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ON THE SEASONAL DEPENDENCE OF THE AIR POLLUTION IN THE CITY OF VOLOS, GREECE

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SUMMARY

In Volos, a city of average size on the western seaboard of Central Greece, from 1987 up to the end of 1994, the concentration values of a series of air pollutants, namely, CO, O_3, NO_2, NO and SO_2, were recorded. The paper describes the climatic and geographical characteristics of the area, which has characteristics representative of the urban and suburban environment in many Greek cities, and examines the statistical attributes of the corresponding time-series with particular attention being paid to those attributes, which are related to seasonality.

KEYWORDS:
Air Pollution, Air Pollutants, Urban Environment, Volos

INTRODUCTION

In Greece, during the last few decades, records reveal that problems with air pollution are all the more intense. They appear more frequently in urban centres and in areas with elevated productive activity with factories and industrial zones, as well as in areas with transportation, such as junctions, harbours etc. In particular, in the atmosphere of the big urban centres of the country and especially in the capital city of Athens, elevated concentrations of air pollutants are frequently encountered, resulting in the appearance of serious disturbances and dangers for a large proportion of the population (1).

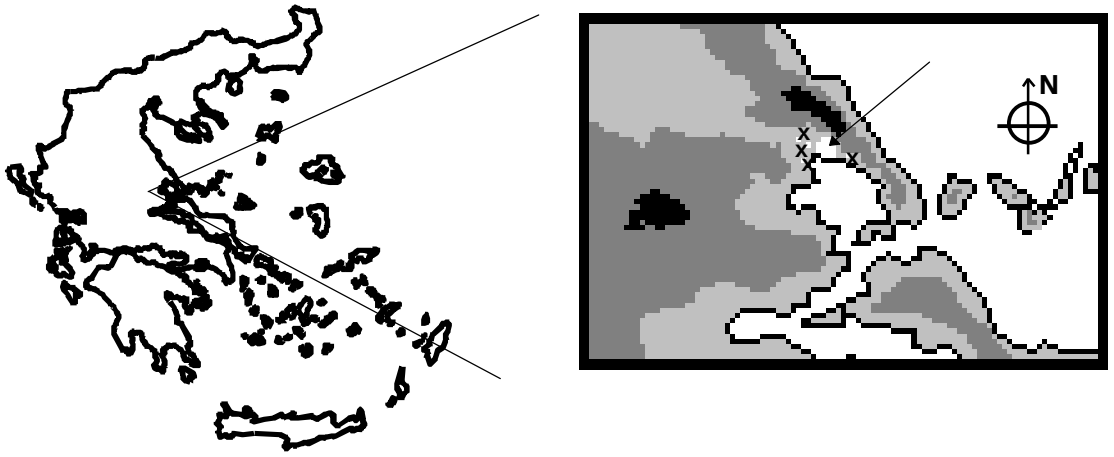
Volos is an average-sized city with a population of approximately 110.000 inhabitants (according to the 1991 census) on the eastern seaboard of Central Greece. The area, within the framework of the general development that has been evident during the last few decades, is already host to a significant number of both large and small industrial units, in addition to being a busy harbour. It also shows strong commercial activity as well as increased traffic and transportation. The aggravation of production activities in combination with deficiencies in land planning and the chaotic urban development, that characterises the area to a large extent, has contributed during the last few decades to the formation of a framework, which by its very nature implies the emergence of environmental problems. The consequences of this framework on air quality has been the subject of several studies that have been based mainly on records from the Air Pollution Monitoring Station, located in the centre of the city, and on those of the local Meteorological Station (2-6). Based on the conclusions of these studies a preliminary picture concerning the sources and the conditions in the area, which create air pollution, as well as the factors that influence them has been formed.

This particular study, without ignoring the results of the previous relevant studies, attempts a more systematic processing of the available data with a view to determining local air pollution conditions and, in particular, by investigating the influence of the climatic seasons on their formation. With this in mind, standard statistical methods of time series analysis are applied. Also, for a more complete documentation of the results, a brief description of the land planning, geographical and climatic characteristics of the area is included. The conclusions drawn from this study concern a large proportion of the Greek population, since the area under study contains many characteristics that can be considered representative of the urban and suburban environment of many other Greek regions.

THE GEOGRAPHICAL AND CLIMATIC CHARACTERISTICS OF THE AREA

The city of Volos extends along the northern part of the cove of the Pagasitikos Gulf on the eastern seaboard of Central Greece (Lat. 39.22 N / Long. 22.56 E). A large part of the city is built amphitheatrically on the western foothills of Pilion Mountain, which extends along the peninsula of Magnesia, from Northwest to Southeast. This mountain, with an altitude of 1548 metres, is the highest in the area and operates as a natural shelter along the arc from the North to the East of the city. To the West, the city is bordered by planes and semi-mountainous areas with heights that do not exceed 500 metres (Figure 1).

FIGURE 1
Simplified map of the greater Volos area with an indication of its position on the Greek peninsula. Elevations contours correspond to 500 and 1000 m. The location of the city is denoted by the white area. 'X' indicates major industrial sites.



Volos, like most Greek cities, is densely populated. Multi-storey block buildings, up to 30 meters high, which are typical of the Greek urban environment are concentrated in the city centre and are mostly utilised as habitations, as offices and as the headquarters of various companies. The city centre, which coincides approximately with the centre of the coastal perimeter zone, has also developed into the main area of commercial activity. Both large and small industrial units are located mainly on the west side of the city, along the two main roads that connect Volos with the national road network. Many industries are located at a distance of a few kilometres to the Northwest of the city in an area, which is an established industrial zone. They are mostly industries that are concerned with the processing of agricultural products, the production and processing of metallurgical products and plastics as well as chemical industries. In addition to this, a large cement production industry is located on the coast, a small distance from the Eastern side of the city. The harbour area is located next to the West end of the city.

The climate in the area of Volos, as in the greater part of Greece, is of the Mediterranean type (7). More specifically, the year can, in general, be divided into the climatically cold and humid season (October - March) on the one hand, and the hot and dry season (April - September) on the other (Figure 2). Between September and October there is a significant drop in temperature (~ 5° C), which continues gradually until January, which is the coldest month of the year (mean temperature: 7.6 °C). Starting in March, the temperature begins to increase until July and August, which are the hottest months of the year (mean temperatures: 26.8 °C and 26.4 °C, respectively). The intervening seasons (autumn and spring) are quite clearly defined and, in particular, autumn is hotter than spring by about 2 - 4 °C. The area has a mean annual isotherm of 17.5°C. The mean extreme temperatures are approximately 3°C in winter and 31°C in summer. The mean annual degree days in the area is about 1506.

The climatic characteristics of the wind, as determined by both the general atmospheric circulation and the prevailing synoptic systems in the area at large, contribute to the predominance of westerly and northerly components and moderate speeds (8). Nevertheless, in combination with these factors, the landscape, which acts as a factor in channelling and mid-scale thermal circulation, plays a dominant role in the determination of the direction and speed of the prevailing winds in the area (4). On a smaller scale, the characteristics of the wind at every point in the city are influenced, in a complex way, by street layout and by the shape and dimensions of the surrounding obstacles, both natural and artificial, such as trees and houses (9).

DATA AND METHODS

The study is based on measurements of air pollutant concentrations conducted during an eight- year period (1987-1994) at the Air Pollution Monitoring Station, which has been in operation since 1986 on the terrace of a four-storey building, in the centre of Volos. The data available include maximum daily concentration values of CO, O_3, NO_2 and SO_2 for the period from 1987 until 1991 and mean hourly concentration values of CO, O_3, NO, NO_2 and SO_2 for the period from 1991 until the end of 1994. The air pollutants included in the study, according to international practice, are considered as being representative of the air quality in urban environments (10).

In particular, most of the pollutants examined are emitted in large quantities during combustion and all of them are toxic (11). The anthropogenic activities that lead to emissions of these pollutants are mainly transportation and combustion for heating facilities e.g. central heating systems, or for industrial purposes. The O_3 in the lower troposphere does not constitute a primary pollutant, but rather is a product of photochemical reactions, which presuppose the presence of Nitrogen Oxides and Hydrocarbons; gases which are emitted by the burning of internal combustion engines.

It is worth noticing that the quality of the data is not fully satisfactory. More precisely, there are gaps in the time series, which in some cases extend to a period of some months and are caused either by instrument failure or by extended periods of decalibration. Furthermore, shorter periods, or isolated values, are clearly erroneous, or at least questionable, obviously due to incidental or systematic errors. Corresponding values were rejected using Chauvenet's criterion. Afterwards, the elaboration of the data was conducted by statistical methods of time series analysis.

RESULTS

Table 1 shows the main statistical parameters of the mean hourly concentration values of the pollutants under study. Separate columns correspond to the data in their totality as well as to their subtotals for cold (October-March) and hot (April-September) periods. From this data, clear differences in conditions of pollution in the area between the cold and the hot periods become apparent. This also means that even the mean values of the corresponding pollutants are not particularly high, while, on the other hand, the maximum values recorded come close to standards of elevated pollution, which, in fact, justifies acceptance of the potentiality for air pollution problems in the area.

FIGURE 2 - The mean monthly values of some basic climatic parameters in the area of Volos.

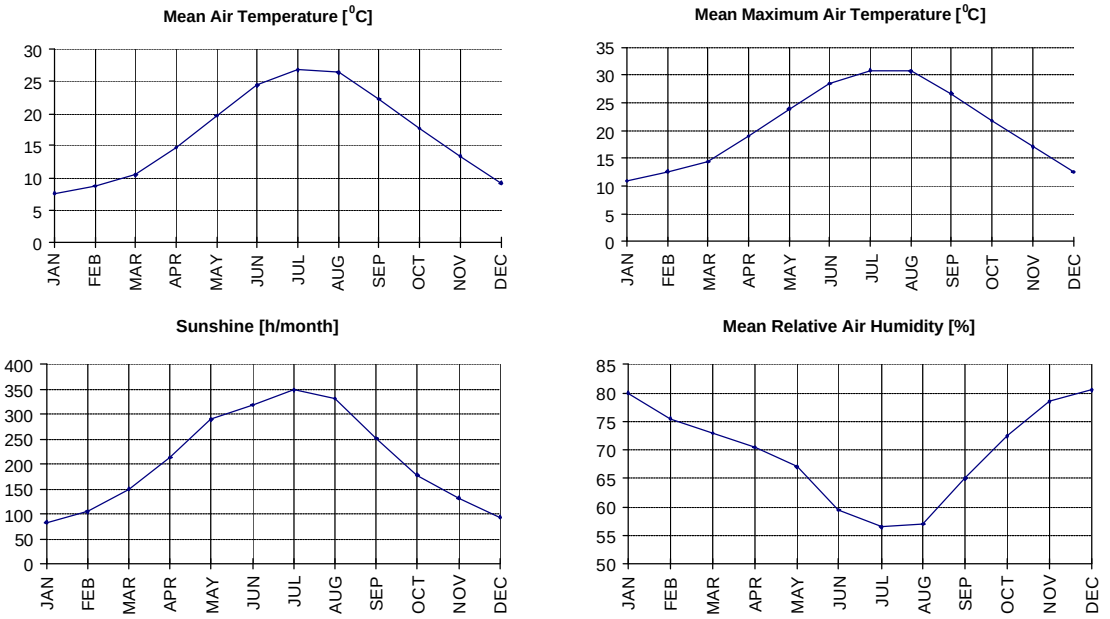


TABLE 1
The main statistical parameters of the mean hourly concentration values of the air pollutants in the area of Volos for the 1991-94 period.

	CO			O_3			NO_2			SO_2		
	All	Cold	Hot	All	Cold	Hot	All	Cold	Hot	All	Cold	Hot
	[mg/m^3]			[μg/m^3]			[μg/m^3]			[μg/m^3]		
Average	2.41	2.59	2.25	58.02	36.43	77.77	41.18	49.46	33.77	38.31	48.82	26.15
Median	1.74	1.85	1.64	46.93	26.45	76.59	34.45	41.82	27.28	20.81	31.72	11.11
Std. Deviation	2.28	2.45	2.11	39.50	24.97	40.01	31.70	34.49	26.89	48.41	53.75	37.89
Maximum	25.64	25.64	22.62	277.8	220.0	277.8	347.3	323.6	347.3	339.1	290.2	339.1
Skewness	2.65	2.56	2.69	0.79	1.72	0.20	2.38	1.92	3.23	3.30	2.80	4.64
Kurtosis	12.66	10.37	15.43	-0.13	7.04	-0.96	12.39	5.60	30.30	17.50	11.41	41.27

FIGURE 3 - The mean and the mean maximum monthly pollutant concentration values.

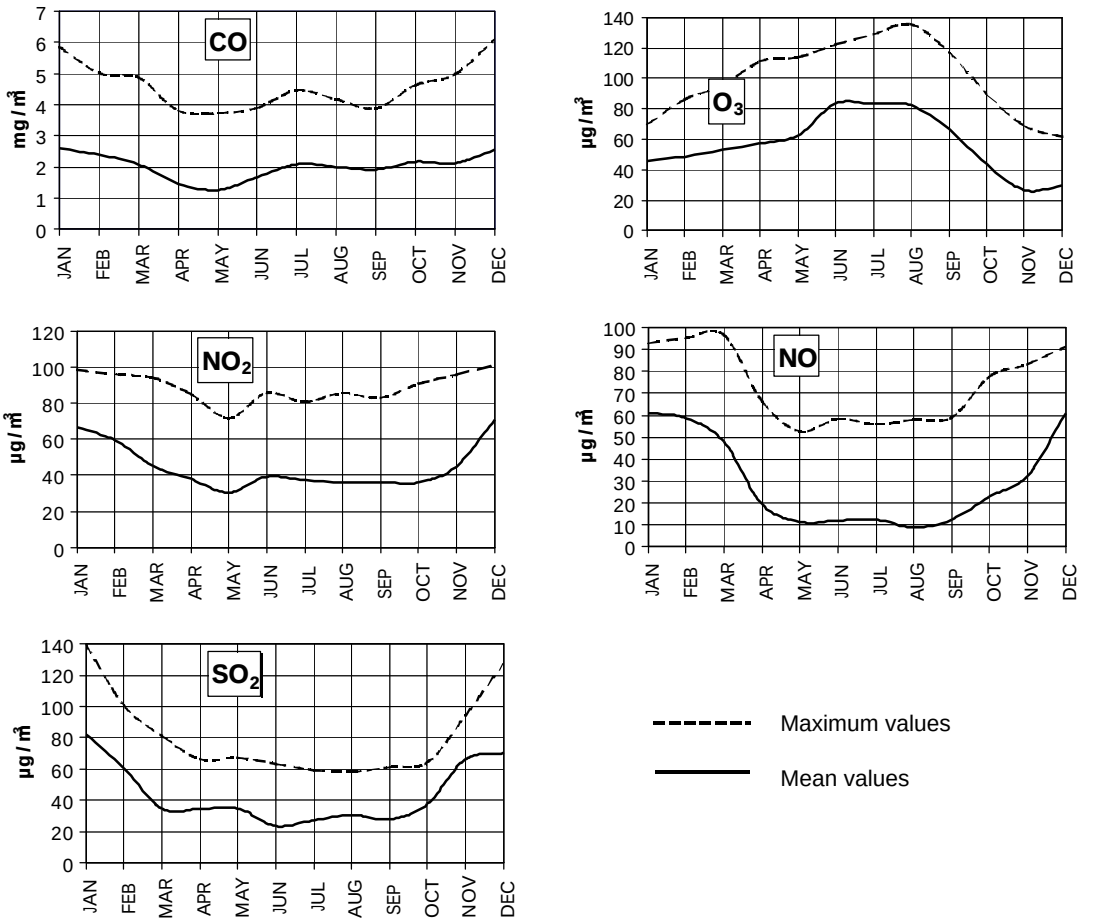


FIGURE 4 - Hourly frequencies of maximum air pollutant concentrations during the 1991-94 period.

