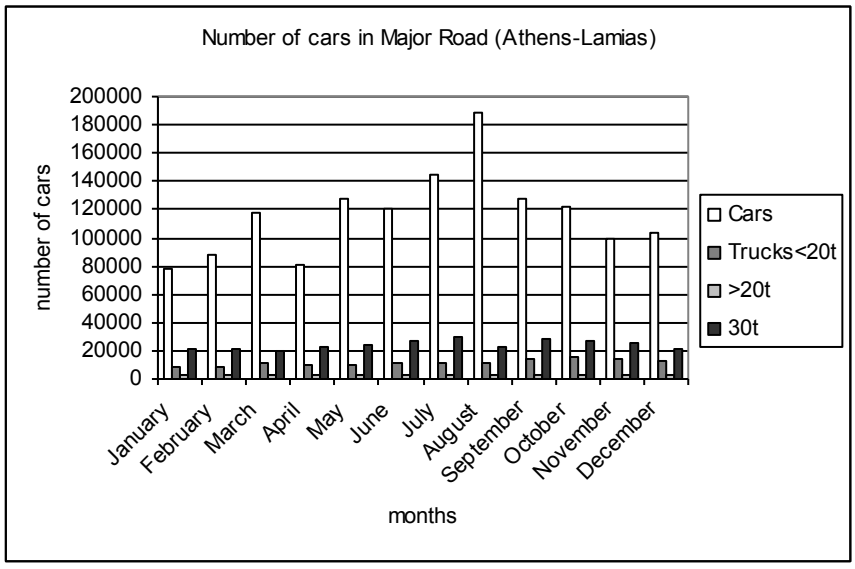

FIGURE 1 - The number of cars on Lamias Major Road in 2002.

Preparation of Vegetation Extracts for the Analysis of Total Metal Concentrations

The following elements were analyzed for the following reasons:

- Pb – previously an additive in petrol
- Cd and Zn – can be derived from tyres
- Cu – from brake linings and wiring
- Cr – from plating
- Al – as a proxy for grain size
- Mn – present in road dust from lorries carrying carbonates

Homogenised vegetation samples of known mass were dried at 105 ^{o}C to constant weight in an oven, and the exact mass (approx. around 1g) recorded. A 5.0 cm^3 aliquot of concentrated nitric acid (Analar) was added to the digestion vessel, and allowed to stand in a fume cupboard for 1 h to initiate gentle digestion. The resulting extracts were heated at 100 ^{o}C for 2 h. An aliquot of conc. perchloric acid (1.0 ml for leaves, 2.0 ml for roots) was added, when the extracts were cooled. Then, the extracts were allowed to digest at 100 ^{o}C for a further 6 h, until a transparent yellow-clear solution was obtained. The extracts were filtered, made up to 50 ml with deionised water, and analysed for total metals using a 'Varian SpectrAA-100' atomic absorption spectrophotometer. The accuracy was checked by analysing reference material, and the precision by analysing duplicates.

Preparation of Soil Extracts for the Analysis of Exchangeable Metal Concentrations

Each homogenised soil sample was ground using a pestle and mortar, and sieved through a125-µm sieve [21]. A known mass of the sieved soil was dried to constant weight in an oven at 105 ^{o}C. A 50-ml aliquot of sodium acetate solution (1 mol L^{-1}) was added to a 5.0 g sample of the dried soil and shaken at 500 oscillations per min at room temperature for 12 h. The resulting mixture was centrifuged at 2,500 rpm for 30 min, the supernatant was collected and analysed for exchangeable metals using the 'Varian SpectrAA-100' atomic absorption spectrophotometer.

Preparation of Soil Samples for pH Analysis

A 25-ml volume of deionised water was added to 10 g of sieved soil, and allowed to equilibrate for 24 h. Measurements of pH were conducted using a standard soil: distilled water ratio of 1:2.5 (w/w) for 24 h at room temperature, and a glass electrode-equipped pH-meter [22].

RESULTS AND DISCUSSION

Table 1 shows the exchangeable metal ion concentrations and the pH values of the soils of *Lycopersicum esculentum* L. within the study area. Tables 2 and 3 show the total metal ion concentrations of the leaves and roots of *Lycopersicum esculentum* L. respectively. Certain specific results are presented graphically, where patterns have been identified. All analyses were performed against blank materials used in the preparations.

TABLE 1
Exchangeable metal ion concentrations (mg/ Kg) of soil samples of *Lycopersicum esculentum* L. taken at various distances from the roadside and at different depths, adjacent to the Lamias road.

Concentrations of heavy metals	Distance from the major road side (m)	Soil sampling depth (cm)		P = 0.05
		0-5	5-15	
Al	0	0.8	1.5	0.361 ns
	5	3.9	5.6	
	10	9.5	8.7	
	20	9.6	10.0	
	50	11.1	10.3	
	100	15.2	12.7	
Pb	0	1.5	1	-1.000ns
	5	0.9	1.3	
	10	0.6	0.9	
	20	0.8	0.9	
	50	0.4	0.6	
	100	0.6	0.9	
Cd	0	0.1	0.1	-0.696ns
	5	0.07	0.09	
	10	0.08	0.08	
	20	0.09	0.07	
	50	0.06	0.06	
	100	0.06	0.09	
Zn	0	0.2	0.2	1.000ns
	5	0.2	0.2	
	10	0.1	0.1	
	20	0.1	0.09	
	50	0.1	0.1	
	100	0.09	0.09	
Cu	0	0.1	0.1	0.889ns
	5	0.09	0.1	
	10	0.09	0.08	
	20	0.07	0.08	
	50	0.1	0.08	
	100	0.1	0.08	
Mn	0	7.1	5.1	2.722*
	5	4.5	5.5	
	10	3.4	0.7	
	20	2.3	1.3	
	50	2.9	0.8	
	100	2.6	0.5	
Cr	0	0.1	0.1	0.791ns
	5	0.1	0.1	
	10	0.3	0.3	
	20	0.6	0.7	
	50	0.7	0.6	
	100	0.8	0.6	
Co	0	0.3	0.3	0.000ns
	5	0.1	0.3	
	10	0.1	0.1	
	20	0.1	0.1	
	50	0.2	0.1	
	100	0.2	0.1	
pH	0	8.05	8.18	0.180ns
	5	8.07	7.87	
	10	8.08	8.02	
	20	7.95	7.82	
	50	8.1	8	
	100	7.89	8.17	

*= $P<0.05$
ns = non significant

TABLE 2- Regression analysis between soil heavy metal concentration (mg/ Kg) and the distance (d) of the sampling point (m) from the highway located in direction perpendicular to the road.

Regression type	Regression equation	R^2	Significance of regression F
Cubic	$Pb= 0.21-1.88\times10^{-6}d^3 +0.00046d -0.0325d$	0.598*	3.981*
Cubic	$Mn=6.15-5.488\times10^{-5} d^3+0.009d\ 2 -0.398d$	0.716*	6.743*
Cubic	$Cu=0.1-3.92\times10^{-7}d^3+6.14\times10^{-5} d^2 - 0.023d$	0.547*	3.220*
Cubic	$Cd=0.093+1.2\times10^{-5}d^2-1.85*10^{-8} d^3-0.00121d$	0.541*	3.155*
Cubic	$Cr=0.01+4.45\times10^{-6} d^3 -0.00079d^2+0.02d$	0.895*	22.893*
Cubic	$Co=0.28-3.07d^3+0.0004d^2-0.019d$	0.594*	3.9*
Cubic	$Al=1.56-0.016d^2+0.0001 d^3+ 0.758d$	0.939*	41.712*
Cubic	$Zn=0.211-1.4\times10^{-6}d^3 +0.00023 d^2 -0.01d$	0.839*	13.939*

* P = 0.05

TABLE 3 - Total metal ion concentrations (mg/ Kg) in the leaves of *Lycopersicum esculentum* L., adjacent to the Lamias road.

Lycopersicum esculentum	Distances from Road (m)	Al	Pb	Cd	Zn	Cu	Mn	Cr	Co
Leaves	0	143.2	0.9	1	103.5	8	117.5	2	2
Leaves	5	90	0.4	0.3	40.2	12.8	72.8	0.7	1.2
Leaves	10	61	0.6	0.6	22.5	12.3	87.4	0.5	1.3
Leaves	20	92	0.6	0.3	62.9	10	85	0.1	1.8
Leaves	50	87.1	0.9	0.5	42.8	11.6	75.6	1.5	2.2
Leaves	100	74.3	0.7	0.5	50.1	11.3	74.8	0.8	2.0

TABLE 4 - Total metal ion concentrations (mg/ Kg) in the roots of *Lycopersicum esculentum* L., adjacent to the Lamias road.

Lycopersicum esculentum	Distances from Road (m)	Al	Pb	Cd	Zn	Cu	Mn	Cr	Co
Roots	0	1741.9	0.6	0.8	31	14	159.4	131.3	13.5
Roots	5	545.2	0.8	0.8	30.7	11.9	77.4	10.1	5.3
Roots	10	1077.6	1.1	0.4	21	10.8	149.6	98	11.4
Roots	20	732.2	<0.1	0.6	22.5	10.4	101	27.3	7.5
Roots	50	595.2	0.6	0.9	28.5	18.7	105.6	19.9	11.4
Roots	100	403.3	0.9	0.2	30.7	10.5	56.6	10.4	4.4

In the following discussion, it should be noted that the levels of elements measured in the soil are *exchangeable* (extractable) amounts, whilst those analyzed in the roots and leaves are *total* amounts.

The following observations have been made when considering the whole data set:

The concentrations of *exchangeable* metals in the soils are very low compared with those of *total* metals in the roots or leaves.

Cadmium is present at <0.1 mg/Kg in the soil, and <1.0 mg/Kg in the roots and leaves. Chromium is present at <1.0 mg/ Kg in the soil and <2.0 mg/Kg in the leaves, whilst it is present between 10-131 mg/Kg in the roots, elucidating a clear distinction between the metal concentrations in leaves and roots. The amount of lead in all samples is generally <1.5 mg/Kg. The concentration of copper in the soil is typically <1 mg/Kg, and it would appear that there is more copper in the roadside samples compared with the field samples.

The copper concentration measured in the vegetation ranged from 10-20 mg/Kg, not demonstrating this clear distinction between the amounts present in roots or leaves. The concentration of zinc measured in roadside and field soil samples is 0.2 mg/Kg or less, whilst the zinc in vegetation ranged from 22-103 mg/Kg, without clear distinction between the concentrations in roots compared to that in leaves. The amount of cobalt in the soil was <0.4 mg/Kg, whilst that in vegetation ranged from 1-14 mg/Kg, a slightly higher level being observed in roots compared to that in leaves. Aluminium is present in the soil at concentrations ranging from 0.8 to 22.9 mg/Kg, with a slightly higher amount being observed in the roadside samples. The concentration of aluminium in the vegetation ranged between 61-1742 mg/Kg, evidencing a marked difference between the concentrations in leaves and roots, the latter being significantly higher.

The amount of manganese in soil is typically less than 10 mg/Kg, whilst that in the vegetation varied from 56-160 mg/Kg, with values being slightly higher for roots.

The pH of the soils is approximately 8, the roadside soils being slightly more acidic.

Figure 2 shows that the highest concentration of *exchangeable* lead was measured at 0 m from the major road, and then tends to decrease with increasing distance from it. There also appears to be a distinction between the levels observed in the 0-5 cm and the 5-15 cm depth fractions, the

highest levels of lead being observed in the 5-15 cm one, except at 0 m, where the pattern is reversed.

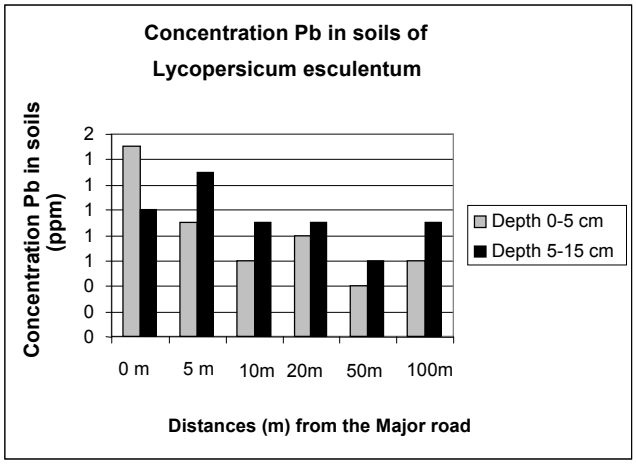

FIGURE 2 - The *exchangeable* concentration of lead (mg/Kg) in soils of *Lycopersicum esculentum* L. at increasing distances from the major road and depths of 0-5 cm and 5-15 cm.

Figure 3 shows the *exchangeable* concentrations of aluminium in the soils. In contrast to the results observed for lead, the highest concentrations of aluminium were measured with increasing distance from the road, the highest being observed at 100 m from the major road. Similar concentrations of aluminium were measured in both depth fractions (0-5 cm and 5-15 cm).

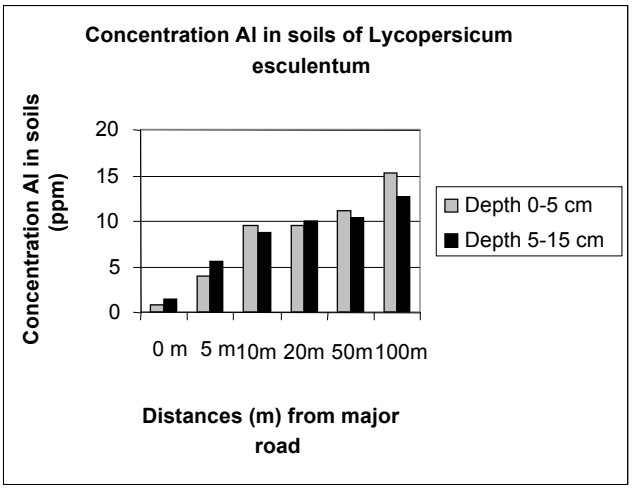

FIGURE 3 - The *exchangeable* concentration of aluminium (mg/Kg) in soils of *Lycopersicum esculentum* L. at increasing distances from the major road and at depths of 0-5 cm and 5-15 cm.

Figure 4 shows the *exchangeable* manganese concentrations in the soils. Like lead, the highest concentrations are observed closest to the major road, at 0 and 5 m, and were lowest further away. There also appears to be a significantly higher level of manganese in the 0-5 cm depth fraction, compared with the 5-15 cm one (except for at 5 m from the road).

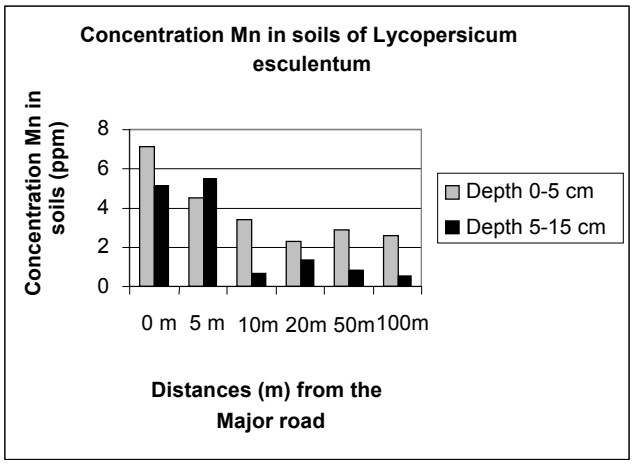

FIGURE 4 - The *exchangeable* concentration of manganese (mg/Kg) in soils of *Lycopersicum esculentum* L. at increasing distances from the major road and at depths of 0-5 cm and 5-15 cm.

The results for exchangeable copper and cadmium are not presented graphically, as very low levels were observed for these metals.

Figure 5 shows the *total* concentrations of lead, cobalt and chromium in the leaves of *Lycopersicum esculentum* at distances of 0, 5, 10, 20, 50 and 100 m from the major road. It was noted that the highest levels of *total* chromium were measured at distances of 0 m, 50 m and 100 m from the road. Higher concentrations of chromium in soil are also observed at 50 m and 100 m from the major road. The highest concentrations of lead and cobalt are again observed at 0 m, 50 m and 100 m from this road. These results in comparison to *total* chromium do not appear to correlate with the levels observed in soil.

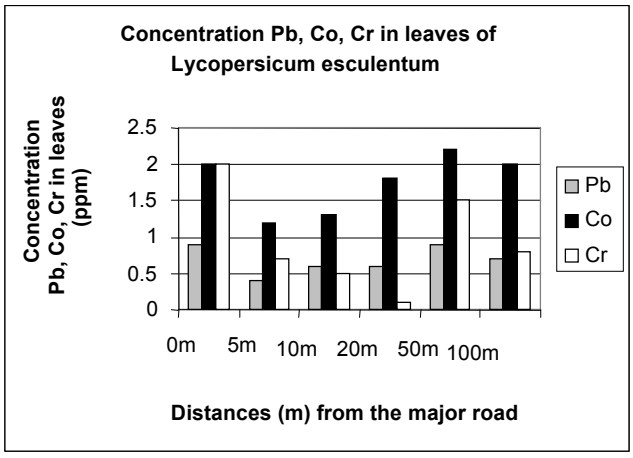

FIGURE 5 - The *total* concentrations of lead, cobalt and chromium (mg/Kg) in the leaves of *Lycopersicum esculentum* L. at increasing distances from the major road.

Figure 6 shows the *total* concentrations of copper, zinc, aluminium and manganese in the leaves of *Lycopersicum*

esculentum at varying distances from the major road. It would appear that the *total* copper levels do not change significantly with increasing distance from the major road, compared with that of zinc, aluminium and manganese, which changed significantly with distance from the road. The highest levels of these three metals were observed in the leaves at 0 m from the road, and typically decreased with increasing distance from the road to about 20 m, where the concentrations tend to stabilize.

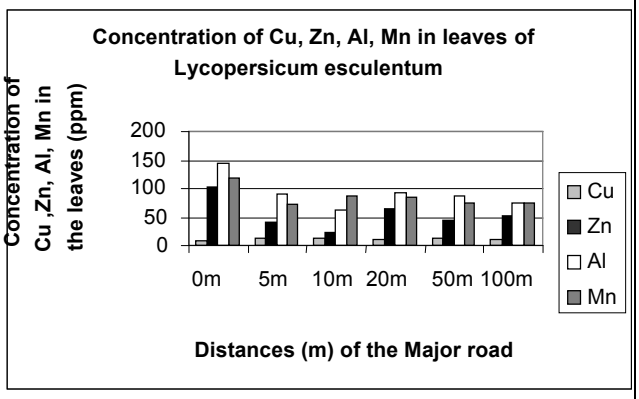

FIGURE 6- The *total* concentrations of copper, zinc, aluminium and manganese (mg/Kg) in the leaves of *Lycopersicum esculentum* L. at increasing distances from the major road.

Figure 7 shows that the *total* concentrations of aluminium and manganese in the roots are highest at 0 m from the road, and then typically decreased with increasing distance.

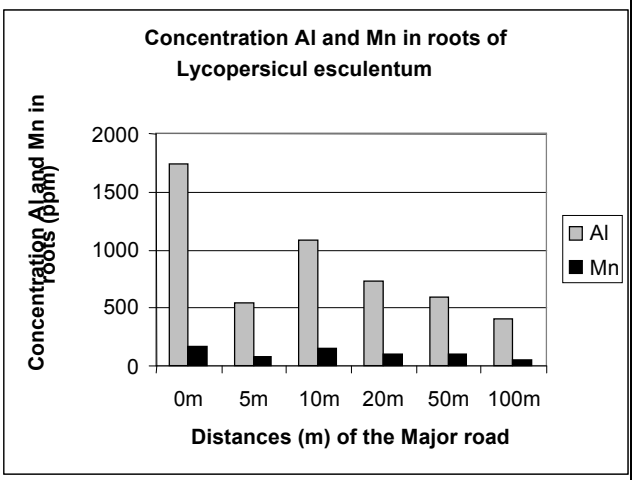

FIGURE 7 - The *total* concentrations of aluminium and manganese (mg/Kg) in the roots of *Lycopersicum esculentum* L. at increasing distances from the major road.

Table 5 expresses the total concentration of each metal in the roots as a ratio of that in the leaves at distances of 0 and 100 m from the major road. We noted that for aluminium, chromium and cobalt, where, at 0 m, more metal was observed in the roots compared to the leaves, there was also found more metal content in roots to the leaves at 100 m. For cadmium and zinc, found in lower amounts in roots than leaves at 0 m, there are again lower levels in roots at 100 m. Contrary, for lead more was observed in the roots with regard to the leaves at 100 m, when compared with the levels at 0 m.

TABLE 5 - Total concentrations of metals in the roots as a ratio of the metal's concentration in the leaves at distances of 0 m and 100 m from the major road.

Metal Ion	Distance from Major Road (m)	Ratio of Total Metals in Roots : Total Metals in Leaves
Al	0	12.2
	100	5.4
Pb	0	0.7
	100	1.3
Cd	0	0.8
	100	0.4
Zn	0	0.3
	100	0.6
Cu	0	1.8
	100	0.9
Mn	0	1.4
	100	0.8
Cr	0	66
	100	13
Co	0	6.8
	100	2.2

CONCLUSIONS

The results of this initial study suggest that there may be an influence of vehicle emissions on *Lycopersicum esculentum* grown adjacent to major roads. Higher concentrations of Al, Pb, Zn, Cu, Mn, Cr, and Co were found in the soils adjacent to the Lamias road, with respect to those collected further away from this road. In addition, higher concentrations of metals were measured in both the road-soil interface and the sub-sur-face soils. The influence of the traffic on the soils could also be identified in the leaves and roots of *Lycopersicum esculentum* L. The influence of metal pollution is more pro-nounced in the case of the roots than in leaves.

Ratios of metal ion concentrations in *Lycopersicum esculentum* L. to those in soils suggested that there may be greater availability of metals at roadside locations.

These findings highlight the need for further extensive sampling, perhaps to include similar study areas at other locations, further appraisals of the most appropriate analytical methodology for investigation, and further research concenrning contamination of other vegetables by roadside emissions.

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RELATIONSHIP BETWEEN HEAVY METALS RESISTANCE AND EXOPOLYSACCHARIDE (EPS) SYNTHESIS OF *PSEUDOMONAS* SPP. STRAINS

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SUMMARY

In this study, we determined the resistance to heavy metals (Zn^{2+} and Cd^{2+}) and exopolysaccharide (EPS) production by 15 *Pseudomonas* spp. EPS yields from these strains ranged between 22-192 mg l^{-1}. In addition, EPS production rates of two strains (G1 and G12), selected on the basis of their high EPS production (192 and 182 mg l^{-1}, respectively), but also heavy metal resistance, were determined in the presence of Zn^{2+} (10-40 mM) and Cd^{2+} (5-20 mM). There was a general increase in EPS production by both strains with increasing heavy metal concentrations. G12 strain showed an increase (approx. 6-fold) in the extent of EPS produced on Pseudomonas Agar P (Difco) medium, supplemented with 2% (v/v) glycerol containing 40 mM Zn^{2+}. By contrast, G1 strain exhibited a lower increase than G12 strain. Furthermore, G12 and G1 strains showed an identical increase (approx. 2-fold) in EPS production on Pseudomonas Agar P (Difco) medium, when supplemented with 2% (v/v) glycerol containing 20 mM Cd^{2+}.

KEYWORDS: *Pseudomonas* spp, heavy metal resistance, exopolysaccharide (EPS).

INTRODUCTION

Microbial polysaccharides are secreted from the cells to form often a considerably thick layer over the surface of the cell wall, for which reason they are known as exopolysaccharides (EPS) [1]. Many microorganisms synthesize EPS that either remain attached to the cell surface or are found in the extracellular medium in the form of amorphous slime [2]. EPS produced by microorganisms are able to concentrate charged organic molecules and inorganic ions [3]. Interactions of heavy metals with microorganisms have been of increasing interest and also metal cations are known to influence EPS biosynthesis [4, 5]. Members of the genus *Pseudomonas* have frequently been reported to produce EPS, which have been shown to bind a number of metal cations [6, 7].

Growing biotechnological interest is being shown towards bacterial EPSs due to their unique functional properties, their diversity and specificity in industrial applications [1]. The role of EPSs in industry is based on their performance as specialized chemicals, which means their capacity to alter the basic properties of water, and their propensity for emulsification, suspension, stabilization, and flocculation. These properties make them suitable for a specific market or application [8].

The aim of this study was to investigate the EPS production of 15 *Pseudomonas* strains, and their resistance against 2 heavy metals most frequently present in polluted aquatic and soil environments (Zn^{2+}, Cd^{2+}). The other objective of this study was to determine whether these two metals were enhanced or not for EPS production in G1 and G12 strains (selected from the 15 *Pseudomonas* spp.)

MATERIALS AND METHODS

Bacterial strains

Fifteen bacteria studied were isolated from polluted soil and biochemically identified by the Analytical Profile Index (API) 20 NE (Biomerieux, Marcy I'Etoile, France). Also, *Pseudomonas aeruginosa* G1 and *Pseudomonas putida* G12 strains were identified by the 16S rRNA gene

sequence analysis, to confirm their biochemical identification. The sequences obtained were searched against the GenBank DNA database using the blast function [9].

Heavy metal resistance of bacterial isolates

The heavy metals were tested as sulfate salts: $ZnSO_4.7H_2O$ and $CdSO_4.8H_2O$. Stock solutions were prepared in distilled water, and sterilized by filtration (with 0.2-µm pore-size filters). Both solutions were kept at 4 ºC (not longer than 1 month), to prepare variously concentrated metal solutions for the tests.

Heavy metal resistance of *Pseudomonas* spp. isolates was determined by agar dilution method [10]. Plates containing 20 ml of one-half strength Pseudomonas Agar P medium and different concentrations of the metals were poured on day of experiments. The ranges of concentrations for heavy metals were as follows (mM): 10, 20, 30, 40 for $Zn^{2+,}$ and 5, 10, 15, 20 for Cd^{2+} [4]. Plates were dried at 37 ^{0}C for 30 min and inoculated with 0.1 ml of exponentially grown cultures. Then they were incubated at 37 ºC for 2 days. Plates containing media without metal addition were inoculated in the same manner to serve as controls.

Isolation of EPS

Isolation of bacterial EPS was done as described by Cérantola et al. [11, 12]. Strains were grown on Pseudomonas Agar P (Difco) medium, supplemented with 2% (v/v) glycerol for 3 days at appropriate temperature (30 or 37 ºC). Agar plate cultures were then washed with saline (0.9% NaCl, w/v) using a glass-rod, and the resulting suspensions were stirred with glass beads in order to detach EPS associated with the bacterial cells. Cells were then removed by centrifugation at 10,000 x *g* for 30 min. The resulting supernatants were precipitated overnight at 4 ºC with 6 volumes of 95% ethanol. Precipitated EPSs were recovered by centrifugation, and the ethanol precipitation step was repeated. After centrifugation (12,000 x *g* for 30 min at 4 ºC), the pellets were dissolved in distilled water. Total EPS (expressed as mg l^{-1}) was estimated in each sample by phenol-sulphuric method [13] using glucose as a standard [14].

Furthermore, *P. aeruginosa* G1 and *P. putida* G12 which were selected due to both high EPS production and resistance to heavy metals (Zn^{2+}, Cd^{2+}), were inoculated on Pseudomonas Agar P (Difco) medium, supplemented with 2% (v/v) glycerol containing different concentrations of Zn^{2+} (10-40 mM) and Cd^{2+} (5-20 mM). Controls included cells grown in the absence of heavy metals. Due to the inhibitory effect of heavy metals on cell growth, the amount of biomass employed was adjusted to 5 ± 0.05 optical density (OD) at 600 nm, in order to calculate EPS-specific production under heavy metal concentrations and controls without metal addition. Then, isolation of bacterial EPS was done as above.

RESULTS

Isolation of EPS and heavy metal resistance

Pseudomonas spp. bacteria have been tested for resistance to Zn^{2+} and Cd^{2+}. These experiments revealed resistance of some *Pseudomonas* strains to Zn^{2+} and Cd^{2+}. Es-pecially, G1 and G12 strains showed resistance against all metal concentrations (Table 1). Additionally, as shown in Table 1, *Pseudomonas* spp. were examined for EPS production on Pseudomonas Agar P (Difco) medium, supplement-ed with 2% (v/v) glycerol. The *P. aeruginosa* G1 and *P. putida* G12 strains produced the highest amounts (192 mg l^{-1} and 182 mg l^{-1}, respectively) of EPS, while *P. putida* G15 and *P. stutzeri* G8 strains produced the lowest ones (22 mg l^{-1} and 23 mg l^{-1}, respectively), and these two strains were also unable to grow in high metal concentrations.

Effects of heavy metals (Zn^{2+} and Cd^{2+}) on EPS synthesis

EPS synthesis by the two strains G1 and G12, which were chosen on the basis of their high EPS production and heavy metal resistance, was determined in the presence of Cd^{2+} (5-20 mM) and Zn^{2+} (10-40 mM). Controls included cells grown in the absence of heavy metals. Our results in-

TABLE 1 - The production of EPS by some *Pseudomonas* species, and heavy metal resistance of *Pseudomonas* species against different concentrations of Cd^{2+} and Zn^{2+}.

		Heavy Metals							
		Zn^{+2}				Cd^{+2}			
		Concentrations (mM)				Concentrations (mM)			
Strains	**EPS (mg l^{-1})**	**10**	**20**	**30**	**40**	**5**	**10**	**15**	**20**
P. aeruginosa G1	192±4	+	+	+	+	+	+	+	+
P. aeruginosa G2	65±2	+	+	-	-	+	-	-	-
P. aeruginosa G3	75±4	+	+	+	-	+	+	+	-
P. fluorescens G4	62±0	+	+	-	-	+	-	-	-
P. fluorescens G5	41±1	+	+	-	-	+	+	-	-

P. fluorescens G6	37±2	+	+	-	-	+	-	-	-
P. fluorescens G7	87±1	+	+	+	-	+	+	-	-
P. stutzeri G8	23±2	+	-	-	-	+	-	-	-
P. stutzeri G9	110±3	+	+	+	-	+	+	-	-
P. stutzeri G10	59±1	+	+	-	-	+	-	-	-
P. stutzeri G11	70±2	+	+	+	-	+	+	-	-
P. putida G12	182±3	+	+	+	+	+	+	+	+
P. cepacia G13	71±1	+	+	+	-	+	+	-	-
P. cepacia G14	51±4	+	+	-	-	+	-	-	-
P. putida G15	22±2	+	-	-	-	+	-	-	-

TABLE 2 - EPS production of G1 and G12 strains at different concentrations of Cd^{2+} and Zn^{2+}.

Strains	Zn^{2+}		Cd^{2+}	
***P. aeruginosa* G1**	**Concentrations (mM)**	***EPS (mg l^{-1})**	**Concentrations (mM)**	***EPS (mg l^{-1})**
	10	33±0	5	49±0
	20	39±2	10	65±2
	30	42±0	15	91±1
	40	72±1	20	113±4
	Control	33±3	**Control**	33±3
***P. putida* G12**	10	61±2	5	68±4
	20	220±5	10	70±5
	30	350±5	15	82±1
	40	370±3	20	123±5
	Control	61±1	**Control**	61±1

* : EPS productions at the same amount of cells ($OD_{600\ nm}$, 5±0.05)

dicate that increased heavy metal concentrations in the medium resulted in an increment EPS synthesis of both strains. G1 strain showed about 2-fold increase in EPS production at both 40 mM Zn^{2+} and 20 mM Cd^{2+} levels, compared to controls, and also G12 strain exhibited almost 2-fold increase in EPS production at 20 mM Cd^{2+}, but significantly larger quantities (6-fold) of EPS at 40 mM Zn^{2+}, with regard to controls. Overall observations are summarized in Table 2.

DISCUSSION

It is well-known that different microorganisms produce various amounts of EPS that may associate with metal ions, divalent cations and other macromolecules [15]. Several researches have reported that EPSs are produced by *Pseudomonas* spp. [11, 16, 17]. Similarly, Osman et al. [18] reported that, yields of EPS produced by *Pseudomonas syringae* pv. glycinea 2159 and K1 strains, were 287 and 80 mg l^{-1} respectively. Fett et al. [19] reported that *P. putida* is capable of producing more than a single acidic EPS, which supports our studies very well.

Many microorganisms have extracellular polymeric layers that confer metal resistance. These polymeric layers are anionic in nature and, thus, attract cationic metals [20]. Also, bacterial EPSs are known to bind and concentrate metal ions and are, therefore, thought to play a role in ameliorating metal toxicity [5]. The toxic effects of Cd^{2+} on microorganisms are well documented, and Cd^{2+} competes with several divalent ions, such as Ca^{2+}, Zn^{2+}, and Mn^{2+}, for metal-binding sites in biological systems [21, 22]. Generally, Gram-negative soil species appear to be more tolerant to heavy metals than the Gram-positive ones [4, 23]. Wang et al. [21] have shown that *Pseudomonas aeruginosa* CW-961 strain is able to grow in concentrations of cadmium upto 5 mM. Hassen et al. [4] have also reported that *P. aeruginosa* has a particular resistance to Zn^{2+} and Cd^{2+}. Malik and Jaiswal [24] indicated that 73.3% of the *Pseudomonas* strains isolated from soil showed resistance to Cd^{2+}, and 71.1% of the isolates exhibited resistance to Zn^{2+}.

Metal ion concentration may be critical for microbial growth and EPS production [2.] Although hyper production of extracellular polysaccharides in response to starvation, dehydration, or antibiotic stress has been reported for *Pseudomonas* spp., the effect of heavy metals on this polymer production is less studied [16]. A number of bacterial strains are also known to exhibit enhanced and/or compositionally altered EPS synthesis, in response to metal ions exposure [7]. Previous observations for different organisms [25, 26] have also elucidated that extracellular anionic polysaccharide-producing bacteria were less susceptible to heavy metals than the non-producing variants, due to a reduction of the free metal ion concentration at the cell surface [27], as we have observed in our experiments. In our study, a correlation was found between EPS synthesis and heavy metal resistance, although other factors may be playing a role in heavy metal resistance.

It is important to note that *P. syringae* strain shows enhanced EPS production, even in the presence of fairly low concentrations of Cu^{2+} (16-100 μM) [16]. Similarly, the exposure of *P. aeruginosa* and *P. fluorescens* to arsenite or steel could enhance EPS synthesis [28, 6]. EPS production is stimulated by Mg^{2+}, Mn^{2+}, and Ca^{2+} in *P. aeruginosa*

sa [7]. Monot and Quinn [29] have exhibited that the presence of magnesium in the culture medium results in a higher EPS yield (9.2 g l^{-1} in the absence of Mg^{2+} l^{-1} and 18.3 g l^{-1} in the presence of 0.44 g Mg^{2+} l^{-1}). Such overproduction of EPS may be due to the heavy metal-mediated generation of free radicals and other toxic molecules, creating oxidative stress for bacterial cells that, in turn, activates EPS gene transcription resulting in enhanced EPS production [30, 31].

The stimulation of EPS synthesis by *Pseudomonas* spp. in the presence of Zn^{2+} and Cd^{2+} heavy metals has not been studied previously. The G1 and G12 strains that we have used in our study have shown that exposure to heavy metals (Zn^{2+} and Cd^{2+}) could lead to an enhance EPS production. Therefore, G1 and G12 strains could represent a potential microbial source for new polymers. Structural and rheological studies on the described EPSs in this study will possibly bring forward many applications in biotechnology.

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ATMOSPHERIC HEAVY METAL DEPOSITION IN DÜZCE PROVINCE BY USING MOSSES AS BIOMONITORS

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SUMMARY

This research was carried out around the industrialised area and D100 highway in Düzce, in order to determine the atmospheric heavy metal deposition by using mosses as biomonitors. Sampling was performed based on the principle that carpet-forming bryophytes (pleurocarpous mosses) at 14 sites distributed over the study area between 2003 and 2004. Dried samples were digested according to the wet digestion method, and the concentrations of heavy metals were determined by graphite–furnace atomic absorption spectrometry (AAS). According to the results of analyses, the heavy metal concentrations in the region are ordered as follows: Fe>Pb>Cu>Co>Cr>Ni>As. Distribution maps were drawn up by using the Geographic Information System (GIS) for each of the studied metals according to their concentration in the mosses. The results were compared with similar studies in Europe, and it was observed that the accumulation ratios of iron, lead, arsenic and cobalt are higher than European rates. In addition, significant correlations were found among Fe-Cu and Co between the results, and control sites were compared by using SPSS statistical programme.

