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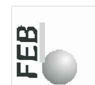

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EFFECT OF H_2O_2 ON CHARACTERISTICS AND BIOLOGICAL TREATMENT OF PETROLEUM REFINERY WASTEWATER

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SUMMARY

The paper presents results on the ability of activated sludge to tolerate increases in hydrogen peroxide concentration due to the introduction of H_2O_2 oxidised wastewater. Effects of hydrogen peroxide in the petroleum refinery effluent directed to further biological treatment is also presented. At low H_2O_2 concentrations the degradation efficiency of total petroleum hydrocarbons (TPH) is relatively high. All H_2O_2 concentrations used inhibited the oxygen uptake rate (OUR) of the activated sludge and, additionally, a reduction of chemical oxygen demand (COD) removal rate was observed. A release of ammonium nitrogen is observed when higher peroxide concentrations are used. When activated sludge has been adapted to peroxide in the oxidised wastewater, the nitrification rate increased up to 0.03% of peroxide applied. Rising H_2O_2 concentration in the wastewater yields an increase of the removal rate of the organic compounds, but is not improving the final biological treatment.

KEYWORDS: Petroleum refinery, wastewater, hydrogen peroxide, activated sludge.

INTRODUCTION

The main contaminants present in the refinery wastewater are light and heavy petroleum fractions, such as n-alkanes, olefins, paraffins, aromatics, asphaltics, phenols and PAHs, but also chlorinated compounds originating from the technological processes [1, 2, 3]. The inorganic fraction of the refinery wastewater consists of mineral suspension, acids and salts, amongst which nitrogen compounds are of special concern. N-compounds originate directly from the crude oil as well as from artificial additives to petroleum such as neutralisers, deemulgators or corrosion inhibitors. Considerable sources of the ammonium nitrogen are reforming processes and stripping waters. In the refinery studied, most of the hydrocarbons are eliminated during physicochemical treatment by separation and coagulation. The secondary effluent is then directed towards biological treatment, where the remaining light fraction of hydrocarbons is degraded and most of the ammonium nitrogen undergoes nitrification.

Stepnowski et al. (4) recently have investigated the photo-degradation process of the contaminants in the refinery wastewater studied and found it to be a very effective pre-treatment technique. In this preliminary study, it was recognised that the degradation efficiency of H_2O_2 is relatively high and that the oxidation of the total petroleum hydrocarbons (TPH) as well as 1,2-dichloroethane and t-butyl methyl ether, additionally present in the wastewater, occurs at low peroxide concentrations. Due to its relatively high stability the degradation rate for dichloromethane appeared to be the lowest one.

It is known that the treatment plants employing biological nitrogen removal encounter problems arising from shock or sustained loads of toxic compounds in the effluents (5, 6, 7). In the present study, the effect of hydrogen peroxide in the effluent directed for further biological treatment is studied, particularly its influence on the performance of the activated sludge.

MATERIALS AND METHODS

Effect on refinery wastewater

Experiments were carried out under room temperature in a 5L laboratory scale batch reactor system with automated aerator and stirrer. The oxygen concentration in the bioreactor was kept in the range of 4.5 – 5 mg L^{-1}. Refinery wastewater, pre-treated physicochemically *in situ* by means of flotation and coagulation, was slightly diluted with domestic sewage and then treated with hydrogen peroxide. The oxidation was studied at four different concentrations of hydrogen peroxide (0.01, 0.03, 0.05 and 0.1%) for 24 h, and samples of the wastewater were taken after 1, 3, 8 and 24 h of the experiment to analyse the total petroleum hydrocarbons (TPHs) using headspace

technique (HP 7694 Headspace sampler) coupled to a GC/FID system (HP 6890 series) equipped with a 50/320/3 SE-54 column. The calculation of the TPHs was done by summarising the total area under resolved and unresolved peaks. Additionally, ammonium-, nitrite- and nitrate-N as well as COD (Spectroquant® Merck Tests) in treated and untreated wastewater were analysed.

Effects on the activated sludge

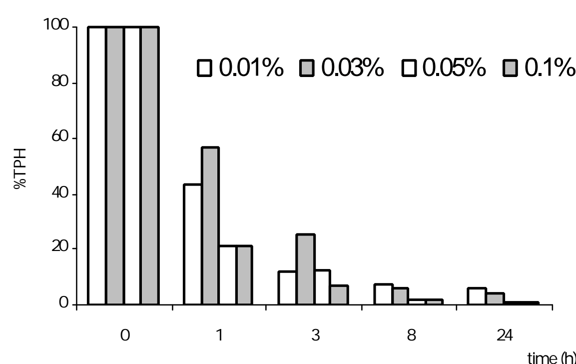
An identical batch reactor system was used and the same conditions were kept. Activated sludge (4.0 – 4.5 kg m^{-3}) was originating from the refinery wastewater treatment plant studied. The ability of unadapted activated sludge to tolerate sudden increases in hydrogen peroxide concentration was determined by measuring the oxygen uptake rate (OUR), nitrification rate and COD removal rate. OUR was measured in 300 mL BOD flasks. If necessary the sample was aerated for up to 3 min. until the dissolved oxygen concentration (DO) reached 5mg L^{-1}. The measurement of DO was performed with a Cell OX 325 electrode (Merck Eurolab), and the DO decrease was monitored until the value of 1mg L^{-1} was reached. The oxygen uptake rate given in [$mgO_2\ g^{-1}\ h^{-1}$] was calculated by dividing the DO decrease in time by the dry mass value of the volatile suspended solids (VSS) in the sample. $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ were analysed in the samples of activated sludge previously filtered (0.45 µm), which were taken every 30 min. for 4 hours of the experimental duration. The nitrification rate given in [$mgN\ g^{-1}\ h^{-1}$] was calculated by dividing the sum of nitrite and nitrate concentration increase in time by VSS of the sample. COD removal rate was calculated from the difference between COD in untreated wastewater and COD of the wastewater treated biologically after the H_2O_2 degradation step. 2

The ability of activated sludge to tolerate increases in hydrogen peroxide concentration due to the influx of H_2O_2 oxidised wastewater was determined by measuring the nitrification rate and COD removal rate. Wastewater was spiked with H_2O_2 with four different concentrations (0.01, 0.03, 0.05 and 0.1%) then added to the bioreactor with the activated sludge (for every concentration fresh activated sludge was grown in the bioreactor) and stirred for 3, 6, 12 and 24h.

RESULTS AND DISCUSSION

Table 1 summarises petroleum refinery wastewater characteristics used in present study. The majority of organic compounds found are dissolved in the liquid phase while those in suspensions are eliminated during the physical treatment step at the studied plant. Both, aliphatic and aromatic hydrocarbons were found as single peaks as well as a part of unresolved peak groups. The concentration of TPH in the studied wastewater was 1.6 mg L^{-1}. Figure 1 compares the effect on TPH concentrations obtained after 1, 3, 8 and 24 h at a H_2O_2 concentration of 0.01, 0.03, 0.05 and 0.1%. At low peroxide concentrations (0.01 – 0.03%) the degradation efficiency is relatively high, although the level of TPH after 24 hrs of treatment is within the range of 3 to 5% of the initial concentration.

FIGURE 1 - Degradation rates (%) of TPHs in the refinery wastewater at different hydrogen peroxide concentrations.



Higher H_2O_2 concentrations (0.05 – 0.1%) yielded a nearly total degradation of TPH after 24 h of the experiment. No considerable effect on ammonium nitrogen concentration was found. Its level was only slightly reduced at the highest concentration of peroxide used.

FIGURE 2 - Inhibition of the oxygen uptake rate of activated sludge by the hydrogen peroxide.

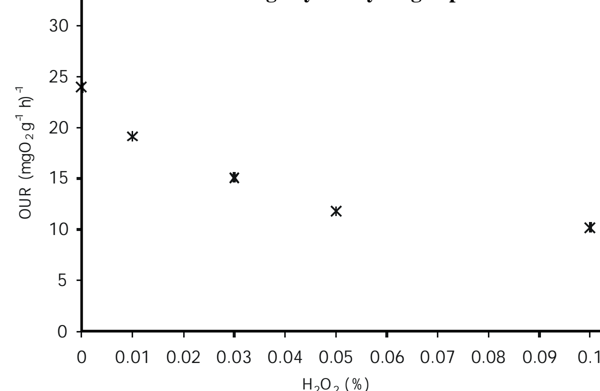
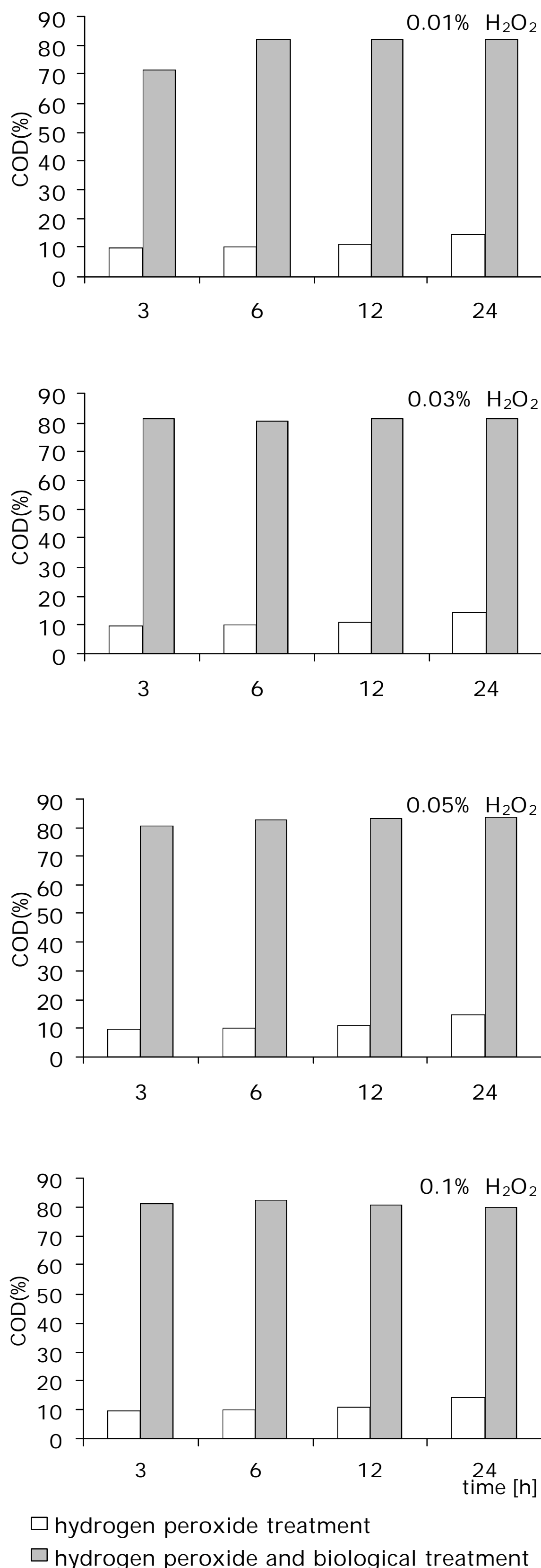


TABLE 1 - Characteristics of the petroleum refinery wastewater.

pH	TCOD	SCOD	BOD_5	Cl^-	SS	N tot	$N\text{-}NH_4^+$	TPH
	$mgO_2\ L^{-1}$	*$mgO_2\ L^{-1}$*	*$mgO_2\ L^{-1}$*	*$mg\ L^{-1}$*	*$mg\ L^{-1}$*	*$mgN\ L^{-1}$*	*$mgN\ L^{-1}$*	*$mg\ L^{-1}$*
7.8	182	169	90.4	327	12	19.1	11.3	1.6

FIGURE 3 - Removal rate of organic matter expressed as COD after treatment with hydrogen peroxide, and hydrogen peroxide followed by biological treatment.



In the experiment carried out to determine the ability of activated sludge to tolerate sudden increases in hydrogen peroxide it was found that all used H_2O_2 concentrations inhibited the OUR, which is illustrated in Figure 2. A reduction of 9 to 16% of COD removal rate was also observed (Figure 3). Even the lowest H_2O_2 concentration induced a relatively high reduction of the nitrification rate (26 %) which is likely due to the high sensitivity of nitrification species in comparison to heterotrophic ones. Higher peroxide concentrations stops the nitrification rate completely not depending on time (Figure 4). Additionally, a release of ammonium nitrogen was observed while higher peroxide concentrations were used, which is most probably a consequence of a direct peroxide interaction with the activated sludge.

FIGURE 4 - Effect of sudden increases in hydrogen peroxide on performance of the activated sludge.

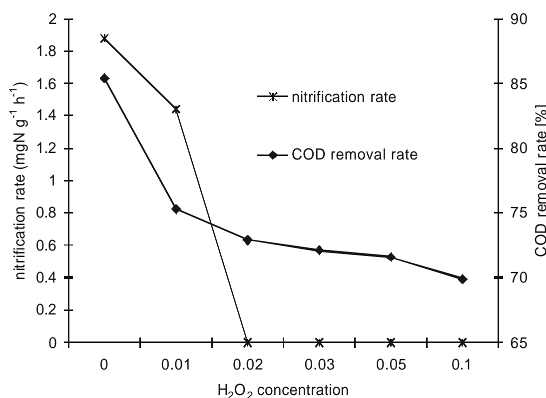
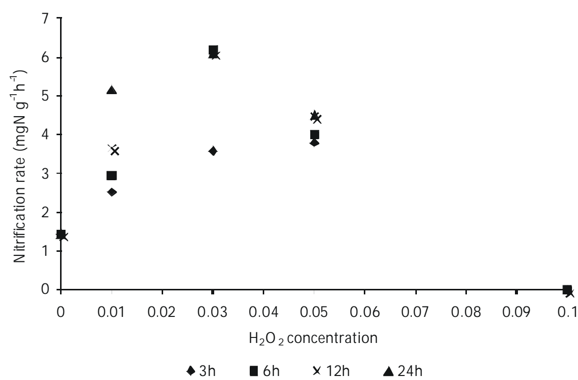


FIGURE 5 - Nitrification rate in the activated sludge acclimated to peroxide through oxidised wastewater.



While examining indirect H_2O_2 effects on the activated sludge which has been adopted to peroxide in the reactor feed (oxidised wastewater), the nitrification rate increases up to 0.03% of peroxide applied and effectively stops when 0.1% of H_2O_2 was used (Figure 5). Although the highest

degradation rate occurs after 24 h, they did not differ significantly from 6 or 12 h duration of the experiment. Increasing H_2O_2 concentration in the wastewater yielded in a removal rate increase of 7 up to 17 % of the organic compounds expressed as the soluble COD (SCOD). The biological treatment did not improve.

Our results show that the oxidative degradation processes of TPHs take place, if relatively low concentrations of H_2O_2 are added to the wastewater. Additionally, it was found that using 0.03 % of H_2O_2 in the wastewater for 6 h before the feeding reactor effected in significant increase in nitrification rate. Using the same peroxide concentration within time range of 8 h, it was possible to reduce TPHs for 97 %.

The oxidation process can be considered as a supplementary pre-treatment step of the secondary effluent in the refinery studied. Applying H_2O_2 to the waste at low concentration before it reaches the bioreactor will improve both, a decrease of the petroleum hydrocarbon concentration and a reduction of nitrogen compounds.

ACKNOWLEDGEMENT

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CRITICAL DISCUSSION ON THE NEED OF SUITABLE PRECONCENTRATION TECHNIQUES FOR THE DETERMINATION OF HEAVY METALS IN WET AND DRY ATMOSPHERIC DEPOSITIONS

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SUMMARY

Two known preconcentration techniques (instrumental multiple injection GF-AAS and solvent extraction followed by back-extraction and GF-AAS determination) have been compared for the purpose of applying them to atmospheric wet and dry depositions collected with a DDAS sampler (Dry Deposition on acquatic surface), in which Cd, Cr, Cu, Ni, Pb and V concentrations are often lower than the detection limit of the commonly adopted analytical techniques. Both preconcentration techniques yielded good results even with respect to matrix influences. The Multiple Injection GF-AAS technique was chosen to be applied to real samples.

KEYWORDS: Heavy metals, atmospheric depositions, preconcentration techniques.

INTRODUCTION

Atmospheric input represents one of the major sources of pollutants to surface waters, soils, and vegetation [1,2]. The knowledge of the sources and the fate of heavy metals is, therefore, extremely important for environmental risk assessment as well as for the planning of emission control policies and heavy metal reduction strategies. To this end, the concept of Critical Load permits to determine a value for the maximum permissible load of pollutants applicable in international abatement scenarios. Atmospheric deposition fluxes represent essential input data for determining that exceeding the limit with respect to the Critical Load. A very accurate sampling and analysis protocol of wet and dry depositions is, therefore, required and such a protocol could be usefully adopted as a reference point for monitoring network planning.

The sampling systems, Wet & **D**ry-**DAS** (**D**ry **D**eposition on **A**quatic **S**urface, MTX-Italy), separately collect atmospheric wet and dry deposition; a rain sensor activates a motor that shuttles a lid. A water layer taken at a constant level of 1 cm is the receptor for atmospheric dry deposition. This device and the whole procedure were both been the subject of previous papers [3, 4].

As for the analytical techniques adopted, the GF-AAS system is not always able to determine the concentration of heavy metals like Cd, Cr, Ni, Pb and V in the soluble fraction of both wet and dry depositions (obtained after filtration on 0.22 µm Millipore filters). Table 1 shows the percentages of samples with a concentration below the detection limit for each element.

TABLE 1 - Annual percentage (%) of samples with concentrations lower than the GF-AAS Detection Limit.

Elements	Detection Limit (mg/L)	Wet Dep. Sol. Fraction			Dry Dep. Sol. Fraction		
		1998	1999	2000	1998	1999	2000
Cd	0.075	66	77	75	96	91	92
Cr	0.75	81	100	94	87	95	89
Cu	0.64	3	0	2	0	0	0
Ni	1.12	75	93	88	78	95	90
Pb	0.65	31	52	50	52	82	75
V	1.4	84	93	93	87	86	88

Dep. = deposition; Sol. = soluble

The contribution of the soluble fraction to total deposition is of great importance not only because of its relevance to monthly flux evaluation but also because it is the fraction that more easily interacts with all environmental compartments and biological systems. An assessment of soluble fraction percentages in total deposition fluxes could, therefore, be very useful. The main purpose of this study was to assess the need and the reliability of proper analytical techniques for the quantification of heavy metal concentration in soluble fraction. For this purpose, two known techniques were tested to determine the most suitable one for our requirements, namely: easiness of application, a preconcentration factor (PF = final /initial concentration) higher than or equal to 20 and a smaller initial sample volume in view of the fact that the volume of the filtered dry deposition is about 0.5 L, while that of the wet deposition sample varies from 0.1 L to 6 L.