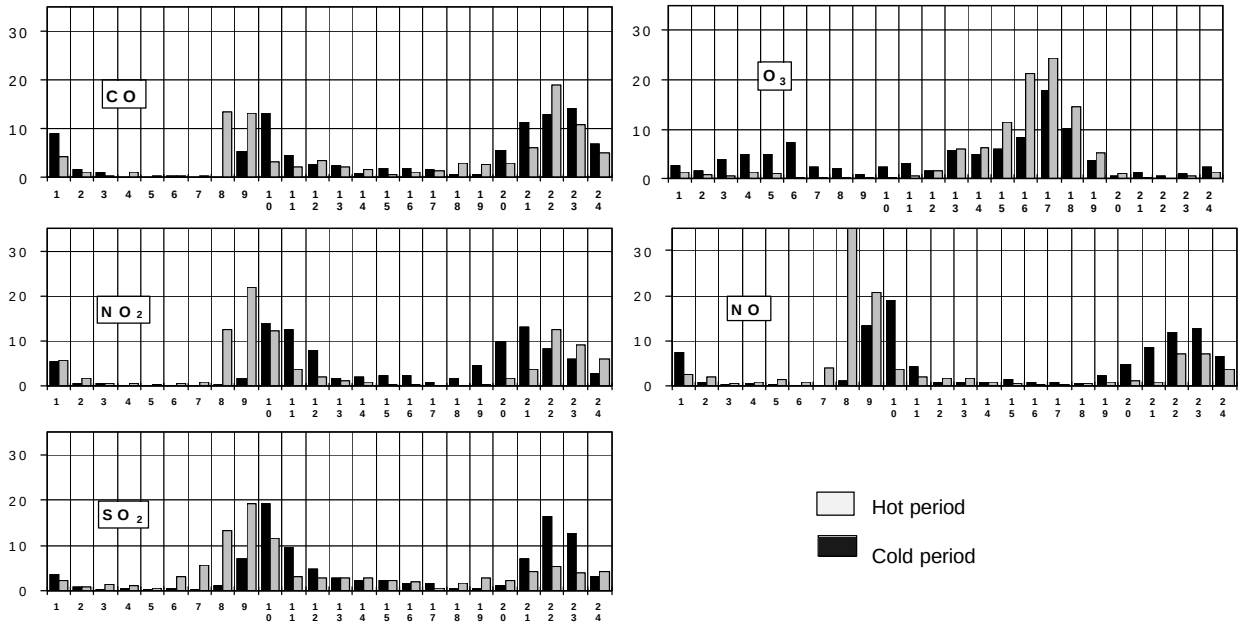
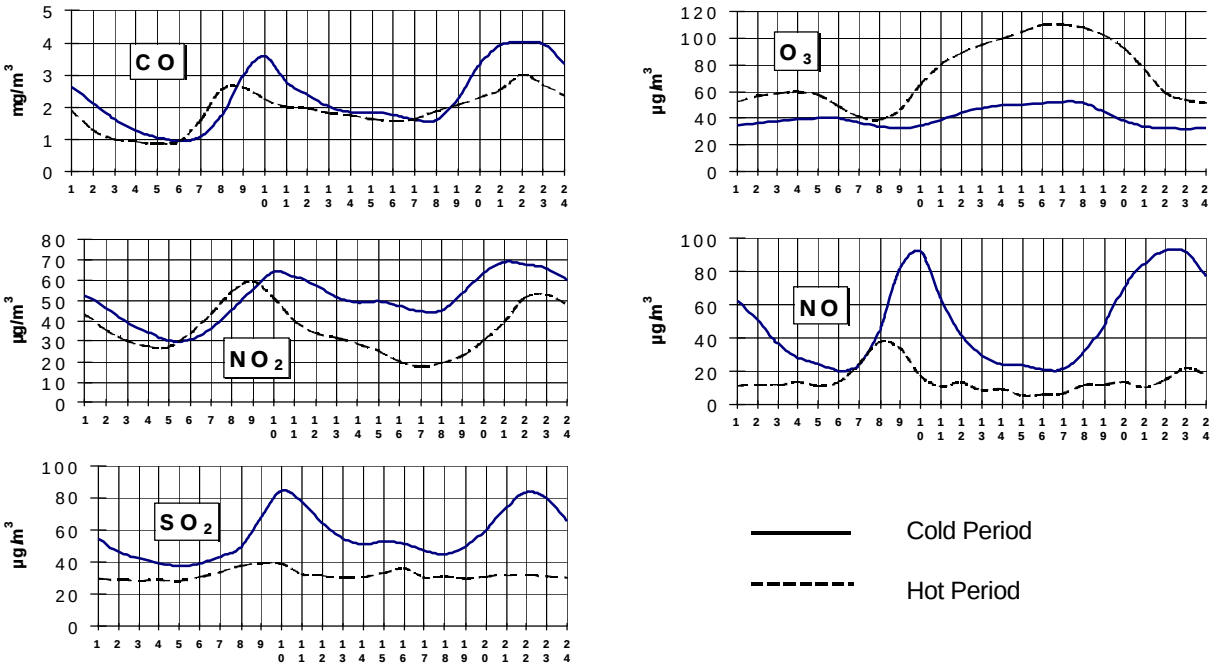


FIGURE 5 - Mean hourly concentration values of pollutants during the 1991-94 period.



The diagrams in Figure 3 show the mean and the mean maximum monthly pollutant concentration values calculated from the mean hourly and the maximum daily concentrations for the periods 1991-1994 and 1987-1994, respectively. In all diagrams it is evident that there is seasonal change in the values, which, in particular, is more apparent for O3, NO, and SO_2 and less apparent, but still clear, for CO and NO_2. In all cases, except for O_3, the corresponding concentrations are higher during the cold period and lower during the hot period. For O_3 the statistics are quite the opposite, namely, the higher values occur during the hot period.

Diagrams in Figure 4 show the hourly frequencies of daily maximum concentrations of the air pollutants during the cold and the hot period. For all air pollutants, except for O_3, two maximums are evident, one between 08:00 and 11:00 in the morning and the other between 20:00 and 24:00 in the evening. The morning maximum is slightly more intense for NO_2, NO and SO_2, while in the case of CO the evening maximum predominates. Differences exist between the hot and the cold period, the most important being the shifting of the maximums during the hot period towards earlier and later hours. In the case of O_3, in essence there exists only one maximum during the afternoon hours, between 14:00 and 18:00 hours, while, in the cold period only, a slight increase in frequencies becomes apparent during the night.

Diagrams in Figure 5 show the mean hourly concentration values of the pollutants during cold and hot periods. In the diagrams for the cold period, in the case of all the pollutants, except for O_3, two maximums become apparent. The first maximum appears during morning hours and the second maximum during evening hours. In the corresponding diagrams for the hot period, the morning maximum also appears, but in the case of NO and SO_2, the evening maximum is notably weakened. In these diagrams, as in the data in Table 1, significant differences between the mean values for cold and hot periods are also evident. For all pollutants, except for O_3, the mean hourly concentrations are higher during the cold period and in some cases they are over double compared to the corresponding readings during the hot period. Also, as in the diagrams in Figure 4, during the hot period there is a noticeable characteristic shifting of the maximum values, by about one hour, towards earlier and later hours compared with corresponding times in the cold period. In the case of O_3, the mean hourly values that appear during the hot period are significantly higher than the corresponding values during the cold period. Also, in the case of O_3, there is, in essence, one maximum during the daytime, which appears both in the cold and the hot periods during afternoon hours between 16:00 and 18:00. During the hot period only a weak maximum appears during the early morning hours, between 03:00 and 05:00.

DISCUSSION AND CONCLUSIONS

From the elaboration of the air pollution data for the 1987-94 period in the city of Volos, the resulting air pollution problems for the area do not seem to be particularly serious. The mean concentration values of the pollutants involved in the study were found to be at low levels, in comparison with the prevailing corresponding limits and, although maximum values exceed the conventional safety thresholds (11), it happens relatively rarely. Nevertheless, apart from the seriousness of the problem,

what the analysis shows is that air pollution conditions in the area are highly influenced by local activities. This conclusion is supported by the fact that the statistically elevated pollutant concentrations, when they occur, are greatly justified by increased anthropogenic polluting activities that, by inference, take place during the corresponding periods of time. Thus, the air pollutant concentrations appear higher during daylight and decrease during the night when, as is reasonable, polluting activities are reduced. The same contrast is also noticeable between the cold and hot periods of the year; the decline in productive activity during the summer, together with the elimination of heating needs during the same period results in a reduction in pollutant emissions and consequently in lower pollutant concentrations in the atmosphere. In relation to the foregoing, the extension of the time of relatively elevated pollutant concentrations is characteristic, which is noticeable during the hot period when the period of daylight is longer and thus logically favours the extension and duration of polluting activities.

Among all the anthropogenic polluting activities, those that seem to be more dynamically involved in the creation of air pollution conditions in the area of Volos are combustion, for heating and for energy production, in general, and for transportation. The significant increase in the concentrations of NO, NO_2 and SO_2, which is noticeable during the cold period, is justified by increased combustion which is needed, mainly, for the heating of buildings during the corresponding period. The pollution from automotive emissions is also evident from the elevated pollutant concentrations, except for O_3, during the hours when traffic is, by inference, high, as for example for transportation between habitations and workplaces. More specifically, the fluctuations in pollutant concentrations coincide with the fluctuations in traffic density.

The unique behaviour of O_3 is justified by the fact that this pollutant is a product of photochemical reactions and their concentrations in the lower troposphere reflect an interplay of emissions of NOx and HCs, meteorology and atmospheric chemistry. The relatively elevated O_3 concentrations, which were detected during the early afternoon hours of the day, are justified by high radiation from sunlight and also by heat during the corresponding period. The same reasons also justify the elevated concentrations during the entire hot period (10).

The above points can be considered reflective of the air pollution conditions that a large part of the Greek population has experienced, since Volos has many of the characteristics, which are representative of the urban and suburban environment in Greece. The enrichment of corresponding findings, in order to become more representative for the urban and suburban environment in Greece, requires additional studies of this type which cover more areas and use longer and more credible time series and deal with more pollutants, if possible. Such studies presuppose the existence of more monitoring stations and additional equipment, which is properly serviced and calibrated.

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DETERMINATION OF VOLATILE SULFUR COMPOUNDS IN WATER SAMPLES BY GC-MS WITH SELECTIVE PRE-CONCENTRATION

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SUMMARY

A GC-MS method for the identification and determination of volatile sulfur compounds (sulfides, polysulfides and thiols) in highly polluted water samples based on membrane extraction in combination with sorption on Ag_2S and thermodesorption is presented. Detection limits between 0.01 and 2.4 µg/L were obtained for the various species. The relative process standard deviations were found to be between 1.5 and 8.2 %. The influence of halogenated hydrocarbons on the determination of sulfur compound was investigated. The method was compared by using Tenax as adsorbent.

KEYWORDS:
Volatile sulfur compound, wastewater, pre-concentration, membrane extraction, thermodesorption, GC-MS

INTRODUCTION

Due to their widespread occurrence in natural products, sulfur species play an important role in various environmental compartments. Their occurrence can be explained as resulting from chemical and biological processes in connection with the natural sulfur cycle as well as by direct anthropogenic influences. Anthropogenic sulfur emissions have risen sharply since the early 20th century due to the human population explosion and rapid industrialization. The majority of emissions are caused by the combustion of fossil fuels. Other sulfurous compounds such as thiols, sulfides, thiophenes, thiazoles, sulfoxides, sulfones and sulfonic acids are released into the environment by several industries (e.g. refineries, the production of detergents, dyestuffs and pesticides). Agriculture is a source of ground and surface water pollution owing to the broad application of pesticides. Some organic sulfur hydrocarbons are known to be toxic and mutagenic.

Despite the existence of numerous published papers on their enrichment, the separation and unambiguous identification of the volatile sulfur compounds are still problematic because of their high reactivity [1–6]. Moreover, reliable analysis is impeded by the broad and varying range of concentrations in different environmental matrices which cause interference problems.

These problems also affect investigations of highly polluted groundwater in the Bitterfeld-Wolfen region (Saxony-Anhalt, Germany). This district has been dominated for more than 100 years by lignite mining and chemical industry. Besides contamination caused by improper handling, the groundwater is also severely polluted by chemical waste products dumped in landfills and open pits. Since mining pits are flooded to create lakes, the rising groundwater levels and the resulting mobilization of pollutants are being calculated under the given local geological conditions. Therefore, the regional groundwater is the object of intense remediation and analytical investigations.

In addition to high level of inorganic contaminants, the water contains high concentrations of halogenated hydrocarbons and sulfur compounds. The identification and determination of these sulfur species in this matrix is similarly very complicated because of interference problems in this matrix.

The objective of this research was to develop a selective method for the enrichment of volatile sulfur compounds (VSCs) from such contaminated water samples for subsequent determination by GC/MS.

Adsorptive enrichment combined with thermodesorption is an established extraction technique for volatile compounds. Various solids, e.g. graphitized carbon, glass materials, molecular sieves, polymers, silicagel, aluminium oxide, metals and metal compounds have been

applied to adsorb VSCs. Particularly, metals and electrophile metal compounds are well known for their strong interactions with nucleophilic sulfur compounds [7–15]. Various methods for enrichment on Au [8, 9, 13, 14], Pd, Pt, Ag, Rh, W, Mo, Sn and Ni [7, 15, 16] have been reported. However, the techniques described therein have several disadvantages. The high reactivity of VSCs, especially those with metal catalytic oxidative features, results in partial decomposition of the compounds owing to the high desorption temperatures required [7, 8]. Moreover, the carrier gas H_2 causes the reduction of some VSCs [9].