KEYWORDS: Trace metals, mosses, bioindicators, digestion, atomic absorption spectrometry (AAS), Düzce-Turkey.

INTRODUCTION

Environmental pollution has been known as one of the most important problems in modern societies. Heavy metals are a major source of environmental pollution, and determining their environmental concentrations is an important part of understanding biogeochemical processes and gauging ecosystem`s health. Currently, metal fluxes cam be attributed to fossil fuel consumption, agricultural dust, and metallurgy [1]. Therefore, it is necessary to maintain a close watch on heavy metal depositions into atmospheric, terrestrial biotic and aquatic environments. The best way to determine the extent of contamination in living organisms is by measurement of the levels of contaminants in the organ-

isms themselves, for which plants have proven to be the most suitable. The use of mosses as biomonitors is a convenient way of determining levels of atmospheric deposition [2]. This is possible because mosses take up nutrients, and also contaminants, directly from the atmosphere. Compared to higher plants, mosses have several advantages which make them more suitable for this kind of study. Mosses lack a root system and a well-developed cuticle, thus, on one hand, the substrate has little influence on the levels of contaminants in their tissues and, on the other hand, they readily take up atmospheric contaminants. Moreover, the cationic exchange capacity in mosses is high and they possess a high surface area to volume ratio, factors which both favor the accumulation of large amounts of pollutants [3]. Bryophytes are especially suitable organisms for purposes of monitoring investigations, because procedures of sampling and chemical analyses are relatively simple and low-cost ones. They are evergreen and perennial plants, so it is easy to collect them throughout the year. Most of their species are widespread, and, thus, heavy metal concentrations of distant areas can also be compared [4, 5].

The use of mosses as biomonitors for atmospheric deposition of metals has been reported in a large number of studies including local investigations [e.g. 6-8], as well as regional or national [e.g. 9-14] and international surveys in different parts of the world [e.g. 15-18]. Regional and temporal variation in atmospheric trace element deposition is frequently monitored with indigenous moss species as bioindicators [19].

The present study is the first attempt to characterize the atmospheric deposition of heavy metals in Düzce province of Turkey by means of mosses. Düzce is located between longitudes 30º 49' and 31º 50' east, and between latitudes 40º 37' and 41º 07' north. This city has approximately 250 industrial foundations, having various sectors in big and small scales [20]. With particular attention, the study area is located on the expressway between the capital Ankara and Istanbul. In addition, this region is surrounded

by industrialized cities, such as Zonguldak to the north, Bolu to the east and Sakarya to the west (Figure 1). Its con-

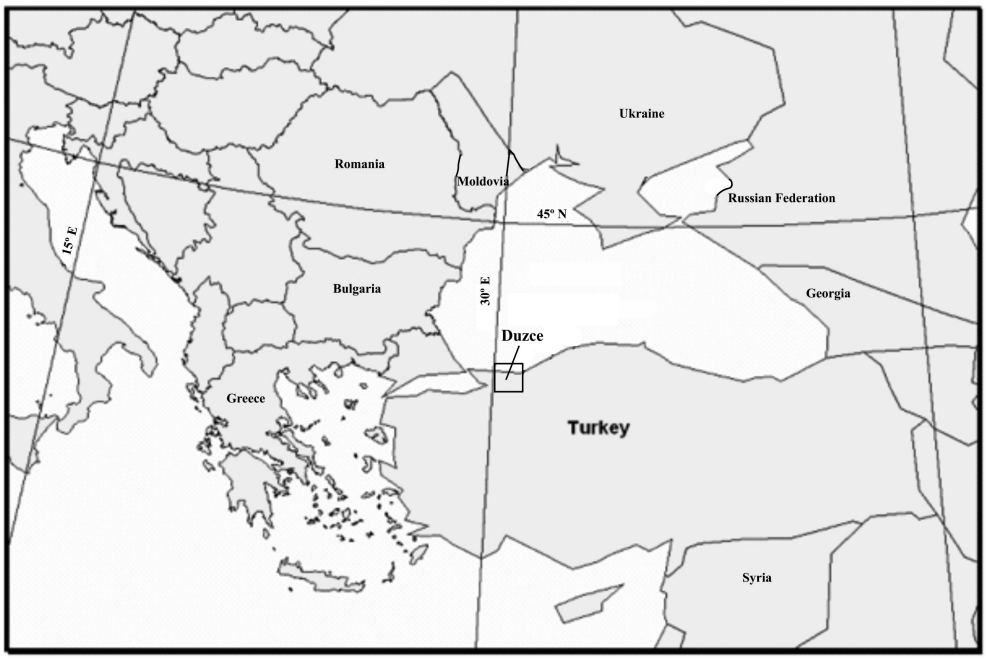

FIGURE 1 - Geographical location of the study area.

ditions encouraged us to conduct a study on atmospheric heavy metal deposition.

The aims of this study were: 1) to quantify heavy metal deposition in the immediate center and surroundings of Düzce, in terms of concentration in mosses as well as by calculated deposition rates, 2) to establish patterns of distribution of the elements analyzed and to identify sources of contamination and contaminated areas, 3) to map and relate the concentration gradients found in this study area with increasing distances from pollution sources, and 4) to make data available that can be compared with results of further moss monitoring studies.

MATERIALS AND METHODS

Our method was largely based on the Scandinavian recommendations [21, 22]. In practice, the guidelines were adapted and supplemented where necessary.

Sampling. Sampling was performed during June 2003 and November 2004 in a relatively dry period at a total of 14 sampling points, with an average density of one site of 130 km^2. Sampling sites were chosen around industrial sites, traffic sites and conspicuous hills enclosing the city centers as well as on the prevailing winds (NE, SW in Düzce, SSE in Akçakoca) (Figure 2). Especially, SW and SSE prevailing winds transport fly-ash from industrial plants to Akçakoca Mountains. The sampling points were at least 300 m from main roads or villages, and 100 m from minor roads and buildings. When necessary, in more densely populated areas, these distances were reduced to 100 m and 50 m, respectively. Moreover, the sampling points in open forest locations lay out-side the area affected by rainwater dripping from the crowns of the trees. At each site, a total sample consisted of sub-samples collected within an area of 50 x 50 m.

Sampling and sample handling were carried out using plastic gloves and bags. Samples of the widespread and most common mosses *Scleropodium purum* (34.5 %), *Hypnum lacunosum* (10.3 %), *Hypnum cupressiforme* (6.9 %), and *Calliergonella cuspidata* (6.9 %) were preferred in Düzce, but when these common species were not available in sampling points, another suitable moss species was chosen. This study is based on the principle that carpet-forming bryophytes (pleurocarpic mosses) can absorb elements and particles from rain, melting snow and dry deposition [23, 24]. Carpet-forming mosses, such as *Scleropodium purum* and *Hypnum cupressiforme,* etc. are not rooted in a substrate, and their uptake of elements from soil is, therefore, quite insignificant or negligible [25].

Preparation of the samples and chemical analysis. All reagents were of analytical grade, unless otherwise stated. All the plastic and quartz-ware was cleaned by soaking it overnight in a 10 % (w/v) nitric acid solution, followed by rinsing with deionized water. Double-deionized water (Milli-Q Millipore 18.2 MΩ cm^{-1}) was used for all dilutions. The samples were cleaned from soil particles, dead material and litter. Only the last three years' growths of moss materials were used without washing for analyses. The samples were processed as described by Perkin-Elmer (1996) for plant wet digestion [26]. In this method, 1 g of ground dried plant sample was put in 100-ml beakers.

10 mL conc. HNO_3 was added, and all was heated carefully on a hot plate until the production of red NO_2 fumes has ceased. After cooling of the solution, 3 mL of $HClO_4$ (70-72 %) was added and heated, till a small part of mixture remained. After that, the solution was filtered using a membrane with 0.45 μm pores, taken in a 50-ml flask, and then demineralized water was added to a total volume of 50 mL. The contents of Fe, Pb, Ni, Cr, As, Cu and Co in the extracts were determined using graphite-furnace AAS (Perkin-Elmer Model SIMAA 6000). Quality control was carried out by parallel analysis of registered reference material SRM (IAEA-336 Lichen). The presence of possible contaminants during the digestion process was controlled by use of blanks, one for every 11 samples, whereby only acid was placed in the Teflon® containers. To control for variations in sampling, extraction and analysis, a total of six replicates of each sample were analysed.

Mapping. The maps, based on the mean values of Inverse Distance Weighting Interpolation (IDW) and Geographic Information System (GIS) techniques, were used in making coloured maps. Colours and scales were chosen that they would clearly illustrate the changes in the heavy-metal concentrations during the period covered by surveys. Interpolation is a mathematical process used to estimate values between known-point observations. Many mathematical formulae can be used to interpolate grid values, and were chosen according to the type of data being examined. The GIS modelling process uses mathematical formulae to estimate the values between known-point observations, and stores the results in a numeric grid. With the purpose of applying interpolation and GIS modelling techniques to our data; a package of programs, MapInfo® Professional 4.1 and Vertical Mapper® Version 1.51, was used.

Statistical analysis. Data were analyzed by using SPSS® for Windows (SPSS Inc. Chicago. IL) computing program. Differences in measured parameters among the four regions were analyzed by a Kruskal-Wallis test (P values less than 0.05 were considered to be significant). In groups, comparisons between regions that present significant values were evaluated with Mann-Whitney U test (significance was attributed to a value of $P<0.05$). A linear correlation test was carried out to investigate the correlations between metal concentrations (significance was attributed to values of $P<0.01$ and $P<0.05$). Two-tailed significance values were used.

RESULTS AND DISCUSSION

The heavy metal mean concentrations in moss samples analyzed are given as μg g^{-1} together with a plant list in Table 1.

Summary statistics were used to obtain standard deviation (S.D.), minimum (Min), maximum (Max) and median concentrations of the different elements (Table 2). All metal concentrations were determined on dry weight basis.

Iron has the highest concentration, followed by lead, copper, cobalt, chromium, nickel, and arsenic, i.e., Fe> Pb>Cu>Co>Cr>Ni>As. The concentration of trace metals in the samples depended on moss species. For example, high metal accumulation levels were found in *Hypnum lacunosum* for Fe and Cr, *Brachythecium glaerosum* for Pb, *Hypnum cupressiforme* for Ni, *Scleropodium purum* for As, Cu and Co, respectively.

TABLE 1 - Heavy metal concentrations (μg g^{-1} dry wt.) of seven trace metals in investigated moss species.

Sampling Point No	Moss Species	Fe (μg g^{-1})	Pb (μg g^{-1})	Ni (μg g^{-1})	Cr (μg g^{-1})	As (μg g^{-1})	Cu (μg g^{-1})	Co (μg g^{-1})
1	*Brachythecium glareosum*	1519.50	63.65	2.83	4.34	0.83	16.03	4.66
2	*Hypnum cupressiforme*	2735.00	55.46	5.33	2.14	0.27	16.80	4.87
3	*Brachythecium oedipodium*	3842.50	63.35	0.44	9.08	0.12	18.96	5.55
4	*Hypnum lacunosum*	4166.50	25.23	4.16	10.67	0.89	10.96	7.04
5	*Hypnum lacunosum*	1808.00	15.18	1.96	2.48	0.56	8.06	2.06
6	*Brachythecium glareosum*	1375.00	12.63	0.21	4.65	0.41	6.41	4.08
7	*Scleropodium purum*	873.00	30.62	1.46	1.52	0.23	4.98	2.45
8	*Scleropodium purum*	1040.00	4.96	1.81	0.19	0.30	8.58	3.20
9	*Eurhynchium speciosum*	1085.20	16.34	1.92	0.20	0.32	6.61	2.86
10	*Scleropodium purum*	2468.50	31.52	1.75	1.96	0.42	5.16	2.98
11	*Scleropodium purum*	936.50	16.08	0.21	0.27	0.66	8.02	2.47
12	*Scleropodium purum*	623.50	45.65	1.43	0.44	0.95	7.86	3.41
13	*Scleropodium purum*	3579.00	7.42	4.17	2.02	0.38	5.58	1.19
14	*Scleropodium purum*	642.00	43.02	0.11	0.33	0.25	22.12	10.18
C1	*Scleropodium purum*	701.00	28.3	4.7	3.14	0.32	0.01	2.23
C2	*Scleropodium purum*	644,0	17,5	2,8	5,04	0,96	0,01	0,39
C3	*Brachythecium glareosum*	415.50	17.9	1.37	0.47	0.74	8.63	0.33
C4	*Scleropodium purum*	119.00	17.7	11.1	3.50	0.51	0.01	0.26
C5	*Hypnum lacunosum*	0.00	12.0	5.0	0.60	0.15	0.01	0.12

C: control site, 0.00: The values are below detection limit of AAS.

TABLE 2 - Mean, standard deviation (S.D.), minimum (Min), maximum (Max) and median concentrations of different elements (μg g^{-1} dry wt.) in moss samples.

	Fe μg g^{-1}	Pb μg g^{-1}	Ni μg g^{-1}	Cr μg g^{-1}	As μg g^{-1}	Cu μg g^{-1}	Co μg g^{-1}
Mean	2003.87	27.67	2.11	2.67	0.43	9.26	3.61
Median	1663.75	20.78	1.86	1.74	0.35	7.94	3.09
Min.	623.50	4.96	0.11	0.19	0.12	0	0
Max.	4166.50	63.65	5.33	10.67	0.95	22.12	10.18
S.D.	1184.79	20.75	1.55	3.13	0.27	6.17	2.52

In general, elevated concentrations of heavy metals in the mosses sampled in a particular region can arise in several ways. Hot spots can be associated with industrial activities, or with large urbanization, whereas widespread effects can be due to widespread sources, particularly vehicle emissions along roads or geological sources, or, too long-range transport of pollution from industrial and vehicle sources. For comparison, data were treated in two ways. For the first run, the territory of Düzce was divided into 3 regions representing (1) traffic sites (TR); along D100 highway and other main roads, (2) industrial sites (IN); near the medium and minor industry establishments, (3) residential sites (RS), and (4) relatively rural sites (RRS); remote from city center regions and roads (Figure 2).

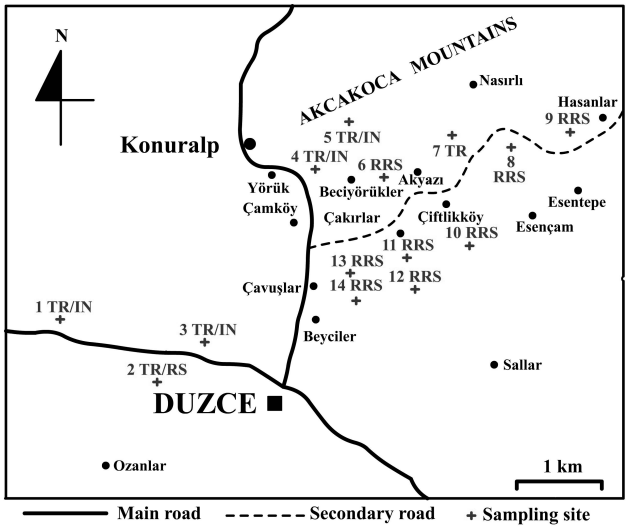

FIGURE 2
A map representing sampling stations and territories of Düzce.

For the second run, cartographic representations of the results were made in order to visualize the distribution of the metal concentrations in the study area (Figs. 3-9).

Iron. It is usually though that this element is bound to soil particles and, thus, the content is not solely due to atmospheric deposition, however, iron is also emitted during combustion of fossil fuels, such as coal [27]. The lowest and highest iron concentrations were found to be 4166 μg g^{-1} in *Hypnum lacunosum* and 623 μg g^{-1} in *Scleropodium purum,* respectively. Iron concentrations in *Hypnum lacunosum* and *Brachythecium oedipodium* species were found to be 35 times and 32 times higher than those of control samples (*Scleropodium purum*), respectively. The mean value for iron was 1980 μg g^{-1}, which was close to the upper limit, compared with the mean European values (259-2070 μg g^{-1}) [3, 9-11, 16, 22, 28]. The iron level in 40 % of the moss samples was higher than 1980 μg g^{-1}. Higher concentrations were measured near residential and intensive traffic regions at SW, N and NNE of the study area (Fig. 3). A potential source of high iron levels may be connected with vehicle emissions along roads or long-range transport routes of pollution from industrial and vehicle sources.

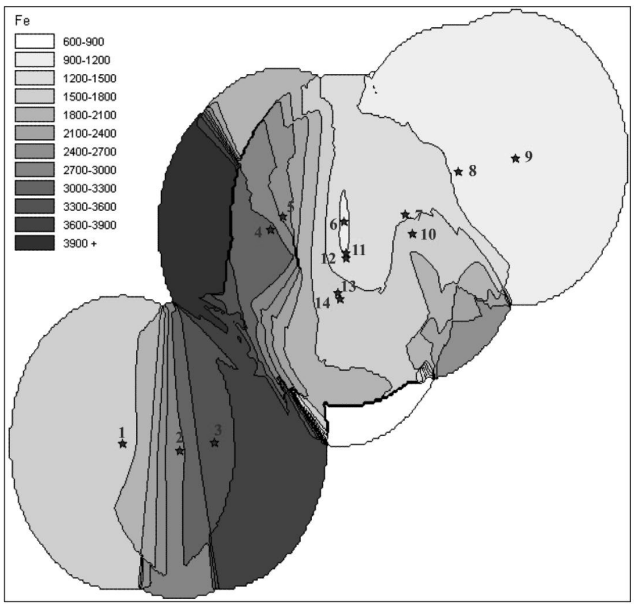

FIGURE 3
Contour map showing iron concentrations (μg g-1 d.w.) in Düzce.

Lead. Leaded fuel is still a main source of lead pollution, together with other sources like metal production and mining [3]. As we measured the background atmospheric deposition in the area, our values were mildly high with a mean of 29.1 μg g^{-1} compared with the European mean values (12.9–20 μg g^{-1}) [22]. Besides, 53 % of the samples exceeded 20.5 μg g^{-1}. Higher concentrations were observed due to emissions of intensive traffic. In addition, coal as a

fossil fuel may also contain considerable amounts of lead. The highest level of Pb (63.6 μg g^{-1} in *Brachythecium glaerosum* was sampled close to the expressway D-100 and residential sites. Secondary high Pb level (45.6 μg g^{-1}) was detected in *Scleropodium purum* from a hill exposed to main winds coming from polluted sites. In turn, the high lead levels in the moss can probably be explained by inadequate filter installations and resulting dust emissions. What is more, coal may also contain considerable amounts of lead. The elevated concentrations found in some regions were similar to the distributions of Cr and Fe (Fig. 4).

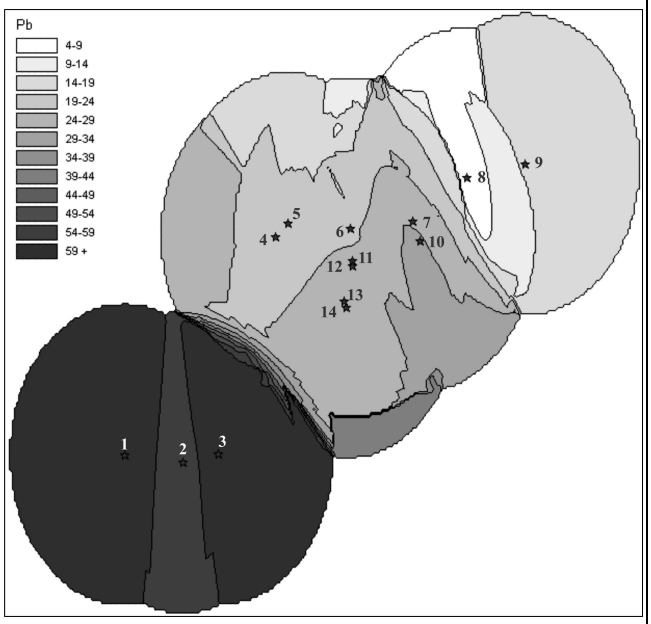

FIGURE 4
Contour map showing lead concentrations (μg g^{-1} d.w.) in Düzce.

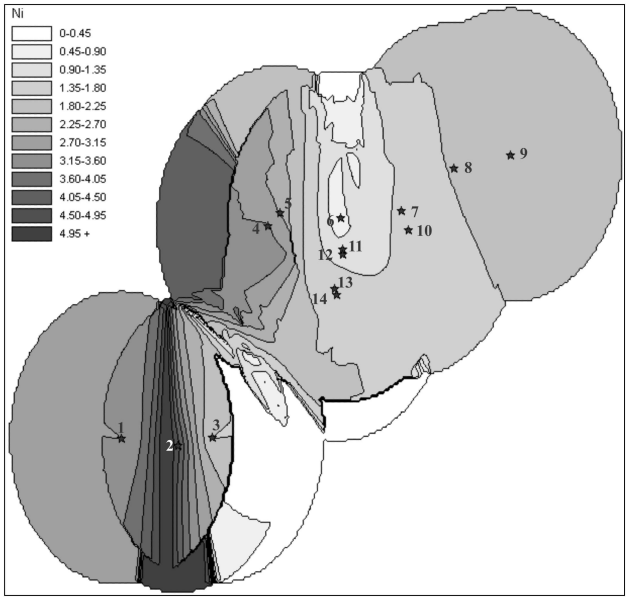

FIGURE 5
Contour map showing nickel concentrations (μg g^{-1} d.w.) in Düzce.

Nickel. Nickel mainly originates from oil and coal burning, steel industry, and smelters [3]. In most European countries, the concentration varied between 2 and 4 μg g^{-1} in mosses [22]. Nickel average levels were found to be 1.96 and 2.83 μg g^{-1} in *Hypnum lacunosum* and *Brachythecium glaerosum,* respectively. Nickel mean level (2.07 μg g^{-1}) was approximately two times higher than that of control sample (1.37 μg g^{-1}) in *Brachythecium glaerosum*. Extremely high emission and accumulation of Ni was observed as 5.33 μg g^{-1} in *Hypnum cupressiforme* at southern border of the study area. This species may be a good indicator for nickel. This distribution could be explained by heavy traffic along D100 highway and exposure to dominant winds (NE) coming from Düzce city center and industrial regions (Fig. 5). In addition, this region is exposed to heavy fume originating from oil and coal burning during winter season. As we measured the background atmospheric deposition in Düzce, our values were similar with a mean of 2.07 μg g^{-1} compared to the values of the European means (1.6–3.7 μg g^{-1}) [3, 9, 28].

Chromium. Above all, higher levels of chromium are associated with emissions from the iron and steel industry. The other emission source can be intensive traffic, especially the transport near the intercity road. All Cr measurements ranged from 0.19 μg g^{-1} to 10.67 μg g^{-1}, with a median of 1.74 μg g^{-1}. Maximum values were measured at the sites close to a standard profile factory, as 10.6 μg g^{-1} in *Hypnum lacunosum*, but also the most urbanised area, as 9.08 μg g^{-1} in *Brachythecium oedipodium*. Other high levels of Cr were measured at the sites of the direction of prevailing winds as 4.65 μg g^{-1} in *Brachythecium glaerosum,* and the sites close to main roads (4.34 μg g^{-1}) in *Brachythecium glaerosum*. The lowest level was found NEE of the study area (Fig. 6). Chromium mean levels (2.67 μg g^{-1}) in Düzce province are mildly elevated when compared with those of Finland (1.25 μg g^{-1}) [11], Galicia in NW Spain (1.2 μg g^{-1}) [10] but are similar to those in Hungary (2.8 μg g^{-1}) [28], North Spain (2.68 μg g^{-1}) [3], Norway (2.6 μg g^{-1}) [9], Germany (2.11 μg g^{-1}) and Poland (2.54 μg g^{-1}) [16].

Arsenic. Arsenic mainly originates from metallurgy, pesticides, detergents, and fossil fuel combustion [27]. The highest concentrations were found in the NE and W part of the study area, usually near the industrial region (IN) and sites close to main roads (TR). High levels of As were also registered in the NEE of the study area, in agricultural regions, the direction of the prevailing winds coming from Düzce industrial region, and the most urbanised region of Düzce province. The mean arsenic concentrations ranged between 0.12 and 0.95 μg g^{-1} in *Brachythecium oedipodium* and *Scleropodium purum*, respectively. Arsenic average levels were found to be 0.45 μg g^{-1} (median 0.27 μg g^{-1}), similar to the mean European values (0.19 μg g^{-1} in Finland [11], 0.40 μg g^{-1} in Spain [3]. 0.56 μg g^{-1} in Austria, and 0.34 μg g^{-1} in Germany [22]). Nevertheless, the mean arse-

nic level in polluted regions is approximately three times higher than that of control sample (*Hypnum lacunosum*). The highest levels of As coincided with industrial activities, motor vehicle emissions and pesticides in agricultural regions (Fig. 7).

Copper. Copper mainly originates from metal industry, mining, coal-fired plants, traffic, and even soil [17]. As it is clearly seen in Fig. 8, copper mainly originated from heavy traffic emissions. The average of Cu level in this region was approximately 9.88 μg g^{-1}, whereas its median concentration was 5.86 μg g^{-1} in Düzce. This value is reasonable compared with European values [22]. The lowest and highest copper values were observed in *Hypnum cupressiforme* and *Scleropodium purum*, respectively. The best bioindicator of moss samples for copper is *Scleropodium purum*, due to the highest copper level found. The elevated concentrations found in some regions were similar to distribution of cobalt and lead ones. High accumulations of Cu were observed at the south part of this area (18.96 μg g^{-1} in *Brachythecium oedipodium* and 16.03 μg g^{-1} in *Brachyhecium glareosum*) because of intensive traffic

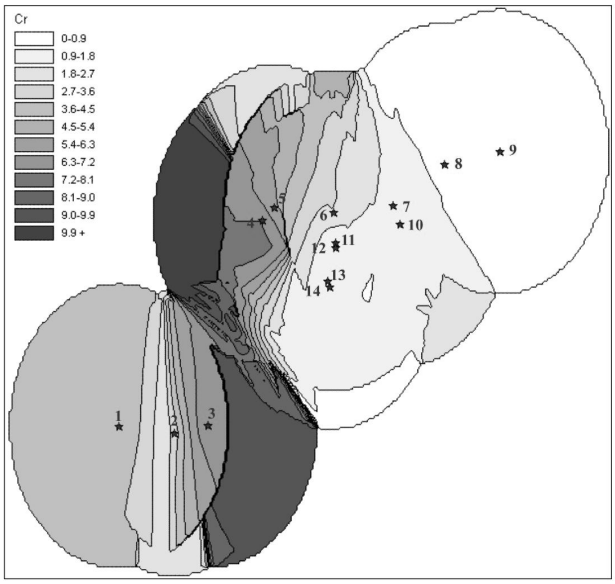

FIGURE 6 - Contour map showing chromium concentrations (μg g^{-1} d.w.) inDüzce.

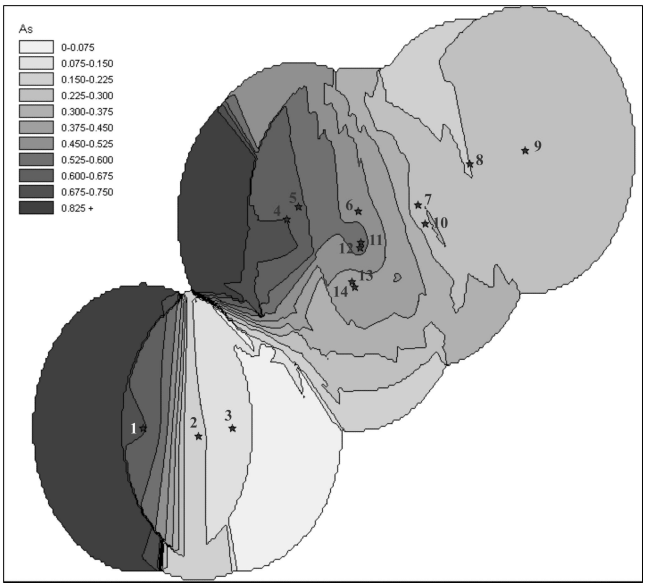

FIGURE 7 - Contour map showing arsenic concentrations (μg g^{-1} d.w.) in Düzce.

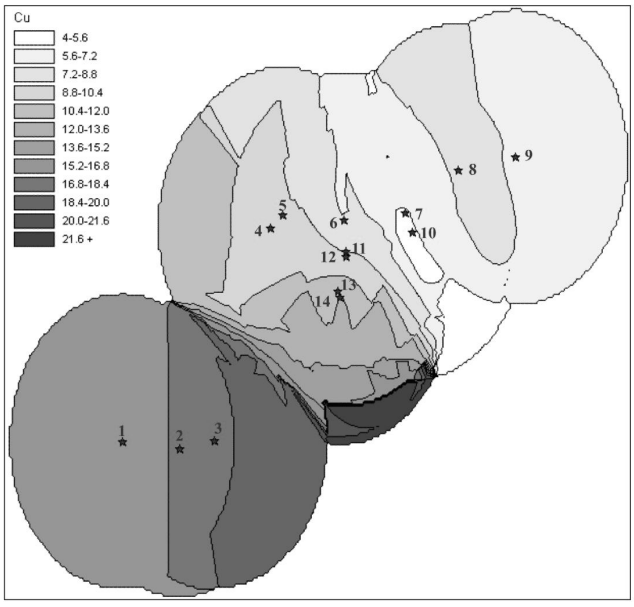

FIGURE 8 - Contour map showing copper concentrations (μg g^{-1} d.w.) in Düzce.

and industrial activities. In addition, an extremely high emission was determined NE of the study area (22.12 μg g^{-1} in *Scleropodium purum*) because of exposure to dominant winds coming from the industrial region and most urban sites. Average copper levels in the whole study area were approximately in the range of 5-9 μg g^{-1}, exceeding 9 μg g^{-1} in 33 % of the samples. The remote regions in the NE part of the study area were the least polluted sites (Fig. 8).

Cobalt. All concentrations of cobalt ranged from 0.69 to 10.18 μg g^{-1} with a median of 2.41 μg g^{-1}, which was highly elevated with respect to the mean European values, 0.37 μg g^{-1} in Norway [9] and 0.60 μg g^{-1} in NW Spain [10]. The lowest and highest cobalt values were observed in *Hypnum cupressiforme* and *Scleropodium purum* species, respectively. Control samples had 0.12 and 2.23 μg g^{-1} cobalt concentrations. Nevertheless, the mean cobalt level (3.61 μg g^{-1}) is approximately nine times higher than that of control sample (0.39 μg g^{-1}) in *Scleropodium purum*. Higher levels of cobalt might be associated with prevailing winds coming from industrialised sites of the region, but also main roads. The NE part of the study area was again the least polluted one (Fig. 9).

Statistical Evaluation. In the statistical analysis, Fe, Cu, and Co elements showed significant correlations between polluted sites and control areas ($p< 0.05$). The other correlations between the other metals and control regions were found not to be significant (Table 3).

Additionally, a linear regression correlation test was carried out to investigate the correlations between metal concentrations. These values are given in Table 4. Correlation analysis (Pearson correlation, 2-tailed) shows good correlations between Fe-Cr and Fe-Co ($p<0.01$). The other correlations between metals were found not to be significant or negative between Pb-Fe, As-Fe, Co-Fe, Cr-Pb, Cu-Pb, Co-Pb, As-Cr, Cu-Cr and Co-As. The correlation between distance to pollution source and element concentration was strong (R^2=0.67) for Fe, but moderate for Co (R^2=0.47).

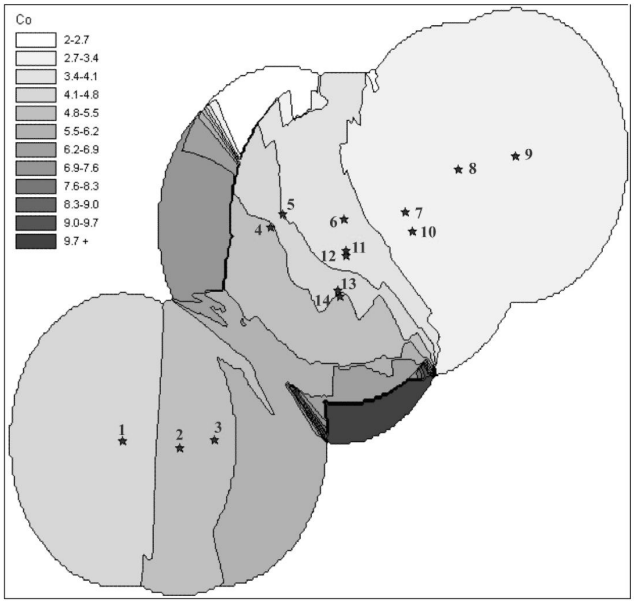

FIGURE 9
Contour map showing cobalt concentrations (μg g^{-1} d.w.) in Düzce.

TABLE 3 - Statistical analysis of metal concentrations.

	Fe * (μg g^{-1})	Pb (μg g^{-1})	Ni (μg g^{-1})	Cr (μg g^{-1})	As (μg g^{-1})	Cu * (μg g^{-1})	Co * (μg g^{-1})
Düzce Industrial Region	Mean (± SD.)	Mean (± SD.)	Mean (± SD.)	Mean (± SD.)	Mean (± SD.)	Mean (± SD.)	Mean (± SD.)
	1980,79 (± 1222,66)	29,16 (± 20,57)	2,07 (± 1,60)	2,78 (± 3,21)	0,45 (± 0,27)	9,88 (± 5,86)	3,84 (± 2,41)
Control sites	375,90 (± 310,82)	18,68 (± 5,92)	4,99 (± 3,72)	2,55 (± 1,97)	0,54 (± 0,32)	1,73 (± 3,85)	0,66 (± 0,88)

($p<0.05$) for comparison of polluted areas and control regions.

TABLE 4 - Correlation between metal concentrations.

	Fe	Pb	Ni	Cr	As	Cu	Co
Fe	1						
Pb	-0.350	1					
Ni	0.272	0.135	1				
Cr	0,673**	-0.055	0.330	1			
As	-0.057	0.009	0.126	-0.341	1		
Cu	0.103	-0.202	0.180	-0.250	0.302	1	
Co	0.479**	-0.062	0.399	0.436	-0.288	0.326	1

** Correlation is significant at the 0.01 level.

CONCLUSION

Effects of the major pollution sources located in industrial zones and near environs can be readily detected, on the basis of moss analysis. Our study showed that the Düzce background concentrations of Pb and Co are significantly higher than the European levels. Nevertheless, Fe and Cr are mildly elevated, in comparison with European averages. The values of other heavy metals found were similar to European averages [3, 9-11, 16, 22, 28]. Düzce is just situated between Ankara and Istanbul, and Ankara is 240 km away to the East, whereas Istanbul is 228 km away to the West. The road of D-100 passes through Düzce, and TEM Highway passes around it. The local emitters in Düzce are the steel, metal, plastic, wooden covering and weapon industries, but also burning of coal and oil or transport. In the study, the highest concentrations of Fe, Co and Cu were detected generally to the northeast of the pollution sources, as a result of prevailing winds` direction. South and southeast of the area, where the highest Pb and Cr contents and a high Ni level were measured, was also influenced by heavy traffic and urbanization. Nevertheless, high levels of As were found near to main roads and agricultural fields in the region. Generally, the highest bioaccumulation values are measured in the direction of prevailing winds, and places close to residential sites, decreasing rapidly with distance.

To summarize, it may be said that chiefly heavy traffic, some industrial activities with low technological standard, and the use of coal and oil for household-heating in this region cause increasing levels of some specific heavy metals, i.e. Pb, Co, Fe and Cr. Our data serve as a reference database for the future, to monitor any changes in background heavy metal deposition. Further investigations are necessary to determine the trace metal pollution trends in this region.

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SUBMERGED MEMBRANE BIOREACTOR AND SECONDARY DIGESTION FOR THE TREATMENT OF WINE DISTILLERY WASTEWATER. PART I: RAW WINE DISTILLERY WASTEWATER DIGESTION.