With an initial sample volume of 0.1 L, the dithiocarbamate solvent extraction method with a back-extraction procedure followed by GF-AAS analysis [5] facilitates achieving the required PF of 150, while with the instrumental multiple injection AAS method far smaller (200 µL – PF 20) initial sample volumes can be used. As Standard Reference Material for rain is not commercially available, precision and accuracy were studied using synthetic rain water spiked with heavy metals and real samples of both wet and dry depositions.

MATERIALS AND METHODS

Spectroscopic measurements were performed using a Perkin-Elmer Model 2100 Atomic Absorption Spectrometer equipped with a deuterium background corrector, a HGA 700 graphite furnace and a burner head. An Ion Chromatograph Dionex 2000i was employed to analyse anion concentrations in synthetic rain.

Nitric acid and all other chemicals used were of Suprapure grade. Standard solutions (1000 mg/L; BDH; England) of Cd, Cr, Ni, Pb and V were employed to prepare the reference solution using water demineralized with a Milli-Q system as a diluting agent. Daily prepared complexing solutions [ammoniun pyrrolidinedithiocarbamate(APDC) plus sodium diethyldithiocarbamate (NaDDC) 0.5 % each and APDC or NaDDC 5%] were purified by 3–fold extraction with 2,6 -dimethyl - 4 heptanone (diisobutyl ketone – DIBK, Fluka). The back-extraction solution was prepared by dissolving 100 mg of $PdCl_2$ (Merck) in 10 mL of HNO_3 in 1L of ultrapure water. A citric acid (0.7 M) – Na citrate (1.2 M) buffer was purified by 3–fold extraction with 100 µL of complexing solution (APDC and NaDDC 0,5% each) and 1 mL of DIBK, while the ammonium acetate buffer, obtained by dissolving 500 g of ammonium acetate (pro analysis, Merck) in 1 L of deionized water, was purified by 3 successive extractions with 2.5 mL of DIBK after adding 10 mL of APDC 5%. The synthetic rain water was prepared in a 3 L flask with ultrapure water by dissolving amounts of NaCl, $(NH_4)_2SO_4$, $NaNO_3$ and Na_2CO_3 to obtain, for each matrix component, the yearly mean values previously determined in the same sampling station [3], which was active since 1995. All alkaline metals were added as Na^+. The solution thus prepared was then spiked with heavy metals, acidified with HNO_3 to reach pH 2, and analysed. Moreover, to get real matrix samples of wet and dry depositions, respectively, equal volumes of real samples were mixed to obtain a final concentration not above the Detection Limit. An average matrix was obtained which was representative of the seasonal variation during the sampling period.

Solvent Extraction and back extraction with a Pd^{2+} solution

100 mL of suprapure water acidified with 100 µl of HNO_3 for blank values and 100 mL of sample solution were treated according to the procedure of Saschsenberg *et al.* [5] to attain a 150 Preconcentration Factor for Cd, Ni and Pb. The complexing solution used was APDC and NaDDC 0,5% each. The only change in the procedure was the use of a citric acid–Na citrate buffer [3].

The preconcentration of V was performed according to the extraction procedure reported by Bone et al. [6] by a 3-fold reduction of the initial volume and the addition of a back-extraction step. An Erlenmeyer flask was filled with 100 mL of sample and 1.5 mL of ammonium acetate buffer; 6.5 mL of APDC (for V and Ni determination) or NaDDC 5% (for Pb and Cd analysis), and 3 mL of DIBK were added. Both complexing solutions have been tested for their suitability. The flask was vigorously shaken by hand for 2 min. After 10 min of phase separation, 750 µL of organic phase were transferred from the Erlenmeyer flask to a PTFE test tube and 500 µL of Pd^{2+} solution were added. Quantitative back-extraction recoveries are achieved upon completion of V-carbamate complex decomposition and the optimal timing sequence was found to be: 5 min shaking; 3 hours resting; 5 min shaking. The enrichment factor in this case was about 50. To have a smaller PF the preconcentration procedure for V was also used for Cd and Pb. This procedure allows to analyse aqueous solutions while offering the advantage of avoiding the problems associated with the limited stability of thiocarbamate complexes and the lack of analytical sensitivity deriving from the organic solvent [5, 6]. Preconcentration was performed during both extraction and back-extraction. The only element that could not be determined was Cr, probably because during back-extraction the exchange reaction between Pd (II) and Cr (VI) does not occur under the same experimental conditions.

Multiple Injection GF-AAS

Multiple Injection GF-AAS was run by increasing the sample volume (from 10 µL to 50 µL) and the number of injections (from 1 to 2 –PF 10 or from 1 to 4 – PF 20). After each injection only evaporation and matrix incineration were carried out in the instrument; atomisation was

carried out after the last injection. This procedure permits to analyse all the elements considered by means of an autosampler, thus reducing manual operations.

RESULTS AND DISCUSSION

Each technique was first tested by analysing blanks and known concentration solutions. It was thus possible to ascertain the viability of the chosen technique for determining the heavy metals often to be found in our environmental samples at low concentrations. Known values and those determined by applying either the solvent extraction-back extraction (Table 2) or multiple injection GF-AAS (Table 3) technique were found to be in good agreement. The recoveries obtained for each element were comparable with those reported in the literature (extraction efficiency: Cd 93%, Pb 95% and V 92% [7]) with an acceptable coefficient of variation in view of the fact that the solvent extraction/back-extraction method requires a lot of steps.

Regarding precision and multiple injection GF-AAS it should be noted that per cent standard deviations were not higher than those typical of the GF-AAS technique. In order to better confirm the applicability of these procedures to real samples they were applied to a synthetic rain sample spiked with heavy metals, characterised in each component (Tables 4 and 5), and to real samples of wet and dry deposition (Table 6). By doing so, matrix influence may thus be properly analysed.

TABLE 2 - Extraction-back extraction preconcentration tests for the determination of Cd, Pb, Ni and V.

Elem.	PF	No. of samples	Known concentration solution (mg/L)	Determined value (mg/L)	Recovery %	Std. Dev. %
Cd	150	9	$(8.23 \pm 0.04)\cdot 10^{-3}$	$(6.8 \pm 0.9)\cdot 10^{-3}$	82 ± 11	13
	50	9	0.01674 ± 0.0008	0.0147 ± 0.0025	88 ± 15	17
Ni	150	10	0.1513 ± 0.0007	0.140 ± 0.028	92 ± 18	20
	100*	5	0.1513 ± 0.0007	0.128 ± 0.013	84 ± 9	11
	100**	5	0.1513 ± 0.0007	0.156 ± 0.,028	103 ±19	18
Pb	150	9	0.2017 ± 0.0009	0.206 ± 0.023	102 ± 11	11
	50	9	0.2017 ± 0.0009	0.23 ± 0.05	113 ± 26	23
V	50	9	0.5030 ± 0.0020	0.52 ± 0.06	103 ± 12	11

PF = preconcentration factor; Std. Dev. = standard deviation; * This PF was obtained with a 2 mL DIBK volume, while during back extraction 1.5 mL of organic phase was shaken with 0.75 mL of Pd (II) solution; **PF obtained by increasing the back-extraction phase volume up to 0.75 mL.

TABLE 3 - Preconcentration test results obtained with multiple injection GF-AAS.

Elem.	PF	No. of samples	Known concentration solutions (mg/L)	Determined value (mg/L)	Std. Dev. %
Cd	20	12	$(5.004 \pm 0.007)\cdot 10^{-2}$	$(5.0 \pm 0.6)\ 10^{-2}$	1.2
Cr	10	12	0.5023 ± 0.0025	0.512 ± 0.017	3.0
Ni	4	8	1.022 ± 0.006	1.48 ± 0.08	5.0
Pb	10	10	0.5023 ± 0.0025	0.57 ± 0.07	12
V	10	10	1.022 ± 0.006	1.07 ± 0.12	11

TABLE 4 - Matrix composition of synthetic rain water.

Matrix Component	Mean year value (mg/L)	Determined value (mg/L)
Na^{+}	1.907	1.973
NH_4^{+}	1.164	0.854
CO_3^{2-}	36.9 (μeq/l)	71.0 (μeq/l)
SO_4^{2-}	2.884	2.884
$N\text{-}NO_3^{-}$	0.605	0.504
Cl^{-}	1.002	0.949

TABLE 5 - Results obtained by applying multiple injections and extraction- retroextraction to the same synthetic rain sample.

Elements	Known concentration solutions (mg/L)	Multiple injections (mg/L)	Extr.- Retroextr. Preconc. (mg/L)
Cd	0.01674 ± 0.00008	0.022 ± 0.003	0.020 ± 0.003
Cr	0.1002 ± 0.0005	0.140 ± 0.009	n.d.
Ni	0.1513 ± 0.0007	0.16 ± 0.016	0.158 ± 0.019
Pb	0.2017 ± 0.0009	0.212 ± 0.011	0.172 ± 0.021
V	0.5030 ± 0.0020	0.41 ± 0.03	0.48 ± 0.05

n.d. = not determined

TABLE 6 - Results obtained by applying multiple injections and extraction- retroextraction to real matrices of wet and dry depositions.

Elem.	Wet deposition sample (µg/L)		Dry deposition sample (µg/L)	
	Multiple injection[a]	**Extr.- Retroextr.[b]**	**Multiple injection[a]**	**Extr.- Retroextr.[b]**
Cd	0.069 ± 0.007	0.062 ± 0.008	0.053 ± 0.003	0.062 ± 0.003
Ni	0.43 ± 0.03	0.32 ± 0.05	0.91 ± 0.04	1.1 ± 0.3
Pb	0.48 ± 0.05	0.44 ± 0.06	0.155 ± 0.014	0.17 ± 0.03
V	0.60 ±0.04	0.67 ±0.05	1.11 ± 0.10	1.02 ± 0.11

[a] Mean values of 6 samples [b] Mean values of 5 samples

As can be seen all values are in good agreement with each other and with the known values in the case of synthetic rain. It may thus be concluded that both Multiple Injection GF-AAS and solvent extraction followed by back–extraction with a Pd (II) solution are suitable for application to real wet and dry deposition samples for determining Cd, Cr, Ni, Pb and V concentrations.

Multiple injection GF-AAS was, however, found to offer additional advantages compared to the other procedure, because it permits to determine all of the elements considered (Cd, Cr, Ni, Pb and V), it allows an initial sample volume small enough (200 µL for each analysis) for the analysis of all the real samples, and it is a simple method entailing less contamination risks as it involves fewer steps than solvent extraction.

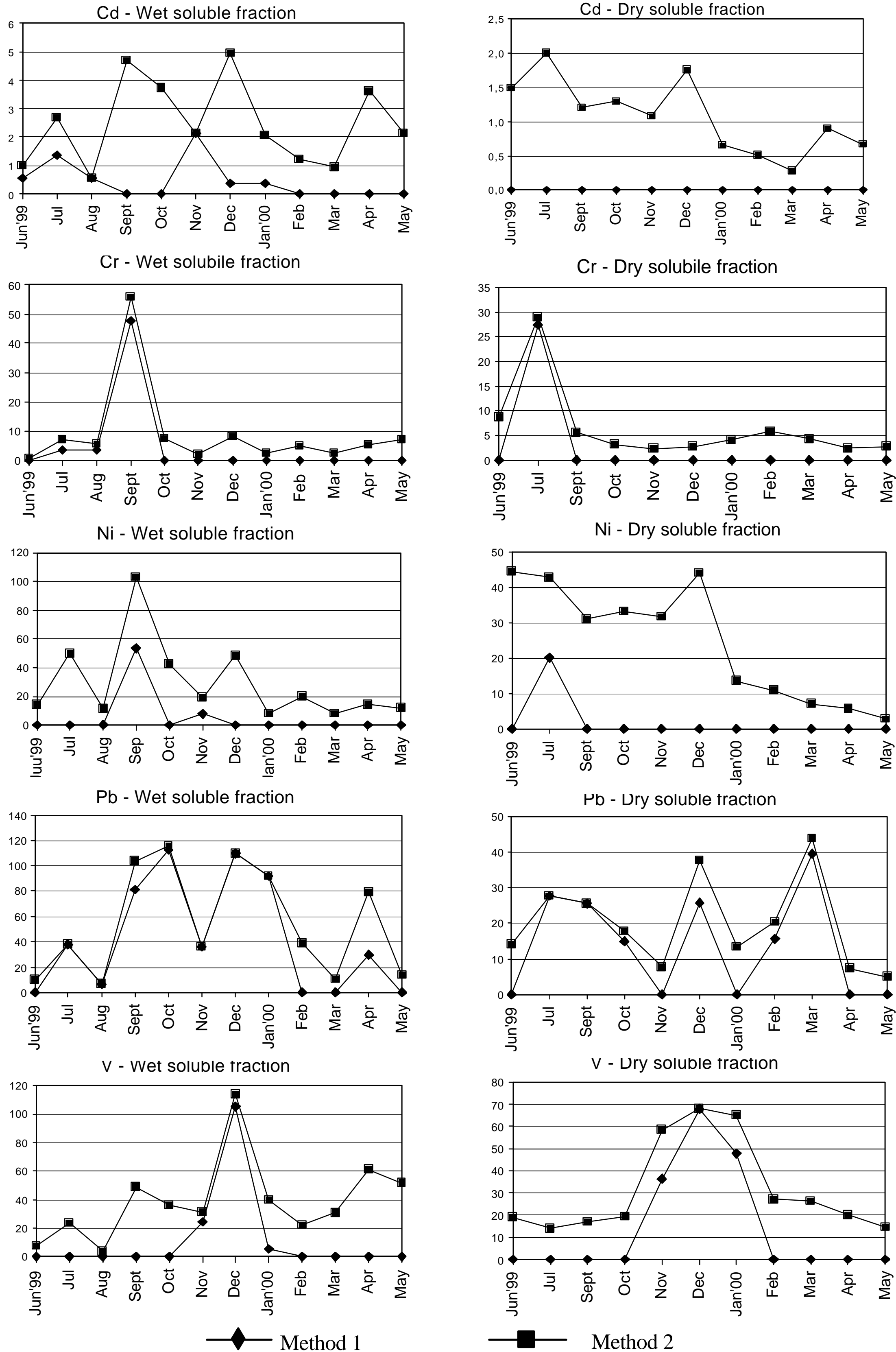
This technique was applied to real samples with concentrations lower than DL related to the period June 1999 - May 2000. The inclusion of the Multiple Injections GF-AAS in the analytical protocol is studied comparing the corresponding monthly deposition fluxes (method 2) with the ones calculated without considering the preconcentration data (method 1). As shown in Fig. 1, for all the elements considered the adoption of a preconcentration technique resulted in more detailed trends than the ones shown by method 1. This is particularly evident in the case of elements like Cd, Ni and V that exhibit concentrations lower than DL in more than 50% of the analysed samples. The incidence on annual fluxes, due to the soluble fractions of wet and dry depositions, revealed by preconcentration techniques, is 29% for Cd, 10% for Cr, 19% for Ni, 20% for V and 3% for Pb.

Another important consequence of this improved accuracy in metal determination is that more reliable correlations and similarities can be found in studying atmospheric depositions. During the considered period, the use of preconcentration techniques permitted to:

- evidence of some important correlations, as between Cd and Ni, Pb and V in wet depositions (correlation coefficient: 0.73, 0.86, 0.79, respectively) and between Cd and Ni in dry ones (correlation coefficient 0.90), that were not shown by results obtained using common analytical techniques;
- find out some false correlations, that in the absence of preconcentration, are given by the lack of accuracy, as between Cr and Ni, both in wet and dry depositions.

All the considerations about seasonal or annual trends and similarity between different months strongly depend on these data. Thus, it can be concluded that an environmental monitoring of wet and dry depositions cannot omit a preconcentration procedure; in particular, Multiple Injection GF-AAS, for the reasons explained above, can be considered the most favourable technique in this field.

FIGURE 1 - Comparison between monthly deposition fluxes of the wet and dry soluble fractions calculated with and without preconcentration.



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EFFECTS OF COPPER AND LEAD ON MICROALGAE (*Isochrysis galbana*) GROWTH

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SUMMARY

Impact of copper (Cu^{2+}) and lead (Pb^{2+}) on the growth of microalgae *Isochrysis galbana* was studied. Algal cultures were exposed to 0.01, 0.1, 1.0 mg/l Cu^{2+} and 0.01, 0.1, 1.0 mg/l Pb^{2+} for a period of 8 days. Biomass growth was estimated by chlorophyll-a measurement. Cu^{2+} concentrations of 1.0 mg/l had significant effects on *Isochrysis galbana* growth rate, while lower concentrations (0.01-0.1mg/l) caused a slow decline in algal growth. Comparable concentrations of lead (Pb^{2+}) were found not to affect the algal growth.

KEYWORDS:
Isochrysis galbana, heavy metals, growth rate, chlorophyll.

INTRODUCTION

Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystems being a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnification in the food-chain [1]. The functional role of trace elements in biological matter has been appreciated recently, even though experimental evidence of their importance was postulated many years ago. Copper, for example, serves as a cofactor in enzymes that regulate redox reactions associated with photosynthesis [2]. Bioassay experiments and studies concerning marine ecosystems have also pointed to copper inhibition of phytoplankton species [3 – 6].

Lead and copper may also damage the plasma membrane permeability leading to uncontrolled leakage of electrolytes [7]. Moreover, heavy metals exert multiple inhibitory effects on photosynthesis at different structural and metabolic levels. Experimental results have shown that some heavy metals (Hg^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}) could substitute the central Mg^{2+} in the chlorophyll molecule [8].

Although knowledge of metal effects on aquatic organisms has grown significantly in recent years, there are still gaps in metal's inhibitory or promoting effects on the aquatic organisms, especially in the lower trophic levels [9].