Earlier work employing various metallic compounds such as Ag_2S, Sb_2O_3, PbS, SnO_2, FeS and GeO_2 was carried out with the aim of finding an adsorbent which enables selective enrichment in combination with reproducible adsorption and desorption reactions [17]. Ag_2S, in particular, exhibited chemical inertness and temperature resistance as well as satisfactory enrichment.

This paper describes the applicability of Ag_2S to adsorb VSCs. Enrichment on Ag_2S is compared with the commonly used adsorbent Tenax. The technique used here was adsorptive enrichment on these materials in combination with membrane extraction followed by thermodesorption and GC/MS detection.

MATERIALS AND METHODS

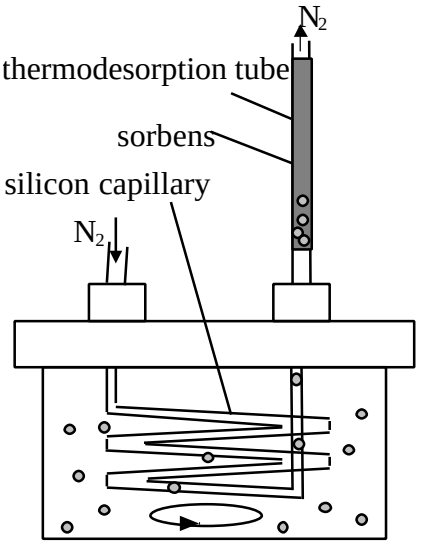
Chemicals: Tetrahydrothiophene, n–propylmercaptan, n–butylmercaptan, n–hexylmercaptan, n–heptylmercaptan, diethyl sulfide, di–n–butyl sulfide, di–n–propyl sulfide, dimethyl sulfide and dimethyl disulfide were purchased from Supelco (Bellefonte, PA, USA). Dimethyl trisulfide was obtained from Promochem (Wesel, Germany). Individual compounds were dissolved in methanol (Merck, Darmstadt, Germany). For aqueous test solutions, 10 µL of methanol solutions of the compounds was diluted in 100 mL distilled water. This results in values between 0.005 and 10 nL/L for each VSC.

Adsorbents: Commercially available adsorption tubes filled with 0.180 g Tenax TA (Gerstel, Mühlheim, Germany) were used. Ag_2S (1 g, Chempur, Karlsruhe, Germany) was fixed in a silanized glass tube (17.8 cm length, 6.4 mm o.d.) using silanized glass wool plugs. The tubes were conditioned at 250°C by purging with nitrogen at 30 mL/min for 2 h. For storage purposes the tubes were placed into PTFE container.

Samples: Groundwater samples were taken from wells in Bitterfeld (GWM19). Besides high concentrations of various chlorinated compounds (HCHs, PCBs, DDX, HCB) [18,19] and PAHs [20], high concentrations of sulfur compounds such as dimethylsulfide, dimethyltrisulfide, tetrachlorthiophene and esters of thiophosphoric acids were identified and determined.

Analytical method: The analytical procedure is based on a combination of membrane extraction, adsorptive enrichment, thermodesorption and GC/MS. The membrane extraction technique was used for the determination of sensitive organic compounds with boiling points up to 220°C in water samples as described in [21]. In this work membrane extraction was combined with trapping on Ag_2S and Tenax for the enrichment of VSCs, as shown in Figure 1.

FIGURE 1 - Setup of membrane



The extraction cell, a glass container, was filled with 100 mL water sample. A constant flow of nitrogen (50–100 mL/min) was passed through a silicon capillary (0.3 m long, i.d. 0.7 mm, o.d. 0.9 mm) placed in the stirred (speed 500 rpm) water sample. Silicon has been described as a suitable material for the extraction of VSCs [22]. Analytes diffused through the hydrophobic silicon capillary and were transported by nitrogen to the adsorbents, which was placed on top of the extraction cell. The volatile compounds separated from aqueous phase were enriched on the sorbents.

The loaded sorbent tubes were transferred to the thermodesorption device. In comparison with simple purging, the extraction method used here, results in a significantly reduced water load on the sorbents. Thermodesorption was carried out using a TDS2/CIS3 unit (Gerstel) connected to an HP 5890 Series II gas chromatograph equipped with an HP5972 mass-selective detector (Hewlett Packard). At the beginning of the desorption process the tube was held at 10°C for 1 min to purge out (He, 42.5 mL/min) most of the contained gas. Analytes were desorbed (10 °C - 2 min, 50 °C/min to 320 °C - 6 min) and transferred (320 °C) to the cold injection system. During desorption the cryo-focussing insert liner (glass wool) was kept at –150°C in order to trap the desorbed compounds. After injection (12°C/min

to 350 °C – 6 min, splitless 1 min) the compounds were separated on an HP-624 capillary column (30 m x 0.25 mm i.d. x 1.4 µm film thickness). The oven program was as follows: 10 °C - 3 min, 10 °C/min to 250 °C - 6 min.

RESULTS AND DISCUSSION

Optimization of the extraction parameters: Both the extraction of VSCs with the silicon capillary (hollow fiber membrane) described and the adsorption of the extracted compounds on Ag_2S and Tenax are affected by the extraction time and nitrogen flow rate. Since both processes depend on each other, they can only be optimized together. Their influence was studied with VSCs spiked into distilled water samples (each 10 nL/L). The extraction time varied between 20 and 60 min at a constant nitrogen flow rate of 100 mL/min. The enrichment of some representative VSCs on Ag_2S and Tenax as a function of the extraction time is shown in Figure 2. When Tenax was used, 40–50 min was found to be optimal time for most of the compounds tested. Only a few compounds, e.g. tetrahydrothiophene and di-n-propyl sulfide, behave differently. These substances were enriched with increased amounts after this time. Notwithstanding the smaller surface, Ag_2S adsorbed the VSCs to a significantly higher extent with ascending concentrations over the total extraction time (surfaces: Ag_2S ca. 0.1 cm^2/g, Tenax 35 m^2/g). To ensure reasonable analysis times, an extraction time of 40 min was chosen in the following investigations for both sorbents.

The flow rate of nitrogen, which strips the extracted analytes from the inner surface of the hollow silicon fiber to the adsorption tube, was varied between 50 and 100 mL/min. The influence of the nitrogen flow rate on the extraction yield was small compared to the influence of the extraction time. The best results were obtained with flow rates of 100 mL/min, and so further experiments were conducted at this flow rate.

Selectivity: The groundwater from the Bitterfeld area is mainly contaminated with halocarbons. These compounds thus account for the majority of the interfering matrix and were the next subject of investigation after VSCs. The groundwater sample was diluted with distilled water (1:50). This dilution (100 mL) was spiked with 10 µL of methanolic VSC solution so that the concentration of each sulfur compound was 0.1 µL/L. The extracted compounds were enriched on Tenax and Ag_2S, respectively, using 100 mL/min nitrogen flow for 40 min.

The abundance of the compounds retained on Tenax and Ag_2S are given in Figure 3. The capacity of Tenax and Ag_2S are characterized by very different particle sizes as well as specific weight and porosities, hence the chromatograms are difficult to compare directly to quantitatively estimate the compounds identified. For interpretation aA semi-quantitative method was selected, which is based on the calculation of relative peak areas. The ratio between the peak area for each compound identified (ppa) and the total peak area (defined to 100 %) was calculated. Peaks with relative areas < 1% were neglected and unidentified peaks not specified, which explains why the sum of all peak areas is less than 100 %.

The detected compounds are shown in Table 1. Although, as expected, the halocarbons were enriched by both Tenax and Ag_2S, in the case of Ag_2S the halocarbons were retained at an essentially lower level.

TABLE 1 - Compounds identified from spiked groundwater sample

Compound	Peak areas (%)	
	Tenax	Ag_2S
CS_2	1	d.
Methylene chloride	4	n.d.
n–Propylmercaptan	3	3
Chloroform	23	d.
Benzene	3	n.d.
Diethyl sulfide	3	6
Trichloroethylene	4	n.d.
n–Butylmercaptan	4	20
Dimethyl disulfide	5	d.
Toluene	2	d.
Trichloroethane	9	d.
Tetrachloroethene	4	d.
Tetrahydrothiophene	2	2
Chlorobenzene	3	d.
Di–n–propyl sulfide	5	47
n–Hexylmercaptan	3	17
Tetrachloroethane	4	d
Dimethyl trisulfide	4	n.d.
n–Heptylmercaptan	d.	1
o–Dichlorobenzene	2	d.
m–Dichlorobenzene	2	d.
Σ ppa	30	96

d. = detected with ppa < 1 %, n.d. = not detectable

Comparison of the relative peak areas shows that VSCs were mainly adsorbed by Ag_2S rather than by halogenated hydrocarbons. 96 % of the peak areas are caused by sulfur compounds. Tenax showed a different enrichment pattern. Only 30 % of the peak area are caused by VSC's. Mainly halogenated compounds were enriched. This illustrates the selective enrichment of VSCs on Ag_2S.

Calibration: Calibration was performed with the optimized extraction parameters (nitrogen flow rate 100 mL/min, extraction time 40 min) based on eight calibration levels. The spiked distilled water samples contained each of the tested VSC between 0.005 and 10 nL/L. The detection limits (LOD) and relative process standard deviations (RPSD) were calculated according to the German regulations DIN 32 645.

FIGURE 2 - Optimization of the extraction time

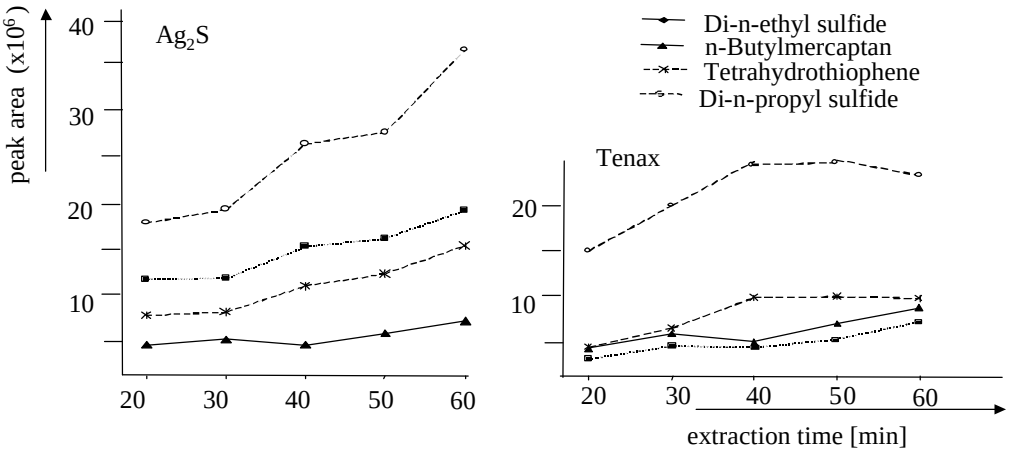


FIGURE 3
GC/MS chromatograms of groundwater samples after membrane extraction, adsorptive enrichment on Tenax or Ag_2S and thermodesorption

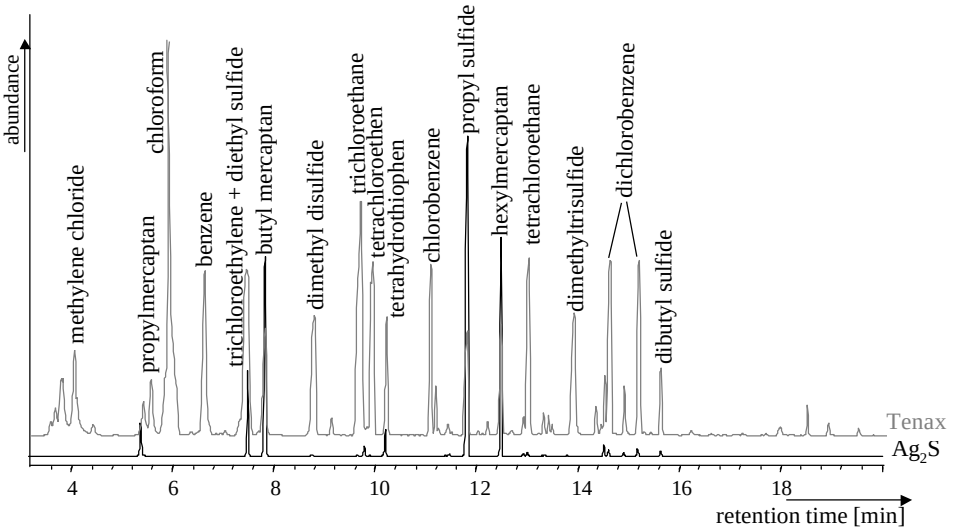


FIGURE 4 - Adsorption isotherm a) common function b) for adsorption inhibited by pressures

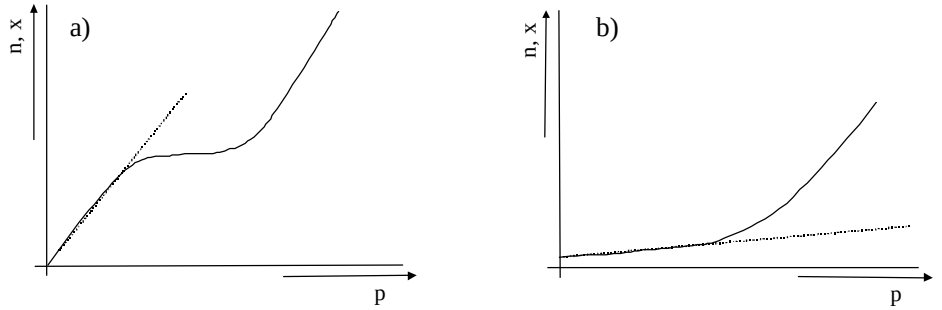


TABLE 2
Calibration parameters of the methods used (membrane extraction, adsorptive enrichment on Ag_2S, thermodesorption, GC/MS)

Compound	Linear range [µg/L]	LOD [µg/L]	Correlation coefficient	RVSD [%]
n–Heptylmercaptan	0.08–8.0	0.7	0.9995	4.3
n–Hexylmercaptan	0.08–8.0	1.6	0.9992	5.5
n–Butylmercaptan	0.08–8.0	2.4	0.9982	8.2
n–Propylmercaptan	0.08–8.0	1.0	0.9997	3.6
Di–n–butyl sulfide	0.004–4.2	0.075	0.9999	1.9
Di–n–propyl sulfide	0.004–0.9	0.01	0.9999	1.7
Diethyl sulfide	0.004–0.8	0.015	0.9994	2.4
Dimethyl sulfide	0.085–0.9	0.1	0.9995	5.7
Dimethyl disulfide	0.05–1.0	0.07	0.9998	3.9
Tetrahydrothiophene	0.005-1	0.009	0.9999	1.5

TABLE 3 - Compounds identified from a Bitterfeld groundwater sample (GWM 19)

Retention time [min]	Compound	Tenax	Ag_2S
3.60	Dimethyl sulfide	x	x
3.86	CS_2	x	x
5.32	Ethyl methyl sulfide	–	x
7.52	Methylthioacetate	–	x
8.70	Dimethyl disulfide	x	x
9.42	Mercaptoacetic acid methylester	–	x
10.73	Methyl ethyl disulfide	x	x
12.02	2,4–Dithiapentane	–	x
13.76	Dimethyl trisulfide	x	x
14.12	1,3–Dithiolane	–	x
14.27	O,O,O–Trimethylthiophosphate	x	x
16.02	Methyl phenyl sulfide	–	x
16.99	Methyl–(methylthio)–methyldisulfide	x	–
17.92	O,O,S–Trimethyldithiophosphate	x	x
18.96	Tetrachlorothiophene	–	x

Linearity exists in the lower concentration ranges. The dependencies shown in Figure 4a were expected. This resembles for low pressures the adsorption isotherm by Langmuir. However, only the analytical data of enrichment on Tenax corresponds to this function. By contrast, enrichment on Ag_2S showed dependence on adsorption curves inhibited by low pressures as depicted in Figure 4b. Such behavior was attributed to the hydrophobicity of adsorbents in the literature, and also occurs in the adsorption of methanol on charcoal [23].