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This paper is dedicated to the memory of Winston Leukes

SUMMARY

A combination of a submerged membrane bioreactor (SMBR) and a secondary digester was tested for the treatment of wine distillery wastewater (WDW). The experimental system, consisting of four individual reactors, was tested during a 30-days study. Buffering of pH was achieved by mixing the feed stream of the system with 1000 mg/l of $CaCO_3$ and K_2HPO_4 for the initial 10 days of the bioreactor system operation, and with 8000 mg/l of $CaCO_3$ and 4000 mg/l of K_2HPO_4 for the remainder of the study. Buffering proved to be significant for optimum performance of the system in removal of soluble chemical oxygen demand (COD_S), and volatile fatty acids (VFAs). Different batches of WDW used for feeding the reactor had variable compositions with respect to concentrations of nitrates, ammonium and the total concentration of phenolic compounds. Am-monium accumulated in the secondary digester after 14 days of treatment system operation, indicating the time required for the establishment of anaerobic conditions in the system. An additional step would be required for removal of phosphates from the effluent of the bioreactor, e.g., reverse osmosis, if the effluent is to be reused in production or other applications.

KEYWORDS: Anaerobic digestion, chemical oxygen demand, polyphenols, submerged membrane bioreactor (SMBR), volatile fatty acids (VFAs), wine distillery wastewater (WDW).

INTRODUCTION

Wine distillery wastewater (WDW) is a by-product from alcoholic distillation operations; its characteristics include dark brown colour, chemical oxygen demand (COD) and five-days biological oxygen demand (BOD_5) values of 25-45 g/l, acidic pH and high concentrations of ammonium and sulphate. Up to 16 m^3 of WDW are produced per ton of ethanol distilled [1]. Although characterised as non-toxic, high concentrations of nutrients make the possible discharge of WDW into water-bodies problematic, causing eutrophication and other adverse environmental effects. In the context of South Africa, this might mean water shortages in areas surrounding alcohol and wine distilleries could be ameliorated by the reuse of treated WDW to replace potable water where possible, e.g. in vineyard irrigation. However, biological wastewater treatment processes, such as activated sludge, have been dogged by operational problems when treating high organic load wastewaters like WDW [1]. These include sludge bulking, high operational and energy costs, and low biomass production. Submerged MBRs make it possible to obtain high biomass concentrations, while anaerobic conditions have been shown to provide more efficient treatment of high B/COD containing wastes [2].

Membrane bioreactors (MBRs) integrate biological degradation with membrane filtration [3], and are increasingly applied to the treatment of industrial wastewaters [4]. The membrane can be submerged in the bioreactor or located externally. If the membrane module is located inside the bioreactor, then the system is called the submerged membrane bioreactor (SMBR). Conversely, if the membrane is located externally to the bioreactor, then the system is called

the external membrane bioreactor (EMBR). In SMBRs permeate is driven through the membrane using a static pressure head of mixed liquor, or by the application of low vacuum on the permeate side of the membrane. One of the key advantages for SMBRs in wastewater treatment is the long sludge retention time (SRT) that can be achieved [5]. This leads to increased mixed liquor suspended solids (MLSS) concentrations, the ability to treat wastewaters with high organic loads, and the selective development of biomass with the ability to efficiently eliminate specific wastewater components. The principal process limitation of SMBRs is membrane fouling, i.e. decrease in membrane permeability with time during system operation. This can be minimised by controlling hydrodynamic conditions inside the bioreactor using bubble aeration [6], backflushing [7], or by keeping the MLSS within 10-20 g/l [8]. Relative robustness of MBRs compared to activated sludge systems has been demonstrated, which concerns the quality of wastewaters that contain <2 mg/l total suspended solids (TSS), and/or 20-30 mg/l chemical oxygen demand (COD) [9].

The usual pore size of SMBR ultrafiltration (UF) membranes is 0.05-0.2 μm [2]. A previous study of the molecular weight (MW) distribution of compounds in the supernatant inside an SMBR and in its permeate found that most of the permeate components had MWs of <30 000 Daltons [9]. This portion constituted 60-70 $\%_W$ of the material, while 10-20 $\%_W$ originated from compounds with MWs of >100 000 Daltons. The relative proportion of the high MW fraction in the permeate increased with operation time. Based on the occurrence of cell lysis in the SMBR and fast depletion of readily biodegradable compounds, it is reasonable to expect that the high MW fraction will contain enzymes and other fractions of dead cells, as well as some inhibitory compounds from the WDW rather than the readily biodegradable ones. As a result, an additional step making use of the potential reactions and biodegradation (with additional inoculation of a secondary digester) in the permeate before effluent discharge or reuse might increase the efficiency of the process. Given the above considerations, a biological wastewater treatment system combining an SMBR with a downstream secondary digester was tested for the treatment of WDW.

MATERIALS AND METHODS

Biological wastewater treatment system

The wastewater treatment system consisted of four individual process units (Figure 1). The feed was pre-mixed and poured into reactor A, which operated as a balancing tank and supply of influent for reactor B. The influent feed was pumped into reactor B using peristaltic pump E (Watson Marlow 505S, Watson Marlow, Falmouth, UK). Reactor B was a 10-l SMBR, which was operated at a SRT of 30 days, and hydraulic retention time (HRT) of 12 hours to ensure a high feed-to-microorganisms ratio. Reactor B was inoculated with 10 $\%_{V/V}$ of methanogenic sludge from an anaerobic digester at Grahamstown municipal wastewater treatment works. The submerged UF membrane module consisted of four tubular ceramic membranes (surface area 55 cm^2, pore size 0.2 μm; Synexa Life Sciences, South Africa). During filtration, the permeate was withdrawn into reactor C (permeate balancing tank) using pump F (Watson Marlow 505S), and then fed to reactor D (the secondary digester) every 48 hours using pump G (Watson Marlow 505S), with outlet I open to drain the overflowing supernatant. Reactor D was a 10-l low rate digester with a 48-hours HRT and 8-days SRT, which was inoculated with sludge from reactor B on day 22 of the study using pump H (Watson Marlow 505S).

During the start-up period, reactor A was filled with deionised water and this was continuously pumped into reactor B for 2 days to deplete all internal carbon sources of the methanogenic sludge in the reactor. Then the study was initiated by feeding reactor B with WDW from Olafbergh Distilleries (Worcester, South Africa) diluted to 30 % with deionised water (v/v). The feed pH was buffered with 1000 mg/l $CaCO_3$ and 1000 mg/l K_2HPO_4 (both AnaLar grade, Merck Chemicals (Pty) Ltd, Johannesburg) for the first 10 days. On day 12, these concentrations were increased to 8000 mg/l $CaCO_3$ and 4000 mg/l K_2HPO_4 (see Results and Discussion) and 50 mg/l $Fe(NO_3)_3$ (AnaLar grade, Merck) was added as a micronutrient, based on previous work [10]. The experimental study lasted 30 days. Samples were collected every 48 hours from reactors A, B, C and D and analysed to determine values of selected operation parameters.

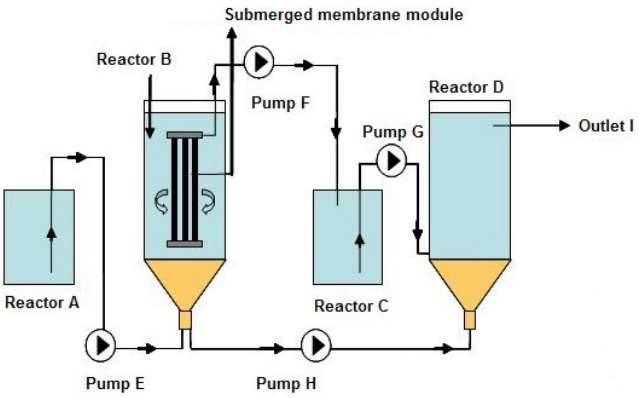

FIGURE 1 - The biological wastewater treatment system used in the study, showing the flow paths between reactors A through D. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

Sample analysis

Several parameters in all four reactors were monitored, including pH and concentrations of COD_S, phosphate, nitrate, ammonia, total phenolics as phenol equivalents and total volatile fatty acids (VFAs). All measurements were performed off-line and after centrifugation of supernatant samples. Measurements of pH were made using a Cyber-

scan 2500 pH-meter (Eutech Instruments, Johannesburg, South Africa). Colorimetric reagent test kits (Merck) based on the principles of *Standard Methods* [11] were used to measure concentrations of COD_S (Spectroquant reagent test 14538/9 analogous to *Standard Method* number 5220-D), nitrates (14773, analogous to 4500-NO_3-E), phosphate (14543, analogous to 4500-P-E), and ammonium (14752, analogous to 4500-NH_3-F). Total VFAs were determined according to a standard titration method [12].

The total concentrations of phenolics in individual samples were measured using a modified version of the Folin-Ciocalteau reaction [13, 14]. For individual samples, 100-µl aliquots were mixed with 1.6 ml of deionised water. The mixture was vortexed for 30 seconds and 250 µl of Folin-Ciocalteau reagent (Merck) added. After vortexing for 30 seconds, 1.5 ml of aqueous solution of Na_2CO_3 (100 g/l; Analar grade, Merck) was added to the mixture and it was vortexed again for 30 seconds. The mixture was then diluted to 10 ml with deionised water in volumetric glassware and incubated at 20 °C in the dark for 60 minutes. The total concentration of phenolics was then determined by measuring the absorbance of the sample at 765 nm, and converting it to phenol equivalents (mg/l). The calibration curve was measured under identical conditions as the samples, and using phenol (Analar grade, Merck) as the standard. All absorbance measurements were performed on a multi-wavelength multi-well plate reader (Power-WaveX, Bio-Tek Instruments Inc., Winoski, VT).

Removal efficiencies of COD_S were calculated using equations (1) and (2):

$$COD_{\text{removal}}(SMBR;\%) = 100 \times \left(1 - \frac{COD_{\text{permeate}}}{COD_{\text{feed}}}\right) \quad (1)$$

$$COD_{\text{removal}}(total;\%) = 100 \times \left(1 - \frac{COD_{\text{effluent reactor D}}}{COD_{\text{feed}}}\right) \quad (2)$$

where:

COD_{removal} = COD_S removal efficiency in the respective part of the system (%)

COD_{permeate} = COD_S in reactor C (mg/l)

COD_{feed} = COD_S in reactor A (mg/l)

$COD_{\text{effluent reactorD}}$ = COD_S of final effluent leaving reactor D (mg/l)

RESULTS AND DISCUSSION

Degassing of wastewater treatment systems contributes significantly to the commissioning costs of a full-scale system, so avoiding it and establishing anaerobic conditions during operation without prior degassing might lead to a decrease in operation costs of the system presented in this study. Consequently, neither the feed nor the contents of any of the reactors were degassed prior to the test period. The pH values inside the reactors over 30 days of system operation are shown in Figure 2. During days 0-10, pH values in all four reactors ranged from 4.38 to 6.37, which is outside of the methanogenic range and indicated the need for better pH buffering. The pH in all four reactors increased after increasing the feed concentrations of $CaCO_3$ to 8000 mg/l, and K_2HPO_4 to 4000 mg/l, respectively on day 12, after which those concentrations were maintained for the remainder of the experiment.

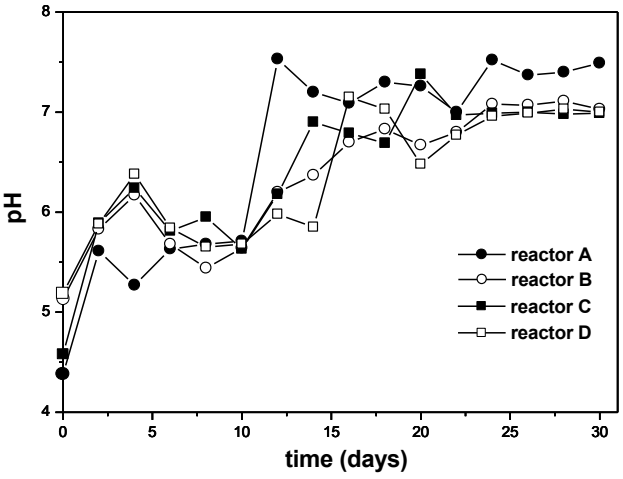

FIGURE 2 - pH of the supernatant in the individual reactors of the bioreactor system as function of time of operation. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

Changes in pH were related to the concentrations of VFAs, shown in Figure 3. Organic material with MW <30 000 Daltons passes through the ceramic membranes with pore diameters of 0.05-0.20 µm [9]. Major components of total VFAs (e.g. acetate, propionate and butyrate), therefore, passed through the UF module inside reactor B. Also, major changes in VFA concentrations that have direct effects on the process performance can be expected to occur in reactors B and D. Based on the above-mentioned facts, the concentrations of VFAs are shown for these two reactors only.

The initial total VFA concentrations were 730 mg/l in reactor B (SMBR) and 820 mg/l in reactor D (secondary digester). After 2 days of operation, the concentrations of VFAs in reactor B dropped to 273 mg/l and in D increased to 1458 mg/l. Taking the pH values for that time interval into account, it appeared that conditions in the system were not yet favourable for methanogenesis. The initial concentrations of oxygen might have caused cell lysis of methanogenic microflora in reactor B, leading to diffusion of enzymes and other cell components into the permeate in reactor C, and then into reactor D. As a result, the concentration of VFAs in reactor D increased, while the lack of active biomass in reactor B might have led to the lack of hydrolysis and acidogenesis, i.e. lack of substrates for VFA production.

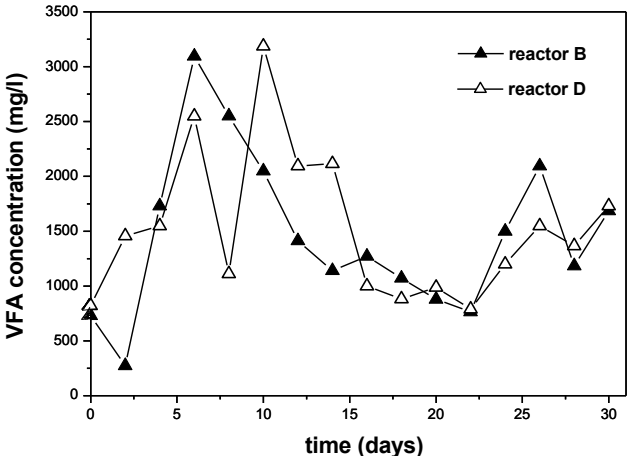

FIGURE 3 - Volatile fatty acid concentrations in reactors B and D of the bioreactor system as function of time of operation. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

From days 2 to 10, the concentrations of VFAs increased to peak values of 3100 mg/l in reactor B on day 6, and 3190 mg/l in reactor D on day 10. If anaerobic conditions were already established, then degradation of readily available WDW compounds, such as carbohydrates or proteins, might have led to stimulation of the hydrolysis and acidogenesis pathways of anaerobic metabolism in reactor B, and thus to an increase in the VFA concentrations observed. After addition of 8000 mg/l of $CaCO_3$ and 4000 mg/l of K_2HPO_4 to the feed from day 12 onwards, pH values in all four reactors stabilised and ranged from 5.98 for reactor D on day 14 to 7.53 in reactor B on day 12. The concentrations of VFAs decreased to minimum values of 764 mg/l in reactor B and 790 mg/l in reactor D on day 22. Decreases in the concentrations of VFAs might have indicated stabilisation of anaerobic conditions inside the system [7]. From this point until the end of the operation period, the concentrations of VFAs ranged from 1200 to 2100 mg/l in both reactors. Soluble COD values of the second batch of WDW used for the feed did not vary significantly from the first batch. As a result, the influence of the changes in composition of WDW on the concentrations of VFAs in reactor B and reactor D could be deemed negligible. Changes in the composition of the microbial community in reactor B and D could, therefore, explain oscillations in the concentrations of VFAs. As individual components of the WDW became depleted sequentially, different portions of the microbial community became active. Therefore, a breakdown of different higher MW compounds from the WDW led to successive accumulation of different concentrations of VFAs in both reactors B and D. At the same time, death of some of the active biomass from reactor B might have provided additional nutrients to the sludge in reactor D.

The average COD_S of the feed in reactor A was 4840 (± 950) mg/l. The COD_S of the original sample of WDW was approximately 16100 mg/l. This is on the lower end of the range for previously published data [8]. The SMBR and the total system COD_S removal efficiencies were calculated to establish whether secondary digestion improved the COD_S removal. During the initial 22 days of operation, before the secondary digester was inoculated, the SMBR COD_S removal efficiency fluctuated widely, between 0 and 76 % (Figure 4). From days 6 to 10, the removal efficiency ranged from 0 to 25 %, which coincided with the maximum concentrations of VFAs and pH values outside the methanogenic range. The system had probably not reached stability for anaerobic removal of COD_S, possibly because of residual oxygen concentrations in the system. A similar trend was observed for the total removal of COD_S, which ranged from 0 to 42 % for the same time period, suggesting that COD_S removal was only marginally caused by reaction of the individual components of the WDW and the methanogenic sludge lysis.

After pH stabilisation using 8000 mg/l $CaCO_3$ and 4000 mg/l K_2HPO_4 began on day 12, COD_S removal in the SMBR reached 76 % on day 14, while the total COD_S removal increased to 72 % on day 16. The lag phases between day 12, when the pH stabilisation was introduced, and the actual peak values of COD_S removals could have been caused by the lag phase of the respective microflora. After the maximum removal had been reached for reactor B (SMBR), there was a sharp drop in COD_S. removal efficiencies. After inoculation of reactor D (secondary digester) on day 22, the total COD_S removal remained higher than that attained by the SMBR alone. This could be explained by the fact that prolonged biomass / substrate contact time was required for the removal of recalcitrant WDW components in reactor D. The two batches of WDW introduced into the feed between days 0-13 and 14-30 showed limited variability in the concentration of total soluble organic compounds as indicated by the lower degree of variability in the COD_S concentrations in comparison to other monitored parameters. The secondary digester (reactor D), together with the pH stabilisation of the system, led to improved and more constant removal efficiencies for COD_S.

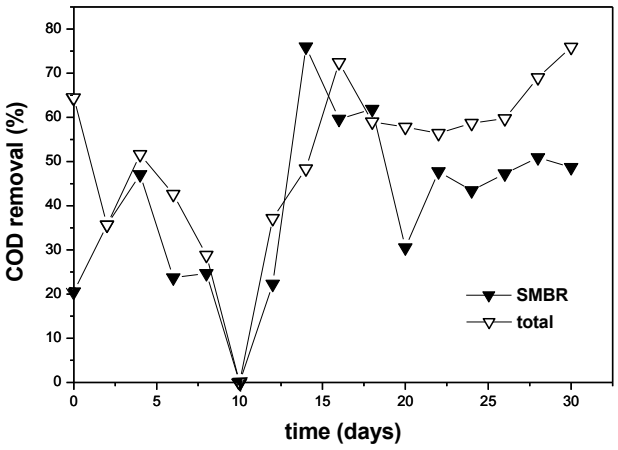

FIGURE 4 - Removal efficiency of COD_S in the SMBR and the total system as a function of time. SMBR = reactor B, total = final effluent from secondary digester (reactor D).

The concentrations of the total phenolic compounds in the system are shown in Figure 5. The concentration of phenolic compounds in the raw WDW in the system varied widely, from 29 mg/l to 503 mg/l phenol equivalents. Several methods have been used to measure the total phenol content (or total polyphenol content) of wines and WDWs [9, 15]. The Folin-Ciocalteau reaction has been shown to be sensitive towards phenol (with one hydroxyl group), as well as towards tannic acid (with 11 phenolic hydroxyl groups) [16]. Molecular weight and the respective value of the molecular absorption coefficient of the used standard influences the measured concentrations of the total phenolic compounds in a particular WDW sample. The results obtained in this study are comparable with some previously reported values [9], but lower than others [17], both of which used gallic acid as the standard. Gallic acid's MW of 170.89 g/mol is higher than that of phenol (94.11 g/mol). When expressing the results in mg/l, the slope of a calibration curve of the Folin-Ciocalteau method based on gallic acid as a standard will be lower than a curve based on phenol as a standard. Consequently, concentrations calculated based on gallic acid will be higher than those calculated on phenol basis. Since the precise mechanism of the reduction of the Folin-Ciocalteau reagent by phenolic compounds is not known, the molar absorption coefficients of standards will be dependent on the particular protocol of the Folin-Ciocalteau method used in the measurements [15]. Published results, therefore, differ based on the exact protocol followed, as well as the phenolic compound standard used for calibrations. The consequence of this is that comparison between published values of the total phenols/polyphenols measured by the Folin-Ciocalteau method should be carried out with caution.

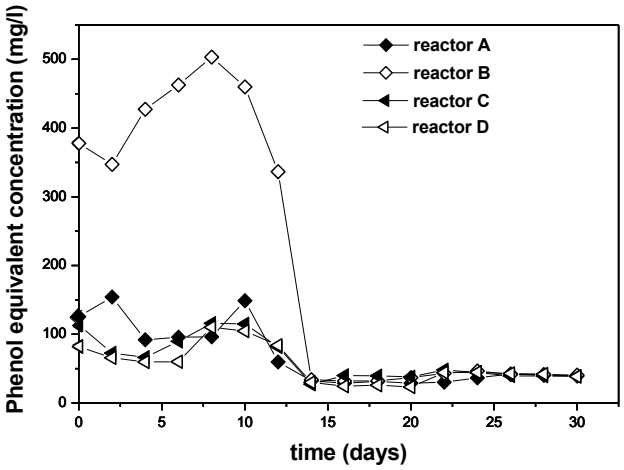

FIGURE 5 - The total concentration of phenolic compounds in the bioreactor system expressed in phenol equivalents as a function of time. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

The total concentrations of phenolics in reactor A ranged from 92 to 154 mg/l during days 0 to 10. After the increase in the concentrations of $CaCO_3$ and K_2HPO_4 on day 12, the total concentration of phenolics in reactor A decreased to 59 mg/l. From day 13 onwards, a new batch of WDW was used for the preparation of the feed in reactor A. There was an immediate decrease in the total concentration of phenolic compounds in reactor A to 29 mg/l, and the values ranged from 30 to 42 mg/l for the remainder of the experiment. The total concentration of phenolics in reactor B (SMBR) ranged from 347 to 503 mg/l between days 0 and 8 of operation. After that, a slight decrease in concentration was recorded with 337 mg/l recorded on day 12, coinciding with increasing concentrations of $CaCO_3$ and K_2HPO_4. After application of the second batch of WDW for feed, there was a sharp decrease in the total concentration of phenolics in reactor B, with in-dividual values ranging from 31 to 46 mg/l.

The total concentrations of phenolics in reactor C (permeate) and reactor D (secondary digester) followed similar trends as reactors A and B. For reactor C, the total concentrations of phenolics ranged from 66 to 116 mg/l between days 0 and 10, decreased to 82 mg/l on day 12, and decreased to 27 mg/l on day 14, when the second batch of WDW was used. The total concentration of phenolics in reactor C ranged from 37 to 48 mg/l for the remainder of the experiment. In the secondary digester (reactor D), the total concentrations of phenolics ranged from 60 to 111 mg/l between days 0 and 10, decreased to 84 mg/l on day 12, decreased further to 27 mg/l on day 14, and ranged between 23 and 45 mg/l from then onwards.

During the initial 10 days of operation, the total concentrations of phenolics fluctuated in all four reactors. This can be explained by mutual transformations of the individual molecules with phenolic groups and detectable by the Folin-Ciocalteau method. These changes could have led to alterations in the number of hydroxyl groups available for the Folin-Ciocalteau reaction, i.e. the more phenyl hydroxyl groups available for the reaction, the higher the concentrations measured. In reactors A (feed), C (permeate) and D (digester) the phenolic molecules probably originated solely from the WDW. However, the concentrations in reactor B (SMBR) were much higher than in the other three reactors during this time. This observation could be explained through the release of additional phenolics from the biomass of the methanogenic sludge that was used as inoculum for reactor B. Increases in the total concentrations of phenolics were not observed in reactor D after its inoculation with mixed liquor from reactor B on day 22. Therefore, the phenolic compounds originating from the methanogenic sludge biomass were degraded by the time reactor D was inoculated.

Changes in the total concentrations of phenolics in the feed in reactor A could be explained by intrinsic activity of native microorganisms present in the WDW. From day 10 to 14, the total concentration of phenolics in the system started to decrease, probably due to the depletion of the readily biodegradable phenolic compounds by the

sludge biomass. After day 14, all the phenolic compounds remaining/ introduced into the system were refractory and resistant to biodegradation, because there were no significant differences between the concentrations of phenolics in all four reactors. Experiments are currently underway to determine changes in MW distribution of phenolic compounds during biodegradation in the system to ascertain their fate in more detail.

The concentrations of nitrogen compounds over time in all four reactors are shown in Figures 6 (nitrates) and 7 (ammonium). The concentrations of nitrates in reactor A ranged from 34.0 to 94.0 mg/l from day 0 until day 12. After the application of the second batch of WDW for feeding the reactor system and the increase in concentration of $CaCO_3$ and K_2HPO_4, the concentrations decreased to the range 8.1-13.7 mg/l from day 14 onwards. The concentrations of nitrates in reactor B ranged from 7.6 to 29.3 mg/l during days 0-10, and decreased to 6.6 mg/l on day 12. After day 14, nitrate concentrations decreased to values ranging from 6.3 to 15.5 mg/l.

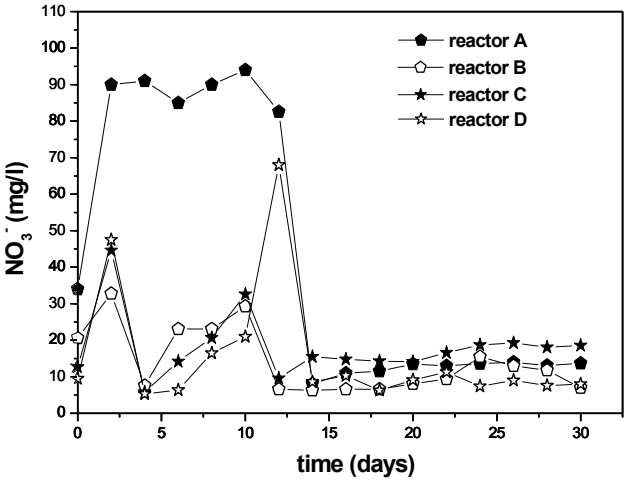

FIGURE 6 - Concentration of nitrates in the system as function of time. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

The decrease in nitrate concentrations between days 0 and 10, in combination with the rate of oxygen mass transfer in the system (data not shown), demonstrated that anaerobic conditions were established in the bioreactor, since it was effective in reducing the nitrate concentrations in WDW by denitrification and dissimilatory reduction of nitrate to ammonium. The concentrations of nitrates in reactor C (permeate) fluctuated from 5.6 to 44.0 mg/l between days 0 to 12. After the application of the second batch of WDW for feeding the system and the increase in concentrations of $CaCO_3$ and 4000 mg/l on day 14, nitrate concentrations decreased to 9.6-19.3 mg/l. The nitrate concentrations in reactor D fluctuated from 5.3 to 68.0 mg/l from days 0 to 12 and were 8.0-11.3 mg/l after day 14.

The concentrations of ammonium in the feed in reactor A ranged from 0.05 to 0.34 mg/l during days 0 to 12 (Figure 7). After day 14, ammonium concentrations decreased to 4.4-7.0 mg/l. The ammonium in reactor B fluctuated between 1.7 and 9.8 mg/l from day 0 to 12, peaking at 9.8 on day 4. After day 14, the ammonium concentrations were 7.6-16.0 mg/l. The concentrations in reactor C were 1.3-8.0 mg/l during days 0 to 12, and 5.4-11.8 mg/l from day 14 onwards. Reactor D (secondary digester) ammonium was 3.0-12.8 mg/l during days 0 to 12, and 18.4-38.0 mg/l from day 14 onwards. Ammonium was observed to accumulate in reactor D after pH stabilisation and introduction of the new batch of WDW into the feed. This indicates that denitrification and dissimilatory assimilation of nitrates (as the terminal acceptor of electrons in anaerobic respiration) were taking place, suggesting that anaerobic (anoxic) conditions were established in the system, i.e. residual oxygen was eliminated from the system within the first 14 days of operation.

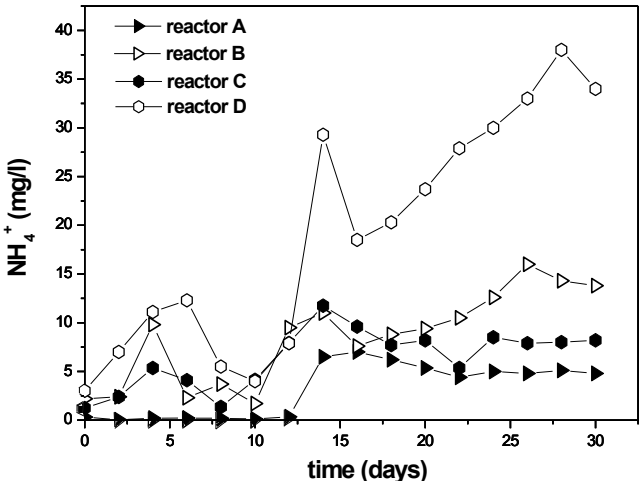

FIGURE 7 - Concentration of ammonium in the system as function of time. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

Concentrations of phosphates in the system were virtually constant in all four reactors over the duration of the experiment, because of the addition of K_2HPO_4 at 1000 or 4000 mg/l into the feed for pH buffering. The overall average concentration of phosphates in the bioreactor system was 100 (± 20) mg/l (data not shown). Possibilities of effluent applications and decrease in concentrations of nitrates and phosphates will be discussed in Part II [18] of this study.

CONCLUSIONS

The experimental system eliminated up to 76 % of the COD_S in the WDW. The residual COD_S levels in the system effluent were ≈1100 mg/l. Secondary digestion downstream of the SMBR, together with pH buffering using 8000 mg/l $CaCO_3$ and 4000 mg/l K_2HPO_4 stabilised COD_S removal. However, the effluent quality did not meet

the standards required for use of the treated wastewater for crop irrigation. To meet regulatory requirements and utilise the efficiency of the bioreactor system in WDW treatment, fungal pretreatment of the raw wastewater was conducted, and the experiments with the system were repeated to further decrease the effluent COD_S (see Part II of this study [18]). Wine distillery wastewater showed variable composition in the concentrations of nitrates, ammonium and the total concentrations of phenolics. Readily biodegradable phenolics were probably removed from the WDW, and it is likely that only recalcitrant compounds with hydroxyl groups passed through the experimental system without significant decrease in their concentrations. Molecular weight changes of the phenolic compounds are currently being evaluated to elucidate the nature of the fate of compounds with different numbers of phenolic hydroxyl groups in the molecule. Additional treatment for the removal of phosphates should be developed to allow for effluent use in irrigation or other applications.

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SUBMERGED MEMBRANE BIOREACTOR AND SECONDARY DIGESTION IN THE TREATMENT OF WINE DISTILLERY WASTEWATER. PART II: THE EFFECT OF FUNGAL PRE-TREATMENT ON WINE DISTILLERY WASTEWATER DIGESTION.

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This paper is dedicated to the memory of Winston Leukes

SUMMARY

The effect of fungal pre-treatment using *Trametes pubescens* on the anaerobic digestion ultrafiltration treatment of wine distillery wastewater (WDW) was studied. The downstream biological treatment system, consisting of four individual reactors, was operated for 30 days. pH buffering was achieved by mixing the pre-treated system feed with $CaCO_3$ and K_2HPO_4; this proved significant for optimum performance of the system in removal of soluble chemical oxygen demand (COD_S). The experimental system was shown to eliminate an average of 86 (± 4) % of COD_S present in the pre-treated WDW. Treatment in a submerged membrane bioreactor (SMBR) and subsequent secondary digester, together with pH buffering using $CaCO_3$ and K_2HPO_4, led to the stabilisation of COD_S removal. The residual COD_S levels in the final effluent were approximately 400 mg/l, significantly lower than the concentrations observed when treating raw WDW, indicating that fungal pre-treatment might have provided additional nutrients for removal of recalcitrant components of the wastewater. The resulting effluent of the system is rich in nitrates and phosphates. Together with the residual organic content it might be used as a fertiliser. Alternatively, if water management of the wine distillery is an issue, a membrane process, such as reverse osmosis or nanofiltration could be applied to bring the parameters of the water to meet the technological needs.

KEYWORDS: Anaerobic digestion, chemical oxygen demand (COD), polyphenols, submerged membrane bioreactor (SMBR), volatile fatty acids (VFAs), wine distillery wastewater (WDW).

INTRODUCTION

The distillation of wine and some waste biological materials such as molasses and sugar cane generates great volumes of wastewater known as wine distillery wastewater (WDW) or vinasse [1]. The pH values of WDWs range from 3.5 to 5.0, depending on the raw material distilled and the chemical oxygen demand (COD) of WDW has been reported to be high as 100 g/l [2]. Several problems have been encountered during biological treatment of WDW, which are linked to its high toxicity and partial inhibition of biodegradation due to the presence of phenolic compounds [3], thus giving WDW antibacterial activity [4]. These problems have resulted in a number of investigations into various pre-treatment / treatment methods, such as chemical oxidation [5]; anaerobic digestion in different reactor configurations [1,6], use of activated sludge systems [7] and dilution of WDW prior to anaerobic digestion [8].

Fungi have been attracting a growing interest for the biological treatment of heavy metals, inorganic and organic compounds [9]. For WDWs, it was shown that fungal pre-treatment under aerobic conditions facilitates the reduction of phenol concentrations by 51 - 100 %; to induce decolourisation of 31-100 %; and reduce the five day biochemical oxygen demand (BOD_5) by up to 85.4 % [9]. In this study a fungal pre-treatment step followed by a submerged membrane bioreactor (SMBR) and secondary di-

gester were used in series to investigate the biological treatment of WDW to obtain reusable water. Fungal pre-treatment of WDW was tested for improvement of the performance of the biological treatment system used in Part I [10] of this study.

MATERIALS AND METHODS

Biological wastewater treatment system

For fungal pre-treatment of WDW, a bubble-lift bioreactor was constructed from fibreglass (shown in Figure 1). The total volume of the bioreactor was 102 l with the following dimensions: height 2.3 m (of which 1.2 m was the lower, V-shaped part), and had a square cross-section with internal dimensions of 0.5 × 0.5 m. The bioreactor was equipped with two ports at the bottom, one for aeration to achieve oxygenation and mixing, and the other port for withdrawal of samples. All fittings were made of PVC and fixed in place with silicone sealant. The bioreactor was sterilised by pumping 5 l of an aqueous solution of formaldehyde (4 $\%_{W/V}$) through the system. After sterilisation, the bioreactor was rinsed twice with 2 l of autoclaved distilled water, and 5 l of autoclaved distilled water was then circulated around the reactor system overnight to rinse out any residual sealant components and leak test the system. In the meantime, an inoculum of *Trametes pubescens* for fungal pre-treatment was grown in a liquid medium of the following composition: malt extract (2 $\%_{W/V}$), glucose (1 $\%_{W/V}$) and yeast extract (0.2 $\%_{W/V}$). The fungus was incubated in 500 ml erlenmeyer flasks placed on a benchtop shaker (Labcon SPL15, Laboratory Marketing Services (Pty) Ltd., Johannesburg) shaking at 150 rpm at 28 ºC and then harvested in the late exponential phase of growth (based on preliminary experiments; data not shown).

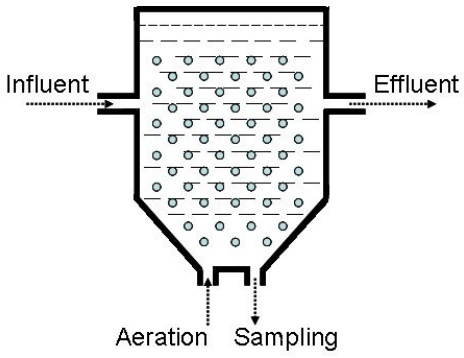

FIGURE 1 - The bioreactor used for fungal pre-treatment of wastewater with floating *T. pubescens* hyphae.