The primary producer, *Isochrysis galbana*, is a unicellar, photosynthetic, golden-brown euryaline flagellate (Class *Prymnesiophyceae*, division *Chrysophyta*). They are used as biomarkers in monitoring programmes due to their responses to contaminants [10]. Danilov et al. [11] suggested that the use of model organisms, often unicellar, which allow for rapid assessment of pollutants, can be of advantage. Moreover, *Isochrysis galbana* cultures are used for larval diet in aquaculture and they possibly may transfer toxicants to higher trophic levels through the food chain [12]. According to Gonzalez [2], laboratory experiments must consider the effects on the uptake of

1. different individual metal ions on each algae species,
2. different metal combination and
3. the effects of temperature, pH and salinity.

In the present study we investigated copper and lead effects on *Isochrysis galbana* biomass growth (measured as chlorophyll).

MATERIALS AND METHODS

Stock cultures of *Isochrysis galbana* (strain 927/1,CCAP) were maintained in sterile nutrient medium F/2, described by Lefley et al. [13] 100 ml of six-days old stock cultures were inoculated into F/2 nutrient medium in 500 ml flasks containing 0.01, 0.1, 1.0 mg/l Cu^{2+} and 0.01, 0.1, 1.0 mg/l Pb^{2+}. For the preparation of metal's solution $CuSO_4.5H_2O$ and $Pb(NO_3)_2$ were used.

Three replicates were prepared for each concentration along with a blank (control).

Flasks were incubated at 17-19 ^{o}C, under aerated conditions, irradiance of 9W. m^{-2} with a 12-h light/ dark cycle.

Chlorophyll-a (Chl-a) concentrations were determined in each flask during an 8 days incubation period spectrophotometrically after acetone extraction of pigment [14].

Phytoplankton growth in cultures was estimated repeatedly at 2 day intervals using the formula: $G=(C_f-C_i)/C_i)$, where C_i and C_f are the initial and final concentration of Chl-a in each culture. The above formula was chosen to offset possible adverse effects caused by the fact that the initial phytoplankton cultures used as starters were not identical in quantity.

To increase the reliability of the experimental results, each treatment was repeated three times including 4 levels of metal concentrations and incubation time. The results were statistically treated by employing a 3-factor ANOVA with the following main effects: metal concentration, incubation time (both fixed effects) and experimental run (random factor). Potential interaction between metal concentration and incubation time was also examined. A visual inspection of the ANOVA residuals for normality and homoscedasticity was preceded the application of ANOVA.

Statistically significant differences between level means were tested by the Student's Newman Keuls test (SNK) of multiple comparisons of means [15].

RESULTS AND DISCUSSION

Experimental results concerning the chlorophyll-a concentrations during exposure period with various concentrations of Cu^{2+} and Pb^{2+} are shown in Figs. 1 and 2 (three replicates of each treatment).

The analysis of variance for chrorophyll-a growth rates at different Cu concentrations revealed statistically significant effects for copper ($p<0.001$), incubation time ($p<0.001$) and their interaction ($p<0.001$). The experimental runs did not differ significantly ($p=0.940$) thus permitting us to consider the treatments as an overall one with three replicates. Examining the growth rates at the various levels of copper and incubation time (Fig. 3) in accordance with the SNK results of comparison of level means we infer that copper caused a gradual slow decline of growth rate at increasing concentrations but had significant effect only on its highest level of 1.0 mg/l, clearly suppressing the growth rate. Bilgrami et al. [7] found that Cu concentration of 0.1 mg/l (as $CuSO_4.5H_2O$) was highly inhibitory on the growth of four phytoplankton species, while lower concentrations, 0.01 mg/l, were found almost non-toxic. Our findings suggest that ion's concentration of 1.0 mg/l reduces significantly the growth rate, while lower concentrations (0.01 and 0.1 mg/l Cu^{2+}) cause a retardation in the growth rate of *Isochrysis galbana*, confirming findings of Moreno-Garrido et al. [16] that *Isochrysis galbana* is very sensitive to copper. Moreover, it seems that the photosynthesis in *I. galbana* is vulnerable to copper toxicity thus making it a suitable parameter for toxicity estimation. The capacity of copper to inhibit photosynthesis has been reported previously for some other algae [17, 18].

FIGURE 1 - Chlorophyll fluctuation in various Cu^{2+} concentrations during the 8 days incubation period (three replicates).

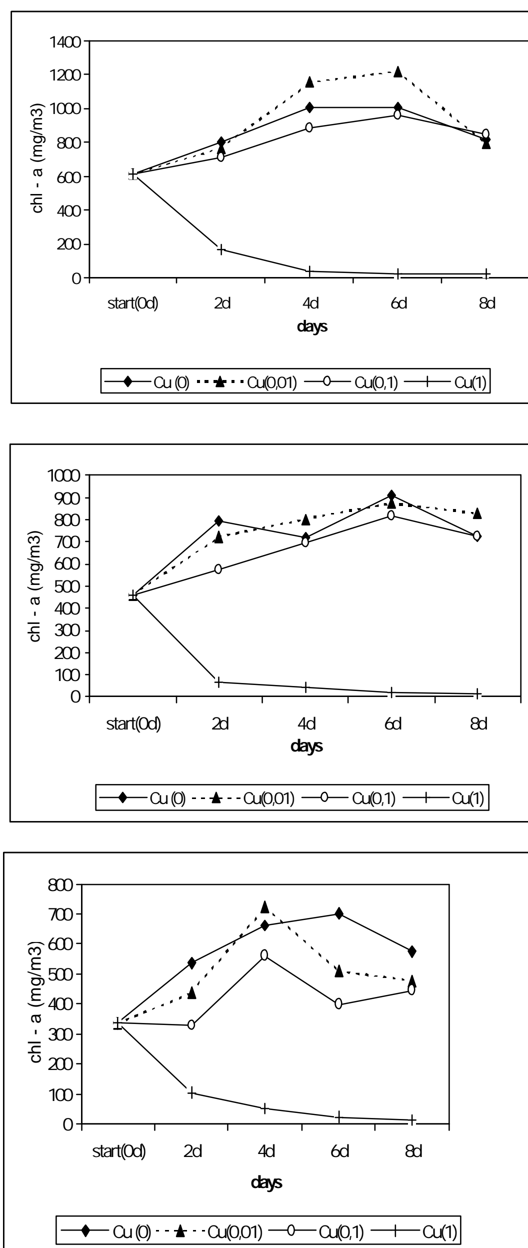
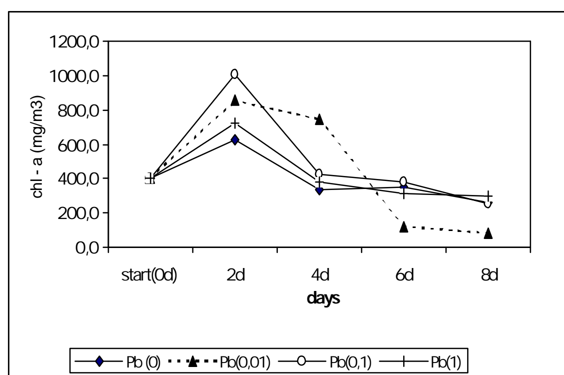
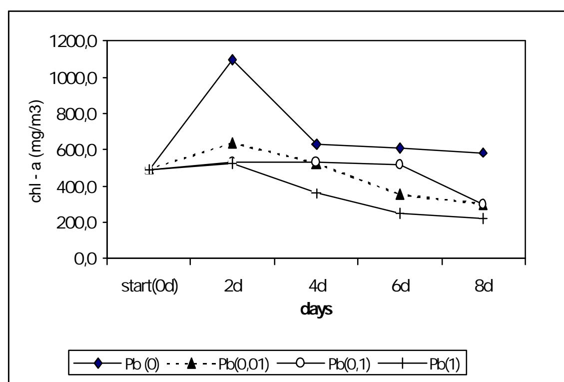
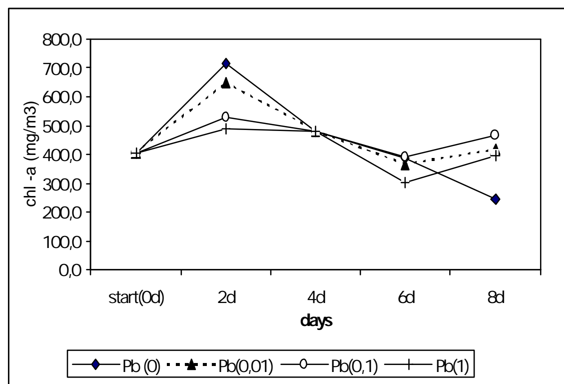


FIGURE 2 - Chlorophyll fluctuation in various Pb^{2+} concentrations during the 8 days incubation period (three replicates).







Generally, the metal can compete for binding sites of enzymes such as urease, acid phosphatase and ATPase, and inhibit other enzymes of nitrogen metabolism and photosynthesis [8]. *Isochrysis galbana* growth sustained higher values in the first 4 days, above the overall growth mean and statistically significant from the lower ones in the day intervals 4-6 and 6-8 (Fig. 3). Growth in the runs was nearly identical. Examination of the growth rates interactively at the 16 levels of copper concentration and incubation time (Fig. 4) reveals two growth trends: a decreasing growth rate by time in a more or less similar manner between copper levels 0, 0.01 and 0.1 mg/l and, interestingly, an increasing rate by time at copper level of 1 mg/l, approaching the final growth levels of the other trials. Presumably, algal cells under the stressing concentration of copper react by diminishing their growth rate down to –75.7 % the first 2 days. Afterwards, however, they demonstrate a remarkable recovery of accelerating growth rate by time. Bilgrami et al. [7] found that growth of four exposed phytoplankton species was 0-21.6% of the control in presence of 1 mg/l Cu (as $CuSO_4.5H_2O$). According to Jones and Gadd [19] these interactions and responses indicate a mechanism to regulate the free metal ion concentration inside the cell as well as for detoxification. Moreover, Giardi et al. [8] suggested that modulation of the photosynthetic apparatus and, especially in the D1 protein of photosystem II, under stress conditions (as metal concentration) is a commonly occurring process.

FIGURE 3 - Chlorophyll growth rates at various levels of copper concentration, incubation time and experimental run (The horizontal line represents the overall mean growth rate).

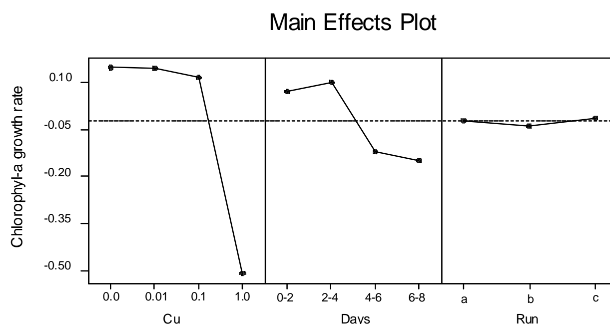
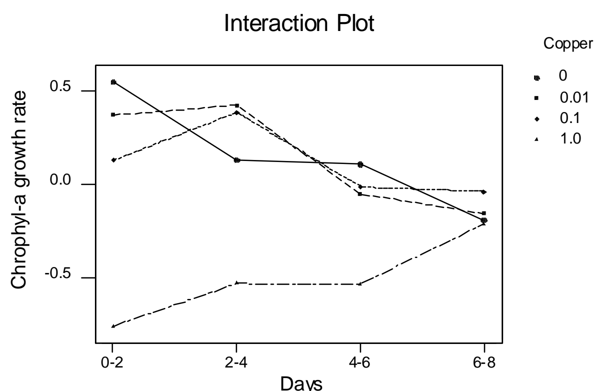


FIGURE 4
Chlorophyll growth rates by time at various copper concentrations.



In the treatments with increasing lead concentration levels the *Isochrysis galbana* growth rates were unaffected ($p = 0.918$, Fig. 5) meaning that these concentrations have no inhibitory effects on the algal growth. These findings are in good agreement with those of Danilov et al. [11]. They reported that Pb^{2+} concentrations between 0.1-2.0 mg/l had only stimulatory effects on the photosynthetic efficiency of *Chlamydomonas reinhardtii* under an exposure duration of 24 h. Moreover, Pb concentrations between 0.1-10.0 mg/l, as $Pb(NO_3)_2$, were found moderately toxic in four algae species after 7-days exposure

time [7]. Incubation time significantly affected the growth ($p<0.001$) causing a sharp, statistically significant decline of growth after the 2nd day interval (Fig. 5). Runs did not differ significantly ($p = 0.796$), increasing the repeatability and reliability of the experimental treatments. No significant effect of interaction between lead concentration and incubation time ($p = 0.537$, Fig. 6) on chlorophyll growth rate was observed, the latter depicting a decreasing rate by time similar to that of the main effects plot (Fig. 5).

FIGURE 5 - Chlorophyll growth rates at various levels of lead concentration, incubation time and experimental run (The horizontal line represents the overall mean growth rate).

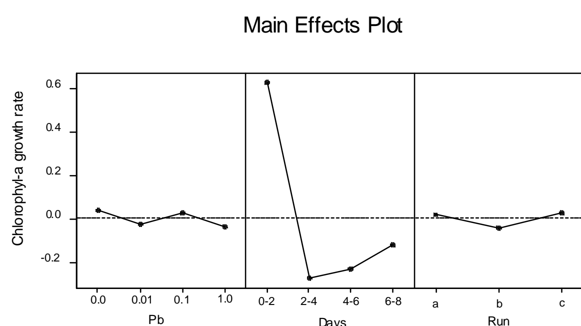
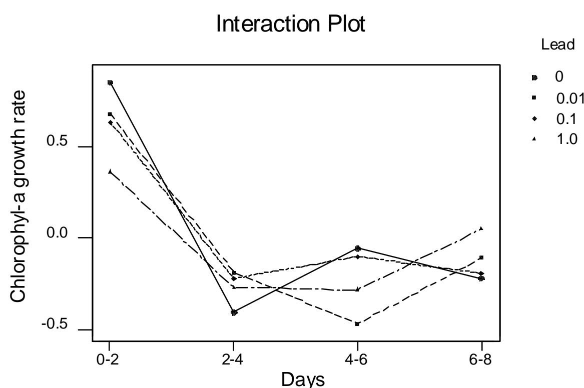


FIGURE 6
Chlorophyll growth rates by time at various lead concentrations.



CONCLUSIONS

Cu^{2+} concentrations of 1.0 mg/l had significant effects on *Isochrysis galbana* growth rate, while lower concentrations (0.01 and 0.1mg/l) caused a slow decline in algal growth. The same concentrations of lead (Pb^{2+}) were found to have no inhibitory effects on the algal growth. According to earlier findings concerning toxicity order for these metals to phytoplankton [20], Cu is more toxic than Pb for certain algal species.

Our findings also suggest that algal biomass (measured as chlorophyll) may be considered as a reliable parameter for toxicity testing. However, more research is needed to describe the photosynthetic apparatus responses of the algae under unfavourable conditions.

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COMPARISON OF PULP MILL EFFLUENT IMPACT ON EROD ACTIVITY AND GENOTOXICITY INDUCTION BETWEEN TWO *Gambusia holbrooki* GROUPS

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SUMMARY

This study concerns the total ethoxyresorufin *O*-deethylase (EROD) activity, acetylcholinesterase (AChE) activity and erythrocytic nuclear abnormality (ENA) frequencies in two female groups – field (F) and laboratory adapted (L) - of *Gambusia holbrooki* exposed to bleached kraft pulp mill effluent (BKPME) contaminated river water. Water samples were collected in the Vouga River (Cacia, Portugal) upstream (R) and downstream (P1, P2, P3) the effluent outlet, and used to expose the fish (2, 4 and 6 hrs). Genotoxicity was documented by the F group exposed to the least diluted BKPME (P1), whereas its EROD activity induction was significant at the most diluted BKPME (P3) after 6-hr exposure. L group failed to exhibit any of the previous responses. The initial physiological condition of each fish group clearly influenced their responses. P1 seems to contain a high concentration of cytotoxic and genotoxic compounds, whereas the dilution factor in P3 seems to be responsible for the EROD induction, previously inhibited in P1.

KEYWORDS: Pulp mill effluent, *Gambusia holbrooki,* EROD, genotoxicity, AChE.

INTRODUCTION

In recent years, an increasing interest has been focused on the impact of bleached kraft pulp mill effluents (BKPMEs) in environmental health. BKPMEs are complex mixtures of environmentally active substances, containing about 300 known chemicals (Nestmann *et al*. [1]), inducing adverse effects in living organisms [2 - 7]. These effluents are one of the major sources of water contamination inducing biochemical and genotoxic effects in several fish species [4, 7 - 9]. A variety of biomarkers have been used to assess the impact of BKPMEs on these organisms.

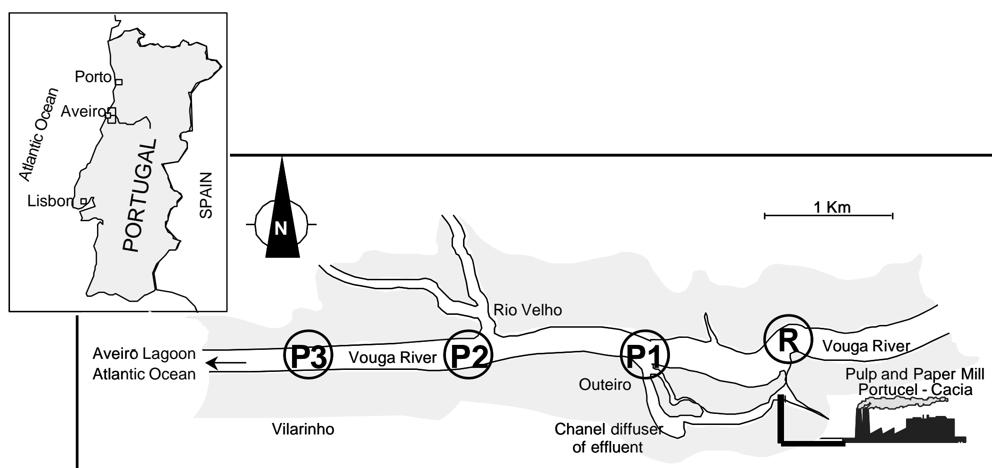
In this context, EROD (7-ethoxyresorufin *O*-deethylase) activity must be included among the most important biochemical effects induced by the pulp mill effluents [7]. Additionally, a few studies have been conducted on mutagenic effects induced in fish: sister chromatid exchange (SCE) (Santos and Pacheco [10]), erythrocytic nuclear abnormalities (ENA) (Pacheco and Santos [7], Gravato and Santos [11]) and DNA strand breaks (Maria *et al*. [12]) in *Anguilla anguilla* and *Dicentrarchus labrax* as well as DNA damage in *Onchorhynchus tshawytscha* (Easton *et al*. [13]).