The linear ranges, LOD, RSD and correlation coefficients are given in Table 2. Good correlation coefficients were obtained for all compounds. As RSD demonstrates, the technique applied produced high reproducibility for the sulfides and tetrahydrothiophen, which resulted in detection limits between 0.009 and 0.1 µg/L for these compounds. Higher deviations were observed for the mercaptanes investigated, especially at concentrations below 0.01 nL/L. This resulted in higher RSD and, therefore, diminished detection limits compared to the sulfides. Considering the main reasons for the higher deviations of the mercaptanes, their adsorption affinity to parts of the equipment such as the column and membrane needs to be taken into account. Mercaptanes are also well known for their reactivity at elevated temperatures, especially in the presence of metals and metal compounds. However, artefacts from catalytic reactions of VSCs on Ag_2S were excluded, because comparative investigations with Tenax revealed deviations within a contrastable range [17].

Analysis of a groundwater sample*:* A groundwater sample from Bitterfeld (GWM 19) diluted with distilled water (1:100) was analyzed using the method introduced here on both Tenax and Ag_2S. The substances identified are listed in Table 3. Thanks to selective enrichment, Ag_2S facilitated the identification of organic sulfur compounds, and so more VSCs were compared with Tenax.

Based on the calibration data, dimethyl sulfide and dimethyl disulfide were quantified. Diluting to 1:1000 and extracting with Ag_2S, it was calculated that the groundwater contained concentrations of 4.18 mg/L (± 0.26 mg/l) of dimethyl sulfide and 5.46 mg/l (± 0.43 mg/l) of dimethyl disulfide.

CONCLUSION

Ag_2S is a suitable adsorbent for the selective adsorption of volatile organic sulfur compounds from water samples. Sulfides, mercaptanes, and esters of thioacetic and thiophosphoric acids are enriched by Ag_2S in combination with membrane extraction. Determination is based on thermodesorption and GC/MS. Ag_2S exhibited a minor affinity to halogenated hydrocarbons compared to Tenax, which considerably facilitates the identification of unknown organic sulfur compounds in highly contaminated water samples.

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BIOPROCESS DEVELOPMENT OF PAPER MILL EFFLUENT'S DECOLORIZATION BY *PHANEROCHAETE CHRYSOSPORIUM* DSMZ 1556

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SUMMARY

In comparative studies on the decolorization of paper mill effluents by *Phanerochaete chrysosporium* DSMZ 1556 using free-cell, repeated-batch and co-immobilization systems were investigated. In repeated-batch decolorization tests a color reduction efficiency of 92.8% was obtained. Co-immobilization was approached by combining the use of adsorbents and cells of *P. chrysosporium* immobilized in a protective permeable barrier to achieve a greater degree of control over the remediation process. The co-immobilized system provided a higher degree of decolorization compared to the other tested systems.

KEYWORDS: *Phanerochaete chrysosporium,* paper mill effluent decolorization, repeated-batch, immobilization, co-immobilization

INTRODUCTION

Biological remediation promises cost-effective, environmentally benign *in situ* clean-up for many polluted sites. Filamentous fungi possess many unique attributes for bioremediation, particularly in terrestrial habitats (Bennett and Faison, 1997). The white rot species *Phanerochaete chrysosporium* has emerged as a model system for studying the fungal degradation of xenobiotics, crystal violet and decolorization of paper mill wastewater (Bumpus et al., 1985; Fernando and Aust, 1994).

In Egypt, large amounts of colored toxic lignin degradation products from paper mill factories are released into the environment and their hazardous effects are noticed by the serious change in color of water and destruction of the sea animal life (Ahmed, 1994). In a previous study, an attempt has been made to optimize cultural conditions leading to maximum decolorization of the effluent using the fungus *P. chrysosporium* DSMZ 1556 (Yusef et al., 1999). However, to be more effective in degrading the dye component by the white rot fungus, a practical treatment system must be developed, within which the fungal cells can grow well and maintain high viability to excrete degrading enzymes for a long term operation (Yang and Yu, 1996). As an extension of our research program, the present study aims to simplify the process, envolving cycling of the decolorized waste in a repeated batch manner, and applying co-immobilization system to increase the waste availability for the growing fungus.

MATERIALS AND METHODS

Effluent source

Combined paper mill effluent was obtained at the final stage of Rakta Pulp and Paper Company. Before use, the brownish yellow turbid effluent was filtered through 0.2 µm pore size filter to remove the suspended particles (Bergbauer et al., 1991).

Microorganism

Phanerochaete chrysosporium DSMZ 1556 used in this work was kindly provided by the Gerrnan Collection of Microorganisms and Cell Cultures.

Preparation of mycelial pellets

Stock culture of the fungus was stored on malt-agar at 4 °C and periodically subcultured. To obtain fungal pellets for inoculation, the fungus was grown in liquid malt extract medium (3%, w/v) at 30 °C continuously under shaking (135 rpm) for three days. Pellets obtained were aseptically harvested by filtration, homogenized and washed with phosphate buffer pH 6.0.

FIGURE 1
Growth pattern and decolorization efficiency of *Phanerochaete chrysosporium* DSMZ 1556 in batch culture in presence of 3 g l^{-1} (solid symbols) and 6 g l^{-1} (open symbols) glucose.

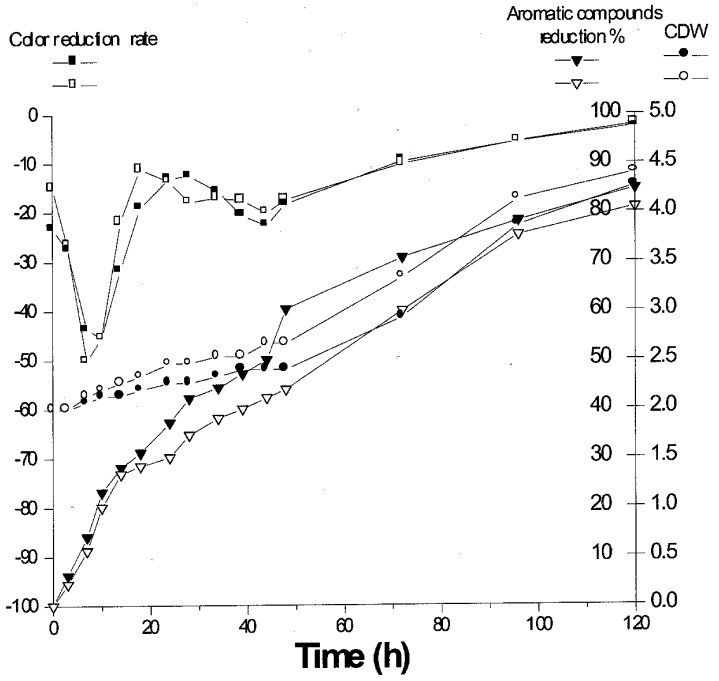


FIGURE 2
Color reduction pattern of Rakta paper mill effluent as affected by growing *Phanerochaete cchrysosporium* DSMZ 1556 in repeated-batch fermentation system.

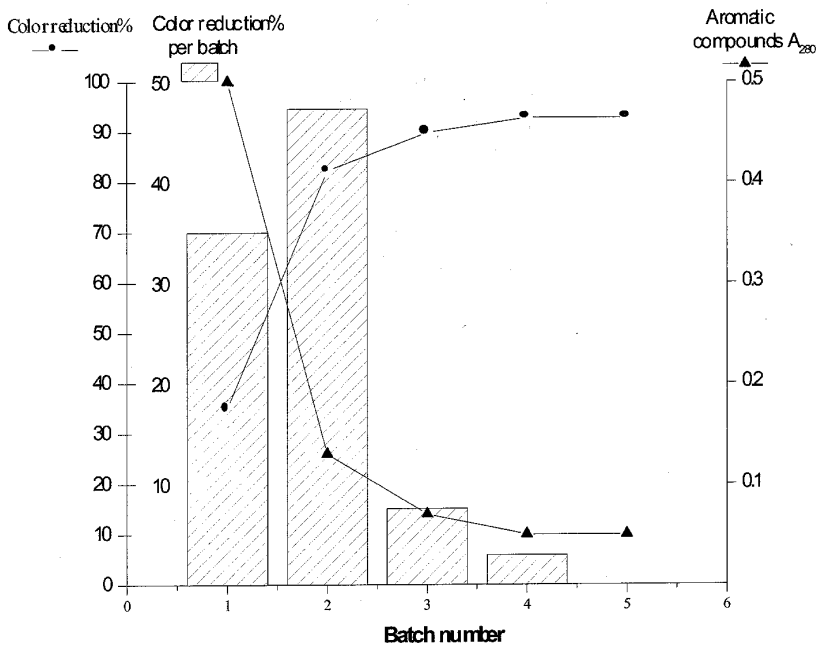
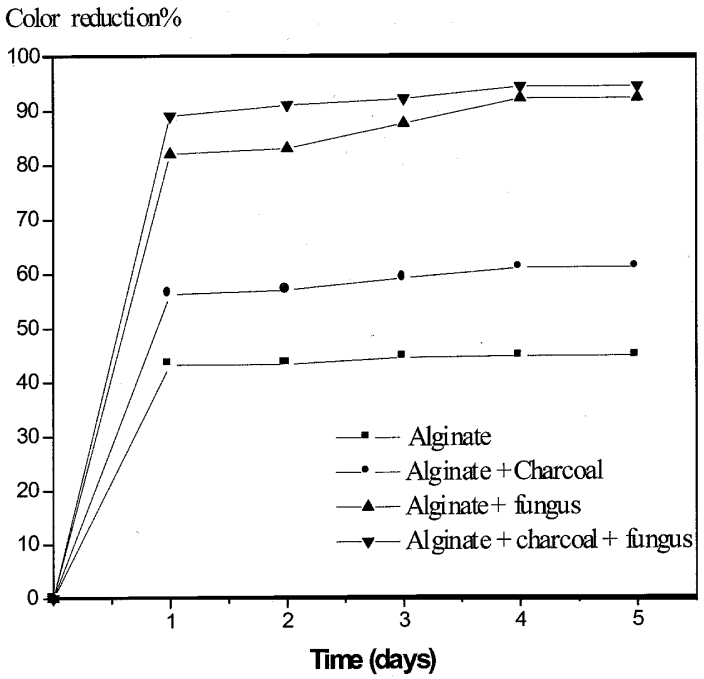


FIGURE 3
Influence of different immobilization strategies on color reduction by *Phanerochaete crysosporium* DSMZ 1556.



Repeated-batch fermentation

Fungal pellets (prepared as described before) equivalent to approximately 2 g l^{-1} were introduced into 250 rnl Erlenmeyer flasks, each containing 75 ml diluted effluent medium (45%). The effluent was adjusted to pH 6.0 and supplemented with 3 or 6 g l^{-1} glucose. Cultures were incubated at 30 °C on rotatory shaker (135 rpm).

For repeated-batch strategy, the ferrnentation broth was decanted after 24 h, added to freshly prepared fungal pellets. The cycle was repeated daily until maximum color removal was achieved.

Preparation of immobilized and co-immobilized capsules

For immobilization, fungal pellets (fresh weight corresponding to 150 mg dry weight) were prepared as previously described, filtered and blended in a Waring blender. The cell suspension was added to a previously sterilized alginate solution to obtain a mixture of 2% (w/v) alginate. The mixture was slowly extruded from a syringe into 2% $CaCl_2$ solution which caused instant formation of gel beads of about 2.5 mm in diameter (Pallerla and Chambers, 1995).

Co-immobilized capsules were prepared by mixing the homogenized pellets with 5 mg of powdered activated carbon and 0.3 M $CaCl_2$ solution to a total of 2.5 ml (for one flask of degradation mixture). This mixture was then dropped from a syringe into a stirred alginate solution (0.5%). Mixing was continued for 2 min. The slurry of the formed capsules and alginate solution was then diluted 5-folds with distilled water to stop the reaction between Ca^{2+} and alginate. Encapsulated activated carbon was prepared by the same procedure replacing homogenized pellets by distilled water. The diameter of co-immobilized capsules and encapsulated carbon was about 3 mm (Siahpush et al., 1992). Beads and capsules were then washed with sterile distilled water and aseptically transferred to 250 ml Erlenmeyer flask containing 75 ml of effluent medium.

Analyses

Color. The pH of each aliquot was measured and adjusted to 7.6 with 1N NaOH. Color was directly determined with a HACH DR/2010 spectrophotometer at 465 nm as described in the manual for the instrument using APHA Platinum-cobalt standard with a range of 0-500 color units (PCU) (Mehna et al., 1995).

Aromatic compounds. Quantitative determination of aromatic compounds was carried out spectrophotometrically at 280 nm with a Perkin-Elmer, 4B UV/VIS Spectrophotometer (Bergbauer et al., 1991).

Dry fungal biomass. Ferrnentation broth was filtered using pre-weighed Whatman No. 1 filter paper that was then dried in an oven at 60 °C until constant weight. Fungal biomass dry weight was then calculated (Mittar et al., 1992).

All analyses were done in triplicates and the mean values have been reported.

RESULTS AND DISCUSSION

Fermentation time course in batch cultures

The time course of batch of freely-suspended cells of *P. chrysosporium* in diluted (45%) effluent medium supplemented with 0.3% or 0.6% glucose is shown in Fig. 1.