For the pre-treatment, the fungal bioreactor was filled with 45 l of autoclaved WDW with the pH adjusted prior to autoclaving to 5.3 with solid Na_2CO_3 (AnaLar grade, Merck Chemicals (Pty) Ltd, Johannesburg). The bioreactor was inoculated with 5 l of the *T. pubescens* inoculum, and fungal pre-treatment was conducted at 25 ºC for 16 days. Upon conclusion of the pre-treatment, the contents were digested inside the SMBR/ secondary digester system described fully in Part I of this study. Briefly, the biological treatment system comprised a balancing tank (reactor A) containing the influent (which consisted of 30 $\%_{V/V}$ WDW and 70 $\%_{V/V}$ deionised water), a SMBR (reactor B), a permeate balancing tank (reactor C) and a secondary low rate digester (reactor D), from which the final effluent escaped via a weir. The SMBR contained a module of four tubular ceramic membranes (surface area 55 cm^2, pore size 0.2 µm; Synexa Life Sciences, South Africa). Flow rates from reactor A through B and C to D were controlled using peristaltic pumps (Watson Marlow 505S, Falmouth, UK). Conditions of the study and system operating parameters were the same as described in Part I, with one exception: for pH buffering, the concentrations of $CaCO_3$ and K_2HPO_4 were kept at 1000 mg/l for the whole duration of the study. This was a consequence of the higher pH of WDW after fungal pre-treatment (see Results and Discussion). Samples were taken from each of the four reactors comprising the system every 48 hours and analysed according to the methods set out in Part I.

RESULTS AND DISCUSSION

The prevailing pH values inside reactors A, B, C and D as a function of time of operation are shown in Figure 2. The individual values ranged from 7.13 to 9.08. Fungal pre-treatment was effective in reducing the extent of pH buffering required, as indicated by the concentrations of $CaCO_3$ and K_2HPO_4 added to the feed for buffering; these were maintained at 1000 mg/l during the whole 30 day operation of the system. Fungal pre-treatment also improved the stability of the system, as indicated by lower concentrations of volatile fatty acids (VFAs) compared to treatment of raw WDW [Part I]. Concentrations of VFAs as a function of time are shown for reactors B and D, and the data are shown in Figure 3. Examination of the molecular weight (MW) distribution of the organic material passing through the ceramic membranes (pore diameter of 0.05 µm) in the permeate suggested that VFAs passed through the membrane, out of reactor B into reactors C and D [11]. It is therefore reasonable to expect that there will be a mutual relationship between the concentrations of the VFAs in reactor B and reactor D.

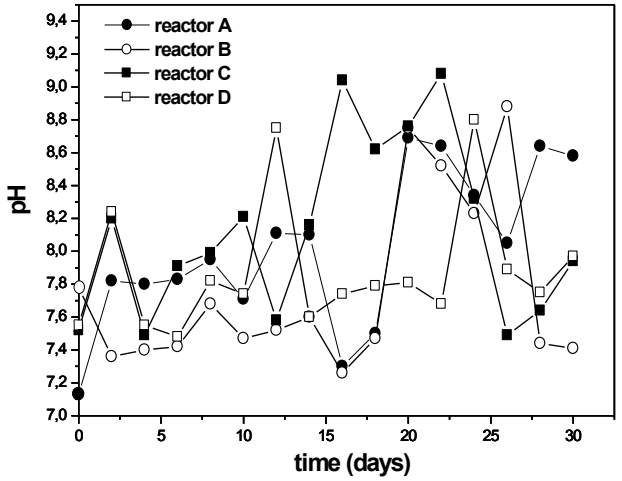

FIGURE 2 - Prevailing pH of the bulk liquid in the individual reactors of the experimental system as function of time of operation of the bioreactor system. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

As in Part I of this study, the major changes in VFA concentrations that have direct effect on the performance of the process were expected to occur in reactors B and D. Therefore the concentrations of VFAs are shown for these two reactors only (Figure 3). The initial VFA concentrations were 228 mg/l in reactor B (SMBR) and 547 mg/l in reactor D (secondary digester), respectively. The concentration of VFAs in reactor B increased to the maximum value of 548 mg/l from day 6 until day 8 of bioreactor system operation. VFA concentrations in reactor D fluctuated between 200 and 474 mg/l during the first 10 days of bioreactor system operation.

From days 10 to 12, the concentration of VFAs decreased in both reactor B (SMBR) and reactor D (secondary digester). In reactor B, the concentrations decreased from 365 to 63 mg/l, while the concentrations dropped from 274 to 51 mg/l in reactor D over the same period. From day 12 until the end of the experiment, the VFA concentrations fluctuated within the range 46 -91 mg/l in reactor B, and 27 - 82 mg/l in reactor D. Only a single batch of the fungally processed WDW was used in the study, and so the observed trends can be confirmed as a result of the WDW processing and not variability in feed composition. Autolysis of the fungal biomass might have provided additional nutrients for the methanogenic sludge used to inoculate the SMBR. As a result, it might have been easier for the sludge microorganisms to degrade components of WDW, as indicated by the lower residual VFA concentrations in the bioreactor system.

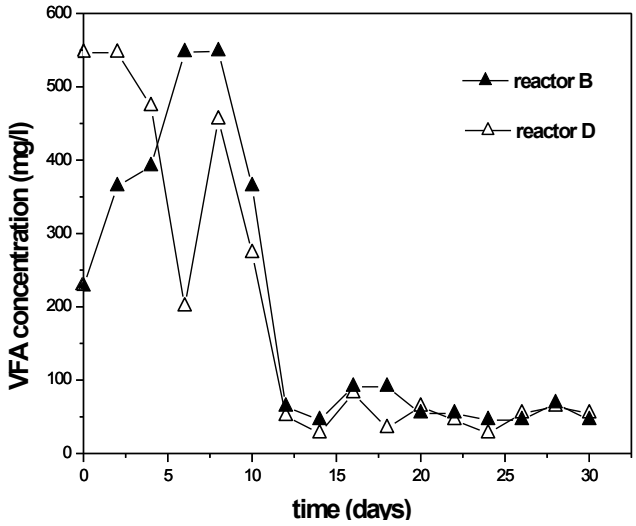

FIGURE 3 - Total VFA concentrations in reactor B (SMBR) and reactor D (secondary digester) as function of time.

The average COD_S of the feed was 4300 (± 1800) mg/l. This value is comparable to the feed used in the treatment of the diluted WDW without fungal pre-treatment [10], but the composition of the COD_S was different due to enrichment of the fungally pre-treated WDW with components of fungal biomass. Removal efficiencies of COD_S were calculated using the method described in Part I [10]. The COD_S removal efficiencies achieved by the SMBR and the total treatment system were calculated to establish whether secondary digestion improved the COD_S removal. The data are presented in Figure 4. Soluble COD removal efficiencies fluctuated in the SMBR: from days 0 to 4 the COD_S removal increased from 0 % to 47 %. After day 4, a sharp decrease to the minimum, 0 %, was recorded on day 10. This could have been caused by the release of an inhibitory lower molecular weight compound from the breakdown of higher molecular weight components of the mixed liquor in reactor B. Subsequently, an increase in COD_S removal for the SMBR was recorded, with the maximum value of 76 % on day 14. From day 16 to 30 the COD_S removal efficiencies for the SMBR fluctuated between 43 and 62 %. The total COD_S removal efficiency was equal to 0 % between days 0 and 2 of system operation. From day 2 onwards, COD_S removal stabilised and became practically independent of time, with the average value equal to 86 (± 4) %.

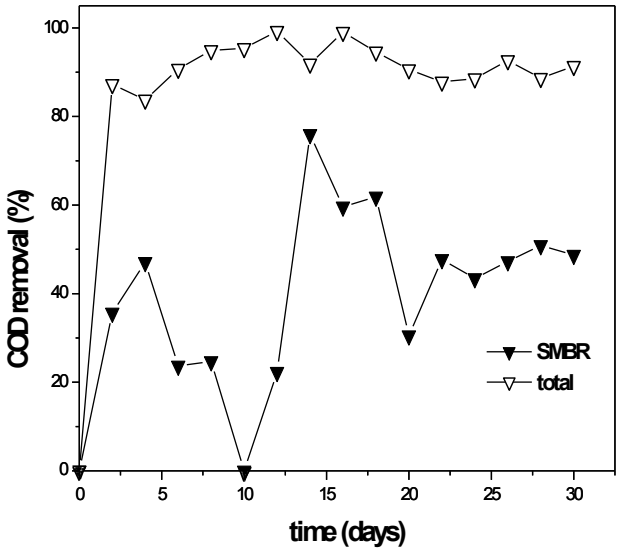

FIGURE 4 - Removal efficiency of COD_S in the SMBR and over the total treatment system as a function of time.

The lack of removal of COD_S by both the SMBR and the bioreactor system as a whole could be explained by the acclimation of the biomass to the new medium. The results indicated that a combination of fungal pre-treatment, SMBR and the secondary digester stabilise the extent of COD_S removal. Fungal pre-treatment and autolysis of fungal biomass probably provide additional nutrients required by the methanogenic sludge for the removal of WDW components, while the secondary digester prolongs the period of effective biodegradation, and thus increases the efficiency of COD_S removal. The residual COD_S levels were around 400 mg/l. These levels could be decreased by further treatment based on a membrane processes (see below).

The total concentrations of phenolic compounds in the bioreactor system as a function of time of operation are shown in Figure 5. The total concentration of phenolic compounds in the raw WDW in the bioreactor system ranged from 1 to 86 mg/l in phenol equivalents.

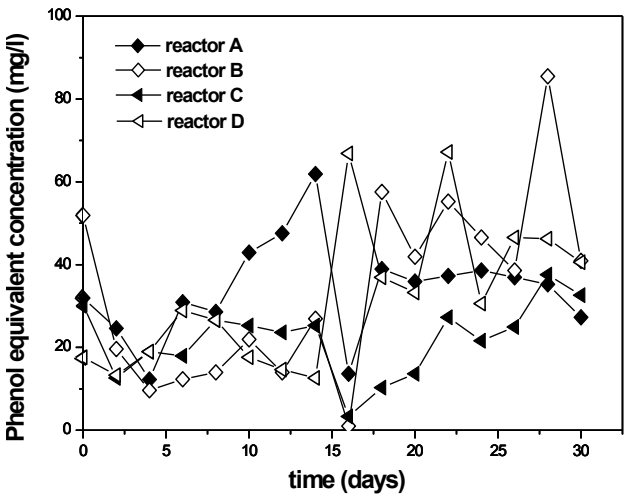

FIGURE 5 - The total concentration of phenolic compounds in the treatment system expressed in phenol equivalents as a function of time operation. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

The total concentrations of phenolics in reactor A fluctuated between 12 and 62 mg/l, and no clear trend with time could be established. At the beginning of the system operation, the total concentration of phenolics in reactor B was 52 mg/l. A decrease to 19 mg/l was recorded on day 2, and the values fluctuated between 9 and 27 mg/l from day 4 until day 14. After that a sharp decrease to 1 mg/l was recorded on day 16, which constituted the minimum value in reactor B and the whole bioreactor system for the entire duration of the experiment. The total concentration of phenolics ranged from 39 to 86 mg/l in reactor B from day 17 onwards.

The total concentrations of phenolics in reactor C fluctuated between 12 and 30 mg/l, and no clear trend with time could be established from day 0 until day 14 of system operation. After that, a sharp decrease to 3 mg/l was recorded on day 16, which constituted the minimum value of the total concentration of phenolics in reactor C. For the remainder of the experiment, the individual values were 10 - 38 mg/l. The total concentrations of phenolics in reactor D fluctuated within the range 13 - 67 mg/l, and no clear trend with time could be established during the entire study period. The trends in the total concentration of phenolics in the individual reactors of the bioreactor system indicate a complex series of (mutual) transformations of phenolic compounds in the experimental system. The total concentrations of phenolics did not vary significantly with time in the experimental system, but the molecular structure of particular compounds underwent changes in the system. A more detailed study of the unit reactions is currently underway.

The concentrations of nitrogen compunds as a function of incubation time in all four reactors of the bioreactor system are shown in Figure 6. The concentrations of nitrates in reactor A ranged from 4.6 to 33.8 mg/l. The concentrations of nitrates in reactor B fluctuated between 5.3 and 12.8 mg/l from day 0 until day 16. A sharp increase in the nitrate concentration to 41.4 mg/l was recorded on day 18, while the nitrate concentrations fluctuated between 13.7 and 33.4 mg/l for the remainder of the experiment. The concentrations of nitrates in reactor C fluctuated between 6.9 and 13.6 mg/l from day 0 until day 16.

A peak in the nitrate concentration of 24.8 mg/l was recorded on day 18, while the nitrate concentrations were in the range 14.1 - 25.2 mg/l for the remainder of the experiment. The concentrations of nitrates in reactor D fluctuated between 4.9 and 9.7 mg/l from day 0 until day 16.

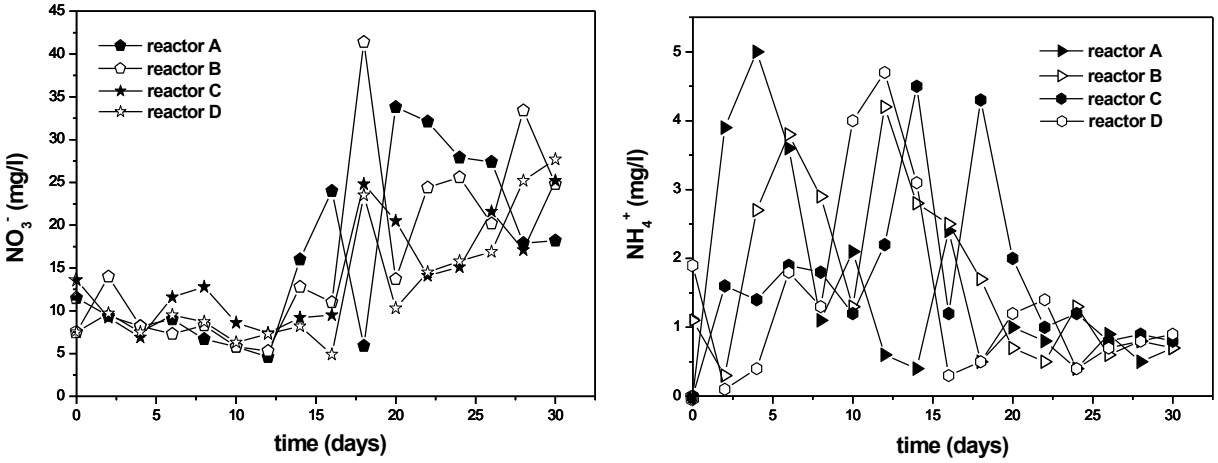

FIGURE 6 - Concentration of nitrates (6a,★ ⬟) and ammonium (6b, ▶ ⬢) in the system as function of incubation time. A = influent tank, B = SMBR, C = permeate balancing tank, D = secondary digester.

A sharp increase in the nitrate concentration, occurred peaking at 23.5 mg/l on day 18. From day 20 onwards, nitrate concentrations increased from 10.3 to 27.8 mg/l. Nitrates were accumulating in the bioreactor system after day 18 of bioreactor operation. This could have been caused by the release of nitrates from the residual fungal components in the feed. No systematic trends in the concentrations of ammonium in all four reactors of the bioreactor system were observed. The values of ammonium concentrations fluctuated with time, and ranged from 0 to 5 mg/l. Based on the data for nitrate and ammonium concentrations, no effective removal of nitrates or ammonium could be observed by SMBR treatment or digestion after fungal pre-treatment of WDW.

Concentrations of phosphates in the system were virtually constant in all four reactors of the bioreactor system throughout the duration of the experiment, because of the addition of K_2HPO_4 at 1000 mg/l to the feed stream for pH buffering. The average concentration of phosphates in the bioreactor system was 107 (± 16) mg/l. Additional treatment of the bioreactor system effluent would be required to meet the water quality guidelines for use in crop irrigation [12]. Membrane processes have been successfully used for treatment of wastewaters [13]. Na_2HPO_4 was present in a simulated wastewater at levels comparable to the residual concentrations in this study. Based on the process used for phosphate removal, the concentrations of phosphates in the wastewater could be reduced to 1.4 - 36.4 mg/l [12]. These levels would allow for the application of the treated effluent as vineyard irrigation water, since phosphates are highly immobile in soils. As a result, the phosphate molecules do not percolate down the soil profile and the risk to the groundwater is reduced to a minimum. The residual COD levels in the final effluent could be reduced by nanofiltration, and the nitrates could be further treated using reverse osmosis [14]. The financial aspect of the resulting process design would the overriding concern in choosing the optimum solution to the additional treatment. Further research on this matter is currently in progress.

CONCLUSIONS

The experimental system has been shown to eliminate up to an average of 86 (± 4) % of COD_S present in the WDW, after fungal pre-treatment. Secondary digestion, together with pH buffering using 1000 mg/l of $CaCO_3$ and K_2HPO_4, led to the stabilisation of COD_S removal. The residual COD_S levels were 400 mg/l, significantly lower than the concentrations measured in Part I of the study (1100 mg/l), indicating that fungal pre-treatment might have provided additional nutrients for removal of recalcitrant components of the wastewater. The resulting effluent was rich in nitrates and phosphates. Together with the residual organic content it might be used as a fertiliser. Alternatively, if water management of the wine distillery is an issue, a membrane process such as reverse osmosis or nanofiltration could be applied to bring the parameters of the water to within acceptable guidelines.

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STUDIES ON REMOVAL OF AMMONIUM IONS FROM SYNTHETIC AQUEOUS SOLUTIONS AND FIELD LEACHATE SAMPLES USING CLINOPTILOLITE

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SUMMARY

The aim of this study is to investigate the capacity of the clinoptilolite for ammonium nitrogen removal from both synthetic solutions and solid waste leachate. Initially, studies were performed in batch reactors using synthetic ammonia nitrogen solutions, in order to establish the applicability of both kinetic sorption rate models as well as the adsorption isotherm models. Subsequently, column studies were carried out with leachate collections from the field to establish the breakthrough time intervals and concentrations, specifically for ammonia nitrogen.

The effect of clinoptilolite sample pre-treatments on removal efficiency was also investigated in a series of batch-wise experiments. The Langmuir, Freundlich and Tempkin equations, which are in common use for describing sorption equilibrium of wastewater-treatment applications, were applied to the experimental data. The sorption kinetics were tested for pseudo-first order, pseudo-second order, Elovich and intra-particle diffusion models, and the rate constants of all kinetic models were calculated and compared. The best correlation coefficient was obtained using the pseudo second-order kinetic model, which shows that ammonium uptake process followed the pseudo-second order rate expression.

The results obtained clearly showed that the zeolitic tuffs of the Çankiri-Çorum Basin in Turkey can be used for removal of ammonium ions both from synthetic aqueous solutions and leachate.

KEYWORDS:
Ammonium removal, leachate, clinoptilolite.

INTRODUCTION

One of the main concerns associated with land-filling of municipal waste is related to the discharge of leachate into the environment, which causes serious pollutions to water reservoir, soil and other components of the natural environment. These effluents contain large amounts of organic matter (both biodegradable and non-biodegradable), ammonia-nitrogen, heavy metals, as well as chlorinated organics and inorganic salts. The discharge of landfill leachate to waterways can result in water quality degradation, such as eutrophication, depletion of dissolved oxygen, chemical toxicity and salinity. This discharge has also been identified as potential source of groundwater contamination, as it may percolate through soil and subsoil. High ammonium ion concentrations originating from landfill leachate decrease oxygen contents of surface water and harm fish and zooplankton [1]. The discharge of water containing ammonium ions is coming under increasingly strict concentration limits. The council of the European Community has set a guide level of 0.5 mg/L of ammonium [2]. In Turkey, the permissible ammonium levels in surface waters vary from 0.2 mg/L to 2 mg/L [3].

There are several biological and physical methods that can be used for ammonium removal from effluents, such as chemical precipitation [4], air stripping [5], nitrification-denitrification [6], ion exchange [7] and photo-oxidation [8]. Zeolites are known to be appropriate material for removing ammonium ions from wastewater. The zeolite minerals are easy to obtain with low prices, compared to the other adsorbents (activated carbon, ion exchange resin etc.), and their exchangeable ions (Na^+, Ca^{2+}, Mg^{2+}, K^+) are relatively harmless [9].

Zeolite is a type of hydrous aluminum-silicate belonging to tectosilicates, in which the SiO_4 tetrahedron forms a 3-dimensional cage-like framework. In zeolite structure, some Si^{4+} ions are replaced by Al^{3+}, resulting in a net-negative charge that needs to be balanced by exchangeable cations. Therefore, naturally formed zeolites have high cation exchange capacity [10, 11]. Clinoptilolite is the most

abundant natural zeolite and has the chemical formula $Na_{0.1}K_{8.57}Ba_{0.04}(Al_{9.31}Si_{26.83}O_{72})$ 19.56 H_2O. It is occurring in the earth crust, and may serve as a cost-effective sorbent for the removal of ammonium ions from wastewater. Although clinoptilolite is commonly used as a cation exchanger, it can be modified in order to enhance sorption of anionic and organic compounds [12]. Several works relating to ammonium removal using clinoptilolite have been already performed [13-16].

The aim of this study is to investigate the removal of ammonium-nitrogen from leachate using clinoptilolized tuffs from Cankiri Çorum Basin, which may be able to remove the Cs, Sr, Cu, Pb, and Cd ions according to previous studies [17-19], and determine the effects of experimental parameters on removal efficiency. The batch and column experiments were conducted for the removal of ammonium ions at different experimental conditions. These parameters would be useful in understanding of ammonium removal from an aqueous environment by clinoptilolite.

MATERIALS AND METHODS

Characterization of clinoptilolite

The clinoptilolite samples used in this study were collected from zeolitized volcanic tuff deposits of the Çankırı Çorum Basin, Turkey. The samples were firstly ground and sieved to sizes of 35-45 mesh, prior to use in the experiments. In order to remove very fine particles from the grain surfaces, the samples were washed with distilled water and then dried at 103-105 ^oC for 2.5 hours. X-ray diffraction (XRD) analysis was carried out to confirm the crystal structure, and the photograph of clinoptilolite sample taken by Scanning Electronic Microscopy (SEM) is given in Figure 1 [20]. As can be seen, the inner parts of the former volcanic shards were replaced by the tabular crystals of clinoptilolite. The chemical composition of clinoptilolitized tuffs by ED-XRF analysis is given in Table 1 [19]. The surface area of the clinoptilolitized tuffs, used in this study, was also measured by the authors in a previous work [21], and estimated to be 18 m^2/g.

FIGURE 1 - The SEM image of the clinoptilolite sample used.

TABLE 1 - Chemical analysis of clinoptilolite samples used.

Content	%
Na_2O	5.6
MgO	1.0
Al_2O_5	13.7
SiO_2	65.0
P_2O_5	0.1
CaO	3.1
TiO_2	0.3
MnO	0.03
K_2O	1.0
$\Sigma FeO + Fe_2O_3$	<0.1

Properties of leachate

The fresh leachate samples were directly taken from the solid waste collection vehicles of the city municipality in the metropolitan city centre of Samsun, Turkey. The composition and concentrations of landfill leachate usually depends on various factors, such as waste composition, age of landfill site, geology, temperature, moisture content, and other seasonal and hydrological factors. So the leachate samples were taken from different geographical areas of the city and mixed to represent the whole city. The fresh leachate samples were analysed five times, and average concentrations are given in Table 2.

TABLE 2 - Some characteristic properties of the leachate used.

Parameters	Concentration (mg/L)
BOD	40.000 – 60.000
COD	70.000 – 80.000
NH_4-N	180–350
Colour	2.000 – 2 500 unit

Chemicals

All chemicals were of analytical grade and used without further treatment. Distilled water was used in all experiments. The synthetic NH_4-N stock solution was prepared by dissolving NH_4-Cl in distilled water, and further diluted to desired concentrations before use. NH_4-N measurements were carried out according to standard methods [22], based on distillation and titration.

Experimental Studies

Batch experiments with synthetic ammonium solution: Kinetic experiments were obtained from mixtures of 4 g clinoptilolite in natural form with 100 mL of ammonium solution in conical flasks. These experiments have been carried out with a constant agitation speed of 100 rpm and particle sizes of 35-45 mesh. In order to evaluate the kinetic data, separate flasks were prepared for each time interval, and only one flask was taken for the desired time. Final ammonium ion concentrations were measured after separating the clinoptilolite by filtration.

The study of sorption kinetics in wastewater treatment is significantly, since it provides valuable insights into the reaction pathways and mechanism of sorption reactions. In order to predict sorption kinetic models of ammonium ions for Elovich, pseudo-first order, pseudo-second order

reaction, and intra-particle diffusion, models were applied corresponding to experimental data.

The equilibrium behaviours, described in terms of equilibrium isotherms, were also determined by mixing 4g of natural clinoptilolite (35-45 mesh) with 100 mL of ammonium solution in conical flasks. The isotherm consisted of eight concentrations varying from 25 to 960 mg/L.

Column experiments with leachate: The scheme of the column experimental system used throughout the experiments is given in Figure 2. It consisted of a glass-pipe (48 cm height, 3.7 cm inside diameter). The base of the reactor, 1.5 cm from the bottom, was fitted with glass wool in order to support the clinoptilolite bed and prevent it from being lost through the column. The total volume of the reactor was about 2 L. The column was packed with 100 g of clinoptilolite at 35-45 mesh size. As known in the literature, Na^+ form is the best one for ammonium removal. Therefore, clinoptilolite samples were conditioned with 2N NaCl for 3 and 24 hours at 70 ^{0}C in a batch-wise experiment to increase cation exchange capacity before the column experiments. A quartz sand-column was included prior to the clinoptilolite column to prevent clogging of the clinoptilolite pores. The columns were operated in a down-flow mode with continuous flow through the leachate. A peristaltic pump was used to feed the influent from the top of the reactors and withdraw effluent from their bottom. Details of the operating conditions of the packed columns are show in Table 3.

Experiments were carried out on the packed columns to determine the effects of two initial concentrations on the columns` ammonium removal efficiency. The leachate solution having concentrations of 183 and 330 mg/L was passed through the column by a peristaltic pump at 5 ml/min flow-rate via a 5-L feeding tank The breakthrough curve is usually illustrated as "S" shape for most sorption processes in wastewater applications. Breakthrough curves were obtained plotting C/C_0 versus time in order to find out the column loading capacity.

TABLE 3 - The operating conditions for the column studies.

Parameter	
Diameter (cm)	3.7
Column Length (cm)	48
Packed Height (cm)	40
Bed volume (L)	45
Influent flow rate (mL/min)	5

The cation exchange capacity measurements (CEC): The cation exchange capacities of clinoptilolite samples prepared from different particle sizes were determined by the following method [23]:

a) 100 ml of 2M NaCl solution was pumped through a column containing approximately 10 g of zeolite, with 5ml/min flow-rate.

b) After this procedure, zeolites were thoroughly washed with distilled water until chloride could not be detected in the washing water with 0.01 N $AgNO_3$ solutions.

c) The procedure in a) was also repeated with 1 M KCl at the same experimental conditions, and then the eluted volume was measured and analysed for its sodium ion content. The cation exchange capacities (C.E.C.) with respect to different particle sizes of zeolites were calculated according to the following equation:

$$C.E.C.\ (meq/g) = (V_{KCl} \times C)/m_z \quad \textbf{(1)}$$

where,

V_{KCl} = volume of KCl solution (L), C = the concentration of Na^+ ions accumulated in the KCl solution, and m_z = the amount of zeolite samples in the column. The results are given in Table 4.

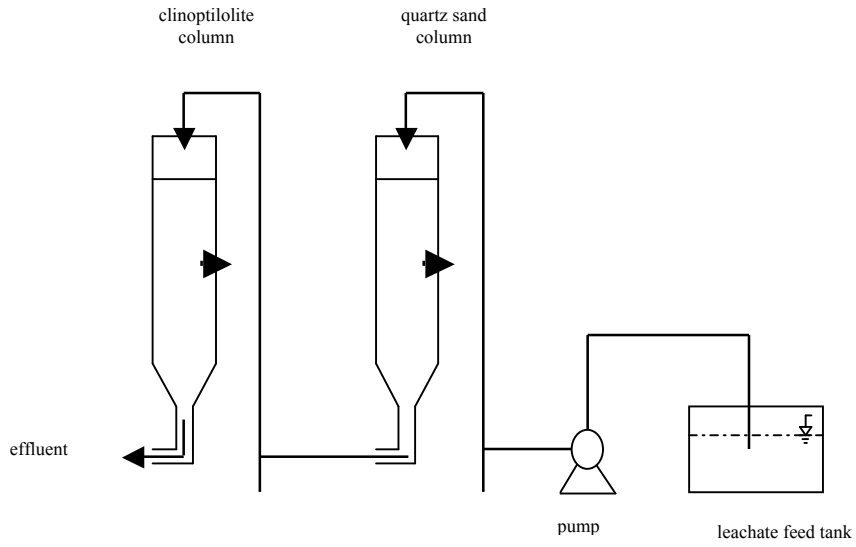

FIGURE 2 - The experimental setup for the column studies.

TABLE 4 - The cation exchange capacities of natural clinoptilolite.

size (mesh)	V_{KCl} (l)	m_z (g)	C (meq/l)	C.E.C. (meq/100g)
16-20	0.1	9,4013	134	142,53
20-35	0.1	9,4907	135	142,25
35-45	0.1	9,2975	135	145,20
45-65	0.1	9,4596	134	141,66

RESULTS AND DISCUSSION

Batch experiments

Figure 3 shows the effects of the agitation time on retention/removal of ammonium ions at an initial concentration of 80 mg/l. It is clearly indicated that the sorption of ammonium ions increases instantly at initial stage, then keeps increasing gradually, and, finally, remains constant. The equilibrium time was selected to be 15 min for the further experiments.

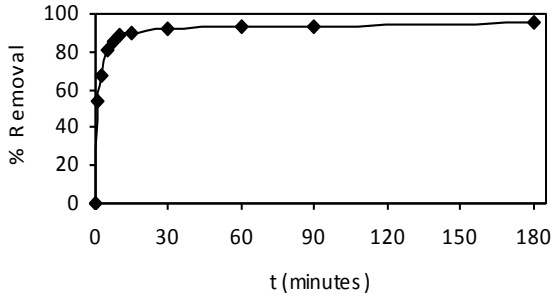

FIGURE 3 - The effect of the agitation time on the removal of ammonium by clinoptilolite.

Kinetic studies

The chemical kinetics describe reaction pathways, along with time to reach the equilibrium, whereas chemical equilibrium gives no information about pathways and reaction rates. The sorption kinetics show large dependence on physical and/or chemical characteristics of sorbent materials, which also influence the sorption mechanism. In order to investigate this mechanism of sorption, four different models have been used under different experimental conditions for sorption processes.

The amount of ammonium ions on the sorbent was calculated using the following mass-balance equation:

$$q_e = \frac{V(C_0 - C)}{m} \quad (2)$$

where,

q_e is the amount of ammonium adsorbed (mg/g of sorbent), C_0 is the initial ammonium concentration (mg/L), C is the final ammonium concentration in solution (mg/L), m is the mass of clinoptilolite (g), and V is the volume of solu-
tion (L).

All these parameters, expect q_e (calculated from mass-balance equation), were measured experimentally.

Pseudo-first order model

This was the first equation for the sorption of the liquid/solid system based on solid capacity [24]. In most cases, the pseudo-first order equation does not fit well for the whole range of contact time. This model may be represented as linearised form:

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2{,}303} t \quad (3)$$

where,

q_t is the amount of solute on the surface of the sorbent at time t, (mg/g), and k_1 is the equilibrium rate constant of pseudo-first sorption (L/min)

In order to obtain the rate constants, the straight line plots of log $(q_e$-$q_t)$ against t for different experimental conditions have been analysed.

Pseudo-second order model

Pseudo-second order reaction model, based on sorption equilibrium capacity, may be expressed in the linearized form as shown below [25]. The equation constants can be determined by plotting t/q_t against t.

$$\frac{t}{q_t} = \frac{1}{k_2' q_e^2} + \frac{1}{q_e} t \quad (4)$$

$$h = k_2' q_e^2$$

where,

k_2' is the equilibrium rate constant of pseudo-second order (g/mg.min), and h is the initial sorption rate (mg/g.min).

Elovich Model

The Elovich equation is given as follows [22]:

$$q = \beta \ln(\alpha) + \beta \ln t \quad (5)$$

Both α (mg g^{-1} min^{-1}) and β (g mg^{-1}) are the equilibrium rate constants for Elovich model.

The equation constants can be obtained from the slope and intercept of a straight line plot of q versus ln t.

Intra particle diffusion model

The intra-particle model is expressed as follows [26]:

$$\log R = \log K_i + \alpha \log t \quad (6)$$

where,

R is the percentage of the ammonium removal, t is the contact time (min), and K_i is the rate constant for intra-particle transport (L/min).

The equation constants can be determined by plotting *log R* against *log t,* and α is the gradient of linear plots de-

picting the sorption mechanisms, whereas the term K_i may be taken as a rate factor. Higher values of *Ki* show an enhancement in the rate of sorption, whereas larger α values illustrate better sorption mechanisms related to an improved bonding between ammonium ions and clinoptilolite particles.

TABLE 5 - A comparison of Pseudo-first order, Pseudo-second order, Elovich kinetic and Intra-particle diffusion model rate constants for ammonia removal by clinoptilolite (100 rpm. 35-45 mesh, 25 °C).

Co (mg/L)	Pseudo-first order		Pseudo-second order		Elovich equation			Intra-particle diffusion Model		
	k_1	r^2	k_2'	r^2	α	β	r^2	K_{id}	α	r^2
80	0.035	0.887	0.047	0.998	7388	0.142	0.779	63.82	0.096	0.734

The straight-line plots of *q* versus ln *t* were tested to obtain the Elovich equation constants. For the pseudo-first sorption rate constant, the straight line plots of log (q_e-q_t) against time for different experimental conditions were analysed. The equilibrium rate constants of pseudo-second order model were determined by plotting t/q_t against *t*. The intra-particle diffusion model constants were obtained from the slope and intercept of a straight line plot of *log R* against *log t,* and α is the gradient of linear plots and depicts the sorption mechanisms and the term K_i may be taken as a rate factor, as already described before.

The values of α less than 0.50 indicate that intra-particle diffusion is not a rate-determining step. The kinetic constants and correlation coefficients of all models are calculated and given in Table 5. Good correlation coefficients were obtained for the pseudo second-order kinetic model, which shows that ammonium uptake process of clinoptilolite follows the pseudo-second order rate expression.

Isotherm studies

The Langmuir and Freundlich equations are in common use for describing adsorption equilibrium for wastewater-treatment applications [27]. The linear forms of Lang-muir and Freundlich isotherms are represented by the following equations, respectively:

$$C_e / q_e = (1/Q^0 b) + (1/Q^0) C_e \quad (7)$$

$$\log q_e = \log K_F + n \log C_e \quad (8)$$

Ce is equilibrium ammonium concentration in solution (mg/L), Q^0 (mg/g) and b (L/mg) are Langmuir isotherm constants, whereas K_F and n are Freundlich isotherm constants. The value of Q^0 gives the maximum sorption capacity of clinoptilolite.

Figure 4 shows the relationship between the amount of ammonium adsorbed per unit mass of clinoptilolite (q_e, mg/g) and its final concentration in the solution (C_e). The increase in the curvature of the isotherms, when it tends to a monolayer, as C_e values increase considerably for the small increase in q_e, which is possibly due to the less active sites being available at the end of the sorption process and/or the difficulty of the edge molecules in penetrating the sorbent, or ammonium ions partially covering the surface sites.

The Tempkin isotherm

Tempkin and Pyzhev [28] considered the effects of some indirect adsorbate/adsorbate interactions on adsorption isotherms and suggested that because of these interactions the heat of adsorption of all the molecules in the layer would decrease linearly with coverage. The Tempkin isotherm has been used in the following form:

$$q_e = RT/b \, (\ln AC_e) \quad (9)$$

Equation (9) can be expressed in its linear form as

$$q_e = RT/b \, (\ln A) + RT/b \, \ln C_e \quad (10)$$

where,

$$B = RT/b \quad (11)$$

The adsorption data can be analyzed according to Eq. 10. A plot of q_e versus $\ln C_e$ enables the determination of the constants A and B. The constant B is related to the heat of adsorption.