The utilisation of pesticides in the forest management may result in the occurrence of organophosphate and carbamate compounds in pulp mill effluents. Since these xenobiotics are known to be neurotoxic, the determination of acetylcholinesterase (AChE) activity may be a useful indicator in order to complete the assessment of BKPME effects.

Studies conducted under field conditions are affected by alterations of environmental parameters such as temperature, dissolved oxygen, conductivity, dilution and dispersion of effluents as well as by predation, competition and diseases. In laboratory experiments, many of these factors can be controlled, and the toxicological response of organisms to the same effluent may be different (Pereira *et al*. [14]). Thus, the responses observed in the test species collected in the field and that acclimated to laboratory environment may differ, as a consequence of their previous physiological condition and biological viability.

The aim of this work is the comparison of total EROD activity and genotoxicity, measured as ENA frequency, between two *Gambusia holbrooki* groups - field and laboratory adapted - exposed to BKPME contaminated river water. Additionally, total AChE activity was assayed and all the water samples were submitted to the Microtox® basic test.

FIGURE 1 - Schematic representation of water sampling sites in the Vouga River, Cacia, Portugal: R - Reference upstream site; P1 - At effluent source (Outeiro); P2 - 1 Km from the effluent source; P3 - 2 Km from effluent source.



MATERIALS AND METHODS

Test animals

Gambusia holbrooki specimens (average weight 200 mg) were collected along the Cértima River (Portugal) left bank during the beginning of the reproductive season (May). This group of fish (L) was kept in laboratory [temperature 20±1 ºC; photoperiod 16 hrs light : 8 hrs dark; Artificial Pond Water (APW)], during five months, until exposure began. Fish were fed with Tetramin® tropical fish food twice daily during this period. Only non-pregnant females were utilised for experimental work. Another group of female fish (F) was collected from the same location prior to experimental exposure, and housed in conditions similar to the L group, during 96 hours. In natural conditions, fish were not exposed to pulp mill effluents.

Effluent characteristics and water samples collection

The primary and secondary-treated BKPME is produced by the pulp and paper mill, Portucel (Cacia, Portugal). The pulp mill production process uses *Eucalyptus globulus* (75%) and *Pinus pinaster* (25%) as wood supply. River water samples were collected upstream [reference (R)] and downstream (P1, P2, P3), the effluent outlet, in the Vouga River (Cacia, Portugal), following a decreasing gradient concentration (see Fig. 1).

Experimental design

L and F fish groups were kept in aerated glass vessels (1.5 L), at 20±1 ºC, photoperiod, 16 hrs light : 8 hrs dark, and no food was supplied during the experimental exposure. The fish were exposed during 2, 4 and 6 hours to water collected from the sampling sites (Fig.1). The controls for groups L and F were APW and field water (Cértima River), respectively. At each sampling point, fish were killed by decapitation, blood smears were prepared and the body immediately frozen in liquid nitrogen and stored at –80 ºC until homogenisation. Five animals were used in each experimental condition.

Enzyme biomarker assays

The whole fish body was homogenized in 0.1 M Tris-HCl buffer pH 7.4, containing 0.15 M KCl and 20% glycerol. Microsomes were obtained by differential centrifugation according to the methods of Lange *et al*. [15] and Monod and Vindimian [16], as adapted by Pacheco and Santos [17]. The resulting microsomal and cytosolic fractions were immediately frozen in liquid nitrogen and stored at -80 ºC until use.

Total (whole body) EROD activity was measured in the microsomal fraction, as described by Burke and Mayer [18]. The reaction was carried out at 25 ºC, in the fluorometer cuvette containing 1 ml 0.5 µM ethoxyresorufin (in the previous Tris-HCl buffer) and 100 µl of microsomal suspension. The reaction was initiated by adding 10 µl of NADPH (10 mM) and the progressive increase in fluorescence, resulting from the resorufin formation, was measured for 3 min (excitation wavelength 530 nm, emission wavelength 585 nm). EROD activity was expressed as picomoles/minute/milligram of microsomal protein.

Total AChE activity was determined in the cytosolic fraction according to the method described by Ellman *et al.* [19]. The reaction was carried out at 25 ºC, in the cuvette containing 3 ml phosphate buffer (pH 7.2)/dithio-bis(nitrobenzoate)(0.26 mM), 100 µl acetylthiocholine (156 mM) and 20 µl of sample. The absorbance change was measured, at 405 nm after exactly 30, 60 and 90 sec. The mean absorbance change per 30 sec was determined and used for the calculation. Enzyme activity was expressed as U/g of cytosolic protein.

Microsomal and cytosolic protein concentrations were determined according to the Biuret method (Gornall *et al*. [20] using bovine serum albumin as the standard.

Mutagenicity assay

The ENA test was carried out in *Gambusia holbrooki* mature erythrocytes according to the procedures adapted by Pacheco and Santos [21]. The nuclear lesions were scored into one of the following categories: micronuclei (M), lobed nuclei (L), dumbbell-shaped or segmented nuclei (S) and kidney-shaped nuclei (K). Cells with nuclear abnormalities were counted on each 1000 mature erythrocytes sample per fish. The final result was expressed as a mean value (‰) of the sum (M+L+S+K) for all the individual lesions observed.

Vibrio fischeri - Microtox®

A basic test was carried out with 5, 15 and 30 min incubation time (Microbics Corp. 1992).

Statistical analysis

All the data were first tested for normality and homogeneity of variance to meet statistical demands. Variance analysis was used to compare the results between control and exposed fish, followed by Dunnet test and Tukey multiple comparison test (Zar [22]).

RESULTS

The EROD results (Fig. 2) revealed a significant increase only for F group after 6 hours exposure to P3 water sample ($P<0.05$). Furthermore, a tendency for increased EROD activity following the gradient of effluent dilution (P1<P2<P3) was also found in the same F group and exposure duration.

FIGURE 2 – Total EROD activity of two *Gambusia holbrooki* groups[(laboratory-adapted (L) and field (F)] after 2, 4 and 6-hr exposure. Vertical bars represent the means with correspondent SE bars. Significant differences between exposed and control fish: *P<0.05.

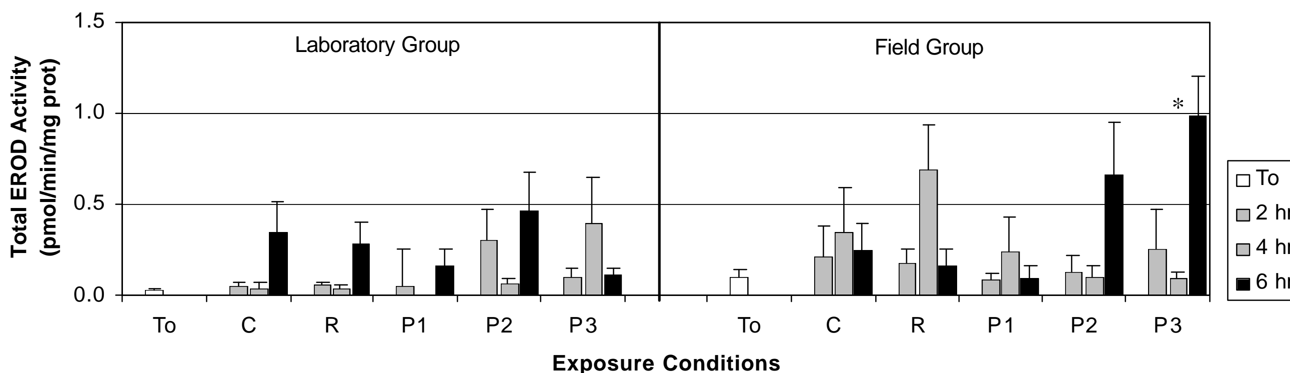


FIGURE 3
Erythrocytic nuclear abnormalities (ENA) frequency of two *Gambusia holbrooki* groups [laboratory-adapted (L) and field (F)] after 2, 4 and 6-hr exposure. Vertical bars represent the means with correspondent SE bars. Significant differences between exposed and control fish: **P<0.01.

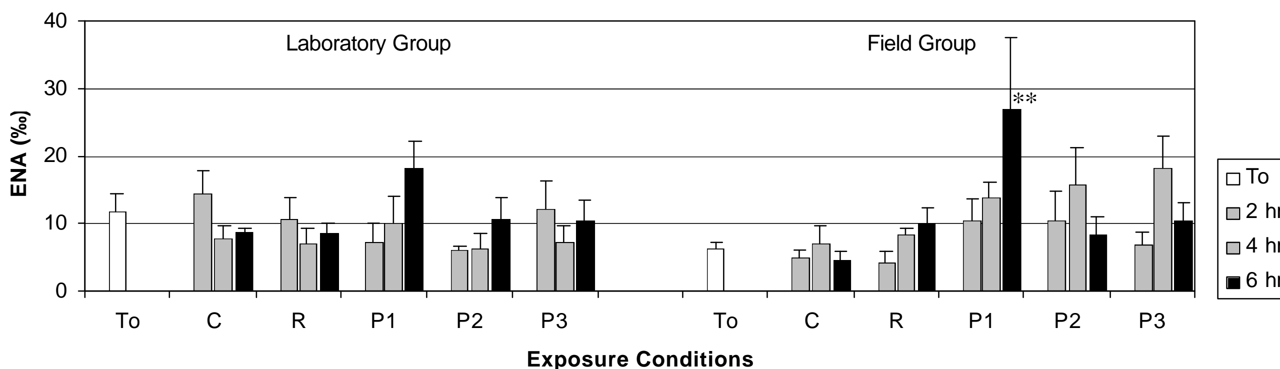
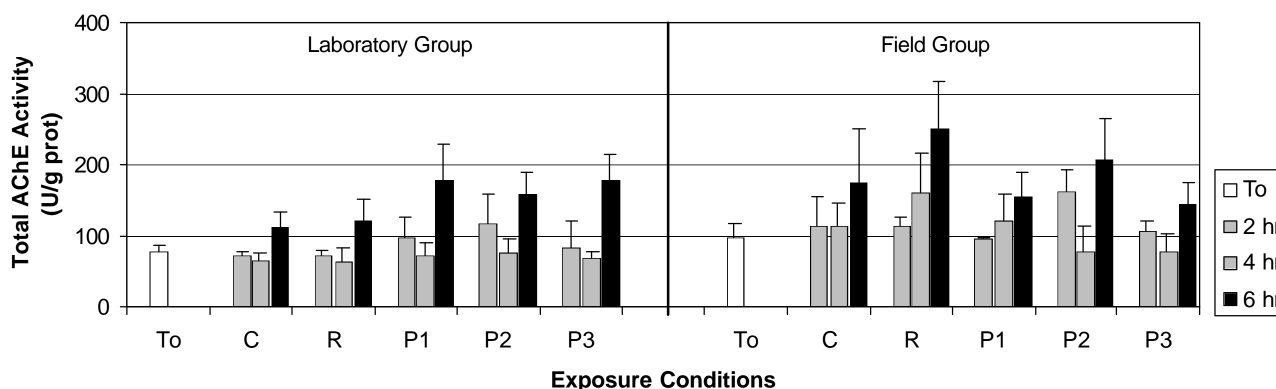


FIGURE 4 – Whole-body AChE activity of two *Gambusia holbrooki* groups [laboratory-adapted (L) and field (F)] after 2, 4 and 6-hr exposure. Vertical bars represent the means with correspondent SE bars.



In terms of genotoxicity (Fig. 3), only the F group displayed significant responses. Thus, ENA frequency was significantly induced ($P<0.01$) after 6-h exposure to P1 water sample. The L group revealed a clear ENA increase in the same exposure conditions, despite the absence of statistical significance.

The results concerning AChE activity (Fig. 4) did not demonstrate any significant alterations in any fish test group.

The Microtox® results did not allow the computation of an EC_{50} for any of the five samples tested and a toxicity pattern related to the exposure time was not observed.

DISCUSSION

A significant genotoxic response was observed in *Gambusia holbrooki* field group exposed to the most contaminated river water sample (P1), whereas its total EROD activity was not altered. Furthermore, the least contaminated river water (P3) induced a significant increase in EROD activity without a correspondent significant genotoxic response, in the field group.

The P1 river water sample seems to contain a high concentration of cytotoxic and genotoxic compounds, whereas the P3 river water sample seems to include EROD inducers rather than genotoxic compounds.

BKPMEs seem to present an EROD inhibition potential resulting either from inhibitor compounds or from EROD inducers which may become inhibitors in extremely high concentrations. The observed tendency for EROD activity rise with increasing distance from the effluent source suggests that the reduction or elimination of the previous inhibitory action was caused by the BKPME dilution.

In general, the present results reinforce previous *in situ* studies carried out in the same area using caged *Anguilla anguilla* [7], confirming the EROD and genotoxic inducing potential of the tested effluent in fish. Additionally, liver EROD activity increased (P1<P2<P3) and ENA decreased (P3<P2<P1) with the increasing distance from the effluent outlet [7].

The current results consolidate the hypothesis presented by Martel *et al.* [23] and Pacheco and Santos [7] that secondary treatment does not eliminate the effluent MFO (mixed function oxygenate) induction potential, present with a considerable capacity to cause EROD induction as well as genetic damage in fish. According to Oikari and Lindström-Seppä [24] the capacity of BKPME to induce EROD activity in fish is due to chlorinated compounds. However, Martel *et al.* [23] and Pacheco and Santos [7, 17] state that resin acids also exhibit this aptitude.

Payne *et al.* [25] found an AChE activity depression in muscle tissues of fish captured in the area of a pulp and paper mill. However, in the current study, the whole body AChE activity was not significantly altered by the BKPME contaminated river water. Our findings agree with a previous caged fish experiment (Oikari *et al.* [26]), where it was demonstrated that BKPME did not work like anticholinesterase agent, since brain AChE activity was not affected. Recently, the use of this endpoint as a specific biomarker for organophosphate and carbamate pesticides has been questioned and Guilhermino *et al.* [27] suggested that, in strongly polluted areas, false diagnostics and wrong conclusions may be achieved, whenever AChE is used as a specific biomarker.

Despite the sensitivity to substances altering metabolic oxidative pathways demonstrated by the bacteria species used in the Microtox® test (Radix *et al.* [28]), the adopted biomarkers showed obvious advantages relatively

to the conventional Microtox® tests since they were able to detect short-term exposures to BKPME, not measurable by Microtox® test, allowing an additional understanding of the toxic mechanisms. According to Kyungho and Meier [29], the sensitivity of Microtox assay is closely related to a specific type of toxicant, and hence its utility in biomonitoring effluents is better evaluated on a case-by-case basis. Therefore, taking into account our results, the relevance of Microtox test in biomonitoring water contamination by BKPMEs seems to be limited.

Our results confirm previous reports (Pacheco and Santos [7, 17]), such as EROD activity and ENA frequency as useful early warning biomarkers, recommending their inclusion in a common biomarker battery towards this class of industrial pollutants. Despite the promising performances of the adopted biomarkers, some problems may arise in the presence of intense pollution, particularly in terms of EROD activity. Therefore, it is very essential to know about biochemical behaviour of each biomarker according to the effluent concentration and each test species.

The present results indicate that F group was clearly more responsive than the L group, since the latter failed to exhibit any significant response once submitted to the same exposure conditions. The different EROD responses observed between F and L groups might be attributed to a lack of some adaptation capabilities in the laboratory fish, resulting from the acclimation conditions, namely the artificial diet and the absence of any environmental stimulation for the last 5 months. In this perspective, the field group will present a more activated metabolism, particularly phase I enzymes.

The effects of BKPME in field studies must take into consideration the initial physiological state of test fish as an important source of variation in the assayed parameters.

In conclusion, the hazards caused in fish by pulp mill effluent contaminated water were confirmed after short-term exposures. In order to select a set of biomarkers to assess the impact of BKPMEs, it is critical to establish their stability under variable physiological conditions of the exposed groups, likely to be encountered over the environmental exposures.

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PRESENT SITUATION OF AIR QUALITY IN THE JINAMAR VALLEY (GRAN CANARIA ISLAND).

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SUMMARY

This paper presents data on the levels of the elements, sodium, potassium, calcium, magnesium, iron, copper, nickel, lead and the polycyclic aromatic hydrocarbon, benzo(a)pyrene, as constituents of total suspended particulate (TSP) together with the gases SO_2 and NO_x present in the environmental air of Jinamar, a small town on the island of Gran Canaria (Spain), which is separated from a thermal power station by a mountain barrier. In order to identify the different sources of the particles, a principal factor analysis was perfomed on the data set.

The average concentrations (range) were 99.30 (79.14–122.20) $\mu g\ m^{-3}$ for TSP, 32.30 (23.50 – 51.00) $\mu g\ m^{-3}$ for SO_2, 8.8 (5.3 – 11.17) $\mu g\ m^{-3}$ for NO and 17.6 (13.7 – 21.7) $\mu g\ m^{-3}$ for NO_2.

KEYWORDS: Aerosols, benzo(a)pyrene, trace elements, nitrogen oxides, sulphur dioxide.

INTRODUCTION

The location of the island of Gran Canaria, between a temperate and a tropical area, in addition to its proximity to the African continent, determines the meteorological conditions for particulate transport and dispersal, namely: (*a*) the Trade Winds, which prevail in the area (particularly in the summer months); (*b*) the atmospheric instability arising from the presence of polar sea air in temperate latitudes, which is responsible for most of the local rainfall; and (*c*) inputs of Saharan air which produces a marked increase in the temperature and a decrease of relative humidity which occasionally drops below 30% near the coast. This atmospheric regime typically produces haze which, on occasions, can thicken to fog [1].

The Jinámar Valley in the NE of the island of Gran Canaria is separated from the coast by a mountain barrier (height: 130 m) which shelters it from the emissions of a thermal power station and a water desalination plant (sea level), both located near the coast. These and the emissions from the nearby motorway are the primary sources of anthropogenic pollution in the area.