The efficient decolorization and aromatic compounds degradation by the fungus in the presence of reducing carbohydrate concentration has been demonstrated (Bergbauer et al., 1991), which is attributed to the fungal growth conditions. In our study, maximum decolorization rates were attained after 7 and 10 hrs in a medium containing 3 or 6 g l^{-1} glucose, respectively. Under these named conditions, the highest specific decolorization rates (0.28 and 0.3 PCU g^{-1} fungal biomass) were achieved in the presence of 3 and 6 g l^{1} glucose , respectively. Therefore, for economic consideration, glucose concentration was reduced to 3 g l^{-1}.

Repeated-batch decolorization test

A further increase in the biotreatment effectiveness was achieved by using repeated-batch strategy with 24 h residence time for 5 successive batches. Data in Fig. 2 indicate that 35% of the effluent color was removed by the first batch, while by the second batch more than 45% of the residual color was removed.

The re-incubation of partially decolorized effluent for three consecutive times led to final reduction of color intensity and aromatic compounds on the order of 93 and 94%, respectively. It can also be seen that exponential increase in decolorization efficiency was obtained after two repeated batches only followed by a lagging effect. These results are in good agreement with those reported by Yang and Yu, 1996. Our finding seems to be interesting in reducing the running cost of the color removal process.

Co-immobilization experiments

The degradation of toxic organic compounds using co-immobilized system combining activated carbon and *P. chrysosporium* has been proposed by Lin et al. (1991). Since adsorbents other than activated carbon can be used in co-immobilized system, the effect of using different adsorbents on the decolorization process was explored. In order to examine the utility of the co-immobilized system to enhance decolorization of Rakta effluent, co-immobilized capsules were compared with immobilized beads. The effect of adsorbent only on the degradation of contaminants was also detected.

The results in Fig. 3 refer to the importance of living cells in the process. A 2-fold increase in color reduction was achieved when the fungus was immobilized in alginate compared to alginate as biosorbent. Moreover, more than 40% increase in color reduction was recorded in cells co-immobilized with alginate and charcoal compared to the alginate, and charcoal used alone as biosorbent.

This result may be ascribed to the ability to influence the concentration of contaminants within the co-immobilized capsules, representing a great potential for degradation of toxic organic compounds. It was reported that the co-immobilized system could enhance migration of toxic compounds from the environment to the adsorbent and provide a controlled micro-environment for subsequent biodegradation (Lin et al, 1991; Sialpush et al, 1992).

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THE EFFECTS OF VINASSE ON SOME GROWTH PARAMETERS OF ALGAE

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SUMMARY

In the present study, the effects of vinasse at various concentrations (0.0; 1; 2; 2.5; 3; 5; 10%) on some growth parameters of *Chlorella sp*. Bejerinck and *Tetraselmis suecica* have been investigated. It was seen that it caused increments in the content of photosynthetic pigments, protein and the cell number of these species at low concentrations (1; 2; 2.5; 3%), but decreased the cell number significantly at higher concentrations (5; 10%) compared with a standard medium. In the light of these findings, it has been discussed whether using vinasse for algal growth and so decreasing its polluting effect is useful or not.

KEYWORDS:
Vinasse, *Chlorella sp.* Bejerinck, *Tetraselmis suecica,* Photosynthetic pigments, Biomass, Cell number, Pollution.

INTRODUCTION

The technology of sugar refining and alcohol fermentation from sugar cane produces several effluents, one of which, vinasse, has been responsible for most pollution problems. The chemical composition of this residue varies with the chemical composition of sugar cane and is also influenced by the fermentation and distillation processes [1]. For example, the waste product of an alcohol factory in Eskitehir, Turkey, has been discharged directly into or at the Porsuk River.

According to some investigators, the BOD and COD loads of vinasses are 45000-65000 mg l^{-1} and 70000-80000 g l^{1}, respectively [2, 3]. Consequently, if vinasse is discharged into the environment directly it will have serious effects both on the growth of plants and the water and soil microflora. However, vinasse has been increasingly used for several purposes [2, 3, 4, 5]. Furthermore, it has been reported that similar wastes can be used as a carbon source for the production of single cell protein (SCP) [6, 7, 8]. But at the end of all these processes, especially in the latter case, the final effluents usually contain significant amounts of inorganic nutrients, in particular, ammonium, some mineral substances, such as Na, K, Ca, Mg, Fe and Zn, and some amino acids and vitamins, which magnify the nutrients loads and eutrophication problems [6, 8, 9]. On the other hand, it was reported that algal cultivation is a means of eliminating residual inorganic nutrients from these wastes and this process represents a potential food source [9].

In this study, the effects of vinasse at various concentrations on the growth, biomass, protein content and photosynthetic pigment contents of a green alga *Chlorella sp*. and *Tetraselmis suecica* have been investigated.

MATERIAL AND METHODS

The cultures of *Chlorella sp*. Bejerinck and *Tetraselmis suecica* were obtained from University of Ege, Faculty of Water Produces. The vinasse used for this study, which contained 9.2% dry matter, 4022 mg l^{-1} total nitrogen, 1705 mg l^{-1} Na, 10580 mg l^{-1} K, 150 mg l^{-1} Mg, 1250 mg l^{-1} Ca, was obtained from the Eskitehir Ethyl Alcohol Factory, Turkey and was stored frozen.

The cultures were grown at 20 ^{o}C on a shaker under continuous illumination (130 W m^{-2}) for 5 days. Algal cell numbers were counted using a haemocytometer (Spencer Improved Neubauer) once a day for 5 days. Chlorophyll and carotenoids were extracted with 80% acetone from the algae after sedimentation of the cells by centrifugation at 6000 g for 5 min. Absorbancies of the extracts were measured spectrophotometrically at 450, 645 and 663 nm. The formulae of Arnon (1949) and Jaspars (1965) were used for the estimation of chlorophyll and carotenoids, respectively [10, 11]. The biomass concentration was determined by centrifugation of the filtered suspension at 6000 g for 10 min. The pellet was washed three times with distilled water and dried at 105 ^{o}C to constant weight [12]. All experiments were replicated three times. Total nitrogen was measured by the Kjeldahl procedure. Crude protein was expressed as total nitrogen multiplied by 6.25 [13].

FIGURE 1 - The effects of vinasse on the cell number of *Chlorella sp.*

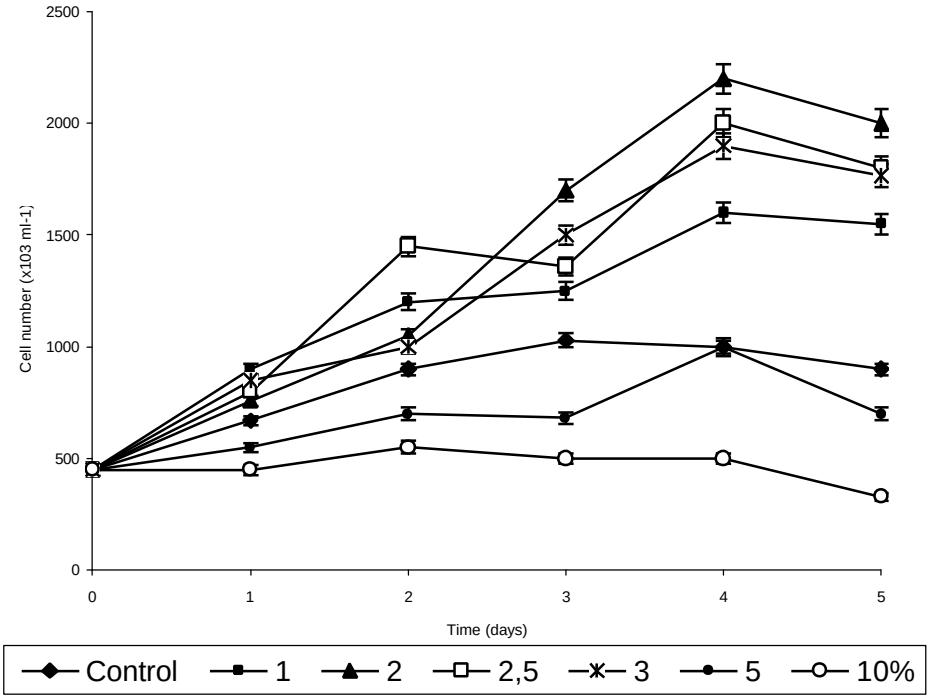

FIGURE 2 - The effects of vinasse on the cell number of *Tetraselmis suecica.*

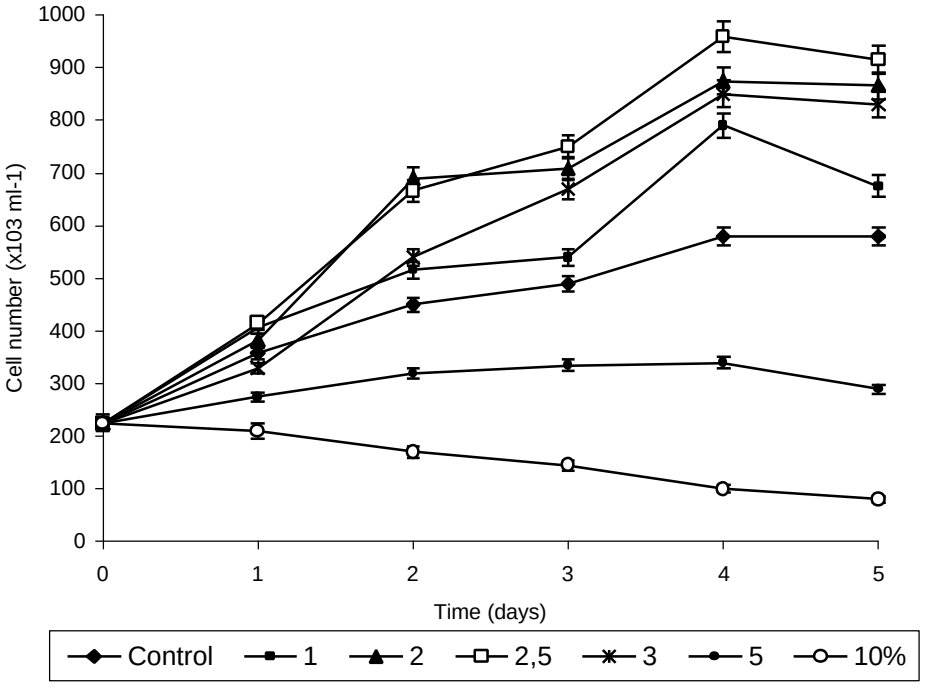

TABLE 1 - The effect of vinasse on the photosynthetic pigment contents of *Chlorella sp.*

		μg pigment per ml culture			
Days	Vinasse Concentration (%)	Chl_a	Chl_b	Carotenoids	Chl_a / Chl_b
1	Control	0.75 ± 0.04	0.50 ± 0.06	0.54 ± 0.04	1.49
	1	1.11 ± 0.15	0.68 ± 0.07	0.64 ± 0.07	1.61
	2	0.86 ± 00.1	0.56 ± 0.01	0.52 ± 0.04	1.51
	2.5	0.91 ± 0.04	0.58 ± 0.02	0.55 ± 0.06	1.56
	3	0.92 ± 0.07	0.59 ± 0.04	0.57 ± 0.09	1.55
	5	0.69 ± 0.11	0.48 ± 0.05	0.44 ± 0.04	1.41
	10%	0.57 ± 0.17	0.43 ± 0.07	0.35 ± 0.06	1.32
2	Control	0.90 ± 0.14	0.59 ± 0.01	0.55 ± 0.24	1.52
	1	2.95 ± 0.26	1.82 ± 0.11	1.71 ± 0.23	1.62
	2	3.44 ± 0.08	2.21 ± 0.02	2.08 ± 0.04	1.55
	2.5	3.51 ± 0.10	2.20 ± 0.05	2.05 ± 0.03	1.59
	3	1.96 ± 0.11	1.24 ± 0.08	1.16 ± 0.06	1.58
	5	2.05 ± 0.09	1.41 ± 0.13	1.29 ± 0.05	1.45
	10%	1.22 ± 0.14	0.93 ± 0.18	0.84 ± 0.04	1.30
3	Control	1.05 ± 0.02	0.66 ± 0.04	0.61 ± 0.04	1.58
	1	3.27 ± 0.08	2.01 ± 0.03	1.91 ± 0.06	1.60
	2	4.75 ± 0.07	2.86 ± 0.08	2.67 ± 0.01	1.66
	2.5	4.26 ± 0.10	2.64 ± 0.07	2.49 ± 0.02	1.61
	3	4.15 ± 0.11	2.56 ± 0.07	2.46 ± 0.03	1.62
	5	2.95 ± 0.09	1.87 ± 0.11	1.85 ± 0.04	1.57
	10%	2.55 ± 0.12	1.79 ± 0.10	1.37 ± 0.03	1.42
4	Control	1.56 ± 0.05	0.97 ± 0.03	0.81 ± 0.08	1.60
	1	3.55 ± 0.15	2.17 ± 0.21	2.21 ± 0.15	1.63
	2	5.76 ± 0.26	3.27 ± 0.13	3.23 ± 0.11	1.76
	2.5	5.11 ± 0.24	3.02 ± 0.12	2.96 ± 0.08	1.69
	3	4.91 ± 0.18	2.97 ± 0.19	2.94 ± 0.07	1.65
	5	3.26 ± 0.17	2.05 ± 0.09	2.08 ± 0.20	1.59
	10%	2.61 ± 0.11	1.77 ± 0.04	1.63 ± 0.10	1.47
5	Control	1.28 ± 0.13	0.78 ± 0.07	0.84 ± 0.06	1.64
	1	3.76 ± 0.31	2.26 ± 0.19	2.32 ± 0.18	1.66
	2	6.45 ± 0.22	3.60 ± 0.14	3.55 ± 0.12	1.79
	2.5	5.73 ± 0.20	3.33 ± 0.15	3.29 ± 0.10	1.72
	3	4.98 ± 0.18	2.94 ± 0.16	3.00 ± 0.09	1.69
	5	3.29 ± 0.13	2.06 ± 0.08	2.08 ± 0.04	1.60
	10%	2.50 ± 0.11	1.68 ± 0.07	1.52 ± 0.03	1.48

Mean standard errors for duplicate samples.

TABLE 2 - The effect of vinasse on the photosynthetic pigment contents of *Tetraselmis suecica*.