B is Tempkin isotherm energy constant (L/g), A is Tempkin isotherm constant, b is Tempkin isotherm energy constant (J/mol, R is the universal gas constant (8,314 J/mol. ^{0}K), and T is temperature (298 ^{0}K).

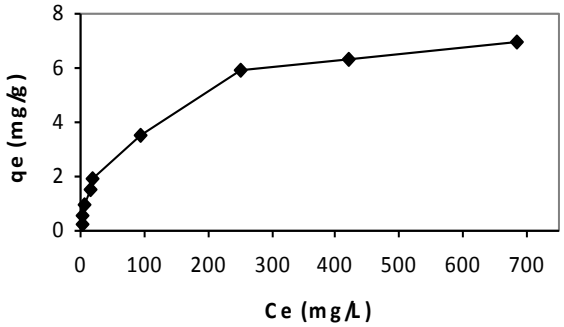

FIGURE 4
Equilibrium isotherm for ammonium removal using clinoptilolite.

The sorption equations were obtained by experimental data and using equations 7, 8 and 10. The isotherm constants were calculated and presented in Table 6. The Lang-

muir equation represents the sorption process very well, the r^2 value is higher for Langmuir isotherm than the Freundlich one. This may be due to homogenous distribution of active sites on clinoptilolite surface. Jorgensen and Weatherley [15] also suggested that the sorption kinetics of ammonium ions onto clinoptilolite was described by the Langmuir equation.

TABLE 6 - A comparison of the Langmuir, Freundlich and Tempkin isotherm constants for ammonia removal by clinoptilolite (100 rpm. 35-45 mesh, 25 °C).

Langmuir Isotherm Constants			Freundlich Isotherm Constants			Tempkin Isotherm Constants		
b	Q^o	r^2	K_F	*n*	r^2	*A*	*B*	r^2
0.016	**7.634**	**0.987**	**6.283**	**2.201**	**0.987**	**3.862**	**0.795**	**0.969**

The values of Q^0, which is defined as the maximum capacity of the sorbent, have been calculated from the Langmuir plot. The maximum capacity of Cankırı-Corum clinoptilolitized tuffs for ammonium ions has been calculated to be 7.44 mg/g under the presented experimental conditions. These values compare favourably with some of those reported. In the work of Demir et al. [29], the removal capacity of raw clinoptilolite for ammonium was given as 5.17 mg/g.

Column studies

The clinoptilolite samples were conditioned in batch-wise experiments to increase cation exchange capacity before the column studies. As can be seen from Figure 5, the removal capacity increased with thermal conditioning and NaCl. The samples conditioned at 70 ^{0}C with 2N NaCl were used for the column experiments. The breakthrough curves were obtained to determine the effects of two initials concentrations on ammonium removal efficiency of the packed clinoptilolite column. The breakthrough curves for each selected initial concentration are given in Figure 6. The breakthrough of ammonium ions occurred after 300 min for both initial concentrations. As seen from the breakthrough point, the maximum ammonium concentrations passing through the column were calculated to be 226 mg and 468 mg for initial ammonium concentrations of 183 and 330 mg/L, respectively. At the same time, in the saturation point (when the flow ratio of C_e/C_0 is equal to 1), ammonium concentrations passing the column were found as 300 mg and 512 mg for initial ammonium concentrations of 183 and 330 mg/L, respectively, and saturation points were reached after 450 and 380 min.

During passage of leachate through the columns, COD parameter was monitored as well. The results are given in Table 7. It can be seen from these results that the value of COD was considerably reduced.

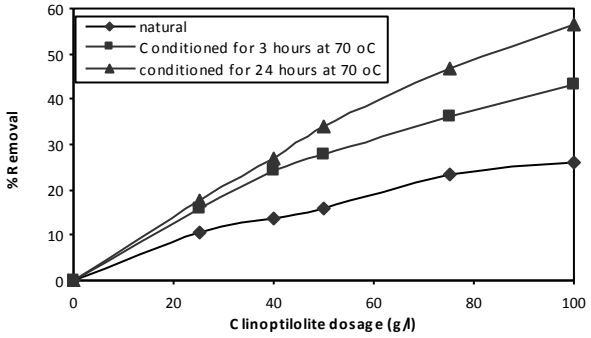

FIGURE 5
The effect of conditioning on ammonium removal process.

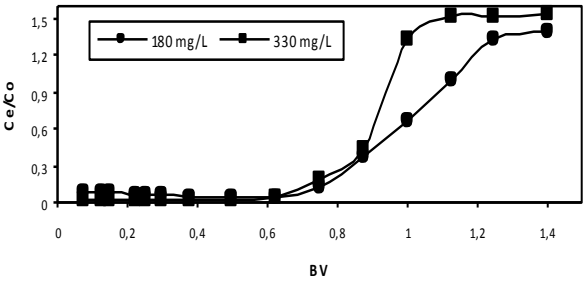

FIGURE 6 - Breakthrough curve for ammonium removal using clinoptilolite in the column experiment.

TABLE 7 - COD concentrations and %-removal using clinoptilolite in column studies (initial NH_3-N concentration: 183 mg/L).

Time (min)	COD (mg/L)	Removal (%)l
0	73.333	-
150	56.650	22.75
300	46.667	36.36
350	46.000	37.27
400	40.000	45.45
450	70.000	4.54

Regeneration studies

Regeneration studies were performed with saturated clinoptilolite after the column studies. NaCl solution passed through the column at three different conditions to determine the effects of regeneration. As can be seen from Table 8, regeneration efficiency increased with increasing concentrations and volumes of NaCl solutions used.

TABLE 8
The results of regeneration studies (clinoptilolite dosage: 100 g/L).

Regeneration type	Released (mg NH_3-N/L)	Removal (%)
Initial	198.24	-
Saturated Zeolite	201.60	-
1 g zeolite/10 mL, 1N NaCl	98.00	50.56
1 g zeolite/10 mL, 2N NaCl	73.92	62.71
1 g zeolite/20 mL, 2N NaCl	60.48	69.49

CONCLUSION

In this study, the removal capacity of the zeolitized volcanic tuffs obtained from the Tertiary sediment of Cankırı-Corum Basin (Turkey) has been investigated. The equilibrium and kinetic data and column studies for ammonium removal using clinoptilolite were presented. The parameters, which have influence on the removal processes, were investigated. The uptake rates were rapid at initial stages, and then kept increasing gradually until the equilibrium was reached. The batch sorption kinetics were tested for first-order reaction, intra-particle diffusion, pseudo-first order and pseudo-second order reaction models. The pseudo-second order kinetic reaction model was found to be the best correlation of the data for ammonium removal from aqueous solution using clinoptilolitized tuffs. The experimental data could be correlated better by the Langmuir isotherm model. The maximum sorption capacity was cal-culated from Langmuir plot as 7.44 mg/g. The breakthrough of ammonium ions occurred after 300 min (0.75 BV) for both initials concentrations. The saturation points were reached after 450 and 380 min for initial ammonium concentrations of 183 and 330 mg/L respectively. The cation exchange capacity was measured to be 142 meq/100 g.

Therefore, it was found that the Cankırı-Corum Basin clinoptilolitized tuff can efficiently remove ammonium nitrogen from leachate.

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AGGRESSIVE BEHAVIOUR IN *Betta splendens* AS A BIO-INDICATOR OF FRESHWATER POLLUTION

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SUMMARY

Male *Betta splendens* are known to be highly territorial and aggressive towards conspecifics. The influence of two metal pollutants, mercuric chloride ($HgCl_2$) and sodium azide (NaN_3), on specific aggressive behaviours of the male *Betta* species was investigated. The aim was to determine if aggressive behaviour can be used as a metal pollution bio-indicator. For this purpose, two males were placed in an aquarium (60 x 25 x 25 cm) that was divided into two equal compartments by a clear, perforated Plexiglas wall. The aquarium was filled with untreated water as control, and with polluted water under experimental conditions. After 24 h, one male (intruder) was placed in the other male's (resident) half of the aquarium. The behaviour of both males, particularly, air gulping rate, latency to first bite and to first opercular expansion, were recorded. The results indicate that the males under treatment conditions were less aggressive than those under control conditions. Thus, the male *Betta splendens* aggressive behaviour can be potentially used as a biological indicator of pollutants in freshwater.

KEYWORDS:
Siamese fighting fish, metal pollutants, mercury, azide.

INTRODUCTION

Metal pollution in freshwater resources is one of the major concerns in a world that is already straining the little freshwater resources available to living organisms [1-3]. Metal pollutants can adversely impact living organisms exposed to them. The pollutants` action is different with regard to various metals and organisms. In animals, they can affect development, the nervous and endocrine systems [4-12]. Sometimes pollutants can act additively or synergistically, thus exaggerating their impact on wildlife [4]. This clearly necessitates the periodic testing of aquatic ecosystems and sources for metals and other pollutants, using different biomarkers and bioindicators [13-15].

Pollutants often have observable effects on the behaviour of animals exposed to them [16-20]. Behaviour has been used frequently as a bio-indicator to test for the presence/ effect of pollutants on animal health [4, 7, 21-24]. Behaviour has the advantage of being non-invasive, inexpensive, and sometimes more powerful than other methods [6, 7, 25-27]. For instance, behaviour could be a better measure than other chemical or physiological parameters, since it is the physical manifestation of an animal's integrated physiological response to its environment [4, 7, 17]. On the other hand, behaviour can be highly variable and difficult to measure [4, 7].

In the present work, the influence of two metal pollutants on the aggressive behaviour of *Betta splendens* was in-vestigated. The two metals tested were sodium azide (NaN_3) and mercuric chloride ($HgCl_2$). Sodium azide is used as a general biocide, and is one of the ingredients used to inflate automobile air bags [28]. The fate of sodium azide in the environment is not known, which makes it problematic, considering the increase of its use over the past few years [28-30]. Mercury is one of the most dangerous and widely used heavy metals. It has many uses and is considered to be a serious pollutant of most ecosystems [31].

Betta splendens, the Siamese fighting fish, belongs to the suborder Anabantoidei, which is characterized by a special respiratory organ, the labyrinth that allows them to breathe air directly from the surface [32]. Male Siamese fighting fish have been long recognized for their territoriality, and the aggressive displays they perform when confronting intruder conspecifics [32, 33]. A male *Betta* will even attack the females that he has just courted, after they have deposited their eggs in his territory [32, 34]. The highly territorial aggression of Siamese males stems from the fact that in this species, males are the sex partner responsible for taking care of the eggs until the fry hatch. They do this by constructing a bubble nest, in which they incubate the eggs [35, 36].

The objective of this study was to determine the possibility of using the aggressive behaviour of male *Betta* as reliable bio-indicator of freshwater metal pollutants.

MATERIAL AND METHODS

Subjects

Naive male *Betta splendens* were purchased from a local supplier. Males were dark-red in colour, and of similar body sizes (measured from the tip of the upper jaw to the caudal peduncle using a Vernier calliper). Males ranged in size from 3.4 cm to 4.9 cm (mean ± s.d. = 4.11 ± 0.51, n = 50). Each male was kept in a glass jar (15 cm diameter x 20 cm height) at 28 °C on a 12-h L: 12-h D cycle for 24-48 h, until it was transferred to the experimental aquarium. Each jar was wrapped in aluminum foil to prevent visual access to other fish. A few Betta® flakes were given to each male every day.

The test aquaria were filled with tap water that had a pH around 8, and a total hardness ($CaCO_3$) between 45-55 mg/l.

General Procedure

Two male *Betta* fish, matched for body length, were placed in a 60 cm x 25 cm x 25 cm aquarium. The aquarium had a clear Plexiglas partition that had holes drilled in it, dividing the aquarium into two equal halves. Males in each half could see each other. Food was not provided for 24 h. Then, one male, assigned as *intruder*, was picked with a fishnet and gently placed in the other male's (*resident*) half. Residents were similarly picked up for 2 sec. The procedure was repeated for nine pairs for each of the different treatments (a total of 18 naïve red males per each treatment; see below).

Data Collection

The following data were collected for each pair of fish. First, the air gulping rate (number of times each male rises and breaks the surface of water with its snout) for both the resident and intruder males, 1 h after placing them into the aquarium, was counted for 10 min (the *before24* test). Then, air gulping rate just before putting the two males together, was counted for a second period of 10 min (the *after24* test). After placing the two males together, and for another 10 min (the *during* test), the air gulping rate, the attempted bite count (i.e., when either male thrashes the opponent fish with its mouth), and the count of opercular expansions (i.e., when either male expanded its opercula when facing the opponent) were determined.

Experimental Treatments

Fish were assigned to one of three treatments. In treatment *control*, fish were placed in water that had no chemicals in it. In treatment *$HgCl_2$*, the water in the aquarium had mercuric chloride added to a concentration of 0.2 mg/l. In treatment *NaN_3*, the water had sodium azide at a concentration of 4 mg/l. The contaminant concentrations were freshly prepared by mixing tap water with the metal powder. Several concentrations were tried, and the ones reported here were chosen because the fish survived them for at least 48 h.

The lab room had five aquaria placed on benches. The fish in different treatments were run simultaneously in the five tanks. For example, on the first day, three tanks were assigned to control treatments and two tanks for the $HgCl_2$ ones. On the next day, after thoroughly cleaning the tanks, the tanks were re-filled. Water and fish prepared for NaN_3 treatment were placed in three tanks, as well as $HgCl_2$ treatment water and fish in two tanks, and so on.

Data Analysis

Data for the three different behavioural measures, air gulping rate, latency to first bite, and latency to first head expansion, were analysed using the ANOVA-Repeated Measures test. Post-hoc analyses were also performed to find out the groups that were significantly different from each other. The analysis was done for each treatment condition, and no attempt for cross comparisons between different conditions was attempted, since we were interested in detecting an effect for each metal on the behaviour of the males.

RESULTS

Treatment Control

During the encounter, both the resident and intruder male Siamese fighting fish had their fins extended for the entire observation period. The males swimming patterns were typically like those described in literature. Basically, the males would cover the entire aquarium, at varying speeds. Fish would come in close proximity many times, where they engage in aggressive displays and manoeuvres.

Overall air gulping rate was not significantly different between the resident and intruder groups (ANOVA, P = 0.8697). Also, air gulping rate was not significantly different among the three tests (before 24, after 24, and during) for the intruder group. The same results were obtained for the resident group, except in the comparison between the after 24 and during tests (Scheffe's post-hoc test, P = 0.033; Fig. 1).

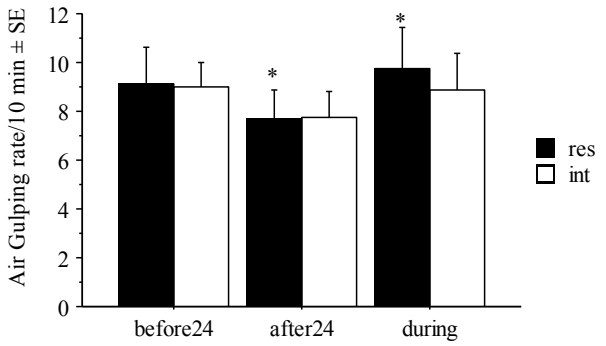

FIGURE 1 - Air gulping rate in the control treatment by both residents and intruders. The comparison between the after 24 and during tests for residents was the only significant result obtained.

The residents expanded their opercula on 8 trials out of the total 9 trials, and were the first to expand their opercula on 7 of these trials. On the other hand, the intruders exhibited opercular expansion on 6 trials, and were the first to expand the opercula on 2 of these trials (Fig. 2). The difference between the two ratios (8/9 vs. 6/9) is not significant ($z = 1.13$, $P = 0.129238$).

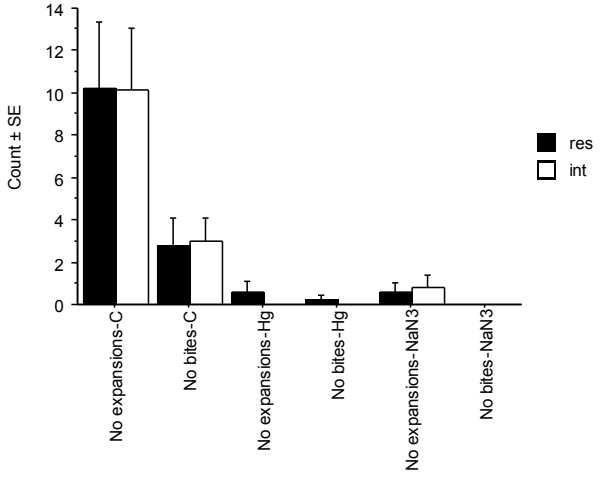

FIGURE 2 - The number (No) of head expansions and bites directed to the opponent in the three different treatments (When a bar is missing, it means the count was zero, such as in the last column).

The residents attempted to bite the opponent in 4 trials out of the total 9 trials. In the other 5 trials, the residents did not make any attempt to bite. The residents were the first to attempt to bite on 2 of the 4 trials. The intruders attempted to bite the resident on 7 trials. The intruders were the first to attempt to bite on 5 of those trials.

From the above results, it is clear that prior residence does not guarantee winning a conflict, at least over the 10-min observation period.

Treatment $HgCl_2$

The general behaviour of the fish was not clearly different from that in the control treatment. The fish were actively swimming, and engaged in fin displays for the entire observation period. The only differences detected were that the males rarely came in close proximity of each other, hardly enough to expand their opercula or deliver a bite.

Overall average air gulping rate differed significantly between residents and intruders (ANOVA, $P = 0.0019$). Both residents and intruders exhibited significant differences in air gulping rate on the before 24-after 24 tests (Scheffe's post-hoc test, $P = 0.0002$ for residents, and $P < 0.0001$ for intruders) and the before 24-during tests (Scheffe's post-hoc test, $P = 0.0031$ for the residents, and $P < 0.0001$ for intruders; Fig. 2). The after 24-during comparisons were not significantly different for either group. Also, residents maintained a higher air gulping rate than intruders over the three tests (ANOVA, $P < 0.0001$).

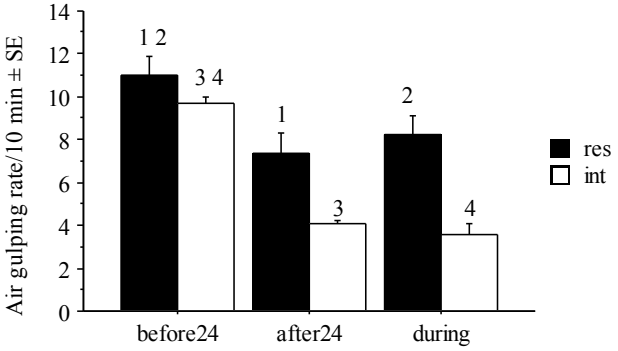

FIGURE 3 - Air gulping rate in the $HgCl_2$ treatment by both residents and intruders (The comparisons between the before 24-during and before 24-after tests for both residents and intruders were significantly different. Number on top of the bars indicate the significant comparisons).

Only one resident male exhibited opercular expansion, and the same male attempted one single bite in that trial. Otherwise, no other males, whether residents or intruders, exhibited opercular expansion or attempted any bites.

Treatment NaN_3

The general behaviour of the fish here was similar to that in the $HgCl_2$ treatment. The fish were actively swimming, and engaged in fin displays for the entire observation period. Again, the only differences detected were that the males rarely came in close proximity of each other, hardly enough to expand their opercula or deliver a bite.

Overall average air gulping rate did not differ significantly between residents and intruders (ANOVA, $P = 0.68$). Both residents and intruders exhibited significant differences for air gulping rate on the before 24-after 24 tests (Scheffe's post hoc test, $P < 0.0001$ for residents, and $P < 0.0001$ for intruders) and the before 24-during tests (Scheffe's post hoc test, $P < 0.0001$ for the residents, and $P < 0.0001$ for intruders; Fig. 4). The after 24-during comparisons were not significantly different for either group.

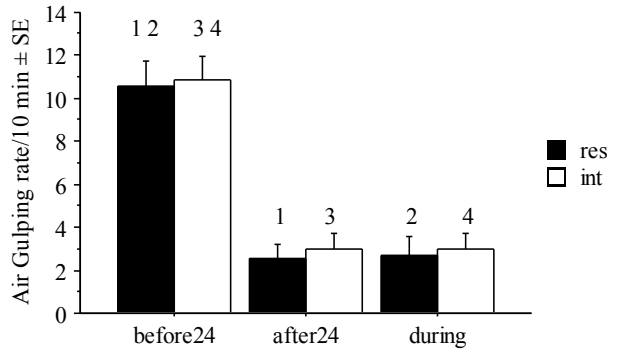

FIGURE 4 - Air gulping rate in the NaN_3 treatment by both residents and intruders (The comparisons between the before 24-during and before 24-after tests for both residents and intruders were significant. Number on top of the bars indicate the significant comparisons).

In one tank, both resident and intruder males exhibited opercular expansion. One other resident male exhibited opercular expansion, and another intruder male, in a different tank, also exhibited opercular expansion. No other males, a total of 14, exhibited opercular expansion.

No male, whether resident or intruder, attempted to bite its opponent for the duration of the observation period, i.e. 10 min.

DISCUSSION

The results can be summarized as follows. In the control treatment, residents and intruders did not significantly differ on any of the three measures, viz. air gulping rate, number of head expansions and bites directed at the opponent. In the $HgCl_2$ condition, residents and intruders had significantly lower air gulping rate after being placed in the polluted water for 24 h, as well as during the encounter, than after spending 1 h in the aquarium. However, the residents maintained higher air gulping rate than the intruders in all tests. Only one resident male attempted to bite, and expanded its opercula to its opponent. No bite attempts or opercula expansion were displayed by any other male. In the NaN_3 treatment, residents and intruders had significantly lower air gulping rate after being placed in the polluted water for 24 h, than after spending 1 h in the aquarium. During the encounter, the air gulping rate did not significantly differ from that just before the encounter. Only one resident male and one intruder male attempted to bite its opponent. Two other different males, a resident and an intruder, exhibited opercular displays.

The results give a very clear evidence of impairment of certain aggressive behavioural measures in male *Betta splendens* when placed in contaminated water. Although the fish swimming activity was not apparently impaired after 24-h exposure to contaminants, and other critically important behaviours were definitely impaired. Air gulping rate, a frequent measure of male *Betta* aggression [32, 33] was sig-nificantly lower after 24 h-exposure to heavy metals in both experimental treatments. Similarly, there was a clear-cut im-pairment of two more behaviours: opercular extension and bite attempts by fish placed in contaminated water. How-ever, this might not be as surprising as it would appear at first. Both opercular expansion and bite attempts appear when fish are mainly in close proximity and/or facing each other [32], which did not happen in most of the experimental trials.

Therefore, it seems that the behaviour of male *Betta splendens*, during aggressive encounters, could be used as a bio-indicator of freshwater metal pollution. In the current experiments, the used contaminant concentrations were less than the LD50/96-h doses reported in the literature for other fish (for mercuric chloride: [37, 38]; for sodium azide: Lyle Lockhart, unpublished data). The ease of carrying out the tests, and the rather inexpensive nature of it, makes *Betta* a good model to use for routine testing of freshwater for contaminants. Since isolating the fish through a transparent barrier should be enough to quantify the behaviour, the tests do not need to involve physical placement of males together and, therefore, need not expose the fish to physical injury [39]. The reported experiments represent a preliminary investigation, which should be followed by more detailed analyses of the chemical pathways disrupted after exposure to more diluted contaminant doses that match those found in freshwater systems.

The use of aggressive behavioural endpoints in *Betta splendens* as a bio-indicator of metal pollution seems to fit with the current research trends in aquatic ecotoxicology. For example, the behavioural endpoints chosen here were definitely disrupted at a lesser concentration than those causing mortality. This makes male *Betta* and their behaviour suitable for assessing water quality, and the effects of pollutants on fish populations [4, 23, 40]. A bio-indicator is considered to be ideal when the effect of metal pollutants in this study can be assessed in the lab 24 h after exposure [2], which is the case in the current study. In addition, the behaviours investigated were very specific and definable, which is always desirable in ecotoxicological research [41]. Another advantage of using male *Betta* aggressive behaviour is the fact that the species has not been used for such purposes before. The use of many species could allow detection of a broader range of pollutants over a wider concentration range, due to fish variability in sensitivity to different toxins [42].

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MOSSES AS INDICATORS OF ATMOSPHERIC HEAVY METAL DEPOSITION AROUND A COAL-FIRED POWER PLANT IN TURKEY

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SUMMARY

This study was carried out from May 2003 to October 2004 in the vicinity of Çatalagzi coal-fired power plant (CATES) located in Zonguldak, North-West Turkey, in order to investigate atmospheric heavy metal depositions by sampling and analysing *Pleurocarp* mosses as biomonitoring plants. Initially, ISC-ST (Industrial Source Complex-Short Term) dispersion models were used to determine theoretically the most polluted sites of CATES. After the modelling, sampling was performed in these theoretically determined grids. Samples were analyzed using graphite–furnace atomic absorption spectrometry (AAS) after wet digestion. In the region, the general order of heavy metal content in samples of mosses was determined to be as follows: Fe>Pb>Ni>Cr>Cu>Co>As. Background mean levels of the metals studied, except Cu, were determined and found to be higher than that of European background. The results are also presented in the form of thematic maps using the Geographic Information System (GIS).

KEYWORDS: Moss-monitoring, heavy metals, coal-fired power plant, Çatalagzi, North-West Turkey.

INTRODUCTION

Heavy metals belong to the most serious air pollutants, which affect our environment. Among the approaches, used to identify these compounds and qualify their influence, is the use of mosses as biomonitors of metal deposition, as illustrated in recent studies [1-3].

Mosses have several advantages as indicator plants: (i) many species have a vast geographical distribution, and they grow abundantly in various natural habitats, even in industrial and urban agglomerations; (ii) they have no epidermis or cuticle, therefore, their cell walls are easily penetrable for metal ions; (iii) due to lack of root systems, they obtain minerals mainly from precipitation; (iv) some species have layer structure and annually produced organic matter forms distinct segments; (v) transport of minerals between segments is poor because of lack of vascular tissues; (vi) mosses accumulate metals in a passive way, acting as ion exchangers; and (vii) mosses show the concentrations of the most metals as a function of the amount of atmospheric deposition [4].

The moss technique has been introduced for large-scale studies of atmospheric deposition of metals in a number of new countries [e.g. 5, 6-10] recently, and the results are summarized in European Atlas edited under the auspices of the UNECE ICP Vegetation [11]. In Turkey, the first data were provided on several moss-monitored metals between 1995 and 1998 [12, 13]. Most recently, the moss technique was carried out to monitor atmospheric heavy metal deposition in Thrace region and the north of Turkey [14-16].

In Turkey, coal-fired thermal power plants have been mainly used in order to produce electricity since 1950, and Zonguldak basin is the major center of hard coal production. Çatalagzi power plant (CATES) is the only plant using bituminous coal, excavated from the Zonguldak coal field in the West Black Sea Region, to produce electricity since 1991. The power plant consists of two separate units equipped with electrostatic filters to control air pollution rising from combustion. It uses 1.500.000 tons hard coal and produces 645.000 tons slag (20% w/w) and fly-ash (80% w/w) in a year [17]. In general, coal ash in a power plant consists of up to 25% bottom-ash and 75% fly-ash [18]. Coal combustion in the power plant gives rise to the emission of primary (direct emissions) and secondary (gas-to-particle conversion) particulate pollutants. Since the emission of pollutants depends on coal quality and com-

bustion technology, and given that transport, transformation and deposition of contaminants depend on regional climatic conditions, specific studies for the power stations should be carried out to evaluate their environmental impact [19].

The aim of this study is to present the regional atmospheric deposition of heavy metals in the vicinity of a coal-fired power plant. This study is the first attempt to characterize the atmospheric deposition of seven heavy metals (Fe, Pb, Ni, Cr, Cu, Co, As) by means of indigenous mosses around the Çatalagzi power plant (CATES) in Zonguldak, Turkey (Fig. 1).

FIGURE 1 - Geographical location of the study area.

MATERIALS AND METHODS

The Dispersion Modelling of Atmospheric Emissions from the Power Plant. Air quality modelling is an essential tool for most air pollution studies. Air dispersion models, i.e. the Industrial Source Complex Short Term (ISC-ST) models, have been extensively used over the past two decades for varied applications [20-26].

The ISCST-3 model developed by US Environmental Protection Agency (EPA) is used to compute the ground level concentrations of the pollutant. The ISCST-3 model for continuous elevated point sources uses the steady-state Gaussian plume equation [27].

The ISCST-3 model employs Briggs formulae to compute plume rise. Pasquill-Gifford curves use the horizontal and vertical dispersion parameters for rural background and empirical relations for urban background, and include also buoyancy-induced dispersion [28]. This model has an option to use rural or urban background. Wind profile law is used to estimate the wind speed at stack height [29].

In this study, in order to decide the sites to be sampled, the ISC-ST modelling was performed using meteorological data given in Table 1, indicating that prevailing wind directions are mainly northern sectors for all months, except November (ESE). The ISC-ST model was used to determine theoretically the most polluted sites before sampling around the CATES. Before the modelling, the study area was divided into 22x16 km^2 grids with 22 grids along the x-axis and 16 grids along y-axis. The parameters employed in the model, such as dust emission of 532 g s^{-1} (single sinter stack), stack height of 120 m, stack diameter of 6.5 m, stack gas temperature of 150 ^oC and stack gas velocity: 12 m s^{-1}, stability classes (compiled from Turner's (1994) table), mixing height (determined using the Holzworth [31] (1967) technique) and mean meteorological values (obtained from the Turkish Meteorological Department) were used as required input data in the modelling [30, 31]. According to the model results, only 48 km^2 of the area were decided to be most polluted sites, consisting of 25 grids as shown in Fig. 2. After the modelling, sampling was performed in these theoretically determined 25 grids from May 2003 to October 2004. Details of the modelling studies were given elsewhere [32].

Sampling. The moss sampling procedure was similar to that summarized in the report of Rühling and Steinnes in their 1995 survey [33]. According to the ISC-ST modelling, sampling was carried out from May 2003 to October 2004 in Çatalagzi province. Nevertheless, some places, such as hills exposed to the plant and sites close to the plant, were theoretically not determined by the model, but were also sampled to get information.

According to model results, sampling should be performed in the theoretically determined 25 grids. However, due to lack of suitable pleurocarpic mosses in some grids for sampling, only 13 grids with 24 points were sampled.

TABLE 1 - Mean meteorological data for Çatalagzi Coal-Fired Power Plant Modelling.

Months	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Temperature	5	6.3	5.8	10.5	15.1	19.4	22	22	18	14	11	8
Wind speed	3.5	3.1	3.3	2.8	2.8	1.7	1.9	2.1	2.8	2.5	2.2	3.3
Wind direction	NNW	NNW	NNE	NNE	NNE	NW	N	N	NNW	NW	ESE	NE
Wind direction in degree	157.5	157.5	202.5	202.5	202.5	135	180	180	157.5	135	292.5	225
Stability	C	B	C	B	B	B	B	B	B	B	B	C
Mixing Layer Height	700	650	700	650	650	650	650	650	650	650	650	700

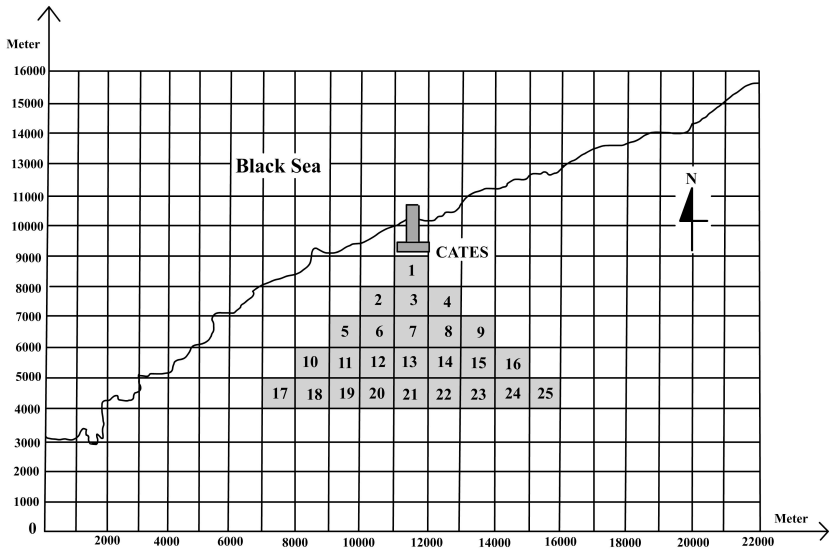

FIGURE 2 - Theoretical grids that determine the most polluted sites predicted by the ISC-ST modeling.

In the study, Çatalagzi region was divided into 4 sub-regions: First region with mainly residential sites (RS); second region in the direction of the prevailing winds (PWS); third region consisting of places near to the plant (NP), and fourth region of control sites (CS) (Fig. 3).

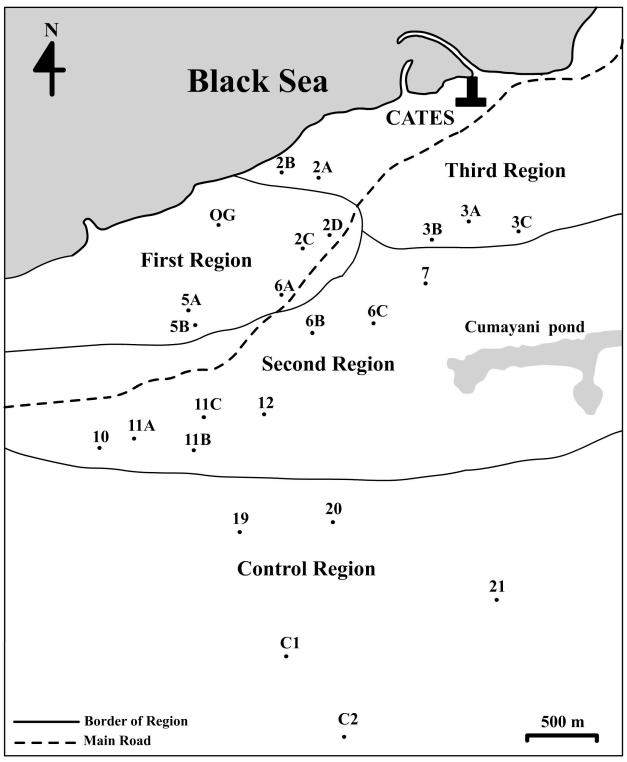

FIGURE 3 - Sampling sites (C1 and C2 stand for two control sites).

The results of the 2003 survey were used to select the most suitable moss species and collection sites for the following survey. Rühling [33] (1994) suggested *Pleurozium schreberi* (Brid.) Mitt., *Hylocomnium splendes* (Hedw.) Schimp., *Hypnum cupressiforme* Hedw. and *Scleropodium purum* (Hedw.) Limpr. as the convenient mosses for biomonitoring. However, between 2003 and 2004, samplings neither *P. schreberi* nor *H. splendes* were found in the 24 sites. Nevertheless, the most abundant species present in this area, *Scleropodium purum* (45 % of all samples collected) and *Hypnum cupressiforme* (25 %) were preferred, but when they were not available in sampling points, another suitable pleurocarpic moss was chosen. The samples were collected at least 300 m from main roads (highways), and, at least, 100 m from smaller roads and houses. When necessary, in more densely populated areas, these distances were reduced to 100 m and 50 m, respectively. In forests or plantations, samples were collected in small open spaces to preclude any effect of canopy drip. Sampling and sample handling were carried out using plastic gloves and bags. Each sample was composed of 5-10 sub-samples collected within an area of 50 m^2. In laboratory, the samples were air-dried at 40 ºC, extraneous plant material was removed, and the upper three segments of each moss plant, representing the last three years of growth, were used for analysis.