The Valley's residents, about 25,000 in number, are routinely exposed to the above-mentioned emissions. Their effects are worsened by both the periods of calm, when the atmospheric pollutants concentrate in the Valley and the episodes of haze, which substantially raise the atmospheric levels of the gases and particulates by thermal surface or low-altitude inversions. These thermal gradient changes close to the ground hinder the vertical motion of the air and thus cause pollutants to concentrate a few hundred meters above ground level. This is severely hazardous to human health, as reflected by the high rate of hospital admissions among the Valley's residents, particularly due to bronchial complaints, and especially among children [2].

In order to assess the quality of the air in the Jinámar Valley, we determined the concentration levels of various pollutants, known to have adverse effects on human health. Such pollutants include NO_2, prolonged exposure to which has been unequivocally correlated to bronchial disease in children [3]; SO_2, which is hazardous when present together with fine particulates (smaller than 2 µm); and other particulates, which may be made up of a large variety of chemical species. The former two have been the subject of many studies over long periods [4 - 6]. Their interest resides in the fact that they are either toxic or allow one to identify the sources of pollution. The following chemicals were determined in this work: Na, K, Ca, Mg, Fe, Cu, Ni and Pb, in addition to benzo(a)pyrene, a polycyclic aromatic hydrocarbon and one of the most potent carcinogens, generally considered to be an indicator and element of assessment of health hazards in combustion-generated polycyclic organic matter, POM, including the risk of PAH contamination.

MATERIALS AND METHODS

Sampling

From July 1999 to July 2000, air samples were taken on the roof of the school building, Pedro Lezcano (P.L.) at a height of about 15 m above the ground. The sampling site was in a residential area located about 2 km from the coast, SW of the power station and the desalination plant. A Monitor Labs model 8840 chemiluminescent analyser provided continuous measurements of environmental NO and NO_x levels, with NO_2 calculated by subtraction. Environmental NO and NO_x levels were taken every 10 s and used to derive 15-min and 1-h averages for subsequent processing/analysis. During the data collection period under consideration, the analyser was calibrated weekly or 2-weekly using commercially-sourced NO in N_2 and NO_2 in air cylinders, carefully cross-checked against laboratory primary standards (permeation tube, static dilution and gas phase titration-derived). The precision of the instrument used is 0.8 ppb (at 100 ppb ambient concentration). Hourly average concentrations of sulphur dioxide were measured using a Monitor Labs 8850 fluorescence analyser. Wind speed (ms^{-1}) and direction (% hourly frequency) were calculated every day.

Extraction and analysis

a) *Total suspended particulates*

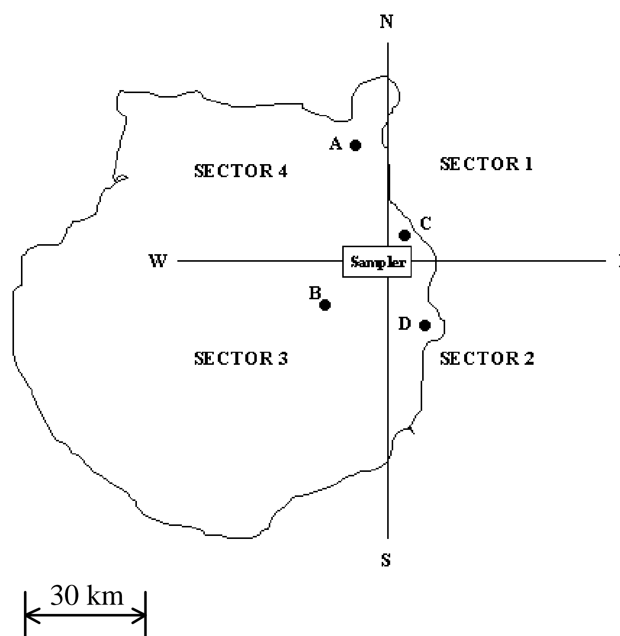
Aerosol samples were collected in 24 h periods, starting at 0800 h with a high-volume pumping system (CAV-P; MCV, Collbató, Spain) equipped with glass-fiber filters, Whatman GF/A (20 x 25 cm), which previously had been fired at 350 ºC, soaked in a solution of 1 N phosphoric acid and extensively washed with distilled water [7] to reduce the blank levels. The concentrations of particulates were determined using pre- and post-sampling filter weights. In order to ensure consistent mass values, the filters were equilibrated prior to weighing under controlled temperature (20-25 ºC) and relative humidity (40 ± 5 %) conditions [8, 9].

b) *Metals*

Heavy metal extraction was carried out by adding 5 mL of nitric acid (Merck Suprapur, 65%), 2 mL of hydrochloric acid (Merck Suprapur, 37%) and 10 mL of ultrapure water (18MΩ cm^{-1} of specific resistance) to the filter portion (1/9 of the sampled filter) in a Pyrex tube and kept at room temperature overnight. Extraction was completed in an oven at 120 ºC for 210 min and the extraction solution was cooled, filtered and diluted to 50 mL with ultrapure water. Samples were kept in precleaned polyethylene bottles in the refrigerator (at 5 ºC) until analysis.

Flame Atomic Absorption Spectroscopy with air-acetylene flame was used to determine Na, K, Ca, Mg, Fe, Cu, Ni and Pb using a Perkin Elmer model 2380 Atomic Absorption Spectrophotometer.

FIGURE 1 - A general perspective of sampling sites and the sectioning of more representative emission sources (A: Las Palmas G.C. city; B: Telde city; C: power station and desalination plant; D: airport).



c) *Benzo(a)pyrene (BaP)*

All the filters from high-volume sampling were stored in glass jars in the dark at -70 ºC, thus ensuring the minimization of PAH losses until extraction and analysis. Samples were placed in a Soxhlet extraction apparatus [10, 11] for at least 20 h and were given more than the minimum 20 cycles required for complete extraction [12]. The solvent was HPLC-grade dichloromethane.

The extracts were concentrated to 2 mL in a rotary evaporator at 35 ºC and 800-810 mbar and fractionated by column chromatography. A column filled with 2 g silica was used. BaP was collected in the second fraction, concentrated to near dryness under a flow of dry nitrogen and redissolved with dichloromethane (20%) and acetonitrile (80%). The recovery factors of this extraction and fractionation process were about 90-100 %.

The samples were analyzed by GC-MS (Shimadzu GC/MS system consisting of a GC-17A gas chromatograph and Shimadzu QP-5000 mass spectrophotometer). A 30 m x 0.25 mm i.d. HP-5MS capillary column (film thickness 0.25 μm) was used. The GC analyses were performed with helium as carrier gas and an oven temperature program from 50 ºC to 300 ºC at 8 ºC/min; injector and transfer line temperatures of 300 and 230 ºC, respectively. The injector was in the splitless mode [13].

Identification and quantification

Compound identification was based on the GC-MS data and co-injection of the authentic standards. The quantification was performed from the GC profiles using the external standard method.

RESULTS AND DISCUSSION

Table 1 shows the mean seasonal and global concentrations of the pollutants studied during the sampling period. The valid samples corresponding to days of measurements were no. 243 for metal species and TSP, no. 352 for NO and NO_2, no. 374 for SO_2, and no. 40 for B(a)P – the last from July to December only.

With the exception of Cu and Pb, the concentrations found in Jinámar were higher than those reported for other European urban regions [14]. The high concentrations of Na, K, Ca, Mg and Fe can be readily understood if one takes into account that these elements usually come from natural sources such as the marine breeze and soil weathering. These concentrations were further increased by the scant rainfall during the sampling period (only 140 mm in 13 months) and by the nearness of the Jinámar valley to the ocean.

This assumption is supported by the correlation coefficients for the previous five elements and those of total suspended matter. According to them, there appear to be two distinct groups of elements, *viz.* one consisting of Ca, Fe and Mg, which exhibits high correlation among the elements and with TSP –thus suggesting a common origin–, and the other consisting of Na and K, of a seemingly different origin.

The nickel levels are higher than those in the areas compared. This may be a result of the proximity to two anthropogenic pollution sources located on the coast (the power station and the water desalination plant). The low levels of lead, below those found in all the other cities compared, suggest a weak influence of the motorway, but we should also bear in mind here the increasing use of unleaded petrol.

TSP concentrations ranged from 79.14 to 122.20 $\mu g\ m^{-3}$ with an average value of 99.3 $\mu g\ m^{-3}$ similar to industrial zones. BaP concentrations with a mean value of 0.159 $\mu g\ m^{-3}$ are lower than those proposed as guideline limits. The mean concentrations of NO and NO_2 suggest that both oxides are carried from distant sources –not so distant, however, as to allow NO to be oxidized to NO_2 to a substantial extent.

Based on the seasonal variations in the pollutant concentrations, the following three groups could be established: (*a*) one consisting of sulphur dioxide, benzo[a] pyrene and nitrogen monoxide, which exhibited high peaks in autumn (October - December); (*b*) another encompassing Ca, Mg, Fe, Cu, Pb, NO_2 and TSP, with winter (January - March) peaks; and (*c*) a third composed of Na and K, with summer (July - September) peaks –K exhibits an additional peak in spring, however.

TABLE 1 - Average value ±standard deviation of component concentration at Jinamar (ng m^{-3}).

Variable	Mean	Summer	Autumn	Winter	Spring
Na	6300 ± 4700	7410 ± 5030	6690 ± 5970	6070 ± 3020	5410 ± 4130
K	1150 ± 1000	1240 ± 1030	1120 ± 1270	930 ± 630	1280 ± 1070
Ca	2270 ± 2320	2330 ± 1650	2190 ± 3410	2950 ± 2880	1700 ± 1280
Mg	890 ± 540	890 ± 410	790 ± 690	1080 ± 680	860 ± 430
Fe	1290 ± 1260	1420 ± 920	1200 ± 1590	1700 ± 1970	950 ± 560
Cu	31 ± 20	26 ± 12	29 ± 17	51 ± 35	26 ± 12
Ni	37 ± 45	28 ± 26	52 ± 64	39 ± 45	28 ± 26
Pb	26 ± 19	29 ± 25	25 ± 10	31 ± 26	29 ± 25
B(a)P	0.159 ± 0.07	0.132 ± 0.04	0.187 ± 0.085	-------	-------
TSP	99300 ± 80100	97700 ± 32300	108300 ± 141400	122200 ± 85600	79140 ± 33430
SO_2	32300 ± 28800	23500 ± 24900	51000 ± 47000	26900 ± 26700	29700 ± 30400
NO	8800 ± 4300	5300 ± 3100	11170 ± 7240	9860 ± 8760	10600 ± 7400
NO_2	17600 ± 7700	13700 ± 6900	18600 ± 9700	21700 ± 14300	19100 ± 9600

TABLE 2 - Monthly maximun and minimum concentrations of NO_2 and wind direction.

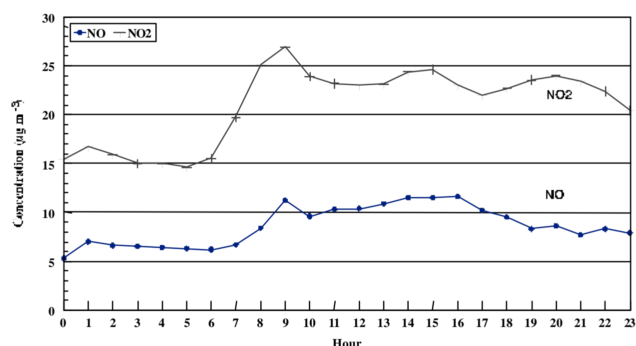
MONTH	MAXIMUM		MINIMUM	
	Concentration	Wind sector	Concentration	Wind sector
July	11.7	1	4.6	4
August	34.6	1	4.0	4
September	23.7	4	4.3	4
October	27.8	1	7.4	4
November	26.6	1	4.3	4
December	40.5	1	10.0	4
January	62.0	1	8.0	1
February	65.6	1	4.0	4
March	26.3	1	11.1	4
April	33.6	1	10.2	4
May	38.7	1	9.4	4
June	37.0	1	6.8	4
July	57.1	1	3.6	4

The autumn peak for the species in the group (*a*) appears to confirm the previous assumption that the power station and desalination plant influence the pollutant concentrations in the Valley as, in fact, this season was the period, during which winds from sector 1 (where the two pollution sources lie) blew for longer times. In fact, the concentrations of Ni (86 ng m^{-3}), SO_2 (44200 ng m^{-3}), B(a)P (0.187 ng m^{-3}) and NO (11300 ng m^{-3}) during the days when winds from this sector prevailed (130 %, 37 %, 40 % and 28%, respectively), were higher than the corresponding mean value for the period.

Prominent among the species which exhibited winter peaks, were Ca, Mg, Fe and Cu, in addition to two anthropogenic pollutants: Pb and NO_2. The last two did not reach their highest values in autumn because the influence of automobile exhaust –not only from the motorway but also from the nearby cities (Las Palmas de Gran Canaria and Telde)– of the Valley seems to be weak. A comparison of the days in each month where the highest and lowest concentrations of NO_2 were recorded with the direction of the prevailing wind for each piece of data (Table 2) reveals that the maxima occurred with winds from sector 1 and the minima with winds from sector 4. The only two exceptions were encountered in September for the maxima and January for the minima. The minimum found in January can be ascribed to the high speed of the wind recorded on the particular date (40% higher than the monthly mean), which favored dispersion of the dioxide. The monthly peak detected in September, with winds from sector 4, may have been the result of winds from sector 1 blowing in the central hours (10 am to 6 pm) that day.

Based on the foregoing data, the mean concentrations of nitrogen oxides, *viz.*8.8 µg m^{-3} (0.0072 ppm) for NO and 17.6 µg m^{-3} (0.0093 ppm) for NO_2, do not appear to be too high. This may be the result of the orography of the land and of the direction of the prevailing winds during the period studied (sector 4), which might have prevented accumulation of pollutants in the Valley.

FIGURE 2 - Diurnal pattern of NO and NO_2 concentrations.



The hourly variation in both oxides is shown in Fig. 2. As can be seen, changes were quite small, which suggests a virtually continuous inflow of both compounds throughout the day probably as a consequence of the fairly constant emissions of the plants over the 24 hours. The coincidence of a maximum for NO and NO_2 at 9.00 am suggests the influence of traffic emissions from the motorway which usually peak at this time together with a change in wind direction due to the effect of the thermal gradient between the land and sea surfaces in the

sea→land direction typical of that time [15]. The concentrations of both oxides decline after 4.00 pm, probably as a result of the opposite effect, the presence of a breeze in the land→sea direction in the absence of appreciable changes in the other factors.

The mean concentration, 32.3 ± 28.8 µg m^{-3}, was slightly higher than the values recommended in the AQG of WHO (30 µg m^{-3}), but lower than the threshold imposed by European legislation (40 µg m^{-3}). A concentration of more than 150 µg m^{-3} (a threshold that should never be exceeded for more than 24 h as per the European legislation) was measured for for seven days. To make things worse, four of the seven days coincided with the heavy desert dust episode of late January, which maintained a very high mean particulate concentration level in the Valley: 713,600 ng m^{-3}.

A comparison of the mean hourly concentrations of SO_2 with the wind direction on the days in question reveals the influence of the coastal pollution sources on the SO_2 levels in the Valley. In fact, although the prevailing winds were WNW winds (*viz*. from sector 4), the concentrations found were the result of the extreme concentrations recorded during the central hours of the day, when winds from sector 1 prevailed.

In order to clarify the relationships between pollutants and establish the influence of the wind direction on concentration levels, a principal factor analysis (PFA) with orthogonal Varimax rotation was performed on the data set (daily 24 hour averages of NO_x and SO_2 were selected for the analysis). One of the most important problems in PFA is the decision of the dimensionality of the model. The decision on how many factors should be retained is often subjective. But it is usually a “rule of thumb” to retain as many factors as there are Eigen values greater than unity. The results of the Varimax rotated factor analysis are shown in Table 3. The five factors together explain 70 % of the total variance.

Factor 1 accounts for 23 % of the total variance and has high loadings for Ca, Mg, Fe and TSP. Soil dust probably corresponds to this factor. The main Fe source is soil dust [16] and according to the literature [17] the most plausible source for Ca and Mg are calcite ($CaCO_3$) and dolomite ($CaCO_3 \cdot MgCO_3$) contained in soil dust. Factor 2 accounts for 19 % of the total variance and contains NO, NO_2, SO_2 and winds from sector 1. This result makes manifest the importance of local sources in this sector.

Factor 3 has high loading for Na and K and represents marine impacts. K enrichments relative to Na have previously been observed in marine aerosols [17]. Ni and Pb, which characterize factor 4, are probably the result of combustion processes. The majority of the total anthropogenic emissions of Pb in Europe comes from car exhausts and the Ni is a typical fossil fuel combustion product. Both elements are correlated to winds from sector 1.

TABLE 3 - Varimax rotated factor loading matrix.

Variable	Communality	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Na	0,837	0.050	- 0.025	0.890	0.038	0.119
K	0.747	0.127	- 0.132	0.839	- 0.021	- 0.090
Ca	0.883	0.921	0.038	0.094	0.003	- 0.017
Mg	0.703	0.814	0.023	0.185	- 0.051	0.046
Fe	0.859	0.906	- 0.020	- 0.020	0,128	- 0.072
Cu	0.300	0.390	0.064	- 0.193	0.074	- 0.173
Ni	0.739	0.165	- 0.018	- 0.191	0.743	- 0.060
Pb	0.529	0.119	0.014	0.065	0.509	- 0.021
TSP	0.878	0.921	0.121	0.041	0.080	0.046
NO	0.721	0.083	0.839	- 0.029	0.069	0.068
NO_2	0.630	0.048	0.769	- 0.143	0.106	- 0.031
SO_2	0.734	0.045	0.847	-0.009	0.052	0.106
Sector 1	0.786	- 0.153	0.525	0.089	0.757	0.015
Sector 2	0.929	- 0.004	0.000	0.011	- 0.213	0.930
Sector 3	0.848	0.116	- 0.027	-0.104	- 0.014	0.018
Sector 4	0.957	0.065	- 0.330	- 0.024	- 0.416	-0.749
Eigen value		3.611	2.811	1.721	1.507	1.144
% variation		23 %	19 %	11 %	10 %	7 %

CONCLUSIONS

In this work, the concentration levels of Na, K, Ca, Mg, Fe, Cu, Ni and Pb as well as benzo(a)pyrene in particulate matter in the vicinity of a thermal power station at Jinamar Valley were determined. The concentration levels of NO, NO_2 and SO_2 and the diurnal variations of NO, NO_2 were also established. NO exhibits a seasonal behaviour very similar to that of Ni and SO_2.