		µg pigment per ml culture			
Days	Vinasse Concentration (%)	Chl_a	Chl_b	Carotenoids	Chl_a / Chl_b
1	Control	0.62 ± 0.02	0.45 ± 0.08	0.47 ± 0.03	1.36
	1	0,74 ± 0.12	0.53 ± 0.10	0.53 ± 0.06	1.39
	2	0.68 ± 00.2	0.47 ± 0.02	0.46 ± 0.05	1.43
	2.5	0.81 ± 0.04	0.57 ± 0.03	0.55 ± 0.07	1.42
	3	0,49 ± 0.06	0.35 ± 0.06	0.33 ± 0.08	1.38
	5	0.45 ± 0.09	0.33 ± 0.04	0.30 ± 0.05	1.34
	10%	0.37 ± 0.13	0.29 ± 0.09	0.26 ± 0.07	1.25
2	Control	0.86 ± 0.12	0.60 ± 0.03	0.61 ± 0.21	1.41
	1	1,48 ± 0.26	1.03 ± 0.09	1.00 ± 0.19	1.43
	2	2,85 ± 0.08	1.97 ± 0.04	1.87 ± 0.03	1.44
	2.5	3,06 ± 0.10	2.02 ± 0.07	1.94 ± 0.04	1.51
	3	1.91 ± 0.10	1.29 ± 0.06	1.21 ± 0.06	1.47
	5	1,21 ± 0.07	0.84 ± 0.10	0.77 ± 0.02	1.43
	10%	0,58 ± 0.11	0.43 ± 0.16	0.42 ± 0.03	1.33
3	Control	1.24 ± 0.03	0.84 ± 0.02	0.80 ± 0.02	1.46
	1	2,03 ± 0.07	1.36 ± 0.01	1.28 ± 0.05	1.49
	2	3.26 ± 0.09	1.98 ± 0.06	1.85 ± 0.02	1.64
	2.5	3.85 ± 0.11	2.43 ± 0.05	2.27 ± 0.03	1.58
	3	2.87 ± 0.12	1.91 ± 0.04	1.73 ± 0.02	1.50
	5	1.51 ± 0.08	1.01 ± 0.13	0.91 ± 0.05	1.49
	10%	0.74 ± 0.10	0.52 ± 0.09	0.49 ± 0.02	1.42
4	Control	1.45 ± 0.06	0.97 ± 0.02	0.98 ± 0.06	1.48
	1	3.15 ± 0.12	2.00 ± 0.18	1.94 ± 0.13	1.57
	2	3.97 ± 0.23	2.45 ± 0.11	2.33 ± 0.11	1.62
	2.5	4.56 ± 0.21	2.68 ± 0.13	2.45 ± 0.07	1.70
	3	3.62 ± 0.14	2.32 ± 0.14	2.16 ± 0.08	1.56
	5	1.81 ± 0.15	1.14 ± 0.07	1.05 ± 0.18	1.58
	10%	0.80 ± 0.10	0.53 ± 0.03	0.49 ± 0.12	1.51
5	Control	1.51 ± 0.11	0.98 ± 0.05	0.97 ± 0.08	1.53
	1	3.20 ± 0.27	2.01 ± 0.17	2.03 ± 0.16	1.59
	2	4.01 ± 0.20	2.48 ± 0.12	2.61 ± 0.10	1.65
	2.5	4.65 ± 0.20	2.71 ± 0.13	2.63 ± 0.12	1.71
	3	3.71 ± 0.13	2.33 ± 0.14	2.24 ± 0.07	1.59
	5	1.85 ± 0.11	1.15 ± 0.06	1.08 ± 0.05	1.61
	10%	0.79 ± 0.09	0.52 ± 0.05	0.50 ± 0.01	1.50

Mean standard errors for duplicate samples.

RESULTS

The effects of vinasse on the growth of *Chlorella sp.* and *Tetraselmis suecica*

The lower concentrations of vinasse had a positive effect on cell number and protein content but at higher concentrations inhibited the growth and protein content of the algae (highest cell numbers and protein content of *Chlorella sp.* and *Tetraselmis suecica* in cultures with 2.5 % vinasse (Figs. 1 and 2). In general, the higher concentrations of vinasse (5 and 10%) decreased the cell number and protein content of *Chlorella sp.* and *Tetraselmis suecica.*

TABLE 3
Biomass, protein and protein contents in biomass of *Chlorella sp.*

Concentrations	Productivity mg/ml	Protein %	Protein in biomass(mg)
Control	265	43,1	114,21
1	288	45,6	131,22
2	428	54,4	232,83
2,5	416	53,5	222,56
3	411	52,3	214,955
5	271	42,4	114,90
10%	215	37,2	79,98

TABLE 4 - Biomass, protein and protein contents in biomass of *Tetraselmis suecica*.

Concentrations	Productivity mg/ml	Protein %	Protein in biomass (mg)
Control	230	43,0	98,90
1	263	45,2	118,87
2	378	51,2	194,29
2,5	386	52,2	201,49
3	365	50,1	182,86
5	214	40,3	86,24
10%	175	34,6	60,55

The effects of vinasse on the contents of photosynthetic pigments of *Chlorella sp.* and *Tetraselmis suecica*

The effects of vinasse on the contents of Chlorophyll a, b, carotenoids and the ratios of chlorophyll a to chlorophyll b (Chl a/b) are shown in Tables 1 and 2. The lower concentrations of vinasse (1, 2, 2.5 and 3 %) enhanced both chlorophyll a and b, and carotenoid contents, while higher concentrations (5 and 10 %) caused a decrease. The Chl a/b ratio showed a gradual increase from the control up to 3 % vinasse, but decreased at higher concentrations of vinasse (Tables 1 and 2). At the end of cultivation the effect of vinasse on biomass yield, protein and protein in biomass of *Chlorella sp* and *Tetraselmis suecica* were similar to that on the cell number (Tables 3 and 4).

DISCUSSION

The vinasse is an effluent rich in both organic and inorganic materials [3]. Some other workers have reported that vinasse has a high BOD and COD load and it causes serious pollution problems near the factory [14, 15]. At the beginning of this research work we supposed that the effluent would have a toxic effect on the growth of *Chlorella sp.* but we were surprised to find that it had a beneficial effect on these species at lower concentrations (especially with 1 and 2% concentrations of vinasse).

As *Chlorella sp* and *Tetraselmis suecica* are much more resistant to adverse environmental conditions, especially to different chemical compounds and heavy metals than the organisms such as bacteria [16], the minerals from vinasse would not be expected to affect the growth of alga adversely.

Furthermore, it was reported that the P uptake rate decreased when P is abundant but N is depleted in *Scenedesmus* cultures, and the higher the N concentrations, the greater the uptake rate of P [17]. Both N and P are present in vinasse [2]. It was also reported that Mg, present in vinasse, has an important effect on the synthesis of photosynthetic pigments [15]. According to the above suggestions, the increasing of cell numbers and the contents of photosynthetic pigments may depend on the presence of these minerals in the cultures at the optimum concentrations.

Although algal cells are resistant to abnormal conditions their long-term incubation under such conditions may cause cell damage and so decrease the cell numbers. Some researchers have reported that vinasse and similar wastes have an osmotic effect on plants [15] because of their high organic - matter content. The present toxic effects of vinasse at higher concentrations may be caused by the same effect. The cell numbers decreased but there was no similar decrease in photosynthetic pigments. This result may depend on the degradation of cells and the passage of photosynthetic pigments from the cytoplasm to the cultures.

It is also possible that some heavy metals such as Zn, Mn and Fe prevent algal growth at higher concentrations of vinasse. Some heavy metal-toxicity has been observed in the case of *Chlorella vulgaris* [18].

The Chl a/b ratio is used as an indicator of adverse environmental conditions [19]. In our experiment, the Chl a/b ratio increased up to 3% concentration of vinasse but decreased at higher concentrations. The results suggest that *Chlorella sp* and *Tetraselmis suecica* might have a use in a system for reduction of the polluting potential of vinasse.

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CONTAMINATION OF HUMAN PLACENTAS WITH ORGANOCHLORINE COMPOUNDS IN FIVE SLOVAK REGIONS RELATED TO DIFFERENT ENVIRONMENTAL CHARACTERISTICS

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SUMMARY

Placental contamination with xenobiotics may act as a biological marker for the exposure of mother, or foetus *via* transplacental transfer. Human placental samples were collected after full-term deliveries in five Slovak regions and analysed for residues of 20 selected organochlorine compounds. The regions were chosen according to their predominant environmental pollution: organic chemical industry, metallurgical industry, agriculture, mining and metallurgy and a rural region devoid of any industry. Placental contents of 12 (1,4+1,3-DCB, 1,3,5-TCB, 1,2,3-TCB, TeCB, PeCB, HCB, α-HCH, ß-HCH, γ-HCH, δ-HCH, p,p´-DDT and PCB 101) out of 20 organochlorine compounds were higher in the region polluted primarily with organic chemicals when compared to the other regions investigated. Concentrations of p,p´-DDE and PCB congeners 28, 52, 138, 153 and 180 were highest in the region with intensive agricultural production. In contrast, the lowest concentrations of 9 organochlorine compounds were found in both regions polluted by the waste derived from iron-ore mining/ processing. Results revealed that placental contamination reflected the regional human activities concerning the production or using of organochlorine compounds.

KEYWORDS:
Human placenta; organochlorine compounds; xenobiotics.

INTRODUCTION

Human placental contamination with organic xenobiotics may act as a biological marker for the exposure of the pregnant woman, or the foetus via transplacental transfer. Organochlorine insecticides and polychlorinated biphenyls (PCBs) are suspected to act in reproductive pathology (1-3). In living organisms they are stored and accumulated mainly in the adipose tissue. Due to their high solubility in fat they are easily transported across the biological membranes (including placental barrier) and they reach developing embryo/foetus. In the Slovak Republic (SR), PCB congeners were detected in human food chain encountering human breast milk, where the PCBs levels were found to be higher comparing to the cow's milk (4,5).

The aim of this study was to compare the level of the contamination of human placentas with selected organochlorine compounds in five Slovak regions in relation to their different regional environmental characteristics and anthropogenic activities.

MATERIALS AND METHODS

Study design: The study was based on 220 full-term deliveries in five selected Slovak regions (44 samples per region), chosen with respect to predominant type of environmental pollution. Mothers were selected according to the following criteria: full-term pregnancy (40 weeks ±2), no occupational exposure to organochlorine compounds, residency in the region at least 3 years before the conception, and informed consent from the mother. The Research Ethics Committee of the Institute of Preventive and Clinical Medicine approved the study.

The study sites were selected according to data published in annual reports of the Ministry of Environment and the Slovak Hydro-meteorological Institute in the SR, and Final Report of PHARE Project (6). The selected regions represented pollution derived from chemical industry, metallurgy, agricultural activity as well as a relatively clean rural region.

Bratislava (Region 1): The capital city of the SR. The main sources of air pollution are derived from chemical industry (i.e. petrol, pesticide, and rubber industries), power generation and traffic-related pollutants. High concentrations of organic compounds were found in water. Soil is polluted mainly by polycyclic aromatic hydrocarbons (PAHs).

TABLE 1 - List of selected persistent organochlorine compounds

A	1,4+1,3-DCB	1,4+1,3-dichlorobenzene
	1,2-DCB	1,2-dichlorobenzene
	1,3,5-TCB	1,3,5-trichlorobenzene
	1,2,4-TCB	1,2,4-trichlorobenzene
	1,2,3-TCB	1,2,3-trichlorobenzene
	TeCB	Σ(1,2,3,5+1,2,4,5)tetrachlorobenzene
	PeCB	pentachlorobenzene
	HCB	hexachlorobenzene
B	α-HCH	alpha-hexachlorocyclohexane
	ß-HCH	beta-hexachlorocyclohexane
	γ-HCH	gamma-hexachlorocyclohexane
	δ-HCH	delta-hexachlorocyclohexane
	p,p´-DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
	p,p´-DDE	1´,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
C	PCB - 28	(2,4,4´- trichlorobiphenyl)
	PCB - 52	(2,2´,5,5´- tetrachlorobiphenyl)
	PCB -101	(2,2´,4,5,5´- pentachlorobiphenyl)
	PCB -138	(2,2´,3,4,4´,5´- hexachlorobiphenyl)
	PCB -153	(2,2´,4,4´,5,5´- hexachlorobiphenyl)
	PCB -180	(2,2´,3,4,4´,5,5´- heptachlorobiphenyl)

A - chlorinated benzenes, B - organochlorine insecticides,
C - polychlorinated biphenyls (indicator congeners according IUPAC)

TABLE 2 - Median and maximum concentrations of selected organochlorine compounds [mg.kg^{-1}.10^{-3}] in human placental samples in 5 Slovak regions

	Organochlorine compounds	Region 1		Region 2		Region 3		Region 4		Region 5	
		med	max	med	max	med	max	med	max	med	max
A	1,4+1,3-DCB	1.4	218.0	0.2	10.2	0.0	45.0	0.0	99.5	0.8	26.9
	1,2-DCB	0.8	46.9	0.1	1.3	0.0	0.2	0.0	0.8	8.1	64.3
	1,3,5-TCB	10.2	310.4	0.0	0.3	0.0	2.2	0.0	0.5	0.7	31.5
	1,2,4-TCB	0.5	41.9	0.0	2.3	0.1	1.1	0.0	2.4	1.9	50.5
	1,2,3-TCB	0.7	3.0	0.0	1.0	0.0	0.9	0.0	1.1	0.5	3.5
	TeCB	0.1	12.7	0.0	19.6	0.0	4.7	0.0	3.2	0.0	10.4
	PeCB	0.4	102.2	0.0	0.3	0.0	0.1	0.0	0.4	0.2	7.0
	HCB	0.6	72.0	0.2	1.6	0.2	8.4	0.3	3.0	0.4	2.0
B	α-HCH	0.2	4.0	0.0	0.1	0.1	1.3	0.0	0.7	0.0	5.4
	ß-HCH	0.3	12.0	0.0	0.3	0.0	1.2	0.2	0.9	0.1	11.2
	γ-HCH	0.6	17.5	0.0	0.2	0.6	2.1	0.3	2.0	0.2	33.1
	δ-HCH	0.3	5.4	0.0	0.0	0.0	0.3	0.0	0.0	0.0	194.6
	p,p´-DDT	0.1	3.5	0.1	10.0	0.0	0.3	0.0	1.1	0.0	5.2
	p,p´-DDE	0.1	2.2	0.3	1.9	0.3	2.0	0.1	2.8	0.1	2.0
C	PCB-28	0.1	4.0	0.0	11.0	0.0	0.3	0.0	1.4	0.0	0.2
	PCB-52	0.1	0.6	1.0	7.4	0.0	1.1	0.0	1.3	0.0	2.0
	PCB-101	0.2	109.0	0.1	6.0	0.0	2.0	0.0	5.0	0.0	8.9
	PCB-138	0.2	7.9	0.2	11.8	0.1	0.4	0.1	2.1	0.0	6.4
	PCB-153	0.2	124.8	0.3	15.5	0.1	0.9	0.0	3.2	0.1	24.4
	PCB-180	0.1	1.9	0.2	11.4	0.0	9.1	0.0	24.6	0.0	0.1

A - chlorinated benzenes, B - organochlorine insecticides, C - polychlorinated biphenyls,
n.d. - below detection limit, med – median concentration, max – maximum concentration
Note: Minimum concentration was equal 0 in every region

Nové Zámky (Region 2): Agricultural region with high usage of fertilizers. Inhabitants of this region have a shorter life expectancy when compared to other Slovak regions. This region belongs to the regions with highest crude mortality rate in the SR.