Preparation of the samples and chemical analysis. All reagents were of analytical grade, unless otherwise stated. All the plastics and quartz wares were cleaned by soaking them overnight in a 10% (w/w) HNO_3 (65 %, Merck) solution, and then by rinsing with deionized water. Double-deionized water (Milli-Q Milli-pore 18.2 MΩ cm^{-1} resistivity) was used for all dilutions. The samples were cleaned from soil particles, dead materials and litters. Only the last three-years growths of moss materials were used without washing for the analyses. The samples were processed as described by Perkin-Elmer (1996) for plant wet-digestion [34]. In this method, 1 g of ground dried plant sample was

put in 100-ml beakers. Ten mL of conc. HNO_3 was added, and all was heated carefully on a hot plate, until the production of red NO_2 fumes has ceased. After cooling of the solution, 3 ml of $HClO_4$ (70-72 %, Merck) was added and heated till a small part of the mixture remained. After that, the solution was filtered using a membrane with 0.45 μm pores (Advantec MFS, Inc., USA), taken into a 50-ml flask, and demineralized water was added to a total volume of 50 ml. The contents of Fe, Pb, Ni, Cr, As, Cu and Co in the extracts were analysed after calibrating with preformulated spectroscopic standards by graphite-furnace AAS (Perkin-Elmer Model SIMAA 6000, detection limit ppb). Blanks (one for every 5 samples) were prepared at the same time and same conditions to control possible contamination during the preparation of sample extracts. Accuracy was checked by parallel analysis of registered reference material SRM (IAEA-336 Lichen). Quality control standard analyses were performed every ten samples, with instrument recalibration every 23 samples. To control for variations in sampling, extraction and also analysis, a total of six replicates of each sample were analyzed. The coefficients of variation ranged between 0.1 and 11% depending on the element analysed. The recovery rates for the heavy metals in the standard reference material ranged from 87% to 110%. The results were expressed as $\mu g\ g^{-1}$.

Mapping. The maps, based on the mean values of Inverse Distance Weighting Interpolation (IDW) and Geographic Information System (GIS) techniques, were used in making coloured maps. Colours and scales were chosen that they would clearly illustrate the changes in the heavy-metal concentrations during the period covered by the surveys. Interpolation is a mathematical process used to estimate values between known point observations. Many mathematical formulae can be used to interpolate grid values, and are chosen according to the type of data being examined. The GIS modelling process uses mathematical formulae to estimate the values between known point observations, and stores the results in a numeric grid. With the purpose of applying interpolation and GIS modelling techniques to our data; a package program, MapInfo® Professional 4.1 and Vertical Mapper® Version 1.51, was used.

Statistical Analysis. Data were analyzed by using SPSS® for Windows (SPSS Inc. Chicago. IL) computing program. Differences in measured parameters among the four regions were analyzed by a Kruskal-Wallis test (P values less than 0.05 were considered to be significant). In the groups, comparisons between regions that present significant values were evaluated with Mann-Whitney U test (significance was attributed to a value of $P<0.05$). A linear correlation test was carried out to investigate the correlations between metal concentrations (significance was attributed to values of $P<0.01$ and $P<0.05$). Two-tailed significance values were used.

RESULTS AND DISCUSSION

Heavy metal concentrations in moss samples analyzed are given as $\mu g\ g^{-1}$ values together with a plant list in Table 2.

TABLE 2 - Heavy metal concentrations ($\mu g\ g^{-1}$ dry wt.) of seven trace metals in the investigated moss species.

Regions	Sampling Points	Moss Species	Fe ($\mu g\ g^{-1}$)	Pb ($\mu g\ g^{-1}$)	Ni ($\mu g\ g^{-1}$)	Cr ($\mu g\ g^{-1}$)	As ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Co ($\mu g\ g^{-1}$)
First Region	2C	*Eurhynchium hians*	3151.0	1.52	3.11	2.52	1.32	12.6	1.76
	2D	*Scleropodium purum*	2159.9	19.11	5.23	2.51	1.11	5.79	1.58
	5A	*Scleropodium purum*	2595.0	41.72	3.22	1.62	1.51	0.02	3.59
	5B	*Homalothecium lutescens*	1300.4	32.60	4.92	17.12	2.40	0.01	2.57
	6A	*Eurhynchium praelongum*	2538.0	56.73	5.14	5.73	1.52	32.00	6.06
	OG	*Hypnum cupressiforme*	5825.00	9.10	3.50	4.18	1.79	0.01	1.39
Second Region	6B	*Brachythecium rivulare*	2312.50	19.14	4.33	4.97	1.63	0.01	1.51
	6C	*Hypnum cupressiforme*	1502.70	13.32	5.28	0.92	1.07	0.01	0.88
	7	*Scleropodium purum*	4184.00	14.28	2.46	1.16	0.84	0.01	1.35
	10	*Scleropodium purum*	857.00	6.01	16.71	3.31	1.00	6.46	1.40
	11A	*Hypnum cupressiforme*	2104.50	10.98	4.55	3.45	1.71	12.03	3.79
	11B	*Scleropodium purum*	2077.80	21.70	24.20	9.26	1.51	0.96	1.30
	11C	*Scleropodium purum*	2440.48	23.22	10.70	10.52	2.24	3.12	2.13
	12	*Scleropodium purum*	3092.20	26.01	12.61	9.50	2.38	2.47	1.92
Third Region	2A	*Hypnum cupressiforme*	2537.00	2.90	3.60	0.01	0.81	0.01	0.52
	2B	*Rhynchostegium megapolitanum*	7586.25	21.15	4.25	2.49	1.03	0.05	2.67
	3A	*Scleropodium purum*	5213.50	50.3	2.71	1.06	0.91	2.41	2.05
	3B	*Hypnum cupressiforme*	2636.00	15.20	3.10	2.76	0.95	0.01	0.43
	3C	*Scleropodium purum*	3564.00	32.60	2.60	1.55	0.77	0.01	0.34
	19	*Ctenidium molluscum*	239.80	23.5	3.10	10.01	1.82	2.27	1.39
	20	*Hypnum cupressiforme*	3.10	8.4	27.81	7.71	0.82	0.03	0.38
	21	*Scleropodium purum*	1720.80	14.9	2.82	1.21	0.88	0.01	0.06
Control Region	C1	*Scleropodium purum*	2289.70	23.6	3.23	3.50	0.70	3.11	0.99
	C2	*Brachythecium rivulare*	2502.30	12.2	2.52	1.44	0.74	0.01	0.06

0.00: The values are below detection limit of AAS.

TABLE 3 - Mean, standard deviation (S.D.), minimum (Min.), maximum (Max.) and median concentrations of different elements (μg g^{-1} dry wt.) in moss samples.

	Fe μg g^{-1}	Pb μg g^{-1}	Ni μg g^{-1}	Cr μg g^{-1}	As μg g^{-1}	Cu μg g^{-1}	Co μg g^{-1}
Mean	2530.8	21.10	6.61	4.81	1.32	3.34	1.65
Median	2312.5	19.11	3.90	3.31	1.07	0.05	1.39
Min.	3.1	1.52	2.46	0.01	0.70	0.01	0.06
Max.	7586.2	56.70	27.75	17.12	2.40	32.00	6.06
S.D.	1755.1	13.71	6.78	4.32	0.52	6.95	1.34

Summary statistics were used to obtain standard deviation (S.D.), minimum (Min), maximum (Max) and median concentrations for different metals (Table 3). All metal concentrations were determined on a dry weight basis.

Thematic mapping is also performed to relate the concentration gradients found in the area, to compare them with results of European countries, and to create a database for future surveys. In order to examine the dispersion of the elements studied, maps of the distribution of each one were figured out (Figs. 4-10).

Iron. The main iron emission sources can be coal-burning and intensive traffic, and there may also be an influence of soil dust, especially in mining regions. This element may be attributed to dry deposition of wind-blown soil particles and dust on the moss. Concentration of Fe varied between 3.1 and 7586 μg g^{-1} in *Hypnum cupressiforme* and *Rhynchostegium megapolitanum* with a median 2312 μg g^{-1}, which was highly elevated compared with the European means (259 μg g^{-1} in Finland [7], 868.2 μg g^{-1} in Spain [35] and 2070 μg g^{-1} in Hungary [6]. The highest concentration of iron was measured as 7586 μg g^{-1} from the third region close to the plant. The iron content exceeded 2530 μg g^{-1} (mean value) in 35 % of the moss samples, which can be due to significant influence of the coal-fired power plant and intensive coal burning for domestic heating in the area. Iron levels in the third region are approximately four times higher than that of control area. Elevated levels of iron pollution in the northeast of the area were associated with the only local source (CATES), and its transportation by prevailing northwesterly winds. Nevertheless, in far away southern control sites, elevated concentrations occurred near coal mining excavating sites (see C1 and C2 in the map). Besides, soil in the sampling area may be rich of iron metal. There are two dominant wind directions in the whole region: north-northwest and north northeast. Due to prevailing wind directions and orographic shapes, the most polluted regions are south southwest and west southwest of the plant. Iron concentrations tended to decrease with distance from the polluted source (Fig. 4).

Arsenic. Arsenic is emitted to the atmosphere mainly from coal combustion and mining. Other emission sources are the use of arsenic-based pesticides and steel production [36]. Contamination by arsenic was similar to the pattern of chromium because of the same emission sources. The highest concentrations of this element were found in the south-

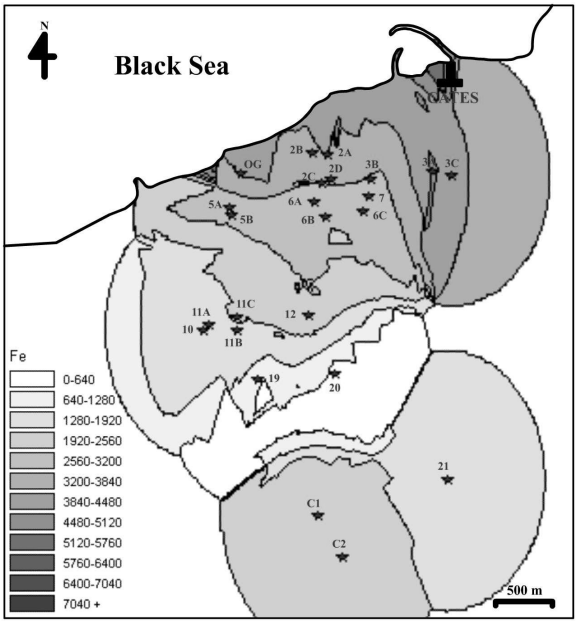

FIGURE 4
Contour map for iron concentrations (μg g^{-1} d.w.) in CATES.

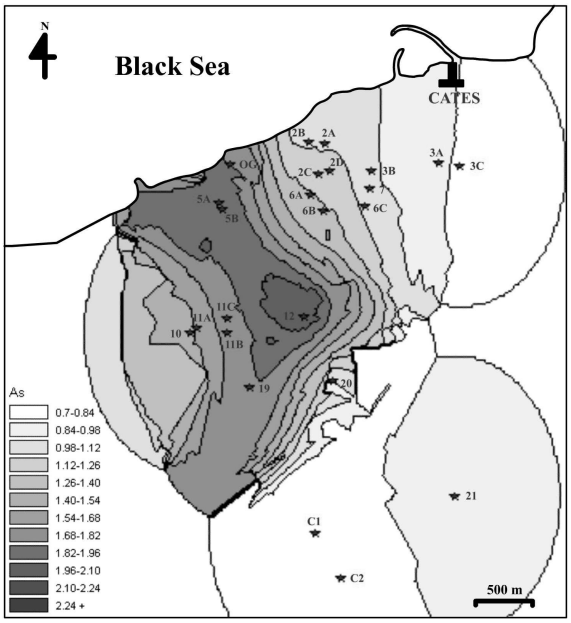

FIGURE 5
Contour map for arsenic concentrations (μg g^{-1} d.w.) in CATES.

western part of the study area. The lowest and highest arsenic concentrations were found to be 0.70 μg g^{-1} in *Scleropodium purum* and 2.40 μg g^{-1} in *Homalothecium lutescens,* respectively. Arsenic average level was found to be 1.32 μg g^{-1} (median 1.07 μg g^{-1}), which was highly elevated compared with the mean European values (0.19 μg g^{-1} in Finland [7], 0.40 μg g^{-1} in Spain [35], 0.56 μg g^{-1} in Austria and 0.34 μg g^{-1} in Germany [37]. In addition, the concentrations of arsenic exceeded 1.0 μg g^{-1} in 50 % of the samples. The peak value near the residential site in the first region, and high local arsenic content in the second region, may be explained by intensive coal utilization in this area. Concentrations tended to decrease from pollution source with distance. The mean arsenic level in polluted regions is approximately two times higher than that of control samples (*Scleropodium purum, Brachythecium rivulare*) (Fig. 5).

Cobalt. Cobalt levels ranged from 0.06 to 6.06 μg g^{-1}, with a median of 1.39 μg g^{-1} which was highly elevated with regard to the mean European values (0.37 μg g^{-1} in Norway [38] and 0.60 μg g^{-1} in NW Spain [39]). Besides, the concentrations of cobalt exceed 1.65 μg g^{-1} (mean value) in 37.5 % of the samples. The lowest and highest cobalt values were observed in *Scleropodium purum* and *Eurhynchium praelongum* species, respectively. Samples collected from the control region have 0.06 and 0.99 μg g^{-1} cobalt concentrations. Higher levels of cobalt can be associated with coal combustions in surrounding areas. The northeast part of the study area was the least polluted one, because there the main wind directions of north and northwest are more frequently than southwest ones. Cobalt amounts in the polluted area for *Scleropodium purum* species were found to be 3.5 times higher than those in control region samples. However, the cobalt level in *Scleropodium purum* near to the plant is identical to that in samples collected from the control region. This situation clearly shows that spreading cobalt emissions from the power station are transported by prevailing winds to the places far away from the source. A potential source of high cobalt levels may be connected to the coal-fired power plant (Fig. 6).

Chromium. Above all, higher levels of chromium are associated with emissions from the coal-fired power plant and coal-mining works. The other emission source may be intensive traffic, especially transport near the intercity road. All Cr measurements ranged from 0.01 μg g^{-1} to 17.12 μg g^{-1}, with a median of 3.31 μg g^{-1}. Maximum values were measured at the sites close to the most urbanised area. Other high levels of Cr were measured at the sites in the direction of prevailing winds (10.52 μg g^{-1} in *Scleropodium purum* and 10.01 μg g^{-1} in *Ctenidium molluscum*). The lowest level (0.01 μg g^{-1}) in *Hypnum cupressiforme* was found in the northeast part of the study area. However, chromium contamination was also observed in rural area (C1, 3.50 μg g^{-1} in *Scleropodium purum*), which might be due to coal-mining in the neighboured fields (Fig. 7). Chromium mean level (4.81 μg g^{-1}) in Çatalagzi province was highly elevated, when compared with the means of Finland (1.25 μg g^{-1}) [7], Galicia in NW Spain (1.2 μg g^{-1}) [39], Hungary (2.8 μg g^{-1}) [6], North Spain (2.68 μg g^{-1}) [35], Norway (2.6 μg g^{-1}) [38], Germany (2.11 μg g^{-1}) and Poland (2.54 μg g^{-1}) [5].

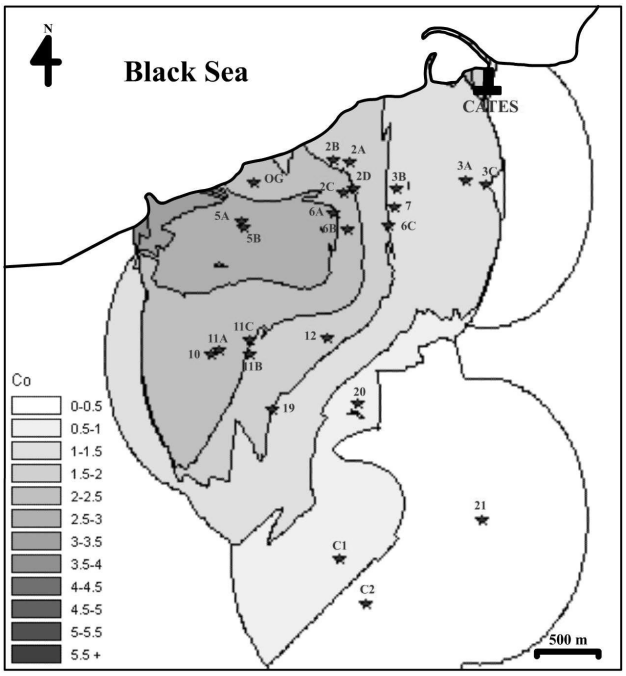

FIGURE 6
Contour map for cobalt concentrations (μg g^{-1} d.w.) in CATES.

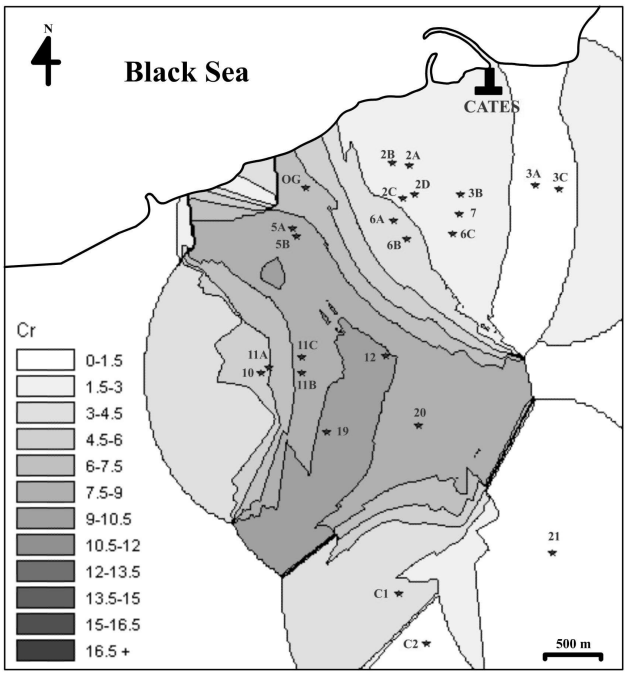

FIGURE 7
Contour map for chromium concentrations (μg g^{-1} d.w.) in CATES.

Copper. Copper mainly originates from metal industry, mining, coal-fired plants, traffic, and even soil [33]. As clearly seen in Fig. 8, the coal–fired power plant and coal-mining works are the main sources. The mean concentration of copper was approximately 3.34 μg g^{-1} in the study area, and similar to European values [6, 7, 35, 38, 39]. The elevated concentrations found in some regions were similar to that of Cobalt distribution. Extremely high emissions and accumulations of Cu were observed southwest of the study area (32 μg g^{-1} in *Eurhynchium praelongum* and 12.03 μg g^{-1} in *Hypnum cupressiforme*), because of exposure to prevailing wind directions. The concentrations of copper did not exceed 0.05 μg g^{-1} in 54 % of the samples. The northeast part of the study area was the least polluted site (Fig. 8).

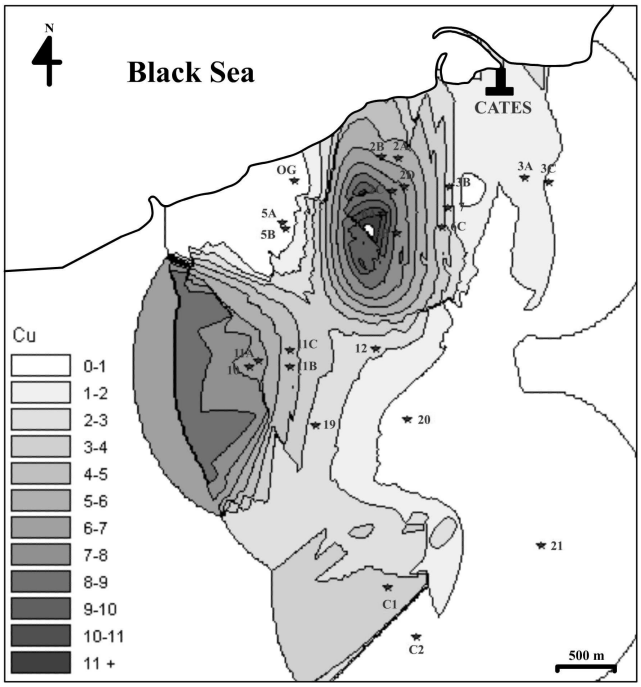

FIGURE 8
Contour map for copper concentrations (μg g^{-1} d.w.) in CATES.

Nickel. Nickel mainly originates from oil and coal burning, steel industry, and smelters [6]. In most European countries, the concentration varied between 2 and 4 μg g^{-1} in mosses [37]. Average nickel levels in the whole study area were approximately in the range of 4 to 6 μg g^{-1}, but concentrations exceeded 5 μg g^{-1} in 33 % of the samples. Nickel mean level (6.61 μg g^{-1}) is three times higher than that of control region samples (2.52 μg g^{-1} for *Brachythecium rivulare* species). Extremely high emission and accumulation of Ni (16.71 μg g^{-1} in *Scleropodium purum*) was observed southwest of the study area. This distribution could be again explained by exposure to prevailing winds from the coal-fired power plant site. In addition, this region is exposed to heavy particles from coal separation processes (Fig. 9). Our values were fairly high with a mean of 6.61 μg g^{-1}, compared with the European means (1.6–3.7 μg g^{-1}) [6, 7, 35, 38].

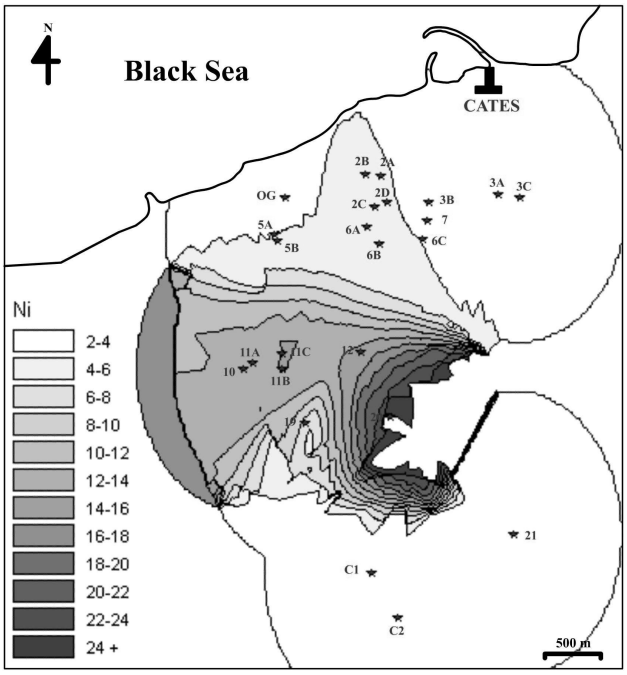

FIGURE 9
Contour map for nickel concentrations (μg g^{-1} d.w.) in CATES.

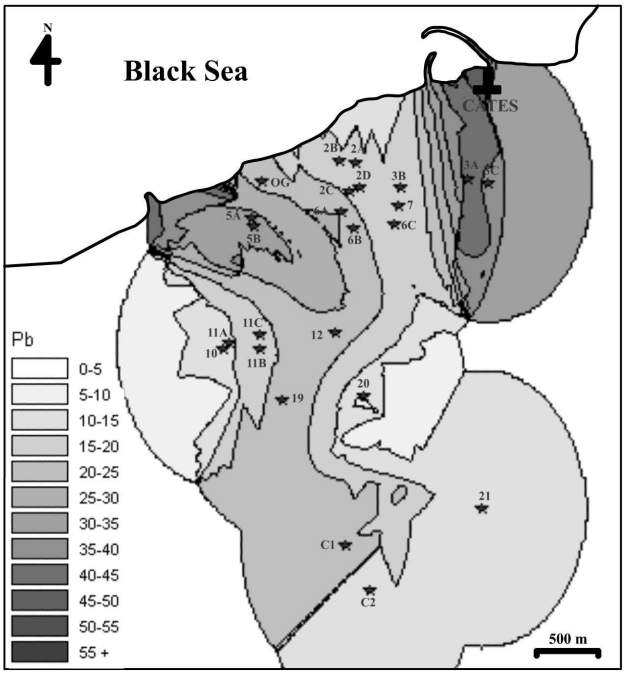

FIGURE 10
Contour map for lead concentrations (μg g^{-1} d.w.) in CATES.

Lead. Combustion of leaded fuel is still a main source of lead pollution, together with metal production and min-

ing sources. The measured values of the background atmospheric deposition in the area were mildly high with a mean of 21.10 μg g^{-1}, with respect to the European mean values (12.9–20 μg g^{-1}) [37], and 41 % of samples exceeded 20 μg g^{-1}. Higher concentrations were observed in the places near to the main road and close to the power plant. In addition, coal as a fossil fuel may also contain considerable amounts of lead. The highest level of Pb, recorded as 56.73 μg g^{-1} in *Eurhynchium praelongum,* was sampled from site 6A in the map, close to the main road, followed by 41.72 μg g^{-1} in *Scleropodium purum* from a hill exposed to main winds coming from the plant site. The elevated concentrations found in some regions were similar to the distributions of Co and Cr (Fig. 10).

Generally, the highest concentrations of Ni, Cr and As were detected in places approximately 3 km away from the pollution source (southwest of the plant) in the direction of prevailing winds (NNW and NNE). This region (named as second region) was also determined to be the most polluted site by the ISC-ST model [32]. In the first region, relatively close to the plant and mainly a residential site, the highest concentrations of Cu and Co were determined. Among all metals studied, Fe and Pb have the highest concentrations and their uptake levels in mosses were also higher than that of the other metals in the vicinity of the power plant (named as third region). In control points, all metal uptake concentrations in mosses were detected to be very low, as expected.

Statistical Evaluation. In the statistical analysis, Fe and Co elements showed significant correlations between each other ($p< 0.05$). Statistically meaningful differences were found for Fe concentrations between second and third region, and between third region and control sites. Furthermore, Co between first region and control sites ($p< 0.05$) was significantly correlated. Significance levels are given in Table 4 for comparison.

The correlation between distance from pollution source and element concentration was strong (R^2=0.72) for Fe and Pb, but moderate for Co (R^2=0.58), and not good for other metals (i.e. R^2=0.30 for As) [32]. As an example, Fig. 11 shows the Fe content versus distance, as plotted from measured values and the best fitting curve.

TABLE 4 - Statistical analysis of metal concentrations.

ÇATES	Fe [b, c] (μg g^{-1})	Pb (μg g^{-1})	Ni (μg g^{-1})	Cr (μg g^{-1})	As (μg g^{-1})	Cu (μg g^{-1})	Co [a] (μg g^{-1})
Regions	Mean (±SD.)	Mean (±SD.)	Mean (±SD.)	Mean (±SD.)	Mean (±SD.)	Mean (±SD.)	Mean (±SD.)
1st region	2348,86 (± 684,61)	30,32 (± 21,13)	4,31 (± 1,07)	5,89 (± 6,45)	1,56 (± 0,49)	10,08 (± 13,30)	3,11 (± 1,83)
2nd region	2321,40 (± 999,91)	16,82 (± 6,80)	10,10 (± 7,51)	5,39 (± 3,86)	1,55 (± 0,56)	3,13 (± 4,22)	1,78 (± 0,90)
3rd region	4560,29 (± 1998,59)	21,87 (± 17,27)	3,29 (± 0,62)	2,01 (± 1,46)	1,04 (± 0,38)	0,42 (± 0,98)	1,23 (± 0,97)
Control region	1351,14 (± 1161,36)	16,52 (± 6,82)	7,88 (± 11,14)	4,77 (± 3,92)	0,99 (± 0,47)	1,09 (± 1,49)	0,57 (± 0,59)

a) ($p<0.05$) for comparison of first and control region. b) ($p<0.05$) for comparison of second and third region. c) ($p<0.05$) for comparison of third and control region.

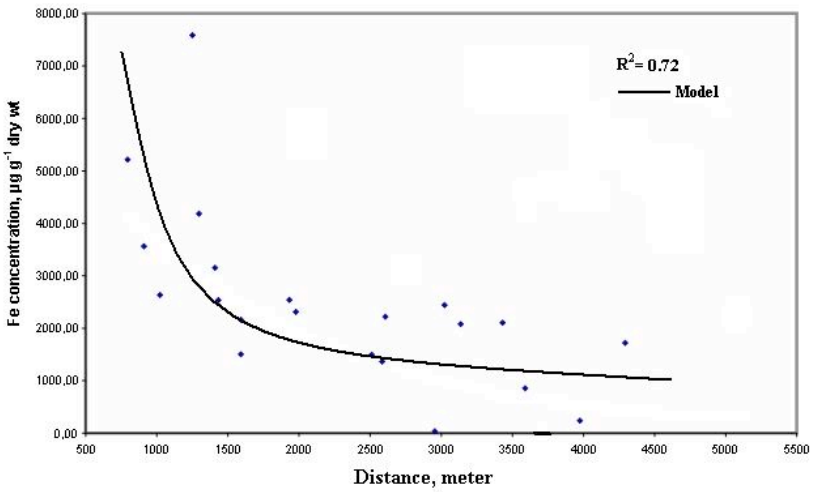

FIGURE 11
Fe concentrations in mosses versus distance from the source.
TABLE 5 - Correlation between metal concentrations.

	Fe	Pb	Ni	Cr	As	Cu	Co
Fe	1						
Pb	0.040	1					
Ni	0.107	-0.045	1				
Cr	**-0.226***	0.196	0.304	1			
As	0.046	0.041	0.115	**0.563****	1		
Cu	-0.091	0.125	-0.168	-0.192	-0.079	1	
Co	**0.371****	**0.299***	-0.085	0.111	**0.256***	**0.608****	1

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

The correlation analysis (Pearson correlation, 2-tailed) between metals is presented in Table 5, indicating a good correlation between Pb-Co and Co-As ($p<0.05$), and high positive correlations ($p<0.01$) between Co-Fe, As-Cr and Cu-Co for all region. The other correlations between metals were found to be insignificant. Negative correlations between Fe and Cr were also found to be significant ($p<0.05$).

Elements in milled coal are redistributed by combustion into bottom-ash, electrostatic precipitator (ESP's) fly-ash, and stack-emitted materials [33, 40]. Table 6 presents the chemical analysis of the fly-ashes obtained from ÇATES for the heavy metals studied, and compares the analysis of fly-ash from the CATES and world coals. As can be seen, iron is the most abundant element, whereas the other metals are present only at trace levels. But if the ash production is taken into consideration (645,000 tons year^{-1} slag and fly-ash), trace elements are significant pollutants in the surrounding area. Fe, Cr, Ni and Cu are the elements originating from the coal combustion, and Ni and Cr concentrations were higher than those of world coals [41-43].

TABLE 6 - Some heavy metals in Çatalağzı Power Plant's fly ashes and world coals.

Components	Contents (μg g^{-1})	
	CATES	World coals
Fe	*2.0	-
Pb	48	2-80
Co	No data	18
As	49	0.5-80
Cu	46	0.5-50
Cr	76	0.5-60
Ni	90	0.5-50

*: percentage (%)

In this study, iron had the highest concentration, followed by lead, chromium, nickel, copper, cobalt, and arsenic. The magnitude of the metal concentrations in indigenous mosses can be ordered as Fe>Pb>Cr>Ni>Cu>Co>As. This is slightly different when compared with Table 6, but may be explained by contamination from other sources, such as traffic and domestic heating. When examining these results, each should keep in mind that the heavy metal concentrations in mosses do not directly reflect their total deposition. There are differences in the accumulation of individual heavy metals in mosses, and their concentrations in mosses are also affected by factors other than atmospheric pollution. The effects of other factors may be considerable, especially in background areas.

Only five elements of environmental concern (As, Cd, Hg, Ni and Pb) are designated as "toxic substances" under the terms of the Canadian Environmental Protection Act CEPA, 1995 [44]). Chromium is added to this list in the present study, due to the possible presence of Cr^{+6}, a carcinogenic form of Cr [45].

CONCLUSION

Metal biomonitoring with naturally growing mosses is a valid and useful technique used in Europe for more than thirty years. In the present study, it was used to describe metal depositions in the environment of a coal-fired power plant in Turkey. It was found that the uptake concentrations of Fe, Co and As are significantly higher compared to other European levels. Nevertheless, Pb (except the mean value of Pb in Poland), Ni and Cr (except the mean values of Ni and Cr in Italy) are mildly elevated in comparison with the European averages. Cu is the only metal showing nearly similar values to the European means.

Generally, the highest bioaccumulation values are measured in the direction of prevailing winds and places close to residential sites, but they were decreasing rapidly with distance, according to a power curve. The effect of traffic compared to the influence of the region`s coal-fired power plant is much milder, since most of the sampling points were not close to the roads, and contamination is rapidly reduced with distance.

In brief, it may be said that the use of coal in the power plant and the high standard of dust emissions in this region cause increase of the levels of some specific heavy metals; i.e. Fe, Pb, As, Co, Cr and Ni. In addition, this study is the first attempt to characterize the atmospheric deposition of seven heavy metals (Fe, Pb, Ni, Cr, Cu, Co, As) by

means of indigenous mosses in the vicinity of the Çatalagzi power plant (CATES) in Zonguldak, Turkey. Our data serve as a reference database for the future studies, to monitor any changes in background heavy metal deposition. Further investigations are necessary to determine the trace metal pollution trends in this region. Important consequences on ecological processes at all levels of organisation, from single organisms to globe, may be foreseen, including possible effects on human health. Therefore, legal measures should be taken to control the amount of contamination in order to protect public health as well as the environment.

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THE USE OF SOME NATURAL PLANT SPECIES FROM THE WESTERN BLACK SEA REGION OF TURKEY FOR LANDSCAPE DESIGN

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SUMMARY

Plant materials have an essential importance in landscape architectural applications. The use of locally natural-growing plants is generally useful, and provides facilities in the selection of plant material. The research for this work was performed in Bartın, Karabük and Zonguldak, located in the Western Black Sea region of Turkey. In this region, two parts of Karabük, named Keltepe and Yenice, have the richest natural vegetation. In this study, 101 woody and 417 herbaceous (a total of 518) plant species in landscape architecture have been investigated. Shape and colour of the flower, blossoming time and its duration, have been observed. In addition, each plant type was evaluated separately for its possible use in landscape planning for the investigated area. A total of 92 of the investigated plants were collected for the first time and identified as new species. The plant species which are naturally distributed in the floral regions are 104 „Euro-Siberian"; 61 „Mediterranean Element"; 34 „Euxine"; 96 „Widespread"; 7 „Hyrcano-Euxine"; 8 „Irano-Turanien"; and 210 unknown species. Here, the Compositae family showed the highest number of genera (41) and species (66), respectively. From 11 endemic plants with defined landscape value, the species *Dianthus setisquamosus, Dianthus kastembeluensis, Centaurea cadmea, Centaurea kilaea,* and *Centaurea inexpectata* were at the top, but the other endemic species should also be taken as being valuable for planting. Later on, the use of *Cardamine* sp., *Silene* sp., *Tamarix* sp., *Pterocarya fraxinifolia, Cercis siliquastrum, Origanum* sp., *Muscari* sp. and *Cyclamen* sp. in landscape regulations should be provided.

KEYWORDS:
Natural plants, natural plants identification, landscape values, species used in landscape architectural applications.

PRELIMINARY REMARKS

The earliest botanical researches in the study area were performed by Rıza and Palibine in 1920 [1], who primarily focussed on weeds and different kinds of woody plants. Other similar studies by Birand (1968) [2] and Kasaplıgil (1947) [3] described in detail the plant species of the Western Black Sea region. The plant families in the forest Karabük-Büyükdüz have been investigated in terms of phytosociology by Aksoy (1978) [4], and the Sakarya-Filyos-river area regarding phytogeography by Yalçınlar in 1985 [5]. The relationship between a great part for the herein investigated plants and the wood species in remote floras was the subject of examinations performed by Browicz in 1988 [6]. In total, 134 representative woody areas of the wood assemblages in the Çitdere-Yenice area have been analysed also for their phytosociology by Özalp (1989) [7]. Paleobotanic information on the investigated area can be found in the report of Aytuğ (1970) [8], and information on the wooden structures in the planned Bartın-Kirazlı area is given by Başaran (1998) [9].