In order to identify the different sources for particles, a principal factor analysis (PFA) with orthogonal varimax rotation was performed on the data set. The results reveal the influence of anthropogenic sources, the power station, the desalination plant and the motorway, on the concentrations of NO, NO_2, SO_2, Ni and Pb. The natural sources are most likely to be the major Ca, Mg and Fe contributors, whereas sodium is associated primarily to sea breeze.

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FEB

EXPOSURE OF SCHOOL CHILDREN TO Pb AND Zn IN AN INDUSTRIALIZED CITY OF TURKEY

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SUMMARY

Blood lead and serum zinc concentrations of school children were determined in Bursa and in a small village nearby. The geometric means of Pb were 4.78 µg/dL, both for the village and city center. Geometric means of zinc were 78.8 µg/dL, and 71.6 µg/dL for the village and city center, respectively. About 8% of the total population had blood lead levels higher than 10 µg/dL, and 28% a serum zinc concentration less than 70 µg/dL, which is below reference concentration. Bivariate analysis indicated a relation between blood Pb, sex of the children and time spent outdoors.

KEYWORDS:
Blood level, serum zinc, pollution, school children.

INTRODUCTION

The potential adverse impact of metals, especially heavy metals, on human health around industrial plants and in areas with heavy traffic and industry has been recognized for a long time. Heavy metals enter in to human body primarily with food, but adsorption in the lungs may also be significant through inhalation [1]. Metals, such as lead and cadmium, are considered to be non-essential and can cause profound biochemical and neurological effects in the human body even at trace levels. Continuous exposure to these toxic heavy metals causes gradual accumulation of these metals in different tissues of the body [2].

There are many studies on the environmental lead exposure of children and and its epidemiology [3,4,5]. In spite of the numerous studies about Pb exposure, the frequency and effects of mild zinc deficiency in unexposed populations have not yet been adequately investigated.

In the present study, the exposure of school children to Pb and Zn was investigated in terms of concentration levels, causes and pathways of exposure. Two different groups of the children living in a big, industrialised city (Bursa) center and a nearby village (Gölyazi) were studied. Data were statistically treated in order to find possible correlations between these two groups and related physical and chemical parameters.

MATERIALS AND METHODS

Study protocol and sampling

Industrialization and activities of about 2 million people living in this area are the major causes of the air pollution observed in Bursa. Motor vehicle emissions also play an important role on air pollution as Bursa is located on the cross roads of east –west and north-south highways connecting eastern and southern cities to Istanbul. [6] Most vehicles still use leaded petroleum with concentrations of 0.15-0.40 g/L, which is the regulatory limit for Turkish Standards for leaded petroleum [7].The concentrations of Pb and Zn in Bursa were measured as 0.088 $\mu g/m^3$, 0.151 $\mu g/m^3$ and 0.29, 0.305 $\mu g/m^3$ in fine fraction (< 2.5 µm) aerosols in summer and winter respectively [8]. Although Pb and Zn concentrations in Bursa atmosphere are not exceeding health limits [9] they are substantially high.

An elementary school located in Bursa city center which is heavily loaded with traffic was chosen to investigate the effect of traffic and industry related pollutants on blood lead and zinc concentrations of children. As a second group, an elementary school in Gölyazi, a small village with 2250 inhabitants and 35 km distant from Bursa city center was chosen. The geographic location of the sampling region and locations of schools are shown in Fig. 1.

Blood samples from school children aged between 8-12 years were studied. Venous blood samples were col-

lected into blue-cap (EDTA containing) Vacutainer Tubes (Becton Dickonson) by the medical practitioners. Serum was carefully separated for zinc analyses immediately after collection. The remaining parts of the blood samples were stored at 5 °C prior to Pb analysis.

Reagents, cleaning the materials and sample preparation

Blood samples were prepared for Pb analysis according to the method described by Bannon et al. [10]. Calibration standards were prepared daily in polyethylene autosampler cups. Standard addition method was used to overcome matrix interferences. A 100 µl of whole blood was diluted tenfold with a matrix modifier solution of $NH_4H_2PO_4$ (2 g/L)+ HNO_3 (2 mL/L)+ Triton X-100 (0.5 mL/L) prepared in deionized water and analyzed against matrix – matched standards.

Analysis of the Samples

Blood Pb concentrations were measured by Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) in our laboratories. A Pye Unicam PU 9200 Atomic Absorption Spectrophotometer with a GF 90 electrothermal atomizer was used. Optimization of furnace parameters were achieved using spiked blood matrix rather than aqueous standard solution. Detection limit of the method (3s), sensitivity and precision were 0.29 ng/mL, 0.44 ng/mL and 4.9 % respectively. Since NISTs "lead in blood standard (SRM 955a)" was not commercially available during this study, the accuracy of the method was checked using Copepod Dried standard reference material of International Atomic Energy Agency (IAEA) standard. The concentration of lead (2.09 ± 0.03µg/g) was in good agreement with the certified concentration (2.10 ± 0.3 µg/g).

Serum zinc concentrations were measured in School of Medicine of Uludag University laboratories by using a Technicon RA-XT analyzer, which is basically a spectrophotometer. Zn complexes of 5-Br-PAPS {2-(5-Bromo-2-pyridylazo)-5-[n-propyl-N-(3-sulfopropyl)amino]phenol); 5-(N-propyl-N-sulfopropyl-amino)phenol disodium salt dihydrate} at pH 8.6 were measured at 560 nm. The interferences, due to oligoelements present in the samples were eliminated using specific masking agents.

RESULTS AND DISCUSSION

General characteristics of data and evaluation of Pb and Zn concentrations with questionnaire parameters

A total of 89 blood samples were collected from children. Out of 89 children, 60 were from the city center and 29 from the village. A questionnaire covering the questions about habits and living standards of children, was designed and applied to the families of the children. The statistical evaluation of the data was done using SG PLUS and SPSS Statistical Package Programs. The responses to the questionnaire and the measured concentrations were evaluated together to investigate the effect of air pollution on school children.

Overall 8 % of the total population revealed blood Pb levels greater than 10 µg/dL, which is the level of Pb recommended by Center for Disease Control [11]. Mean Pb for combined population was 5.37 µg/dL and most of the children had blood Pb concentrations around the average. Out of 89 children 26 (29%) had serum zinc levels lower than 69.9, which is the lower limit for serum zinc (69.9-149.7 µg/dL) [12].

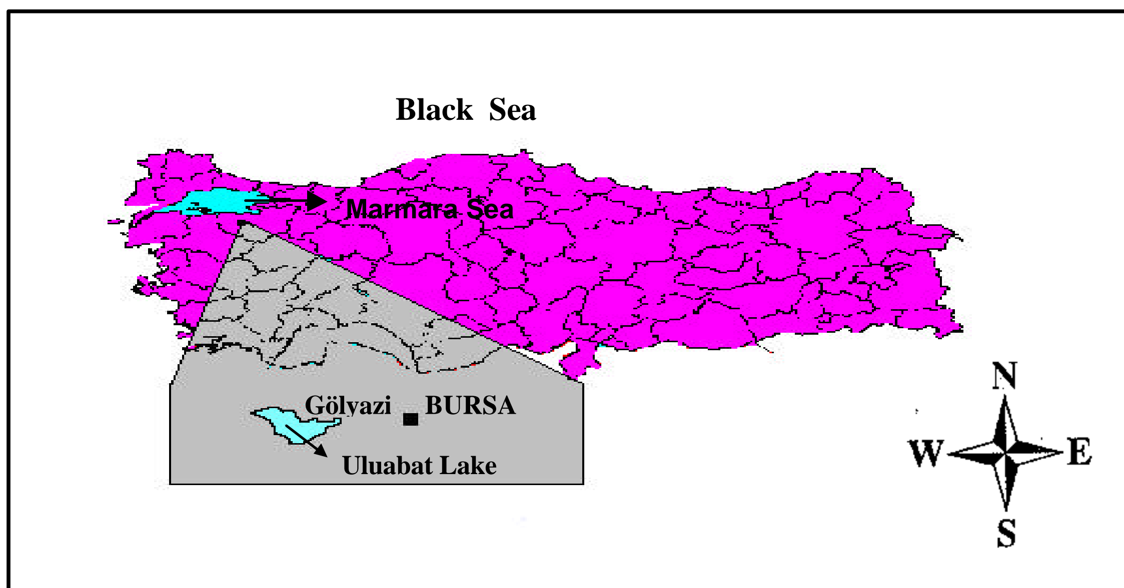
A city center and small village school were chosen with the idea that air pollution impact can be easily evaluated by comparing two different populations. However, results of analyses showed that they are exposed to almost the same Pb and Zn levels and even village population is worse. Since the village Gölyazi has minimal air pollution from motor vehicles, other factors may play a greater role on children's exposure. Therefore, data are subjected to other statistical tests in order to explain the behaviour of these metals for two socioeconomically different populations.

As a first step of statistical analyses, the distribution of concentrations was examined by using SGPLUS statistical package program. Distributions of Pb and Zn were tested by Chi-Square, Shapiro-Wilks , standardized skewness and standardized Kurtosis tests and found to be not Gaussian. The lowest p values were less than 0.01 for Pb and 0.05 for Zn distributions at 99% and 95 % confidence levels respectively.

TABLE 1 - Summary statistics for blood lead and serum zinc.

	Pb concentration (µg/dL)		Zn concentration (µg/dL)	
	Bursa city center	Gölyazi village	Bursa city center	Gölyazi village
Sample size	60	29	60	29
Average	5.12	5.61	72.2	79.9
Std. deviation	1.92	3.36	8.98	14.0
Minimum	1.43	1.52	57.3	57.2
Maximum	11.9	13.8	102	116
Geomean	4.78	4.78	71.6	78.8
Median	4.74	4.48	71.7	77.2

FIGURE 1 - Map of Bursa city.



In order to see the differences between the two data sets, basic statistics were calculated for both metals and listed in Table 1. As can be seen from Table 1, village Pb data have higher standard deviations.

Since blood lead levels do not show normal distribution, comparison of medians were prefered to investigate the difference between these two groups. According to Mann- Whitney (Wilconson) W test there was no statistically significant difference between the medians of blood lead of the two groups at the 95 % confidence level.

The Gölyazi village, which is 35 km far from city center, is located at the coast of the Uluabat lake. (Fig. 1). People living in the village make up rather a close society with similar income levels. Because of the insufficient agricultural land, major income for villagers is from fishing from Uluabat Lake, which shows a dramatic decrease in fish population as a result of pollution in the last few years. There are direct discharges of wastewaters from several industries including metal industry. It was, hence, thought that the major reason for high blood lead of village children was due to their diets, as fish is the most frequently consumed food in these families. Even though these children were living in relatively clean air environment they were exposed to the same level of Pb as children living in the center of a big industralized city. Unfortunately, the questionnaire responses of parents about the nutrition related questions were not in support of this hypothesis. But, when the data for Zn were examined it was observed that Zn concentrations measured in the blood serum of Gölyazi children were significantly higher than those living in the city center ($p=0.01$). This observation can also be explained by fish consumption, as zinc is also an element easily accumulated on the fish tissue. The discrepancy between our observations and the results of questionnaire indicated that the parents of the village children answered the questions incorrectly, which is expected considering low their educational level. However, because of the unreliable data in the village, meaningful results were not obtained from other statistical tests, too. That is why further statistical tests were applied to the data obtained from the children living in the city center of Bursa. As a first test, correlation analyses were performed and Spearman correlation coefficients were determined by SPSS package program. A strong correlation was observed between the sex of the children and their blood Pb concentrations ($r= 0.331$, $p= 0.01$).

The blood lead levels were significantly higher among boys compared to girls and may be related to differences in their activities ($p=0.011$). However, there was no difference in blood Pb and Zn and sex for the Gölyazi village children.

Reasonably good correlation was found between the blood lead and the time children spent outdoors ($r = 0.417$, $p=0.001$). As it can be seen from Fig. 2, boys spent more time outdoors than girls. So they are potentially more exposed to lead, an observation supported by the higher concentration of lead in boys compared to girls.

Another important parameter to be considered is the age of the exposed individuals, as young children are at greater risk to toxicity, because of higher lead intake relative to body size and high rate of absorption from the gastrointestinal tract [13]. Blood lead levels increase from about six months of age to four years of age and, thereaf-

ter, decline with age [14]. Therefore, age of a child is a strong predictor of increased blood lead levels. In this study no statistically significant correlation was observed between Pb concentration and age of the children. The mean lead levels for children of different ages are shown in Fig. 3. Highest concentration of Pb is observed for children aged 10, but comparable values also with ages 9 and 11. Approximately 20 % of these children had blood Pb levels between 3-6 μg/dL. On the other hand, the lowest concentrations are observed in the blood of 8 years old children.

FIGURE 2 - Blood lead vs. time spent outdoors.

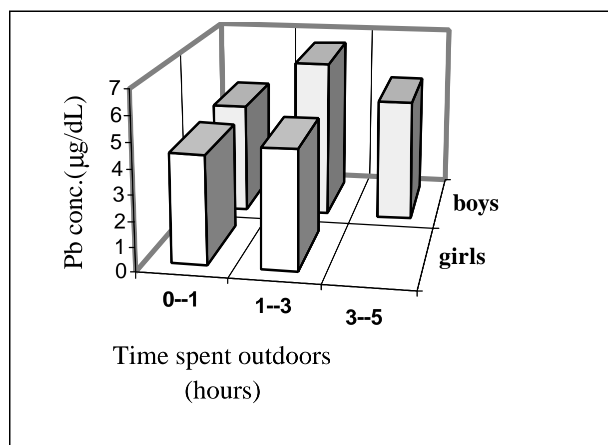
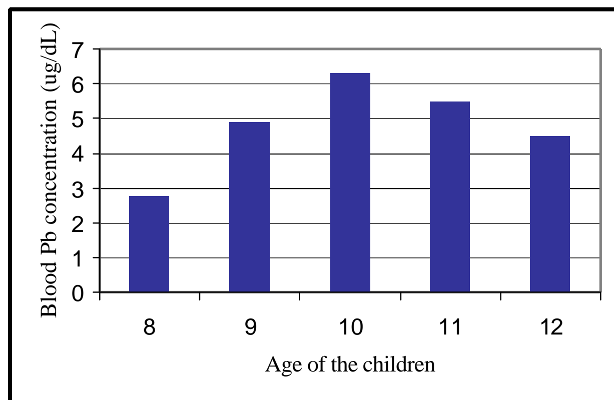


FIGURE 3 - Blood lead distribution with respect to age.



As the major aim of this study was to find out reasons for Pb exposure of children in their living environment, we looked at the relations of Pb concentrations with other environmental parameters. Possible sources of lead in the household and outdoors include lead paint, automotive and industrial lead emissions, and lead in food and water [15]. No correlation was observed between blood lead levels and the household environment of the children (type, age and paint of residential place) in both Gölyazi and Bursa. Actually the residential places in both Bursa and Gölyazi are not older than 30 years. The older buildings were generally destroyed and new buildings constructed, and it is known that, after regulations were set in the 1960's, Pb content of paints used decreased all over the country. Also no statistically significant relations were found between the following variables and blood lead level: eating nail, past occupational exposure to Pb of any parent, education level of parents, distance of the child's home from heavy traffic, drinking water supply, transportation of child to school. All the above-mentioned observations indicate that the time spent outdoors which, in turn, means exposure to air pollution, is the major reason for the observed blood Pb levels of children living in city center of Bursa.

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DECOLORIZATION OF METHYLENE BLUE BY WHITE ROT FUNGUS *Coriolus versicolor*

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SUMMARY

Decolorization of Methylene Blue in cultures of the white rot fungus, *Coriolus versicolor*, was demonstrated. *C. versicolor* was found to effectively decolorize media containing 5 and 10 mg L^{-1} methylene blue. The effects of various glucose (5.5 and 27.7 mM) and $NH_4H_2PO_4$ (4.34, 0.43 and 0.04 μM) concentrations on the decolorization of methylene blue by *C. versicolor* were investigated. Decolorizing was found higher in media where the C/N ratio was adjusted to 2:1. Maximum decolorizing activity was achieved during the secondary metabolic phase.

KEYWORDS:
Decolorization, *Coriolus versicolor*, Methylene blue.

INTRODUCTION

Dyes and pigments of 10000 different types are approximately produced annually world wide and used extensively in dyeing and printing industries. It is estimated that about 10% are lost in industrial effluent (1), which might be transferred to carcinogenic compounds under anaerobic conditions (2).

Ligninolytic fungi have been reported to degrade xenobiotic compounds such as azo, heterocyclic, reactive and polymeric dyes (3-7). Most of the research on dye decolorization by white rot fungi has been focused on *Phanerochaete chrysosporium* (3,5,8-11). On the other hand, there are few studies of dye decolorization by *Trametes versicolor* (12-14).

Biodegradation of dyestuff has been ascribed to extracellular oxidation by the non-specific, lignin-degrading enzymes, such as lignin peroxidase (LP), manganese peroxidase (MnP) and laccases. The ligninolytic enzymes of the white rot fungi are thought to be expressed during secondary metabolism (15) when carbon and/or nitrogen (N) becomes limiting (13).

In this paper, the effects of carbon and/or nitrogen (N) concentrations on the decolorization of methylene blue were investigated. This dye, which is highly soluble and hence represents a high-risk contaminant of ground water, is degraded by *Coriolus versicolor* (16).

MATERIALS AND METHODS

Organism and Chemicals

The culture of Coriolus versicolor was obtained from Inonu University, Department of Biology, Turkey. It was maintained on Sabouraud dextrose agar (SDA, Oxoid) slants and stored at 4°C. Dyestuffs, Azure A (CI.52005), Azure B (CI.52010), Azure C (CI.52002) and Thionine (CI.52000) were obtained from Sigma. Methylene blue (CI.52015) and all the other chemicals were obtained from Merck.