Spisska Nova Ves (Region 3): Air, soil and water are polluted mainly due to the release of heavy metals into environment from the mining of iron-ore, the mercury recycling plant and copper smelter, although there was a decline in metal mining after 1992. Other sources of pollution are power generation and local heating. High concentrations of mercury, arsenic, cadmium, lead, copper and zinc were found in water and soil.

Košice (Region 4): The city is known for its metallurgic industry (East-Slovakian Iron Works). Additional sources of pollution include power generation and traffic. In the past, it was home to a magnesite-processing plant. The city belongs among the most polluted regions in the SR.

Stará Lubovna (Region 5): It is predominantly a rural region, only partially agricultural. Although there is no source of industrial pollution in this area, the cross-country traffic through the region has been increasing over the last several years.

Placental samples collection: Samples of full-term placentas (about 30 g of full-thickness tissue) were uniformly excised in a triangular shape (from periumbilical to marginal zones), and kept at -20 °C until their processing in a laboratory.

Organochlorine compounds determination in placental samples: Placental residues of 20 organochlorine compounds (chlorinated benzenes, organochlorine insecticides and congeners of polychlorinated biphenyls) were analyzed. The list of analytes is given in Table 1.

A sample preparation comprised three steps: 1) sample homogenisation, 2) Soxhlet extraction, and 3) clean-up procedure. For analytical identification and determination of the compounds, the method of capillary gas chromatography using ^{63}Ni electron capture detection was used. The following chemicals were used: Solvents: acetone, n-hexane, n-heptane and dichloromethane were purchased from Merck, Bratislava, Slovakia. All solvents were pesticide grade. Standards: The standards of PCB indicator congeners IUPAC No. 28, 52, 101, 138, 153 and 180 were purchased from the Slovak Institute for Metrology, Bratislava, Slovakia. The standard mixtures of organochlorine insecticides and chlorinated benzenes were purchased from Supelco SA, Gland, Switzerland, and from Aldrich, Steinheim, Germany. Sorbents: Florisil (60/100 mesh) was washed with bidistilled water, acetone and n-hexane. Anhydrous sodium sulphate was Soxhlet-extracted with n-hexane to avoid possible interference.

Sample preparation and extraction: A sample of approximately 10 g of placental tissue was homogenised with sodium sulfate in a grinder to give a pulverised consistency. Then the sample was quantitatively replaced in a Soxhlet apparatus and allowed for 5 hours of Soxhlet extraction with 250 ml of n-hexane. The extract was evaporated on a vacuum rotary evaporator to a volume of 5 ml. The clean-up and gas chromatography procedures used in this study were based on the methods published earlier (5,7).

Validation of method: The method was validated by six parallel recovery experiments using spiked placental tissue samples at the level of 1 ug.kg^{-1}. The recoveries for chlorinated benzenes and insecticides were from 83.2 ± 4.2 % to 94.2 ± 1.8 %. For polychlorinated biphenyls, the recoveries varied between 90.3 and 92.5 ± 5 %. The limit of quantification (LOQ) was 0.1 ug.kg^{-1} .

Statistical analysis: Statistical analysis was carried out using SPSS for Windows (SPSS, Inc., Chicago, USA). Kolmogorov-Smirnov's test to assess the normality of data distribution and non-parametric Kruskal-Willis and Wilcoxon's tests corrected for ties for placental organochlorine compounds' comparison among the selected regions were used. Results of $p<0.05$ were considered as statistically significant.

RESULTS

Table 1 shows the List of organochlorine compounds analysed in the human placental samples collected in the five selected regions on the territory of Slovakia. The median and maximum concentrations of 20 selected organochlorine compounds are shown in Table 2.

In Table 3, the comparison of the rate of negative human placental samples among five Slovak regions is demonstrated.

The statistical analysis of the placental organochlorine compound concentrations revealed that the contents of 12 (e.g. 1,4+1,3-DCB, 1,3,5-TCB, 1,2,3-TCB, TeCB, PeCB, HCB, α-HCH, ß-HCH, γ-HCH, δ-HCH, p,p'-DDT and PCB 101) out of 20 organochlorine compounds analysed were higher in the Region 1 polluted primarily by chemical industry when compared to the other investigated regions. However, statistically significant differences with respect to Regions 2, 3, 4 and 5 was found for the following substances: 1,3,5-TCB, HCB, α-HCH and δ-HCH ($P<0.05$ and $P<0.001$, respectively).

The contents of p,p´-DDE and PCB congeners 52 and 180 were found to be significantly highest ($P< 0.05$ and $P < 0.001$) in the agricultural Region 2. The levels of PCB congeners 28, 138 and 153 were comparable between Region 1 and Region 2, but significantly higher ($P < 0.05$ and $P < 0.001$) in comparison with Region 3 - 5.

TABLE 3
Organochlorine compounds in human placental samples below detection limits: comparison among 5 Slovak regions

	Organochlorine compounds	Regional frequency of negative samples [% n.d.]				
		Region 1	Region 2	Region 3	Region 4	Region 5
A	1,4+1,3-DCB	19	45	66	60	21
	1,2-DCB	18	25	90	90	18
	1,3,5-TCB	11	80	94	96	14
	1,2,4-TCB	14	57	46	52	13
	1,2,3-TCB	14	72	64	52	32
	TeCB	28	53	70	94	68
	PeCB	23	82	94	86	32
	HCB	2	12	28	30	25
B	α-HCH	19	96	44	64	70
	ß-HCH	12	71	60	18	59
	γ-HCH	5	71	12	32	40
	δ-HCH	25	100	94	100	65
	p,p´-DDT	26	39	96	90	54
	p,p´-DDE	12	4	26	44	24
C	PCB - 28	14	31	78	86	73
	PCB - 52	12	4	90	94	67
	PCB - 101	26	29	56	54	76
	PCB - 138	5	8	46	44	60
	PCB - 153	9	4	48	68	46
	PCB - 180	32	31	60	54	81

A - chlorinated benzenes, B - organochlorine insecticides, C - polychlorinated biphenyls (indicator congeners).
n.d. - below detection limit (negative placental samples

It was of interest, that among the investigated regions, the lower concentrations of 9 organochlorine compounds (e.g. 1,2-DCB, 1,3,5-TCB, TeCB, PeCB, p.p´-DDT, PCB 28, PCB 52, PCB 138 and PCB 153) were found to be in the both regions polluted chiefly by iron-ore mining (Region 3) or iron-ore metallurgy (Region 4). Furthermore, the higher rate of negative placental samples (e.g. samples with concentrations of organochlorine compounds below detection limits) among the regions was found also in these two regions (Table 3).

DISCUSSION

Human exposure to organochlorine compounds in the environment can be determined in samples from various tissues, encountered foetal and placental tissues. Morphological changes in foetal and placental tissue following exposure of pregnant minks to PCBs demonstrated predominantly by degenerative changes in maternal endothelium and extensive placental infarction were observed (3). Experimental findings revealed that organochlorine compounds may induce oxidative stress in fetal and placental tissue resulting in tissue damage. For example, in C57BL/6J and DBA/2J pregnant mice, following oral administration (on day 12 of gestation) of pesticides (lindane and endrin), production of superoxide anion and lipid peroxidation, and DNA-single strand breaks were found (8). Other experiments have pointed to possible growth retardation or fetal death in mink. Additionally, morphological changes in fetal and placental tissue have been observed, as demonstrated by degenerative changes and extensive placental infarction. Lectin staining has revealed the effects of PCB toxicity, shown by increased injury to maternal endothelium and severe trophoblastic damage (3). Normal human placenta secretes lipid peroxides that can circulate in pregnant women (9). It suggests that the mother's environmental exposure to organochlorine compounds might evoke the enhancement of lipid peroxides secretion in human placenta. Lipid peroxides are responsible for endothelial cell impairment and vasoconstriction, leading to preeclampsia and risk pregnancy. Oxidative stress might be evoked by drugs, hormones, and various environmental chemicals, such as organochlorine pesticides (10). These findings are of extraordinary interest if related to the data on the increased inflammatory cytokines production in the human placenta under the hypoxic conditions (11).

In our previous work we have found the higher proportion of pathological microstructural deviations in the human placental samples collected from the Region 1

polluted by organic chemical industry in comparison to the rural Region 5 (12). Coincidentally, in the Region 1 we demonstrated the higher proportion of the newborns with the elevated total immunoglobulin E (uIgE) in the umbilical blood (used as a biomarker of intrauterine allergic sensitisation) when compared to the Region 5. We have demonstrated a significant interindividual positive correlation between the p,p´-DDE placental concentration and the total uIgE in the same cohort (13). Our findings demonstrated the global exposure of pregnant women to organochlorine compounds with respect to a predominant type of pollutants in their environment. From the analytical point of view, the assessment of the human placental contamination with organochlorine compounds should be careful due to their low values determined in the placental tissue (due to a low fat content in the placenta), when compared to concentrations in mother's or cow's milk. On the other hand, the differences in the placental contamination found among the selected regions clearly reflected the major regional human activities aimed at a production or using of organochlorine compounds. The period of intrauterine development of the embryo/foetus is highly susceptible to adverse impacts of organochlorine compounds, hence further research focused on the risk of pregnancy, prenatal pathology and immunology is highly needed.

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REMOVAL OF 3(5)-AMINO-1,2,4-TRIAZOLE HERBICIDE FROM AQUEOUS SOLUTION BY TITANIUM DIOXIDE TiO_2 COUPLED TO SIMULATED SUNLIGHT

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SUMMARY

We investigated in preliminary studies the removal of 3(5)-amino-1,2,4-triazole in aqueous solution by the photodegradation processes in the presence of titranium dioxide (TiO_2) at different ratios of herbicide/TiO_2 (weight/ weight) coupled with simulated sunlight. This photocatalytic degradation was evaluated at pH values 5, 6 and 7.

Irradiation was carried out with polychromatic light using heraeus apparatus equipped with xenon lamp having a spectral energy distribution similar to solar irradiation (> 290 nm).

The concentration of remaining herbicide was monitored using a high pressure liquid chromatograph (HPLC) equipped with UV detector at 205 nm.

The presence of TiO_2 induced an increase of the photodegradation of the pesticide with respect to pure water solution. By varying the pH in the range 5 - 7, no effect on rate of photodegradation of 3-amino-1,2,4-triazole with TiO_2 (ratio 1/5) was observed.

KEYWORDS:
3-amino-1,2,4-triazole, removal, TiO_2, pH, aqueous solution

INTRODUCTION

3-amino-1,2,4-triazole (named amitrol, ATA, amizol) is a non selective systemic herbicide (1,2). It is used on non-cropland for control of annual grasses and perennial and annual broadleaf weeds, for poison ivy control and for control of aquatic weeds in marshes and drainage ditches (3).

3-amino-1,2,4-triazole is also an interesting compound in materials science, since it has been proposed as raw material in synthesis of ferromagnetic materials through formation of binuclear complexes (4). It is readily soluble in water and does not adsorb strongly to soil particles (5). In aquatic environment, this pesticide does not break down by hydrolysis or photolysis (6). However, concentration of 3-amino-1,2,4-triazole, which would be found in surface and groundwater, will exceed the CE limit (0,1µ/L) and will be a potential pollutant of water.

In recent years, interest has focused on the use of photocatalyst for the destruction of such pollutant. UV plus semiconductor photocatalyst titanium dioxide may be a very efficient tool for removing these polluants in the final stages of water treatment (7). The catalyst is not consumed during the reaction and can carry out oxidations and reductions simultaneously.

In this paper, we have investigated the use of TiO_2, coupled with simulated sunlight, to remove 3-amino-1,2,4-triazole from aqueous solution.

MATERIALS AND METHODS

Chemicals

3-amino-1,2,4-triazole was purchased from commercial source at purity greater than 99%. Ultra pure water was used, HPLC reagents were from Riedel de Haen and TiO_2 was from Degussa. Concentration of 50 mg/L of 3-amino-1,2,4-triazole was used. Experiments were carried out at pH = 5, 6 and 7 (pH of environmental interest). The pH 7 was buffered with a mixture of K_2HPO_4 $3.9.10^{-4}$ mol/L and KH_2PO_4 $6.1.10^{-4}$ mol/L (1/1; v/v). The other values of pH 5 and 6 were adjusted by concentrated H_3PO_4 and NaOH.

Physical properties (8)

Chemical name : 3-amino-1,2,4-triazole

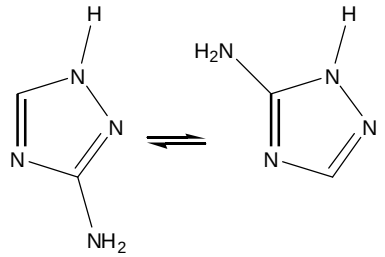
Molecular formula : $C_2H_4N_4$

Molecular weight : 84,1

Melting Point : 159°C

Solubility: Soluble in water (28% at 25°C) and ethanol (26% at 75°C), slightly soluble in chloroforme and methylene chloride.

Structure:



Analytical procedure

UV-VIS absorption spectra were recorded using Uvikon 930 spectrophotometer. The disappearance of 3-amino-1,2,4-triazole at various illumination times was determined by HPLC system equipped with UV detector at 205 nm and C_{18} DS_2 column (25 × 4.6 mm i.d). The mobile phase was composed of 50% water + 50% acetonitrile adjusted to pH ~ 3 by concentred H_3PO_4. The flux rate was 1ml/min. The retention time under the chromatographic conditions described was 4.2 min.

Photochemical procedure

The photodegradation experiments were performed using a cylindrical photoreactor placed in suntest heraeus apparatus. The irradiation source was a Xenon lamp (T = 20°C) which provides a good simulation of solar light. The lamp was placed above the surface of the solution. The solution was stirred with magnetic stirrer. In order to ensure steady state irradiation condition, the apparatus was switched on at least 30 min before starting the experiment.

Procedure

Fifty ml of 3-amino-1,2,4-triazole was exposed for photodegradation in the presence of TiO_2 at various illumination times. The catalyst which was not consumed, was filtred through a millipore filter, before a HPLC analysis.

RESULTS AND DISCUSSION

The herbicide 3-Amino-1,2,4-triazole does not absorb wavelengths above 290 nm (Fig. 1) and it is not expected to be photodegraded and removed by direct photolytic processes in aqueous solution.

The first stages of the degradation and removal of this herbicide were investigated in the presence of TiO_2 at different ratios of herbicide/TiO_2 (weight/weight). The wavelength which appears to be most effective is 365 nm, but we are now looking for at what degree the sunlight could be used to contribute to this effect.

FIGURE 1 - Scanned UV spectrum of 3-amino-1,2,4-triazole in buffered solutions at pH = 5, 6 and 7.