As the basis for identification of the plant species described herein relied upon the volumes of the "Flora of Turkey and East Aegean Islands", edited by Davis (1964-1985) [10], as well as on the reports by Bonnier (1912-1934) [11], and Tutin and Heywood (1964-1980) [12]. Also considered in this regard were the works of Arnal (1996) [13] and Noordhuis (1996) [14], as well as that of Yaltırık and Efe (1989) [15]. Yaltırık (1991) [16] and Acartürk (1997) [17] served further as botanical reference for this investigation, and also Mayer and Aksoy (1998) [18] were consulted for the determination of habitat-characteristics of the respective wood-taxa. Aspects concerning the use of natural plant species in landscaping were discussed by Koç (1977) [19] and Ayaşlıgil (1989) [20]. The work of Kostak (1998) [21] was additionally considered due to its relevance concerning handling and storage of ornament plants that occurred naturally in the flora of Turkey. In some Turkish cities, plants from natural habitats were planted/ used through landscape design measures, which have been described also in the literature (Erik et al. (1998) for Ankara

[22], Yücel (1992) for Eskişehir [23], Yaltırık (1996) for İstanbul [24], and Karaer and Kılınç (1993) for the Sinop-peninsula at the Turkish Black Sea coast [25]. In this regard, the work of Özgen (1987) [26] has also gained attention. Finally, the practical hints on more than 1,500 plants stated in Ferguson (1978) [27] served as a source for orientation.

INTRODUCTION

During the last few decades, the rapid, unplanned, and unhealthy expansion of urban areas in Turkey occurred at the expense of lands previously used for agriculture, forestry, as well as grazing and other green open places. Qualitative and quantitative reduction of vegetation in these areas lead to severe environmental and social consequences. With the help of landscape measures, cities and communities have created natural places for recuperation, and re-established disturbed relationships between humans and the nature. The use of plants as found in their natural habitats in urban open spaces can contribute to the diversity of green areas that have previously been set aside for monocultures. Native plants offer better ecological and economical benefits, since they tend to have higher survival rates, in contrast to exotic plants, which may be imported from different ecological regions. The study area, which reaches the provincial borders of the cities Zonguldak, Bartın and Karabük, possesses a wide floristic spectrum of species, especially in the region of Yenice and Karabük-Keltepe, where numerous endemic species can be found. This area has rather attractive countrysides, which makes it predestined for inspirational landscaping concepts (Fig. 1).

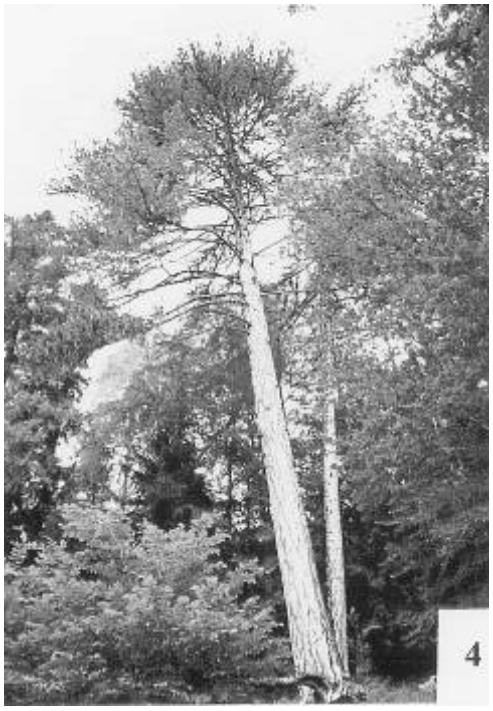

FIGURE 1 - Countryside close to the research forest Büyükdüz.

The first investigation in the research area has been exerted by Yatgın in 1996 [28], for which the author examined 57 different wooden and weed species, and scrutinized further possible applications of the species in terms of landscaping. Another study was performed in the flora of Bartın-Amasra by Topay and Kaya in 1998 [29], attempting to evaluate some weed-like ornamental plants for landscape architecture and possible applications in urban open spaces. Another investigation, exerted by Sarıbaş (1998) [30], eval-uated 77 taxa of Angiospermae, in terms of their feasibility as ornamental plants and landscaping characteristics. More recently, in 2002, within a research project as to the biodiversity of the province Zonguldak, Sarıbaş et al. [31] examined 597 plant species.

MATERIALS AND METHODS

The materials investigated for this research were taken from the plant area of the provinces Zonguldak, Karabük and Bartın. When selecting the wooden species, emphasis was laid on species particularly feasible for landscaping performances. For setting up a herbarium, exemplary plants were collected at spots and surroundings as shown in Fig. 2. In total, 59 locations were chosen at the district towns: Kozcağız, Ulus, Kurucaşile and Amasra (province Bartın); Karabük-city, Keltepe, Yenice, Eflani and Safranbolu (province Karabük); Zonguldak-city, Çaycuma, Gökçebey, Ereğli, Alaplı, Devrek and Dirgine (province Karabük). The collected plants were identified in herbariums of the Bartın Faculty of Forestry, whereupon a file was prepared for each identified plant, containing information about family, species, location, flora region, as well as habitat and morphological characteristics. A general evaluation of individual plants followed according to their form, blossom colour, flowering period, and feasibility in landscaping.

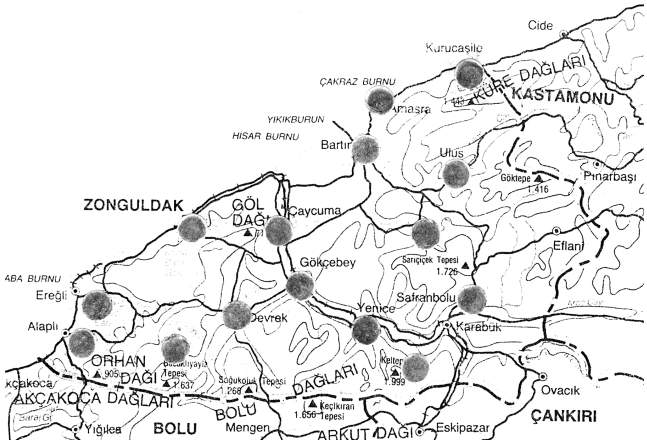

FIGURE 2
Sampling places shown as spots (●) in the study area.

Geographical location of the investigated area

The research area comprised the entire province of the city Zonguldak. It should be noted that, in 1992, the prov-

ince Zonguldak has been parted in three provinces, named Zonguldak, Bartın and Karabük. The province Bartın covers in total 1266 km^2 and is surrounded in the east and south by mountains with averagely 1000 m in altitude. The hydrology of the Bartın region is predominantly influenced by the Bartın river. The province Zonguldak, on the other hand, shows a relatively parted character due to some river valleys. The highest point given here is the mountain Orhan-Dağ with 900 m in altitude. (Fig. 3)

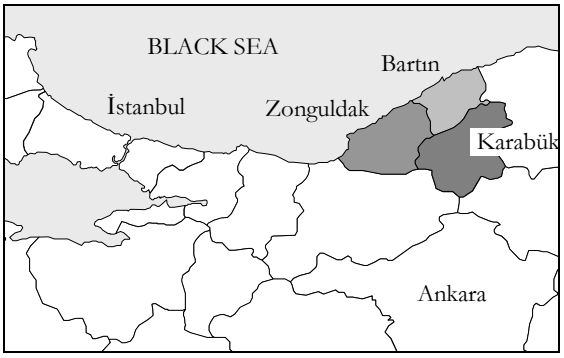

FIGURE 3 - Geographical location of the investigated area, showing the provinces Zonguldak, Bartın and Karabük.

Geology and soils of the investigated area

Tertiary and quaternary-formations are the primary geological structures of the study area. In the regions of Çaycuma, Devrek, Yenice and Kozcağız, also palaeozoic layers are observed. According to Ketin (1983) [32], soil types can be described primarily as black forest and sour soil, alluvial soil, chestnut coloured soil, and black veld soil ones.

Climate of the study area

According to different meteorological stations located in this region, the climate can be characterized as being predominantly the Western Black Sea - Type IIc (Erinç, 1969) [33]. In the Yenice-Çitdere region, however, a local mesothermal microclimate without water deficiency prevails, which can also be designated as Oceanic climate type, B4-b1-r3 (Sarıbaş, 1989) [34].

The forests of the study area

The forest types of this area can be described as Euxine deciduous mixed forests. There is a short border along the East with a Hyrcanian region, which displays a relatively weak presence. In the South of the area, the region is separated by the sub-Euxine zone and the central Anatolian veld, showing two clearly distinguished climate and vegetation regions. According to Zohary (1973) [35], the xero-Euxine region can generally be characterized as sub-Mediterranean Quercus pubescens-region.

The use of the investigated plants for landscaping purposes

Plants of the study area may be used in landscape design as solitaire trees, in ground floors, in stone and water gardens, in urban parks, for planting vegetation on roads, for natural arrangements, for compositions in natural colours at open spaces, as hedge plants, and as clamberers on facades.

RESULTS AND DISCUSSION

In total, 518 species (101 woody and 417 herbaceous ones), as listed in Table 1, were collected and examined in terms of their feasibility in landscape architecture. 92 of them were recorded for the first time, and registered as new pictures for the A4-quadrat. The identified plants can be categorized according to their distributions in the flora regions of Turkey as follows [10]: 104 „Euro-Siberian“, 61 „Mediterranian Element“, 34 „Euxine“, 96 „widely spread“, 7 „Hyrcano-Euxine“ and 8 „Irano-Turanien“ species. In this relation, 210 species could not be assigned. The family comprising the highest number of genera and species is the Compositae family (41 genera and 66 species), followed by Leguminosae (29 genera, 44 species), and Rosaceae (20 genera, 30 species). 11 out of the 518 investigated species were of endemic nature: *Abies nordmanniana* subsp. *bornmülleriana, Dianthus castambeluensis, Dianthus setisquamosus, Rhamnus thymifolius, Euonymus latifolius* subsp. *cauconis, Crataegus tanacetifolia, Crataegus dikmensis, Centaurea cadmea, Centaurea kilaea, Centaurea inexpectata*, and *Galanthus pilicatus* subsp. *byzantinus*. Figs. 4 and 5 give two examples of soil covering plant species observed in the research area.

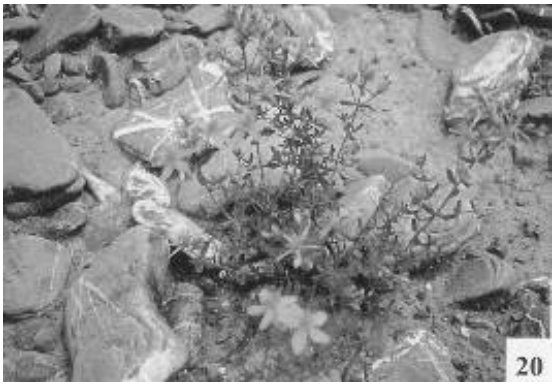

FIGURE 4 - *Hypericum perforatum* L.

FIGURE 5 - *Sedum album* L.

TABLE 1 - Morphology and usage of plant taxa observed in the investigated area (the numbers indicate the following: Species 1: shrub, 2: sporophyte, 3: tree, 4: weed; Possibilities of usage – 1: solitary tree, 2: ground floor flowers, 3: in stone gardens, 4: in water gardens, 5: in places inside, 6: in urban parks, 7: on road borders, 8: for natural landscaping, 9: as colour compositing elements, 10: as ground floor coverage, 11: in groups of plants, 12: on acclivities, 13: as hedge plants, 14: as clamberers).

No.	Plant taxa	Species	Blossom colour	Flowering Duration (months)	Usage possibilities
1	*Ephedra major*	1	yellow	4-8	1, 3
2	*Equisetum arvense*	2	yellow	4-8	2, 4
3	*Equisetum telmeteia*	2	yellow	4-8	2, 4
4	*Equisetum pallustre*	2	yellow	4-8	2, 4, 11
5	*Equisetum ramossisimum*	2	yellow	4-8	2, 4, 11
6	*Pteridium aquilinum*	2	yellow	4-8	5, 3
7	*Asplenium trichomanes*	2	yellow	4-8	3
8	*Asplenium adiantum nigrum*	2	yellow	4-8	3
9	*Ceterach officinarum*	2	yellow	4-8	5, 3
10	*Phytillitis scolopendrium*	2	yellow	4-8	10, 4
11	*Athyrium distentifolium*	2	yellow	4-8	2, 3
12	*Polystichum setiferum*	2	yellow	4-8	10, 3
13	*Polystichum aculeatum*	2	yellow	4-8	10, 3
14	*Dryopteris abreviata*	2	yellow	4-8	10, 2
15	*Dryopteris filix-max*	2	yellow	4-8	8, 11
16	*Polypodium vulgare* ssp. *vulgare*	2	yellow	4-8	3, 8
17	*Pinus brutia*	3	yellow	4-5	1, 12, 6
18	*Pinus nigra*	3	yellow	4-5	1, 6, 12
19	*Pinus pinea*	3	yellow	4-5	1, 7, 12
20	*Pinus silvestris*	3	yellow	4-6	1, 6, 7
21	*Pinus nigra* var. *yaltirik*	3	yellow	5-6	6, 7
22	*Abies nordmanniana* ssp. *bornmülleriana*	3	yellow	3-5	1, 6
23	*Taxus baccata*	1	yellow	2-3	1, 11, 13
24	*Juniperus sabina*	1	yellow	2-3	10
25	*Juniperus communis* ssp. *nana*	1	yellow	2-3	10
26	*Ranunculus constantinopolis*	4	yellow	3-5	1, 6
27	*Ranunculus muricatus*	4	pale-yellow	3-5	1, 11
28	*Ranunculus ficaria* ssp. *ficariformis*	4	yellow	3-4	1
29	*Ranunculus marjinatus* var. *marjinatus*	4	yellow	4-6	1, 11
30	*Ranunculus repens*	4	yellow	5-7	1, 11
31	*Ranunculus gracilis*	4	yellow	4	1
32	*Ranunculus chius*	4	yellow	3-4	4, 12
33	*Ranunculus ophioglossifolius*	4	yellow	3-4	2, 4
34	*Adonis aestivalis*	4	red	5	2, 11
35	*Anemon nomerosa*	4	pink	3-4	2, 11
36	*Helleborus orientalis*	4	white	3-5	2, 11
37	*Thalictrum aquilegiofolium*	4	white	5-7	2, 4
38	*Thalictrum lucidum*	4	white	6-7	8
39	*Thalictrum flavum*	4	white	6-7	8
40	*Clematis vitalba*	4	white	6-9	14
41	*Epimedium pubigerum*	4	pink	5-6	2
42	*Berberis crataegina*	1	pink	5-6	2, 11, 13
43	*Papaver rhoas*	4	red	3-8	2
44	*Papaver commutatum*	4	dark red	5-6	9
45	*Papaver dubium*	4	bright red	4-6	9
46	*Chelidonum majus*	4	yellow	4-8	2, 11
47	*Arabis causasica.* ssp. *causasica*	4	white	3-8	10
48	*Nasturtium officinale*	4	white	3-7	2, 11
49	*Barbarea vulgaris*	4	yellow	4-5	2, 11
50	*Cardamine quinquefolia*	4	bright blue	3-5	2, 11
51	*Cardamine bulbifera*	4	purple	4-6	2, 11
52	*Cardamine impatiens* var. *pectinata*	4	white	6-8	2, 11
53	*Cardamine hirsuta*	4	white	3-4	2, 11
54	*Cakile maritima*	4	violet-red	6-8	3, 15
55	*Capsella bursa-pastoris*	4	white	1-11	8
56	*Lobularia maritima*	4	white	6-7	2, 11
57	*Alliaria petiolata*	4	white	4-6	2, 11
58	*Raphanus raphanistrum* ssp. *maritimus*	4	white	3-5	2, 8
59	*Aurinia saxatilis* ssp. *orientalis*	4	yellow	4-5	2, 8
60	*Brassica elongata*	4	yellow	4-6	2
61	*Hirsfeldia incana*	4	yellow	5-7	2, 8

No.	Plant taxa	Species	Blossom colour	Flowering Duration (months)	Usage possibilities
62	*Cistus salviifolius*	1	white	3-6	3
63	*Cistus creticus*	1	bright red	5-8	3
64	*Helianthemum nummularium* ssp. *num.*	4	yellow	4-8	2, 11
65	*Viola sieheana*	4	blue-white	3-6	2, 5
66	*Viola odorata*	4	violet	4-5	2, 11
67	*Polygala vulgaris*	4	blue-pink	5-8	2, 11
68	*Polygala pruniosa*	4	blue-violet	4-7	2
69	*Portulaca oleraceae*	4	yellow	3-5	10
70	*Phytolacca americana*	4	white	6-9	1
71	*Sagina procumbens*	4	white	6-7	10
72	*Silene italica*	4	white	5-6	2, 11
73	*Silene vulgaris*	4	white	5-8	2, 11
74	*Silene otites*	4	yellow	6-7	2, 11
75	*Sagina apetala*	4	white	5-8	2
76	*Dianthus kastembeluensis*	4	pink	8	2, 11
77	*Dianthus setisquamosus*	4	pink	7-8	2, 16
78	*Dianthus gigantheus*	4	red	6-9	2, 11
79	*Cerastium glomeratum*	4	white	6-7	2
80	*Polygonum persicaria*	4	pink	8-12	2, 10
81	*Polygonum arenastrum*	4	pink	6-11	2, 10
82	*Polygonum lapathifolium*	4	pink	6-8	4
83	*Polygonum aviculare*	4	yellow	7-11	4
84	*Polygonum hidropiper*	4	pink	8	4
85	*Rumex hidrolapathum*	4	yellow	6-8	4
86	*Rumex crispus*	4	white	5-7	4
87	*Chenopodium foliosum*	4	red	5-7	2, 11
88	*Tamarix smirnensis*	1	pink	4-8	1, 11
89	*Hypericum calicinum*	4	yellow	5-10	12
90	*Hypericum androcaemum*	4	yellow	6-7	8
91	*Hypericum bithynicum*	4	yellow	5-10	11
92	*Hypericum perforatum*	4	yellow	4-9	6, 12
93	*Malva alcea*	4	pink	5-7	2
94	*Malva silvestris*	4	pink-violet	5-10	1, 11
95	*Melisa officinalis* ssp. *officinalis*	4	white	6-9	2
96	*Alcea pallida*	4	violet	5-10	2, 11
97	*Lavatera cretaica*	4	pink	4-5	2
98	*Tilia argentea*	3	white	6-7	6
99	*Tilia rubra*	3	yellow	6-8	6, 7
100	*Tilia grandifolia*	3	yellow	6-8	6, 7
101	*Linum bienne*	1	yellow	4-8	3, 1
102	*Linum usitatissimum*	4	blue	3-5	2, 8
103	*Geranium collinum*	4	purple	6-8	2, 11
104	*Geranium purpureum*	4	pink	3-4	8
105	*Geranium lucidum*	4	pink	3-5	2, 11
106	*Geranium redundifolium*	4	purple	3-5	2, 8
107	*Geranium molle* ssp. *molle*	4	pink	3-4	2, 10
108	*Geranium dissectum*	4	pink	3-4	10, 11
109	*Geranium robertianum*	4	pink	4-8	2, 8
110	*Geranium pussilum*	4	bright-violet	5-6	2, 11
111	*Geranium columbinum*	4	violet-blue	4-6	2, 11
112	*Erodium cicutaria* ssp. *cicutaria*	4	pink	4-5	2, 11
113	*Oxalis acetocella*	4	white	4	2
114	*Oxalis corniculata*	4	yellow	3-8	2, 11
115	*Acer campestre* ssp. *campestre*	3	yellow	4-5	6, 7
116	*Acer trautvetteri*	3	yellow-green	5-6	6, 7, 8
117	*Acer platanoides*	3	yellow-green	4-5	1
118	*Dictamnus albus*	3	white	5-6	2, 12
119	*Ruta graveolens*	3	yellow	6-8	3, 12
120	*Staphylea pinnata*	1	yellow	4-5	1, 6
121	*Staphylea colchica*	1	white	4-5	1, 6
122	*Frangula alnus* ssp. *alnus*	1	white	6-7	1
123	*Paliurus spina-christii*	1	yellow	5-6	12
124	*Rhamnus thymifolius*	1	yellow	4-6	3
125	*Ilex aquifolium* ssp. *colchica*	1	white	6-7	3
126	*Pistacia terebinthus* ssp. *terebinthus*	3	white	4-5	6, 7, 12
127	*Rhus coriaria*	3	red-brown	4-5	1, 7, 10
128	*Cotynus coggyria*	3	violet	4-5	1, 11
129	*Euonymus latifolius* ssp. *latifolius*	3	green-white	5	1

No.	Plant taxa	Species	Blossom colour	Flowering Duration (months)	Usage possibilities
130	*Euonymus latifolius* ssp. *cauconis*	3	white	5-6	1, 11
131	*Psorolea bituminosae*	4	violet-blue	5-8	10
132	*Calycotome villosa*	4	yellow	3-6	2, 11
133	*Dorycnium graceum*	4	white	4-8	2, 11
134	*Genista tinctoria*	3	yellow	4-7	3, 10
135	*Trifolium campestre*	3	yellow	2-4	10
136	*Trifolium resupinatum* var. *resupinatum*	3	pale-blue	5	10
137	*Cytisus supinus*	3	yellow	4-6	3, 12
138	*Rostraria hispida*	3	red	4-6	8, 10
139	*Scorpiurus muricatus* var. *subvillosus*	3	yellow	4-5	2, 10
140	*Medicago arabica*	3	blue	3-5	2, 11
141	*Medicago polimorpha*	3	yellow	3-5	2, 11
142	*Medicago littoralis*	3	yellow	4-6	2
143	*Melilotus alba*	3	yellow	2-5	2, 11
144	*Colutea cilicica*	1	yellow	6-8	1
145	*Lotus corniculatus* var. *corniculatus*	4	yellow	5-9	2
146	***Dorycnium pentaphyllum* ssp. *herbaceum***	4	violet	5-8	2
147	***Sophora jaubertii***	1	yellow-white	5-7	1, 2
148	*Melilotus officinalis*	4	yellow	5-9	2, 11
149	*Lathyrus hirsutus*	4	violet	5-7	14
150	*Coronilla varia* ssp. *varia*	4	white-pink	5-8	10
151	*Cercis siliquastrum* ssp. *siliquastrum*	3	scarlet	4-5	1, 11
152	*Agyrolobium biebersteinii*	4	yellow	6-8	3, 10
153	*Spartium junceum*	1	bright-yellow	6-8	1, 3
154	*Trifolium angustifolium* var. *angustifolium*	4	violet-white	3-4	10
155	*Trifolium hibridum* var. *hibridum*	4	pink	5-9	7, 12
156	*Trifolium pratense* var. *pratense*	4	violet	5-9	10
157	*Trifolium fragiferum* var. *fragiferum*	4	white	3-8	10, 12
158	*Trifolium ocroleucum*	4	cream-colour	5-8	10, 12
159	*Trifolium subterranum*	4	yellow	5-8	10, 12
160	*Trifolium arvense* var. *arvense*	4	yellow	5-8	10, 12
161	*Vicia lathyroides*	4	pink-red	5-6	10
162	*Vicia sativa*	4	violet-pink	5-6	10
163	*Vicia cracca* ssp. *sterophylla*	4	violet blue	5-6	10
164	*Medicago marina*	4	yellow	2-6	10
165	*Medicago orbicularis*	4	yellow	2-6	10
166	*Medicago falcata*	4	yellow	5-8	3, 10, 11
167	*Medicago lupulina*	4	yellow	5-8	3, 10
168	*Medicago varia*	4	cream-colour	5-8	2, 10, 11
169	*Medicago repens*	4	bright-violet	5-8	10
170	*Hypocrepis unisiliqua* ssp. *unisiliqua*	4	yellow	3-5	2, 11
171	*Ononis spinosa* ssp. *leiospermae*	4	yellow	5-8	2, 12
172	*Glycyrrhiza glabra* var. *glandulifera*	4	blue-violet	5-6	10, 12
173	*Lathyrus laxiflorus* ssp. *laxiflorus*	3	violet-blue	5-7	10, 14
174	*Anthyllis vulneraria* ssp. *boissieri*	3	yellow	6-7	3
175	*Tetragonolobus maritimus*	3	sulfur-yellow	6-7	2, 11
176	*Mespilus germanica*	4	white	5-6	1, 8
177	*Potentilla argentea*	4	yellow	6-8	10
178	*Cynodon oblonga*	1	white	5-6	1
179	*Persica vulgaris*	1	pink	3-4	1, 6, 8
180	*Potentilla reptans*	4	yellow	5-8	10
181	*Rubus sanctus*	1	pink	6-8	12, 13
182	***Rubus hirta***	1	white	6-7	12, 13
183	*Rubus canescens* var. *canescens*	1	pink-red	6-7	12, 13
184	***Rubus caesius***	1	white	3-8	12, 13
185	*Agrimonia eupotaria*	1	yellow	5-9	2, 11
186	*Prunus x domestica*	3	white	3-4	1
187	*Pink canina*	1	pink-white	5-6	2, 3, 12
188	*Sorbus aucuparia*	3	white	5	1, 7, 11
189	*Sorbus torminalis* var. *torminalis*	3	white	5-6	1, 7, 11
190	*Crataegus orientalis* var. *orientalis*	1	white	6	12, 13
191	*Crataegus curvisepala*	1	white	5-6	1, 12
192	*Crataegus microphylla*	1	white	4-5	1, 12
193	*Crataegus szovitsii*	1	white	6-7	1, 12
194	*Crataegus tanacetifolia*	1	white	4-6	1, 12
195	*Crataegus dikmensis*	1	white	4-5	1, 12
196	*Crataegus pentagyna*	1	white	5-6	1, 12
197	*Crataegus monogyna* ssp. *monogyna*	1	white	5-6	1, 12

No.	Plant taxa	Species	Blossom colour	Flowering Duration (months)	Usage possibilities
198	***Pyracantha coccinea***	1	white	4-7	1, 11, 12
199	*Pyrus communis* ssp. *communis*	1	white	4-5	1, 8
200	*Malus silvestris* ssp. *orientalis*	3	white	3-8	1, 11
201	*Prunus spinosa* ssp. *dasyphylla*	3	white	3-5	1, 11
202	*Sanguisorba officinalis*	4	chestnut-brown	6-8	2
203	*Potentilla recta*	4	yellow	5-7	2, 11
204	*Prunus avium*	3	white	4-5	1, 7, 11
205	*Laurocerasus officinalis*	1	white	4	1, 13
206	*Myrtus communis* ssp. *communis*	1	white	4-5	3, 8
207	*Epilobium lanceolatum*	4	pink	6-8	2
208	*Epilobium hirsutum*	4	pink	7-9	2
209	*Epilobium tetragonium* ssp. *tetragonium*	4	pink	6-8	2, 11
210	*Datisca cannabina*	4	bright-yellow	5-6	8
211	*Sedum album*	4	white	6-9	1, 3, 5
212	*Sedum pallidum*	4	white-pink	6-8	3, 10
213	*Sedum hispanicum* var. *hispanicum*	4	pink	4-7	3
214	*Sedum stoloniferum*	4	bright-pink	7-8	3
215	*Sedum acre*	4	yellow	6-7	3
216	*Saxifraga redundifolia*	4	white	4-9	3
217	*Saxifraga adscendes* ssp. *adscendes*	4	white	6-7	10
218	*Saxifraga tridactylites*	4	white	3-7	1
219	*Oenanthe pimpinelloides*	4	white-red	4-7	8
220	*Daucus careda*	4	pink-yellow	6-9	8
221	*Bifora radians*	4	white	5-8	8
222	*Sison ammomum*	4	white	5-8	8
223	*Torilis japonica*	4	white	4-5	2, 11
224	*Ammi visnaga*	4	white	5-8	1, 8
225	*Hedera helix*	4	yellow	5-6	14
226	*Cornus mas*	1	yellow	2-4	1, 13
227	*Cornus sanguinea*	3	white	3-5	1, 11
228	*Sambucus nigra*	1	cream-colour	5-8	1
229	*Sambucus ebulus*	4	white	6-8	12
230	*Valerianella muricata*	4	blue	4-6	8
231	*Scabiosa atropurpurea* ssp. *maritima*	4	pink	5-8	2
232	***Scabiosa columbaria* ssp. *columbaria***	4	white	6-9	2, 8
233	*Scabiosa argentea*	4	white	5-9	2, 11
234	*Scabiosa columbaria* var. *columbaria*	4	pink	6-9	2, 8
235	*Scabiosa micrantha*	4	violet	5-7	2, 11
236	*Calendula arvensis*	4	orange	1-6	2
237	*Calendula officinalis*	4	orange	1-5	2, 11
238	*Calendula suffruticosa*	4	yellow	1-6	2, 11
239	*Carlina intermedia*	4	yellow	6-8	2, 11
240	***Cnicus benedictus* var. *benedictus***	4	yellow	5-8	8
241	*Lapsana communis*	4	yellow	5-10	2, 11
242	*Doronicum orientale*	4	yellow	3-7	2
243	*Lactuca serriola*	4	yellow	7-9	2, 3
244	*Tussilago farfara*	4	yellow	1-3	2, 3
245	*Pulicaria dysenterica*	4	yellow	7-9	10
246	***Cirsium arvense***	4	yellow	7-9	2
247	*Cirsium vulgare*	4	yellow	7-9	2
248	*Eupotarium cannabibum*	4	yellow	7-10	2, 11
249	*Bellis perennis*	4	white	3-8	2, 11
250	*Agrimonia eupotaria*	4	yellow	5-9	2, 11
251	*Senecio vulgaris*	4	yellow	4-8	8
252	*Senecio aquaticus* ssp. *erraticus*	4	yellow	6-10	2, 8
253	*Senecio vernalis*	4	yellow	3-8	2, 8
254	***Anthemis tinctoria* var. *tinctoria***	4	cream-colour	3-6	2, 7
255	*Anthemis cretica* ssp. *pontica*	4	bright-yellow	4-7	2, 3
256	*Anthemis cretica* ssp. *tenuiloba*	4	cream-colour	5-6	3
257	*Anthemis tinctoria* var. *pallida*	4	beige	5-10	2, 3
258	*Centaurea salicifolia* ssp. *salicifolia*	4	red	7-8	2, 11
259	*Centaurea diffusa*	4	white	7-8	2, 11
260	*Centaurea iberica*	4	violet-pink	6-8	2, 11
261	***Centaurea stenolepis***	4	pale-yellow	7-8	2, 11
262	*Centaurea kilaea*	4	pink	7-8	2, 11
263	*Inula helenium* ssp. *turcorasemosa*	4	yellow	7-9	8
264	*Inula graveolens*	4	red-brown.	8-10	2, 11
265	*Inula britannica*	4	yellow	6-10	2, 11

No.	Plant taxa	Species	Blossom colour	Flowering Duration (months)	Usage possibilities
266	*Potentilla micrantha*	4	white	3-7	10
267	*Chondrilla juncea*	4	pale-yellow	8-9	8
268	*Conyza canadensis*	4	pinkred	7-10	12
269	*Achillea biebersteinii*	4	yellow	5-9	2
270	*Xanthium spinosum*	4	white	8-10	12
271	*Logfia gallica*	4	yellow	4-6	2, 8
272	*Mycelis muralis*	4	yellow	6-9	2, 8
273	*Taraxacum serredinum*	4	yellow	4-6	10
274	*Filago pyramidata*	4	yellow	4-6	2, 8
275	*Hieracium pannosum*	4	yellow	7-8	2, 8
276	*Pulicaria odora*	4	yellow	5-6	2
277	*Crepis foetida* ssp. *rhoedifolia*	4	yellow-red.	5-10	2, 8
278	*Sonchus oleraceus*	4	yellow	3-5	2, 8
279	*Sonchus aspera* ssp. *glaucescens*	4	yellow	6-8	2, 8
280	***Tanacetum vulgare***	4	bright-yellow	3-8	8
281	*Tanacetum corymbosum*	4	bright-yellow	7-9	2, 8
282	*Bidens tripartita*	4	bright-yellow	6-8	2, 8
283	*Hypochoeris achyrophorus*	4	bright-yellow	4-10	2, 8
284	*Conyza canadensis*	4	white	7-11	2, 8
285	*Filago gallica*	4	yellow	4-6	2, 8
286	*Senecio vulgaris*	4	yellow	3-9	2, 8
287	*Phylosella piloselloides* ssp. *piloselloides*	4	yellow	6-8	2, 8
288	*Crepis setosa*	4	yellow	5-7	2, 11
289	*Crepis sancta*	4	yellow	6-7	2, 8
290	***Matricaria chamomilla* var. *chamomilla***	4	white	3-4	2, 8
291	*Tripleurospermum elongatum*	4	yellow	3-7	2, 8
292	*Leontodon saxatilis*	4	yellow	10-11	8, 10
293	***Centaurea depressa***	4	dark-violet	5-7	2, 8
294	*Petasites hybridus*	4	scarlet	5-6	2, 10
295	*Tanacetum parthenium*	4	white	5-9	2, 8
296	*Centaurea inexpectata*	4	dark-violet	8-9	2, 8
297	*Centaurea solsitialis* ssp. *solsitialis*	4	yellow	6-8	2, 8
298	*Xeranthemum annum*	4	pale-blue	6-9	3
299	*Centaurea cadmea*	4	red-violet	5-8	2, 3
300	*Hieracium medianiforme*	4	yellow	5-7	8
301	*Xanthium strumarium* ssp. *strumarium*	4	yellow	6-7	8
302	*Cichorium inthybus*	4	violet	6-9	8
303	*Arctium minus*	4	violet	7-8	2, 8
304	*Campanula glomerata* ssp. *hispida*	4	violet-blue	5-9	2, 3
305	***Campanula lactiflora***	4	bright-blue	6-8	2, 11
306	*Campanula percifolia*	4	blue	6-8	2, 11
307	*Campanula olympica*	4	blue	5-9	2, 11
308	*Campanula alliarifolia*	4	cream-colour	6-9	2, 11
309	*Campanula rapunculoides* ssp. *cordifolia*	4	blue-violet	7-8	2, 8
310	*Campanula rapunculoides* ssp. *rapunc.*	4	scarlet	7-9	2, 11
311	*Campanula tridentata*	4	bright-violet	5-8	2, 11
312	*Campanula trachelium* ssp. *athoa*	4	violet	4-7	8
313	*Campanula lyrata* ssp. *lyrata*	4	violet-blue	4-7	2, 8
314	*Legousia speculum-veneris*	4	violet-blue	4-6	2, 8
315	*Asyneuma limoniifolium*	4	white	3-6	2
316	*Rhododendron ponticum* ssp. *ponticum*	1	violet	5-6	2, 12
317	*Erica arborea*	1	pink	3-7	2, 3, 12
318	***Arbutus unedo***	1	bright-pink	9-11	1, 11
319	*Vaccinium arctostaphylos*	1	white	5-6	1, 11
320	*Anagallis arvensis* var. *arvensis*	4	blue	3-9	2, 11
321	*Ligustrum vulgare*	1	white	5-6	12, 13
322	*Primula vulgaris* ssp. *sibtorphii*	1	red	3-6	2, 5
323	*Primula vulgaris* ssp. *vulgaris*	1	yellow	3-6	2, 5
324	*Anagallis foemina*	1	blue	6-10	2, 8
325	*Lysimachia verticillaris*	1	white	6-9	2, 11
326	*Fraxinus angustifolia* ssp. *oxycarpa*	3	yellow	4-5	1, 11
327	*Fraxinus excelsior* ssp. *excelsior*	3	yellow	4-5	1, 11
328	*Phylleria latifolia*	1	white	4	1, 11
329	*Vinca herbacea*	4	purple-violet	3-8	2
330	*Periploca gracea*	4	yellow	7-8	4
331	*Cionura erecta*	4	white	4-9	1
332	*Buddleia davidii*	1	violet	4-8	1, 15
333	*Blackstonia perfoliata*	4	yellow	4-10	2, 11