Culture Conditions

C. versicolor was grown on SDA plates at 30°C. Two 1 cm^2 discs cut from the growing zone of the fungus on a SDA plate were transferred to stock medium containing 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L $CaCl_2.2H_2O$, 20.0 g/L KH_2PO_4, 27.7 mM glucose and 4.34 mM $NH_4H_2PO_4$ in pH 5.0 phosphate buffer. The stock medium was homogenized by a homogeniser (9000 rpm, 5 sec) at the end of seven days of incubation at 30°C. Each of different types of medium (Table 1) was inoculated with 1 mL micelial suspension (approx. 28 mg/30 mL dry weight) and then incubated under static conditions at 30°C±1. The pH of each medium was adjusted to 5.0 with phosphate buffer. Controls lacking inoculum were also incubated under the same conditions.

TABLE 1 - Composition of the Culture Media

Medium	Methylene Blue (mg L^{-1})	Glucose (mM)	$NH_4(H_2PO_4)$ (μM)	C / N Ratio (w / w)
I	5.0	27.7	0.0434	1000:1
II	5.0	27.7	0.4340	100:1
III	5.0	27.7	4.3400	10:1
IV	5.0	5.5	4.3400	2:1
V	10.0	5.5	4.3400	2:1
VI	10.0	27.7	4.3400	10:1

(All media consisted of $MgSO_4.7H_2O$ 0.5 g/L, $CaCl.2H_2O$ 0.5 g/L, 20,0 g/L KH_2PO_4)

Assays

The decolorization rate of the dye was measured spectrophotometrically (Shimadzu UV visible 160-A) at 664.0 nm of spectral maxima together with all shifts in the visible range. The methylene blue concentration was calculated from the calibration curve (17).

The dry weight of the fungal mass was obtained by filtering the contents of each flask (30 mL) through pre-weighed Whatmann no: 1 filter paper and then drying to a constant weight at 70°C. Fungal dry weight was expressed as g of biomass per 30 mL of culture (18).

The amount of the total sugar in the medium was estimated using the Anthrone method (19).

Metabolite formation was examined by thin-layer chromatography (TLC) according to Kling and Neto (20).

RESULTS AND DISCUSSION

The fungi consume and grow on readily available carbon sources. The most readily used one by white rot fungi is glucose. Decolorization ability of *Coriolus (Trametes) versicolor* was increased when glucose was used as a sole carbon source. Kapdan et. al. (12) reported that glucose is the most effective carbon source for the decolorization of Everzol Turquoise Blue G when compared to fructose, starch and molasses. Also, in most researches glucose was used for the decolorization of different chemicals, which are hazardous to the environment (6, 8, 21-22).

Different glucose concentrations were used (5.5 and 27.7 mM) to find the optimum for the decolorization of methylene blue by *Coriolus versicolor*. 5 mg L^{-1} methylene blue was decolorized up to 98% in 8 days incubated cultures (medium IV). Similar result was obtained when 10 mg L^{-1} dye was present (Medium V, up to 93%). The decolorization activity of fungus was 89% and 52% respectively, in Medium III and VI with the glucose concentration adjusted to 27.7 mM (Fig. 1).

The glucose consumption rate was higher during the first 4-6 days of incubation than that of 6-12 days. Fungal mass increased tenfold as compared to its initial weight during high glucose consumption period and then showed no further significant increase. These results suggested that secondary metabolic phase started at the end of the 4-6 days of incubation.

The most widely used nitrogen sources for the fungal decolorization of the dyestuffs are ammonium salts (9, 10, 12, 23). Three different concentrations of $NH_4H_2PO_4$ (Medium I, II, III) were used to find the effect of the nitrogen concentration on the decolorization rate of the dye. In all of the media, decolorization up to 97% was achieved at the end of 12 days of incubation. However, different decolorization values of methylene blue were observed at the end of 8 days of incubation. At the end of 8 days, approximately 96 % and 89 % decolorization were achieved in medium II and III, respectively. Medium II contained tenfold less $NH_4H_2PO_4$ concentration than medium III. On the other hand, a 57% decolorization was achieved in medium I at the end of 8 days of incubation, which contained tenfold less $NH_4H_2PO_4$ than medium II. This low decolorization suggested that this concentration of nitrogen might be insufficient. These results showed that, not only glucose concentration but also nitrogen concentration affects the decolorization of methylene blue. This is in line with the reports, claiming that the carbon and nitrogen sources affected the decolorization of Orange II (23). However, the decolorization of xenobiotic compounds (25), including Crystal violet (9), by *Phanerochaete chrysosporium* is known to occur under non-ligninolytic conditions, suggesting that, in addition to the normal ligninase, another mechanism may exist for dye decolorization. In another study, it was reported that hydrolyzed Reactive Violet 5 was decolorized 95 % after 6 days by *B. adusta* and *T. versicolor* (26) when C/N (w/w) ratio was adjusted to 2:1. In our study, the decolorizing activity of fungus increased as the C/N (w/w) ratio reached 2:1. The decolorizing activity was found higher in medium IV and V (98 and 93%, respectively) in which the C/N (w/w) ratio was adjusted to 2:1 as against that in medium III and VI with 10:1 C/N (w/w) ratio.

FIGURE 1 - Effect of media composition on methylene blue decolorization by *Coriolus versicolor* (Control: without inoculum, FDW: fungus dry weight)

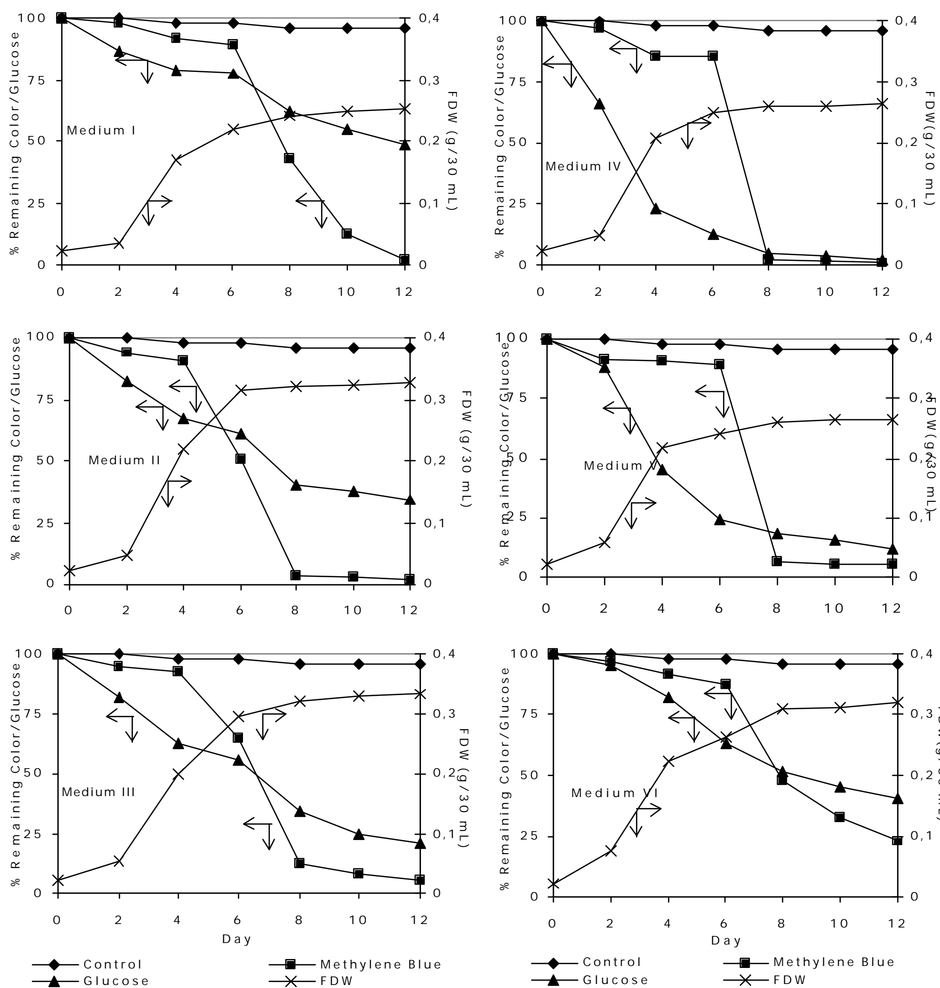

FIGURE 2 - Changes in absorption spectrum of methylene blue during the incubation period.

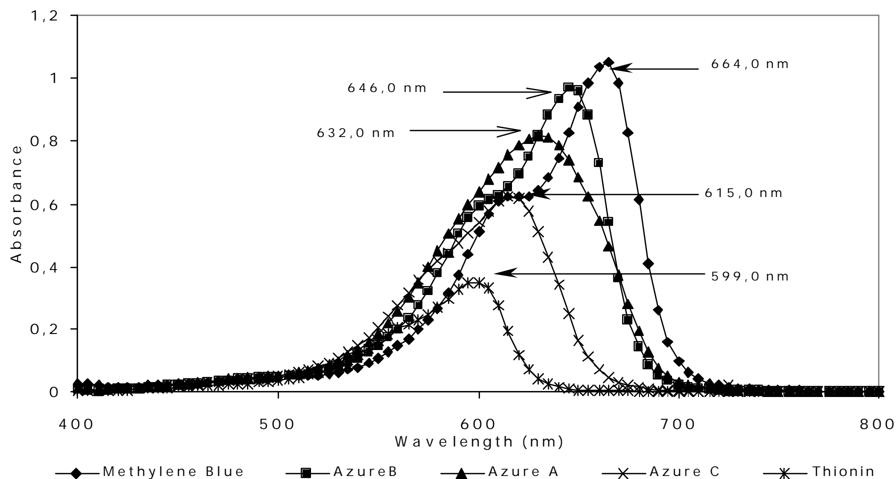

TABLE 2
The maximum wavelength and R_f values* of methylene blue and decolorization products of methylene blue.

Dyes	Standards		Decolorization Products	
	R_f	λ max	R_f	λ max
Methylene Blue	0,39	664,0	-	-
Azure B	0,50	647,0	0,50	646,0
Azure A	0,59	631,5	0,59	632,0
Azure C	0,64	616,0	0,62	615,0
Thionine	0,70	599,0	0,70	599,0

(*A solvent system of ethanol: HCl (99:1 v/v) was used (20).)

The temporal effects of medium composition on the decolorization of methylene blue cultures of *C. versicolor* are shown in Fig 1. Decolorization started after an initial lag period of approximately 2 days and then continued through the 12 days of incubation period in all of the experiments. The fungal dry weight (FDW) increased for the first 6 days during the primary metabolic phase and then, entering the secondary metabolic phase, remained constant throughout. Methylene blue decolorization reached a peak at about the 8th day of incubation. At the same time, the absorbance maximum underwent a hypsochromic shift from 664.0 to 559.0 nm (Fig. 2) and the color of media changed from blue to purple. Some of the dye appeared to be bound to the mycelium of *C. versicolor*. However, even this material was decolorized at the end of the incubation period. No color removal was observed in the control flasks lacking the fungus.

The shift in absorption maxima of methylene blue during decolorization to that of the dyes Azure B, Azure C, Azure A and Thionine, suggested that the decolorization products of this dye might resemble these mentioned dyes. Therefore, the cultures of *C. versicolor* incubated with methylene blue were analyzed by TLC, whenever the hypsochromic shift was detected by the spectrophotometer. TLC analysis showed that four metabolites were formed in media during decolorization (Table 2). These metabolites were determined as Azure B, Azure C, Azure A and Thionine. All of these metabolites were also decolorized at the end of the incubation period like methylene blue.

The decolorization of methylene blue in crude extracellular extract of the white rot fungus *Phanerochaete chrysosporium* was demonstrated by changes of the visible spectra of absorption and final product of decolorization was identified as Azure C by TLC analysis (20). Cripps et al. (10) reported that an LP-containing supernatant from a *Phanerochaete chrysosporium* culture contained four colored degradation products of Azure B. *T. versicolor* LP-Azure B reaction showed that the absorption maxima shifted from 647.0 to 617.0 nm. It was also reported that both Azure A and Azure C dyes underwent similar hypsochromic shifts in response to LP oxidation (27).

CONCLUSION

The effects of various C and/ or N concentrations on the decolorization of methylene blue by *C. versicolor* were investigated. Decolorizing was found higher in medium IV and V with a C/N ratio of 2:1. Decolorizing activity was maximum in secondary metabolism.

Methylene blue was decolorized by *C. versicolor*. It was shown that, upon decolorization four metabolites were formed and appeared in the media that were detected by both spectrophotometer and TLC analysis. These metabolites were found to be Azure A, Azure B, Azure C and Thionine, respectively.

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THE EFFECTS OF HEAVY METAL IONS ON THE GROWTH OF *Rhizopus arrhizus*

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SUMMARY

In this study, the effect of heavy metal ions on the biomass increase of *Rhizopus arrhizus* was investigated as a function of pH, temperature, metal ion concentration and initial sucrose concentration. The optimum pH values were found as 5.0, 3.5, 4.0, 4.5 and optimum temperatures as 30, 30, 25, 30 ^oC for control, cadmium(II), lead(II) and copper(II) ions, respectively. The biomass concentration increased with increasing initial sucrose concentration up to 30 mg L^{-1} and decreased with increasing metal ion concentration. The adsorbed cadmium(II), lead(II) and copper(II) amounts at the end of growth period of *R. arrhizus* were found as 8.7, 14.4 and 11.2 mg g $cell^{-1}$, respectively, for 50 mg L^{-1} initial metal ion concentration.

KEYWORDS:
Biosorption, heavy metal ions , *Rhizopus arrhizus*, cell growth.

INTRODUCTION

Many microorganisms can take up dissolved heavy metal ions from their surroundings. Adsorption or accumulation of heavy metals by microorganisms have received much attention recently due to their potential use in waste treatment processes involving removal of heavy metal pollutants from a contaminated environment. Typical biological processes utilize viable cells to degrade or remove toxic substances [1].

Many aquatic microorganisms, including bacteria, yeast and algae, can adsorb dissolved heavy metals and radioactive elements from their surroundings, and may, hence, be used to remediate wastewater contaminated with heavy metals.

Biological treatments are feasible alternatives to chemical methods, since a wide range of microorganisms metabolize such chemicals. Biosorption is generally used for the treatment of heavy metal pollutants in wastewaters, but may also be used for wastewaters containing metal ions. Biosorption in natural or uncontrolled situations typically involves a combination of active and passive transport mechanisms starting with the diffusion of the metal ion to the surface of the microbial cell [2]. Once the metal ion has diffused to the cell surface, it binds to the sites on the cell surface exhibiting some chemical affinity for the metal. Biosorption is often followed by a slower metal binding process, often irreversibly, when an additional metal ion is bound [1,2,3].

The scientific literature reveals two distinct approaches to these problems: use of living organisms and use of a non-viable biomass. There are significant practical limitations to the methods that employ living microbial systems. Perhaps the most significant limitation is that microbial growth is inhibited, when the metal ion concentration is too high or significant amounts of metal ions are adsorbed by the microorganisms [3].

The filamentous fungus *R. arrhizus* is effective in the removal of heavy metals. The cell wall of *R. arrhizus* essentially consists of various organic compounds including chitin, acidic polysaccharides, lipids, amino acids and other cellular components. The ability of chitin to complex metal ions has been confirmed in the literature [4]. The fungus *R.arrhizus* has been shown to have high uptake capacities for variety of common metal ions, and the metal uptake characteristics of free *R.arrhizus* biomass, both living and dead, are well documented [5,6].

Metal uptake by microbial cells is moderated by a number of factors, such as the chemical and physical properties of the metals, cellular physiology, and the ambient conditions, pH and temperature [2,6,7]. In addition, metabolism-dependent uptake of metal ions can be influenced by the presence of competing metal cations or lack of an avaliable energy source [7]. Therefore, the objective of this study was to investigate the effects of cadmium (II), lead (II) and copper (II) ions on the growth of the *Rhizopus arrhizus* cells and their adsorption to growing cells.

MATERIALS AND METHODS

Growth of microorganism: *Rhizopus arrhizus* was grown in a shaker incubator at 30 °C and 150 rpm. The liquid medium used for growth contained 2.0 g yeast extract, 30.0 g sucrose, 0.2 g $MgSO_4.7H_2O$, 0.5 g K_2HPO_4 and 0.5 g KH_2PO_4 per liter of distilled water. The pH of the medium was adjusted to 5.0 with 1 mol L^{-1} HNO_3 and NaOH.

Determination of biomass: The amount of *R. arrhizus* growth was determined by weighing .

Preparation of heavy metal solutions: 1.0 g L^{-1} stock solutions of cadmium (II), lead (II) and copper (II) were prepared by dissolving weighed quantities of $CdCl_2.3H_2O$, Pb $(NO_3)_2$ and Cu $(NO_3)_2$, respectively, and then diluted to the desired concentration. The pH of cadmium solutions was adjusted with 1 mol L^{-1} HCl and NaOH and that of lead and copper solutions with 1 mol L^{-1} HNO_3 and NaOH.

Desorption: Following the metal sorption batch experiment, the biomass loaded with metal ions was separated by filtration and returned into 250 mL Erlenmeyer flasks containig 100 mL of the corresponding eluent solution (0.5 M HNO_3 for lead(II) and copper(II) ions and 0.5 M HCl for cadmium(II) ions). The desorption was carried out on a shaker (30 °C, 150 rpm) for 2 hrs or longer to let equilibrium established. The concentrations of the metal ions released into the solution were determined by an atomic absorption spectrophotometer, UNICAM 929 model.

Analysis of heavy metal ions: The residual concentrations of cadmium (II), lead (II) and copper(II) in the growth and biosorption media were also determined by AAS analysis.

RESULTS AND DISCUSSION

Effect of the environmental conditions on *R. arrhizus* growth

The mean cell size and size distribution of a population of microbial cells are known to be influenced by the growth phase of the culture and the environment in which the cells are growing [8]. The initial pH of the growth medium is an important parameter affecting the microbial growth and the metal uptake process. *R. arrhizus* cells were grown at different initial pH values. The biomass concentrations at the end of growth period were plotted against the initial pH (Figure 1). As seen in Figure 1, the maximum growth of fungal cells was observed in the control medium (the medium without metal ions) at pH 5. At 50 mg L^{-1} initial metal ion concentration (for cadmium (II), lead (II) and copper (II) ions each), the optimum initial pH values of *R. arrhizus* cell growth for cadmium (II), lead (II) and copper (II) were found to be 3.5, 4.0 and 4.5, respectively. At low metal ion concentrations (2.5-20 mg L^{-1}), the optimum pH values for these metal ions were similar to that obtained at 50 mg L^{-1} initial metal concentration [9].

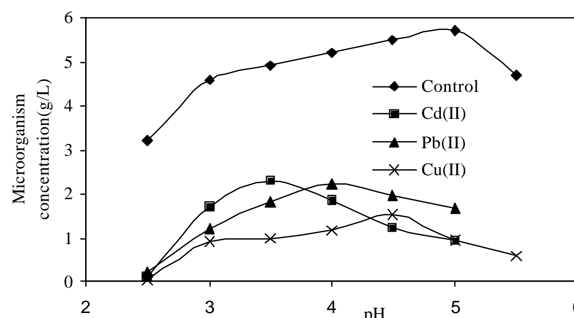

FIGURE 1 - The effect of initial pH on the *R. arrhizus* growth (S_o 30 g L^{-1}; C_o 50 mg L^{-1};agitation rate 150 rpm; temperature 30 °C, for Cd(II) and Cu(II) ions; temperature 25 °C for Pb(II) ions)

The temperature related effects do not appear to be particularly pronounced over the range investigated (20-40 °C). Figure 2 shows the final biomass concentrations plotted against temperature. Optimum temperature values for microbial growth in the presence of cadmium (II), lead (II) and copper (II) ions were found to be 30, 25, 30 °C, respectively. At low temperatures lower biomass concentration were attained. The metabolic yields of magnesium, potassium, phosphourous and carbon decrease at lower temperatures resulting in reduction of their energy yields, which, in turn, results in lower biomass concentration. Structural denaturation caused by increase of toxic wastes in growth medium at high temperatures results in early cell division, which might explain the decrease in biomass concentration [10].

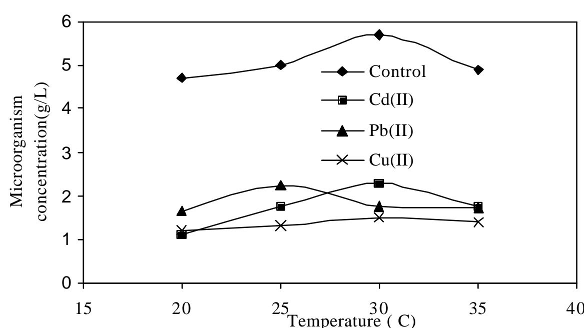

FIGURE 2 - The effect of temperature on the *R. arrhizus* growth (S_o 30 g L^{-1}; Co 50 mg L^{-1}; agitation rate 150 rpm; pH 3.5 for Cd(II) ions, pH 4.0 for Pb(II) ions; pH 4.5 for Cu(II) ions)

Figure 3 shows the variation of the final biomass concentration with sucrose concentration for control, cadmium(II), lead(II) and copper(II) ions. The biomass concentration increased with increasing initial sucrose concen-

tration (S_o) up to 30 g L^{-1} and then did not change further, because of the beginning inhibition effect of sucrose. The biomass concentrations for the control medium, cadmium(II), lead(II) and copper(II) ions (at 50 mg L^{-1} initial metal ion concentration) were found to be 5.7, 2.3, 2.23 and 1.53 g L^{-1}, respectively, at 30 g L^{-1} of sucrose concentration. The extent of inhibition of the metal ions on growth depends on the type of the heavy metal ion (Figure 3).

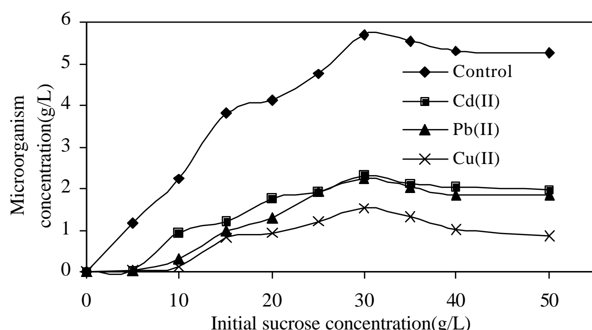

FIGURE 3 - The effect of the initial sucrose concentration on *R. arrhizus* growth (C_o 50 mg L^{-1}; agitation rate 150 rpm; the optimum pH and temperature values for Cd(II) and Pb(II) and Cu(II) ions)

Metal ions present in the growth medium affected the biomass increase of *R. arrhizus* (Figure 4). When grown in the control medium lacking any metal ion, almost no lag phase was observed. The cell concentration reached its maximum value 72 hours after inoculation. On the other hand, metal ions significantly reduced the biomass increase of the cells. For all of the ions, after a lag phase of 20 hours, cells entered the logarithmic growth phase, after which a stationary phase was observed 52 hours after inoculation. The reverse effect of copper(II) ions was more pronounced over that of cadmium(II) and lead(II) ions. It was observed that the growth of fungal cell was decreased with increasing metal ion concentration because of their toxicities [9].

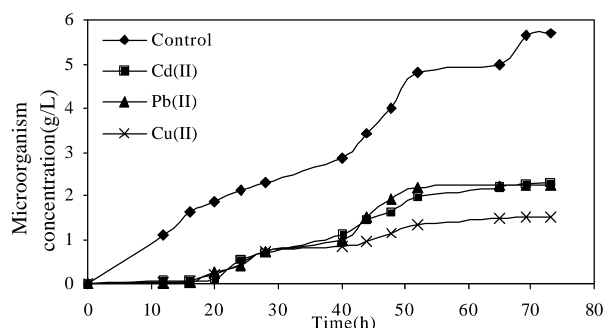

FIGURE 4 - Effect of the initial metal ion concentration (S_o 30 g L^{-1}; C_o 50 mg L^{-1}; agitation rate 150 rpm; pH 3.5 for Cd(II) ions, temperature 30 °C; pH 4.0 for Pb(II) ions, temperature 25 °C; pH 4.5 for Cu(II) ions, temperature 30 °C)

Adsorption of the heavy metal ions to growing *R. arrhizus* cells:

The initial metal ion concentrations played a major role for the growth and metal ion uptake properties of fungal *R. arrhizus.* Figure 5 shows cadmium(II) uptake and desorption during the growth period of *R. arrhizus* cells. The cadmium(II) ion uptake started during the lag phase and then increased throughout the logarithmic phase. This increase was due to the increase of the biomass concentration in the growth medium. Although cadmium(II) ions inhibited the *R. arrhizus* cell growth at the same time, an increase in uptake was observed. This might be due to the possible contribution of the metal ion uptake by inactive cells. In fact, previously it has been demonstrated that the dead cells accumulated heavy metals to the same or greater extent than living cells [11-13]. The adsorbed cadmium(II) ion concentration was determined to be 20 mg L^{-1}, while the desorbed cadmium(II) ion concentration was 17.5 mg L^{-1} at the end of the growth period. The non-desorbed cadmium(II) ions might be chemically bound by the fungal cells.

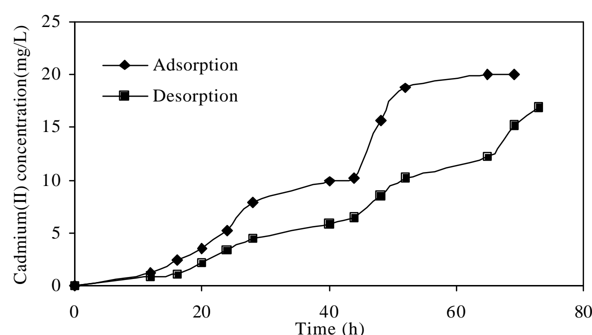

FIGURE 5 - Adsorbed and desorbed cadmium(II) ion concentrations during the *R. arrhizus* growth period (S_o 30 g L^{-1}; C_o 50 mg L^{-1}; agitation rate 150 rpm; pH 3.5, temperature 30 °C)

The adsorbed lead(II) ion concentrations during the growth period are presented in Figure 6. An initial lag period with relatively low lead(II) uptake was followed by a sharp increase in lead(II) binding capacity up to a level of 25-30 mg L^{-1}. Metal binding reached a peak at the end of the growth period. The adsorbed and desorbed lead(II) ion concentrations at the end of the growth period were found to be 32 and 27.5 mg L^{-1}, respectively.

Figure 7 shows the adsorbed and desorbed copper(II) ion concentrations during the growth of active *R. arrhizus* cells. At 50 mg L^{-1} copper(II) ion concentration, the adsorbed and desorbed metal ion concentrations were found to be 17 and 13 mg L^{-1}, respectively. The difference between adsorbed and desorbed copper(II) ion concentrations could be attributed to the metabolic activities of *R. arrhizus* cells.

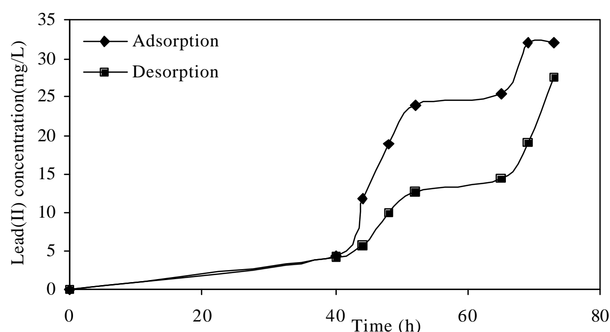

FIGURE 6 - Adsorbed and desorbed lead(ll) ion concentrations during the *R. arrhizus* growth period (S_o 30 g L^{-1}; C_o 50 mg L^{-1}; agitation rate 150 rpm; pH 4.0; temperature 25 ºC)

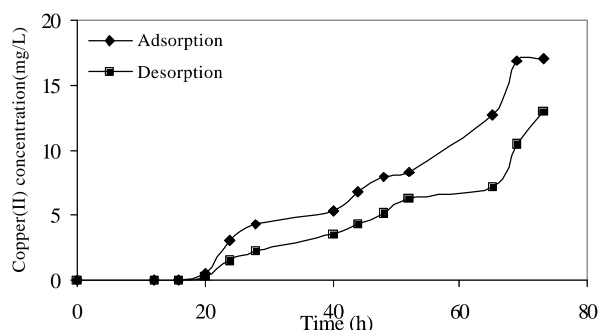

FIGURE 7 - Adsorbed and desorbed copper(ll) ion concentrations during the *R. arrhizus* growth period (S_o 30 g L^{-1}; C_o 50 mg L^{-1}; agitation rate 150 rpm , pH 4.5; temperature 30 ºC)

The amounts of adsorbed metal ions per unit weight of biomass were calculated as 8.7, 14.4 and 11.2 mg g $cell^{-1}$ for cadmium(II), lead(II) and copper(II), respectively (Figs. 3, 5, 6 and 7). As a conclusion, the active *R. arrhizus* cells exhibited a somewhat higher selectivity for lead(II) ions when compared to cadmium(II) and copper(II) ions.

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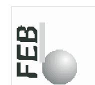

1st International Forum ANALYTICS and ANALYSTS 2 – 6 June 2003, Voronezh - Russia

Russian Academy of Sciences, Scientific Committee on Analytical Chemistry of RAS,Russian Chemical Society named after D. I. Mendeleev with participation of Polish Chemical Society and Czech Chemical Society, Russian Federation Ministry of Education, Russian Federation Ministry of Industry, Science and Technology, Administration of Voronezh Region, Voronezh State Technological Academy, International Educational and Cultural Services, Inc. (USA).

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Discussion of new ideas and projects in educational and computer technologies, perspectives, and general trends.

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   5b) medicine (biochemistry, pharmacology, including veterinary medicine),
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   5e) hydrochemistry, geochemistry, petrochemistry, coke chemistry, dendrochemistry,
   5f) chemistry of building materials, household chemistry,
   5g) criminology, judicial chemistry
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Dr. A.H. Heineken Prize for Environmental Sciences 2002

The Royal Netherlands Academy of Arts and Sciences has awarded the Dr A.H. Heineken Prize for Environmental Sciences 2002 (USD 150,000) to **Professor Lonnie G. Thompson**, Byrd Polar Research Center, Ohio State University, United States for his pioneering work in research into ice cores in the polar regions and the tropics.

THE SUBJECT

Lonnie Thompson is convinced that ice forms the best archive of the earth's climate. And that frozen history is located not only at the North and South Poles, but also in the tropics – for example at the peaks of Mt. Kilimanjaro, where the ice caps are in fact melting rapidly. Thompson was one of the first to realise that global warming poses a threat to a number of the world's ice archives. Partly because of this, gathering data is high on his list of priorities. He has often moved heaven and earth to gain permission to work with his drilling team in a particular location. Under the most extreme conditions, at altitudes where even mountaineers can barely survive, he has succeeded in collecting ice cores. His ice samples come from all over the world: from Bolivia, Peru, China and a host of other locations. The freezers in his laboratory, where Thompson analyses the ice, are now full to overflowing.

The information on the climate and the atmosphere which is stored in the ice can go back 700,000 years. The ice contains a clear record of phenomena such as El Niño and the Asian Monsoon, for example; in a somewhat similar way to tree rings, except that the ice history goes much further back in time and contains much more information. Thompson's research provides an insight into natural climate change, ultimately making it possible to assess the effects of human beings on the earth's climate, something which has been a source of heated debate among researchers for many years.

Lonnie Thompson was born in 1948 in Huntington, West Virginia and graduated in geology in 1973 from Ohio State University, to which he has remained attached since then. He obtained his doctorate there in 1976 (based on research into micro-particles in ice and the climate), and in 1994 he became a professor in the Department of Geological Sciences. Thompson is also closely involved in the research of the Byrd Polar Research Center at his university. He is very productive, and the results of his research regularly appear in the journals *Nature and Science.*

Thompson also sits on a number of advisory bodies in the field of the climate; he is a member of the editorial team of several journals, a member of a number of international partnerships, and leads one or more research expeditions every year. In 2001 *Time Magazine* and CNN added his name to the list of 'America's Best in Science and Medicine'. Thompson also works hard to ensure that the findings of his research are brought to the attention of politicians and the public at large.

More information can be found at the website of The Ice Group and via the website of *Times Magazine* and CNN on America's Best in Science and Medicine.

FURTHER READING

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THE PRIZE

The Dr A.H. Heineken Prize for Environmental Sciences has been awarded every two years since 1990. Previous Prize-winners have included James Lovelock, Colin Bibby (from the organisation Birdlife International) and Paul Ehrlich.

THE AWARDS CEREMONY

The Heineken Prizes are presented every two years during a special session of the Academy. This year's awards ceremony will take place on Tuesday, 24 September 2002 in the Beurs van Berlage building in Amsterdam.

GEORGE KVESITADZE

Main scientific directions of the Durmishidze Institute of Biochemistry and Biotechnology, Tbilisi, Georgia 150 co-workers, 12 professors, 33 Ph. D.:

- Investigation of plant genome, plant genetic engineering,
- Isolation, characterization and biological activity of natural compounds of Caucasian flora,
- Investigation of plants and microorganisms detoxificational potential directed to the metabolical utilization of xenobiotics,
- Investigation of structure and regulatory functions of enzymes of carbohydrates, nitrogen and energy metabolism and oxidative enzymes participating in degradation of xenobiotics
- Secondary metabolites of plant and microbial origin.

On May 30th, 2002 George Kvesitadze Ph. D., Dr. Sc., Professor of Tbilisi State University and Georgian Technical University, Director of the Durmishidze Institute of Biochemistry and Biotechnology, member of Academy of Sciences of Georgia celebrates his 60th birthday.

A postgraduate of Georgian Agricultural University, he defended in 1969 and in 1980 his candidate and doctoral thesis at the Bach Institute of Biochemistry, Russian Academy of Sciences. The themes of the theses were related to syntheses, isolation and characterization of amylolitic enzymes of microscopic fungi. He received mutant strains- active producers of enzymes and investigated in great details the physical-chemical and technological characteristics of regular, acid-stable and heat-stable amylases.

In 1973, the method of purification of these enzymes developed by G. Kvesitadze was patented in the USA and in 1974 in Germany and Switzerland.

Since the middle 70's in the lab headed by G. Kvesitadze the investigation of stable enzymes produced by extremophilic microorganisms had a target direction. Quite a few industrially important mutant and fusant strains have been received and investigated the specificities of heat-, acid- and alkali-stable enzymes. Based on the analysis of literary data and own experimental results, he supposed the existence of some unification in stable enzymes' characteristics depending on the degree of extremophilicity of microorganisms primarily expressed in the weak manifestation of allosteric and regulatory properties characteristic of their mezophilic analogues.

G. Kvesitadze was a visiting scientist at the University of Pennsylvania (USA) in 1977-1978, working with the enzymes of carbohydrates in the group of professor K. Pay. He was the only reporter in 1986 at the 42nd reading, devoted in memory of Professor Bach (Bakhovskye Chtenia) in Moscow, by presenting the lecture: "Enzymes of microorganisms, living in extreme conditions".

Close relationship with the world-known Ecological Chemist Professor F. Korte and Professor S. Durmishidze, a pioneer in plant xenobiochemistry, in the middle 80's favored to start investigations in evaluation of detoxificational potential of plants and microorganisms of different taxonomic groups. In this traditional field the contribution of Professor G. Kvesitadze for his institute consists in acquiring technological direction to further investigation. Based on the detoxificational abilities of plants and microorganisms, together with his experienced co-workers and colleagues he is on the way to complete new ecological concept. Nowadays, he is leader of three international ecobiotechnological projects supported by INTAS and ISTC.

G. Kvesitadze is the author of more than 250 publications, 4 international and 42 former USSR patents and 7 books. He is associated member of NATO life science and technologies Panel; expert of Eurounion in the field of Biotechnology; member of the Editorial boards of three international journals: 'Fresenius Environmental Eulletin', 'Ecotoxicology and Environmental Safety' and 'Journal of Biological Physics and Chemistry', two Journals of Russian Academy of Sciences: 'Biochemistry', 'Applied Biochemistry and Microbiology'.

In 1985-1987 Professor G. Kvesitadze was Deputy Chairman of the Science and Technology Department and in 1992-1994 - Minister of Agriculture and Food Processing of Georgia.

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