No.	Plant taxa	Species	Blossom colour	Flowering Duration (months)	Usage possibilities
334	*Centaurium erythreae* ssp. *erythreae*	4	pink	5-8	2, 11
335	*Convolvulus catabrica*	4	pink	4-8	2
336	*Convolvulus arvensis*	4	white	5-7	14
337	*Calystegia sepium* ssp. *sepium*	4	white	4-8	4, 8
338	*Calystegia silvatica*	4	white	4-7	4, 7
339	*Myosotis alpestris* ssp. *alpestris*	4	blue-violet	5-8	5, 8
340	***Myosotis stricta***	4	Pale blue	5-9	5, 9
341	*Myosotis silvatica*	4	blue	5-9	5, 9
342	*Myosotis lithospermifolia*	4	blue	5-8	5, 8
343	*Myosotis ramossisimum* ssp. *ramoss.*	4	blue	4-8	4, 8
344	***Cynoglossum montanum***	4	dark-pink	4-8	4, 8
345	*Trachystemon orientalis*	4	violet-blue	3-5	3, 5
346	*Echium vulgare*	4	dark-blue	5-9	5, 9
347	*Echium angustifolium*	4	blue	3-8	3, 8
348	*Lithospermum officinale*	4	white	5-7	5, 7
349	*Lithospermum purpureo-caeruleum*	4	blue	3-6	3, 6
350	*Anchusa azurea*	4	dark-blue	4-8	4, 8
351	*Cynoglossum creticum*	4	red	4	4
352	*Cynoglossum officinale*	4	scarlet	4-5	4, 5
353	*Buglossoides arvensis*	4	white-blue	2-6	2, 6
354	*Lappula barbata*	4	blue-white	5-7	5, 7
355	*Moltkia coerulea*	4	blue	4-6	4, 6
356	*Myosotis arvensis* ssp. *arvensis*	4	blue	4-7	4, 7
357	***Cynoglossum montanum***	4	red-pink	4-7	4, 7
358	*Heliotropium suaveolens*	4	white	6-8	6, 8
359	*Solanum dulcamara*	4	violet	5-9	5, 9
360	*Solanum nigrum* ssp. *nigrum*	4	white	5-6	5, 6
361	*Scrophularia scopoli* var. *scopoli*	4	red	4-8	4, 8
362	*Antirrhinum majus* ssp. *tortuosum*	4	pink	5-7	5, 7
363	*Scrophularia scopoli*	4	violet	4-8	2
364	*Melampyrum arvense* var. *arvense*	4	white-pink	5-9	2
365	*Parentucellia viscosa*	4	yellow	6-9	2, 11
366	*Digitalis ferruginea* ssp. *ferruginea*	4	yellow	6-9	2, 11
367	*Parentucellia latifolia*	4	violet, red	5-6	8
368	*Veronica filiformis*	4	white	4-7	2, 8
369	*Verbascum sinuata* var. *sinuata*	4	yellow	8-10	2, 11
370	*Verbascum blattaria*	4	yellow	5-8	2, 11
371	*Verbascum gnapphaloides*	4	yellow	5-9	2, 11
372	*Veronica chamaedris*	4	blue	4-7	2, 11
373	*Digitalis ferruginea* ssp. *schischikinii*	4	yellow	6-9	8, 12
374	*Melampyrum arvense*	4	violet	5-8	2, 11
375	*Verbena officinalis*	4	purple	6-8	2, 11
376	*Ajuga reptans*	4	blue-violet	3-5	2, 8
377	*Ajuga chamaepitys* ssp. *chia*	4	yellow	4-7	2, 11
378	*Satureja spicigera*	4	white	8-9	2, 11
379	*Satureja hortensis*	4	bright-violet	6-9	2, 11
380	*Teucrium polium*	4	yellow	6-9	3
381	*Teucrium scordium* ssp. *scordium*	4	red, violet	4-6	3
382	*Lamium purpureum* var. *purpureum*	4	pale-blue	3-5	2, 11
383	*Lamium album*	4	white	5-8	2, 8
384	*Lamium amplexicaule*	4	violet	2-11	2
385	*Stachys annua* ssp. *annua*	4	white	6-7	2, 11
386	*Stachys thirkei*	4	red	5-9	2, 11
387	*Prunella lanata*	4	violet	5-7	2, 11
388	*Prunella vulgaris*	4	violet	5-9	2, 16
389	*Origanum vulgare* ssp. *vulgare*	4	white	5-10	2, 16
390	*Origanum laevigatum*	4	violet	5-9	10
391	*Origanum onites*	4	white	5-9	2, 11
392	*Origanum parviflorum*	4	white	5-10	2, 12
393	*Clinopodium vulgare* ssp. *vulgare*	4	violet	6-10	8
394	*Calamintha nepeta* ssp. *glandulosa*	4	violet-red	8-9	2, 8
395	*Thimbra spicata* var. *spicata*	4	scarlet	6-7	2, 8
396	*Mentha x piperita*	4	pink	6-9	2, 10
397	*Mentha longifolia* ssp. *longifolia*	4	pink	6-8	2, 11
398	*Mentha aquatica*	4	violet	8-10	4
399	*Mentha pulegium*	4	purple	6-9	10
400	*Lycopus europaeus*	4	dark-violet	6-10	8
401	*Salvia tomentosa*	4	violet	8-9	12

No.	Plant taxa	Species	Blossom colour	Flowering Duration (months)	Usage possibilities
402	*Salvia virgata*	4	blue-violet	8-9	2, 12
403	*Salvia verbenaca*	4	purple	3-4	2, 11
404	*Salvia forskahlei*	4	violet-red	6-9	2, 12
405	*Salvia verticillata* ssp. *amaciaka*	4	blueish-purple	5-9	2, 11
406	*Ocimum basiliqum*	4	white-pink	8-11	2, 11
407	*Tetragonolobus requennii*	4	yellow	4	2, 11
408	*Sideritis montana* ssp. *remota*	4	white	4-6	2, 11
409	*Scutellaria galericulata*	4	yellow-violet	4-7	10
410	*Plantago lanceolata*	4	brown	4-10	10
411	*Plantago major* ssp. *major*	4	yellow	4-9	2
412	*Plantago lagopus*	4	bright-yellow	4-8	2, 11
413	*Plantago argentea*	4	white	6-7	2, 11
414	*Daphne pontica*	1	bright-yellow	5	2, 11
415	*Laurus nobilis*	1	green-white	3-5	1, 13
416	*Aristolochia hirta*	4	violet	5-6	1
417	*Euphorbia amygdaloides*	4	green-yellow	3-8	1
418	*Euphorbia sequieriana* ssp. *niciciana*	4	green-yellow	3-10	1
419	*Euphorbia peplus* var. *peplus*	4	yellow	4-7	1, 7
420	*Euphorbia brittingeri*	4	yellow	4-9	2, 3
421	*Euphorbia taurinensis*	4	yellow	3-8	3
422	*Euphorbia paralias*	4	yellow	4-9	3
423	*Euphorbia myrsinites*	4	yellow	4-8	3
424	*Euphorbia helioscopia*	4	yellow	2-6	3, 12
425	*Euphorbia stricta*	4	yellow	4-8	2, 11
426	*Buxus sempervirens*	1	white	3-5	1
427	*Cannabis sativa*	4	yellow	6-9	6, 7
428	*Ficus carica* ssp. *carica*	3	bright-yellow	?	6, 7
429	*Ulmus montana*	3	violet	3	1, 6
430	*Ulmus minor* ssp. *minor*	3	green-white	2-4	1, 7
431	*Juglans regia*	3	yellow	3-5	7
432	*Pterocarya fraxinifolia*	3	yellow	4-5	6, 7
433	*Platanus orientalis*	3	yellow	3-5	1, 6
434	*Fagus orientalis*	3	yellow	3-5	7, 12
435	*Castanea sativa*	3	yellow	3-5	7, 12
436	*Quercus petrea* ssp. *iberica*	3	yellow	4-5	1, 6
437	*Quercus robur* ssp. *robur*	3	yellow	3-4	1, 7
438	***Quercus ithaburensis* ssp. *macrolepis***	3	yellow	3-4	6
439	*Quercus virgiliana*	3	yellow	3-5	7, 13
440	*Quercus infectoria* ssp. *infectoria*	3	yellow	4-5	1, 13
441	*Carpinus betulus*	3	yellow	4-5	1, 6
442	*Carpinus orientalis*	3	yellow	4-5	1, 6
443	*Corylus avellana*	1	yellow	1-3	6
444	*Corylus colurna*		yellow	1-4	1, 12
445	*Alnus glutinosa* ssp. *glutinosa*	3	violet	2-4	1, 12
446	*Ostrya carpinifolia*	3	yellow	3-4	12
447	*Salix caprea*	1	yellow	2-4	12, 17
448	*Salix amplexicaulis*	3	yellow	2-4	6, 7
449	*Salix alba*	3	yellow	2-4	1, 7
450	*Populus x canescens*	3	white	2-4	1, 7
451	*Populus tremula*	3	yellow	2-4	1, 11
452	*Populus alba*	3	yellow	2-5	1, 11
453	*Sherardia arvensis*	4	pink	3-9	1, 8
454	*Galium paschale*	4	white	6-8	3, 12
455	*Galium aparine*	4	white	4-7	1, 10
456	*Galium incanum* ssp. *incanum*	4	white	5-8	1, 11
457	*Galium palustre*	4	white	5-8	14
458	*Galium verum*	4	gold-yellow	6-8	2, 11
459	*Rubai peregrina*	4	yellowish-green	6-7	4
460	*Crucianella bithynica*	4	yellow	6-7	2, 11
461	*Alisma plantago-aquatica*	4	white	4-5	2
462	*Arum maculatum*	4	violet-yellow	4-6	14
463	*Arum orientale* ssp. *orientale*	4	pale-green	4-6	2
464	*Smilax excelsa*	4	pale-green	4-5	14
465	*Ruscus aculeatus* ssp. *aculeatus*	4	green-white	2-6	10, 12
466	*Ruscus hypoglossum*	4	green-white	3-5	10
467	*Asparagus aphyllus* ssp. *orientalis*	4	white	8-9	16
468	*Asparagus acutifolius*	4	white	8-10	2, 12
469	*Allium cepa*	4	green-white	6-8	11

No.	Plant taxa	Species	Blossom colour	Flowering Duration (months)	Usage possibilities
470	*Allium sativum*	4	grey-white	6-8	2
471	*Allium porrum*	4	pink	6-7	2, 11
472	*Allium flauvum* ssp. *tauricum*	4	pink	5-8	2
473	*Scilla bithynica*	4	blue	3-4	2, 3
474	*Ornithogalum narbonense*	4	white	3-4	2, 11
475	*Ornithogalum armeniacum*	4	white	4-8	2
476	*Ornithogalum wiedemannii*	4	white	4-8	2, 11
477	*Ornithogalum umbellatum*	4	white	3-5	2
478	*Ornithogalum fimbriatum*	4	white	4-7	2
479	*Fritillaria pontica*	4	red-brown	4-5	2, 11
480	*Pancratium maritimum*	4	white	6-10	2, 11
481	*Colchicum automnale*	4	white-pink	9-11	2, 11
482	*Muscari comosum*	4	violet-blue	3-7	2
483	*Muscari armeniacum*	4	violet-blue	3-5	2
484	*Lilium martagon*	4	pink	6-7	2, 3
485	*Crocus sativus*	4	yellow-violet	10-11	3
486	*Narcissus pseudonarcissus*	4	dark-yellow	3-7	2, 3
487	*Leucojum aestivum*	4	white	3-6	8, 9
488	*Ornithogalum narb.*	4	white	3-4	2, 11
489	*Galanthus plicatus* ssp. *byzantinus*	4	white	1-4	2
490	*Iris germanica*	4	violet	4-5	2, 16
491	*Iris pseudocorus*	4	yellow	4-5	16
492	*Crocus speciosus* ssp. *speciosus*	4	yellow	9-11	2
493	***Crocus olivieri* ssp. *olivieri***	4	scarlet	2-4	2
494	*Orchis laxiflora*	4	violet-red	5-6	2
495	*Ophyris*	4	red	5-7	2, 11
496	*Ophyris oestrifera* ssp. *oestrifera*	4	red-violet	3-6	3
497	*Anacamptis pyramidalis*	4	red	3-7	2, 11
498	*Dactylorhiza romana*	4	red	3-6	2
499	*Dactylorhiza saccifera*	4	white	3-6	2
500	*Spirantes spiralis*	4	white	3-6	2
501	*Juncus effusus*	4	sporophyte	-	4
502	*Orchis pallustris*	4	pink	3-6	16
503	*Orchis morio* ssp. *morio*	4	violet, pink	3-6	2
504	*Typha angustifolia*	4	sporophyte	-	4
505	*Juncus inflexus*	4	sporophyte	-	4
506	*Juncus buffonius*	4	sporophyte	-	4
507	*Juncus articulatus*	4	sporophyte	-	4
508	*Briza maxima*	4	violet	4-5	10
509	*Luzula forsteri*	4	violet-pink	-	10
510	*Luzula campestris*	4	violet-pink	-	8
511	*Carex flacca* ssp. *serrulata*	4	yellow	4-5	10
512	*Carex remota*	4	dark-yellow	3-5	10
513	*Festuca heterophylla*	4	dark-yellow	7-8	10
514	***Elymus hispidus***	4	yellow	6-7	2
515	*Agrostis giganthea*	4	violet	7-8	2, 3
516	*Agrostis stolonifera*	4	violet	6-8	8
517	*Bromus sterilis*	4	white	4-10	10
518	*Cynodon dactylon*	4	violet	4-9	10

The correct choice of the plant material is of utmost importance in landscape architectural design and planning. The functional application of native plants can have many advantages, such as a better accommodation on local conditions, which additionally keeps the expenses low. As already mentioned, richness of the species in the research area is clearly abundant, with distinctive regions concerning endemic species, such as the places in and around Karabük-Keltepe and Yenice. The WWF (World Wildlife Fund) in 1999 has added the forests of Yenice to the Biodiversity List, containing the one hundred most valuable and to be protected forests of Europe [36].

Studies have already been performed in different regions of Turkey with similar aims as the research work presented herein. Particularly in the metropolitan areas of Turkey, naturally occurring plants are used frequently in landscape design. However, this may still not be sufficient. In many regions of Turkey, natural Rhododendron species remain disregarded for landscaping, although the species enjoy great popularity in many European cities as ornamental elements.

RECOMMENDATIONS FOR APPLICATION IN PRAXIS

The woody species, such as *Cercis siliquastrum L.* ssp. *siliquastrum and Cotynus coggyria* Scop.*, Staphylea pinnata* L.*, Pterocarya fraxinifolia* Scop.*,* and *Euonymus* sp.*,* evaluated in this study, stand out in terms of form and colours, and can be highly recommended for the use in urban parks. Species like *Origanum laevigatum* Boiss.*, Cardamine hirsuta* L.*, Cardamine bulbifera* (L.) Crantz*,* which accord more and more importance to pharmacology, could be applied to all intents and purposes in landscape design due to their decorative feature. Many of the cultivated forms of the Silene taxa have already found their place in landscaping. The Silene species found in this work were *Silene otites* (L.) Wibb*, Silene vulgaris (*Moench*)* Gerche*, Silene italica* (L.) Pers*, and Muscari comosum* (L.) Mill, which can be recommended as well for the use in landscape architecture. The Lily-Sand species *Pancratium maritimum* Boiss. and Scilla *bithynica* Boiss. would also serve well in landscaping. The Hypericum species with their flamboyant blossoms may well be applied on acclivities at line routings.

For all endemic plant species, especially for those threatened with extinction, protection measures are urgently necessary. It may further be useful to support industrial production of these species to facilitate the wide-spread usage of them in landscape architecture. For other vegetation areas of Turkey, further respective dendrological studies are necessary to gain more knowledge about the feasibility of naturally occurring plants in landscape design. In addition, hybridation experiments for the production of ornamental plants of natural origin are to be supported. In this respect, an industrial production of endemic ornamental plants with outstanding aesthetic properties, which may also stand up against global business competition, seems to be useful.

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THE EFFECTS OF THE LARGEST IRRIGATION OF GAP PROJECT ON GROUNDWATER QUALITY, ŞANLIURFA – HARRAN PLAIN, TURKEY

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SUMMARY

The Harran Plain which is the largest plain of the Southeastern Anatolia Project (known as the GAP project in Turkey) in Turkey comprises 141.500 ha irrigable land. In 1995, besides the introduction to the surface irrigation; significant environmental problems aroused at the plain as a result of intensive agricultural practices, excessive and uncontrolled irrigation, insufficient drainage systems, salinity and domestic and other wastes that were disposed of to the plain. At some parts of the plain the groundwater level reached 0.0 – 2.0 m.

The aim of this study was to determine the effects of the aforementioned surface irrigation problems on the area's groundwater quality. Therefore, groundwater samples were taken monthly from 24 representative observation wells during 2006-water year, and electrical conductivity (EC), temperature, pH and groundwater level were measured in-situ immediately after the sampling. The results were evaluated in accordance with: directive 98/83/EC of the EU (European Union) and standard 266 of the Turkish Standards Institution (TSE) regarding the quality of water intended for human consumption, and the Guidelines for Drinking-Water Quality of World Health Organization (WHO). They were also compared with previous studies.

The mean temperature of groundwater was approximately 20 ^{o}C. The average pH values, which ranged from 7.00 to 7.80, are within the limits accepted by the TSE (266) standard, the WHO guidelines and the EU directive. The average EC values (1317 - 2935 µS/cm) were found to be fairly above the TSE (266) (guide level = 650 µS/cm) standard and the WHO (max. admissible concentration = 250 µS/cm) guidelines.

KEYWORDS:
Salinity, irrigation, groundwater quality, Harran Plain, GAP, Turkey.

INTRODUCTION

Along the history, people have used groundwater for drinking everywhere around the world. Even today, more than half of the world's population use groundwater to survive. Thus, the groundwater is an important source to drink, to use and to supply water for the industry. In some regions, it is the main source for the river flow. Meanwhile, groundwaters are also necessary for the variety, sustainablity and liveliness of the ecosystem.

Salinity in irrigated areas of semi-arid climates affects not only the yield, but also soil and water salinization and groundwater quality [1]. Also, salinized soils are a major constraint for agricultural production in the irrigated arid as well as in semi-arid regions. Periodical monitoring of the salt-affected areas are a must for developing reclamation and management strategies. The most important factors that effect soil productivity are: soil salinity, alkalinity and shallow groundwater levels. Salty water movement towards the topsoil and excessive evapotranspiration can cause salt accumulation at different soil horizons [2, 3]. Excessive groundwater irrigation without construction of drainage systems and solving drainage discharge problems significantly increased the salinity levels. Another reason for high salinity is drainage water use returning from irrigation in areas where insufficient water resources are found [4].

In this study, the wells from which the samples were taken (before irrigation) are used for drinking, usage and irrigation. At the same time, almost all of the drinking water supply of the Şanlıurfa city and its districts were supplied from these wells. Nowadays, an important part of the city, its districts and its towns are connected to the drinking water network, only usage water is supplied from those wells.

MATERIALS AND METHODS

Description of the Study Area

The Harran Plain which is the largest plain of the Southeastern Anatolia Project (GAP) in Turkey comprises 141.500 ha irrigable land [5] (Figure 1). The study area is approximately between latitudes 36^{0} 43' to 37^{0} 10' North

and longitudes 38^0 47' to 39^0 10' East (Figure 2). It has a drainage area of 3700 km² and a plain area of 1500 km². The plain has a semi-arid climate with almost no precipitation between June and September. In the plain, the long-term mean annual precipitation is 284.2 mm, temperature is 18 °C and evaporation is 1884 mm.

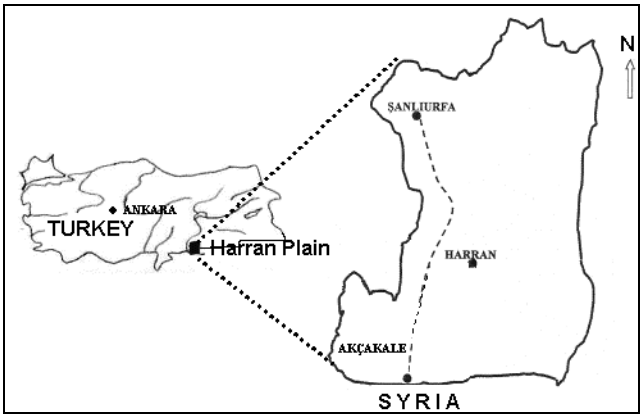

FIGURE 1 - Location map of the study area.

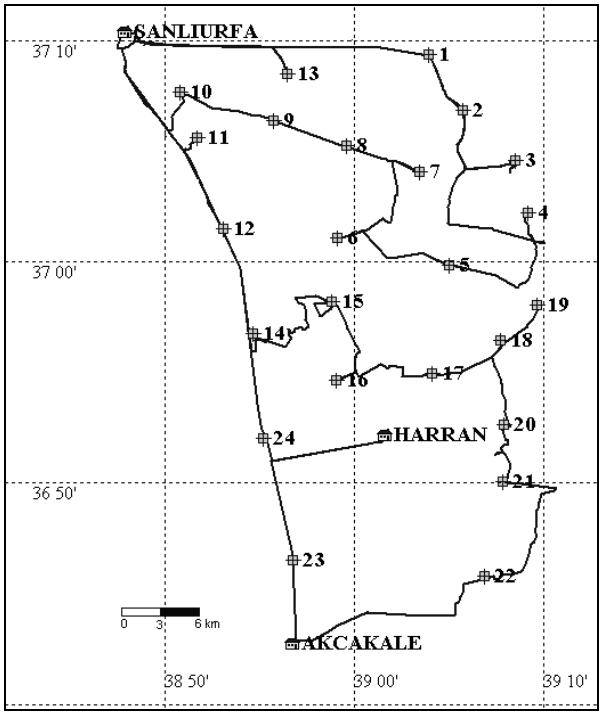

FIGURE 2 - Study area showing location of wells sampled for groundwater measurements.

Topography of the Study Area

The general direction of the slope in the plain is from north to south and varies between 0% and 2%. Surface slope is rather flat around the towns of Harran and Akçakale and low-lying areas cause outlet problem. Slopes in the northern parts are mostly higher than that of the southern parts of the plain and surface and sub-surface drainage problems are rarely encountered [6].

Main Geological, Hydrogeological and Soil Characteristics of the Study Area

Geological units outcropping in the study area, from the bottom to the top, are comprised of Eocene, Pliocene and Pleistocene. The Eocene aged unit is composed of karstic, jointed and fractured limestones, and its thickness is about 300 m. This unit outcrops in the north, west and east of the study area, and is a deep and confined aquifer. It is overlaid by the Pliocene. The Pliocene is composed of clay containing gypsum locally and its thickness is roughly 200 m. This unit has not formed an aquifer. It forms impermeable barrier for the Pleistocene aged unit and is overlaid by the Pleistocene. The Pleistocene is composed of clay, sand and gravel, and its thickness is approximately 60 m. This unit is a/an shallow/unconfined aquifer. Hundreds of shallow wells on this unit are drilled. For this work, groundwater samples were taken from this unit [7].

The soils of the Harran Plain are mainly clays and pH values are neutral (pH = 7.50 – 8 .00). According to the results of the permeability tests on the plain, the minimum permeability value is measured at 0.22 m/day and the maximum is 3.51 m/day [8]. Soil profiles are deeper than 150 cm in 77% of the area and in the rest of the area, they are shallow and underlying materials are sand, gravel, lime and rock. 95% of soils have heavy texture in tillage layer and only 5% of soils have medium texture; also, sub-layers of the soils are heavy textured. Soil colors are mostly brown and reddish brown [7-9].

Field Measurements

Especially, in the sections that possess significant environmental problems, groundwater samples were taken from the selected sampling points that represent the whole plain in order to measure, assess and discuss temperature, pH, EC, and groundwater level at the field during the 2006-water year (October 01, 2005-September 30, 2006). "The water year used in Turkey and in the entire northern hemisphere starts on October 1 of a year and ends on September 30 of the next year, and takes the name of the latter year [10]".

The EC, temperature, pH and groundwater level were measured in-situ by using YSI 6600 sonde, SevenGo pro-SG7 conductivity meter, a portable pH meter and an electric contact meter immediately after sampling. Sampling and measurement procedures were carried out in accordance with:

- D4448-01 Standard Guide for Sampling Ground-Water Monitoring Wells [11].
- Water Quality-Sampling-Part 2: Guidance on sampling techniques [12].
- Groundwater Well Sampling [13].

The results were evaluated in accordance with:

- Standard 266 of the Turkish Standards Institution (TSE) regarding the quality of water intended for human consumption [14].

- The Guidelines for Drinking-Water Quality of World Health Organization (WHO) [15]
- Directive 98/83/EC of the EU (European Union) regarding the quality of water intended for human consumption [16].

RESULTS

Since the 1980s, the DSI (The General Directorate of State Hydraulic Works) conducts groundwater level measurements on different parts of the plain. Along with the surface irrigation since 1995, the groundwater level of some wells raised from 30 meters to 2 meters (1995-2003). For instance, in 2002, though the quantity of the water conveyed in the plain by irrigation was $1048x10^6$ m^3 and that of the water brought by rainfalls was $223x10^6$ m^3, the quantity of the evapotranspiration was $1021x10^6$ m^3 and that of the drained water was $128x10^6$ m^3. Consequently, the quantity of the stored water was $121x10^6$ m^3. Although the Harran soils are clay-textured, their water transmission capacity is considerably high [8]. For this reason, in some sections, the groundwater level was closer to the land surface because of uncontrolled irrigation and insufficient drainage. In this study, the average groundwater level in 24 wells that were observed for 12 months were at their lowest level during summer months (June, July and August) and September (Figure 3). In spite of an increase at the centimetric level in the following months, it is in stable situation during the whole year. The average EC values were the highest in all wells in fall months (excluding September) and December. The average EC values stay stable in the following months (Figure 4).

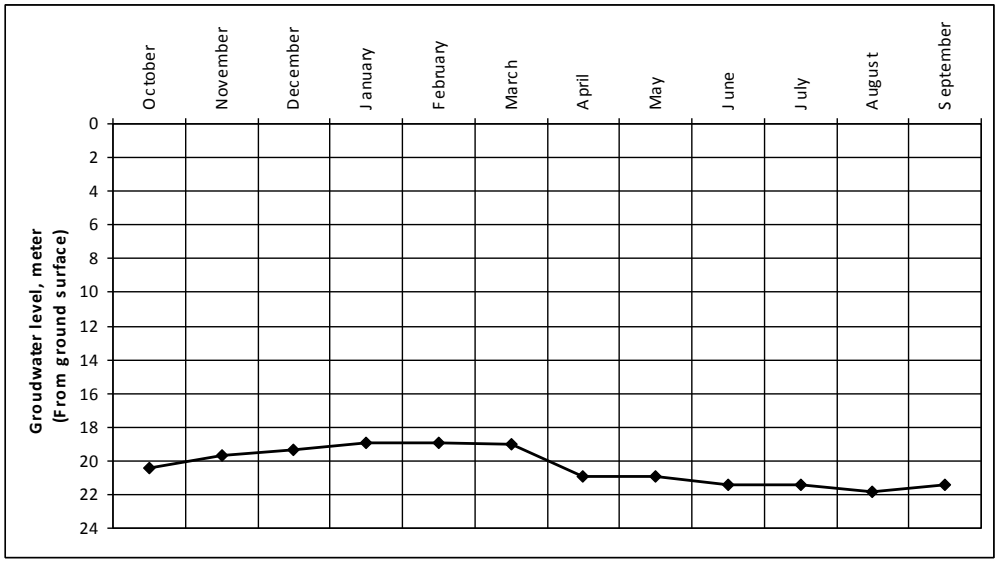

FIGURE 3 - Monthly variation of the average groundwater level in the study area.

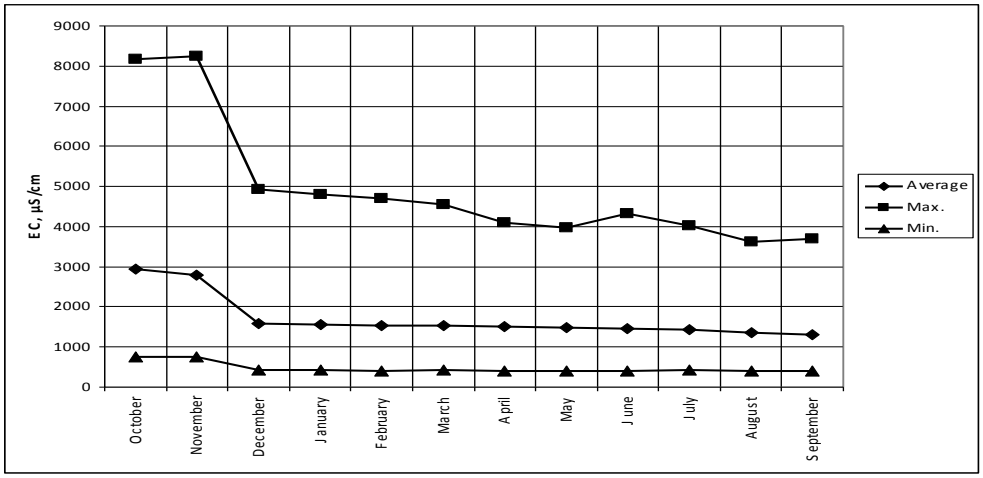

FIGURE 4 - Monthly variation of the average, max and min EC in the groundwater of the study area.

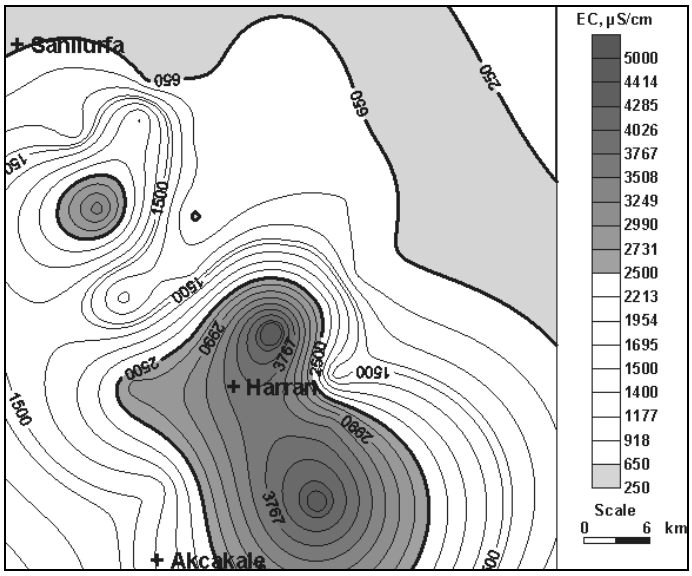

FIGURE 5 - Spatial distribution of EC in the groundwater of the study area during 2006-water year.

As can be seen in Figure 5, the highest EC distributions are generally noticed in the vicinity of Harran, in the northeastern part of Akçakale and in the southeastern part of Şanlıurfa. It can be explained by the slope of the lands in these regions nearly equal to zero, its depression-like shape, and the intense salinization of soil.

There was no significant seasonal variation in the groundwater temperature during this sampling period. The annual mean temperature was approximately 20 ^{o}C (Figure 6). The average pH of the groundwater (7.00 – 7.80) is within the limits accepted by the TSE standard (266), the WHO guidelines and the EU directive (Figure 7). The EC has been used a criterion in classification of drinking and irrigation waters [17]. The average EC values (1317 - 2935 µS/cm) are fairly above the TSE standard (266) (guide level = 650 µS/cm) and the WHO (max. admissible concentration = 250 µS/cm) guidelines. According to a study conducted between 2000 – 2003 in the exit mouth of the Şanlıurfa Tunnes [18], the average EC of the water before being conveyed into the plain was 380 µS/cm, and the irrigation water quality was found to be in the "very good to good" class in the Wilcox diagram, and the C2-S1 class in the USA salinity diagram. Thus, these waters were found to be suitable for all types of irrigation [18]. These results have shown that the irrigation water quality has no negative impact on the groundwater. But, in another study in the Harran Plain, after irrigation, the irrigation water quality of the groundwater was found to be in the C3-S1 and C4-S1 class in the USA salinity diagram [19].

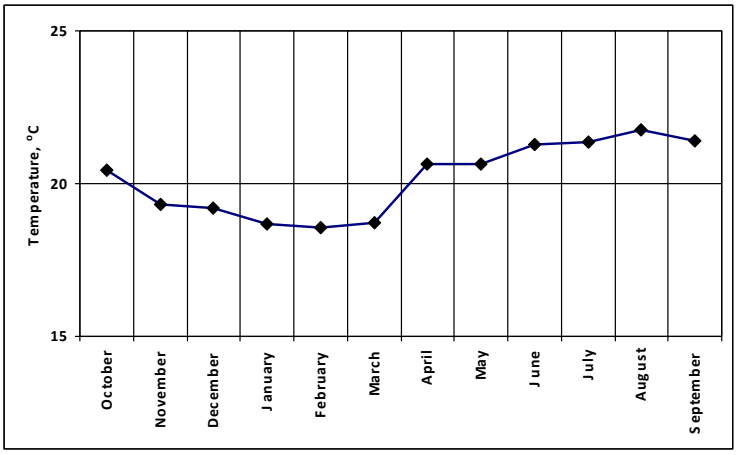

FIGURE 6 - Monthly variation of the average temperature in the groundwater of the study area.

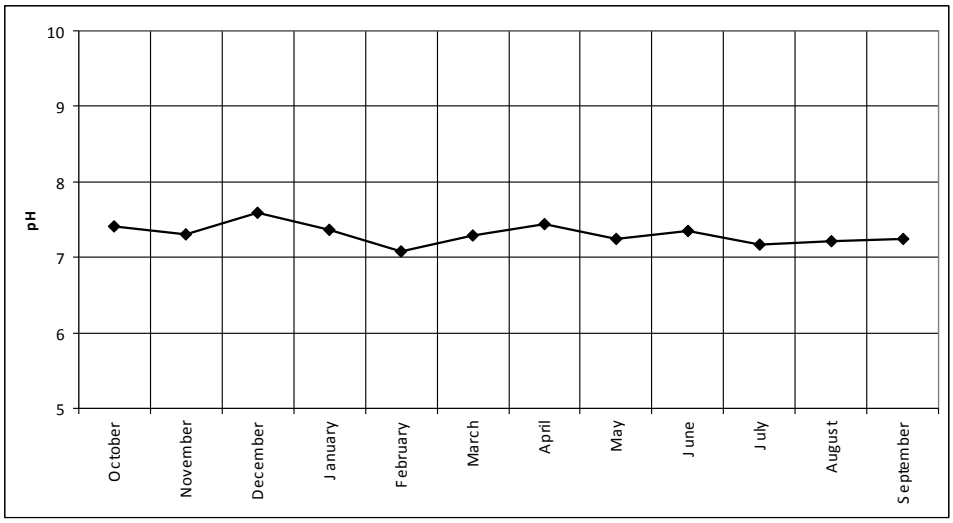

FIGURE 7 - Monthly variation of the average pH in the groundwater of the study area.

CONCLUSIONS AND RECOMMENDATIONS

As a result of the monitoring of 24 wells that represent the general plain, it has been determined that the groundwater level is rising. This raise caused salinization and an approach to the critical threshold value for plants. The rise of the EC value rendered the wells out of use that were previously used for drinking and usage waters. At the same time, this situation resulted in the pollution possibility of the deep aquifer providing abundant and good quality water at the lower part. Unfortunately, the quality of the largest groundwater reservoir of the Middle East has been deteriorated. If the experiences obtained from this project or similar to that are discussed in scientific and technical environments and the results are published, they would provide a reference for the construction of this sort of facility in the future. In this context, the implementation of the following recommendations is of vital importance:

- The monitoring process presented in this study must be immediately applied in more parameters by raising the number of wells, including deep wells.
- Especially, the nitrate level of groundwater should be monitored.
- Vulnerability and contamination maps of groundwater should be prepared immediately.
- Extension education is necessary for water users in irrigated agriculture.
- Main drainage network must be examined and developed if necessary.
- Sub-surface drainage system should be constructed wherever it is necessary.
- Infrastructures such as land leveling and surface inlets must be completed.

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