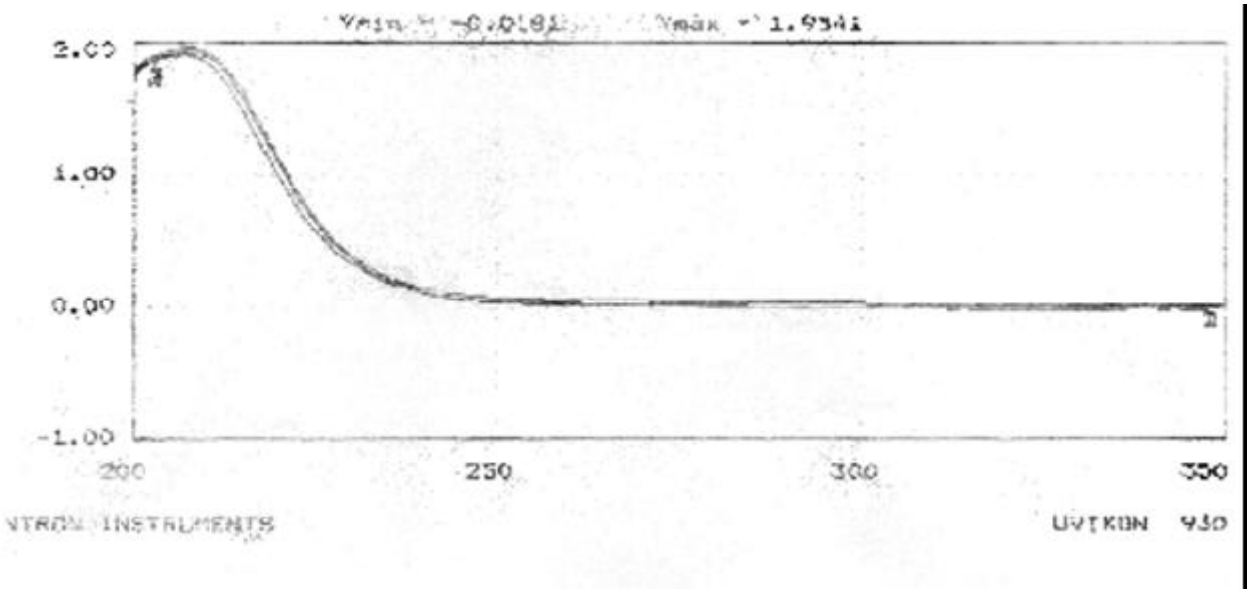


FIGURE 2 – Disappearance of 3-amino-1,2,4- triazole in the presence of TiO_2 at different ratios (a) without TiO_2, (b) with TiO_2: 1/1, (c) with TiO_2: 1/3, (d) with TiO_2: 1/5

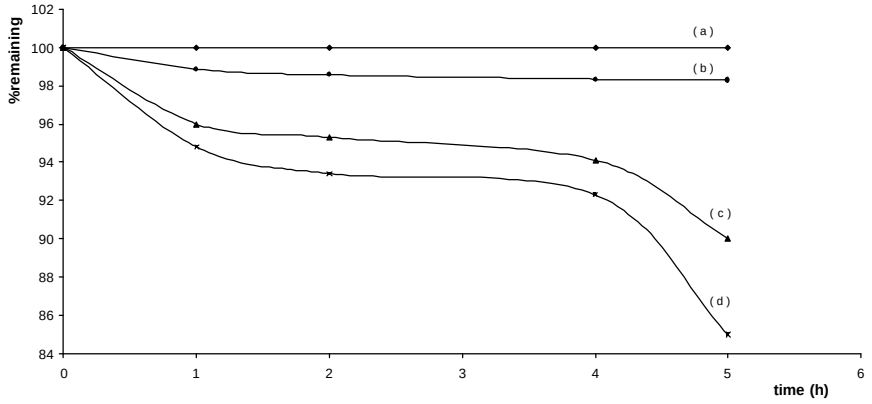


FIGURE 3 – Disappearance of 3-amino-1,2,4-triazole and its metabolite in the presence of TiO_2 (a) 3-amino-1,2,4-triazole without TiO_2, (b) 3-amino-1,2,4-triazole with TiO_2 ratio 1/5, (c) the metabolite

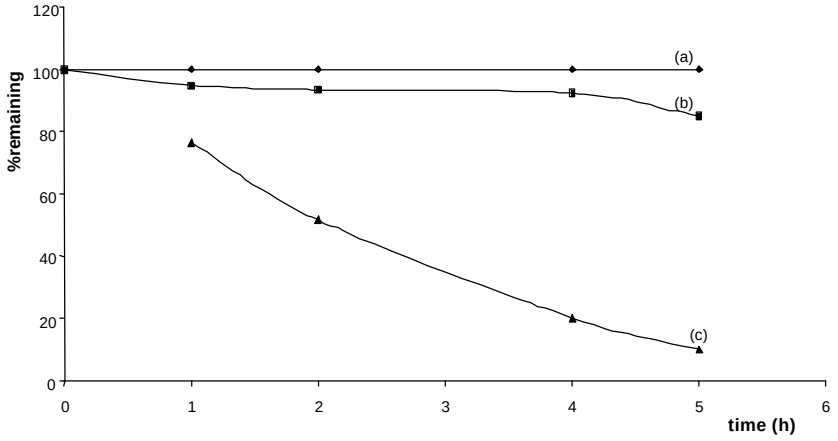
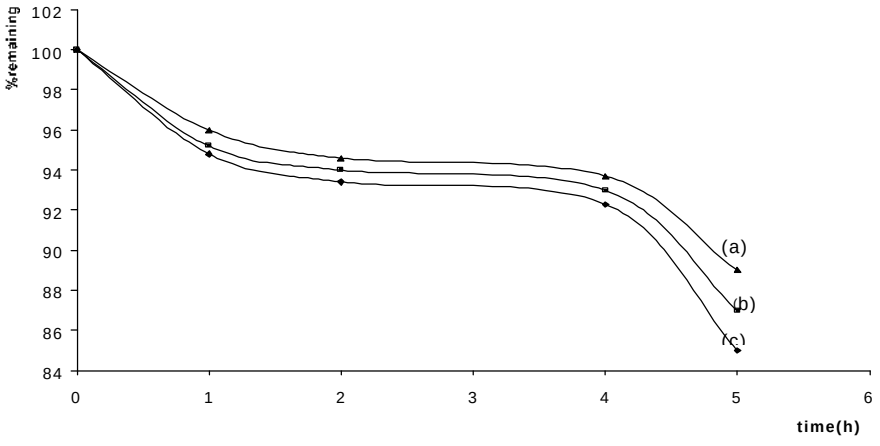


FIGURE 4 – Comparison of photolysis of 3-amino-1,2,4-triazole in the presence of TiO2 at three pH values (a) pH = 5, (b) pH = 6, (c) pH = 7



In Figure 2, the disappearance of 3-amino-1,2,4-triazole in the presence of TiO_2 at pH = 7 is reported.

The investigation was performed on a series of herbicide/TiO_2 ratios. The concentrations of titanium dioxide used to evaluate the photocatalytic destruction of 3-amino-1,2,4-triazole greatly exceeded that which occurs naturally. More destruction of the herbicide was observed for the ratio 1/5 herbicide /TiO_2 (weight/weight) (Fig. 2).

During irradiation TiO_2 generates $OH^{\cdot}$ species, and these radicals then oxidise the polluting molecule.

The sample that was irradiated without TiO_2 showed no degradation (Fig. 2), because of the negligible absorptivity of the 3-amino-1,2,4-triazole in the UV region at wavelengths greater than 290 nm.

During the HPLC analysis, one main metabolite was detected (Fig. 3). It is appeared after 1 hour of irradiation of 1/5 ratio solution. After 5 hours of irradiation, only 10% of this metabolite remained in the medium. This does not mean that this metabolite is not formed, because it can quickly be decomposed by $OH^{\cdot}$ generated during the irradiation.

pH effect on photocatalysis of 3-amino-1,2,4-triazole

In this part we studied the effect of pH on photocatalytic degradation of 3-amino-1,2,4-triazole. The pH's 5 and 6 were choosen since from pH = 5 to pH = 9 is the range of pH of environmental interest.

In Figure 4 the disappearance of the herbicide (herbicide/TiO_2 ratio: 1/5) in buffered solution is shown. No effect on rate of photocatalysis of 3-amino-1,2,4-triazole was observed for all pH's.

CONCLUSION

Preliminary studies on sunlight induced degradation show that the semiconductor TiO_2 plays a fundamental role on the removal of 3-amino-1,2,4-triazole from aqueous solution.

The photodegradation of this herbicide has been found to be efficiently improved by TiO_2 coupled with simulated sunlight. As a result, photocatalysis by TiO_2 and solar light can be considered as one of the abiotic pathways leading to the degradation and removal of 3-amino-1,2,4-triazole in the environment.

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NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NITRITE IN WATER

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SUMMARY

A selective and rapid spectrophotometric method for the determination of nitrite is presented. It relies on the reaction of nitrite with p-aminoacetophenone to form a diazonium ion, which is coupled with citrazinic acid in a basic medium to form an azo dye, which shows an absorption maximum at 495 nm. The colour is stable for 3h. Beer's law is obeyed in the range of 0.5 to 12 µg of nitrite in a final volume of 10 ml, with a molar absorptivity and its Sandell's sensitivity are 2.9×10^4 l mol^{-1} cm^{-1} and 16 ng cm-^{2}, respectively. The optimum reaction conditions and other analytical parameters are evaluated. The method has been successfully applied to the determination of nitrite in natural waters.

KEYWORDS: Spectrophotometry, p-Aminoacetophenone, Citrazic acid, Nitrite determination

INTRODUCTION

Nitrite in water is either due to oxidation of ammonium compounds or due to reduction of nitrate. As an intermediate stage in the nitrogen cycle, it is unstable. Usual concentration of nitrite in natural waters are in the range of some tenths of mg/litre. Higher concentrations are present in industrial water, sewage and in biologically purified effluents and in polluted streams. Very high nitrite levels are usually associated with water of unsatisfactory microbiological activity. The determination of nitrite is of considerable interest because of many reasons. Nitrite is the actual etiologic agent of methemoglobinemia. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines to form nitrosamines, many of which are known to be carcinogens[1].

General procedures for the determination of nitrites are usually based upon some form of diazotization reaction. Many spectrophotometric methods[2-11] and spectrofluorimetric methods[12,13] have been reported for the determination of trace amounts of nitrite based on diazotization reaction, and subsequent coupling to form an azo dye, often involve close control of pH and temperature during diazotization step as well as relatively long coupling times. Some methods often involve carcinogenic materials for the determination of nitrite, as the naphthylamines. Other methods, such as liquid chromatography[14, 15], voltammetry [16, 17] and the use of chemically modified electrodes[18, 19] and also a number of kinetic methods have been reported for nitrite determination[20-22]. However, some of these methods are characterized by non–linear calibration graphs, whereas others lack the high sensitivity and involves rigorous experimental conditions.

The purpose of this work is to provide a facile, rapid and selective method for the determination of nitrite in natural waters without involving complicated steps, using citrazinic acid as a new coupling agent.

EXPERIMENTAL

Apparatus

All absorbance measurements were made with a Jasco model UVIDEC - 610 and Elico model CL – 27 spectrophotometers with 1cm matched cells.

Reagents

All chemicals used were of analytical reagent grade, and distilled water was used for preparing the reagent solutions.

Standard nitrite solution (1 mg/ml). Prepared by dissolving 0.15 g sodium nitrite in water and diluting to 100 ml with water. Working standards were prepared by appropriate dilution.

p-Aminoacetophenone (0.05%). Prepared by dissolving 0.125 g of p-aminoacetophenone in 55.3 ml concentrated HCl and diluting to 250 ml with water.

Citrazinic acid (0.1%). Freshly prepared by dissolving 0.1 g of citrazinic acid in 2 ml of 4 M NaOH and diluting to 100 ml with water.

Sodium hydroxide solution (4M, 1M). Prepared by dissolving 16g/4g of NaOH in water and diluting to 100 ml with water.

EDTA (0.2M). Prepared by dissolving 7.44 g EDTA in 100 ml water.

Procedures

General procedure for the determination of nitrite:

An aliquot of the sample solution containing 0.5 to 12 μg of nitrite was transferred into a series of 10 ml calibrated flasks. To this solution, was added 0.5 ml of 0.05% p-aminoaceto-phenone and the solution was shaken thoroughly for 2 min to allow the diazotization reaction to go to completion. Then, a volume of 1.5 ml of 0.1% citrazinic acid and 3 ml of 4 M sodium hydroxide solution were added and contents were diluted with distilled water and mixed well. After 5 min, absorbance of the coloured azo dye was measured at 495 nm against the reagent blank.

Procedure for the determination of nitrite in water samples:

An aliquot (≤ 4 ml) of the sample containing not more than 12 μg of nitrite was treated with 0.5 ml 1M NaOH and 0.5 ml 0.2 M EDTA. The solution was mixed and centrifuged to remove any precipitate formed. The centrifugate was transferred to 10 ml calibrated flask and treated with 0.5 ml of 0.05% p-aminoacetophenone, mixed well, and allowed to stand for 2 min. Then, 1.5 ml of 0.1% citrazinic acid and 3 ml of 4 M NaOH were added and the contents were diluted to the mark with distilled water and mixed well. After 5 min, absorbance of the coloured azo dye was measured at 495 nm against reagent blank. Concentration of nitrite was established by reference to the calibration graph prepared by using 0–12 μg of nitrite in 10 ml standard flask using distilled water.

RESULTS AND DISCUSSION

Preliminary studies were carried out using 10 μg of standard solution in a final volume of 10 ml. The absorption spectra of the coloured azo dye shows maximum absorption at 495 nm (Figure. 1). Under the recommended conditions the dye is stable for 3 h.

Effect of acid concentration and temperature on diazotization.

Diazotization was carried out at room temperature (ca 25 ± 5 °C), and cooling to 0-5 °C was not necessary. The effect of acidity on the diazotization reaction was studied in the range 0.1–0.5 M hydrochloric acid, and constant absorbance was observed in this range. Above this range, a decrease in the absorbance was observed. The optimum acidity for the diazotization was fixed to be 0.125 M and minimum time for complete diazotization was found to be 2 min.

Effect of p-aminoacetophenone concentration

The effect of the p-aminoacetophenone concentration on the colour intensity was studied using the proposed procedure and adding 0.5 ml of 0.025-0.1% solutions of the p-aminoacetophenone in hydrochloric acid (0.125 M) to a series of nitirite solutions. The results showed that a 0.05% of p-aminoacetophenone solution was sufficient for complete colour development. Higher concentration did not enhance the absorbance further, and a lower concentration did not give good results.

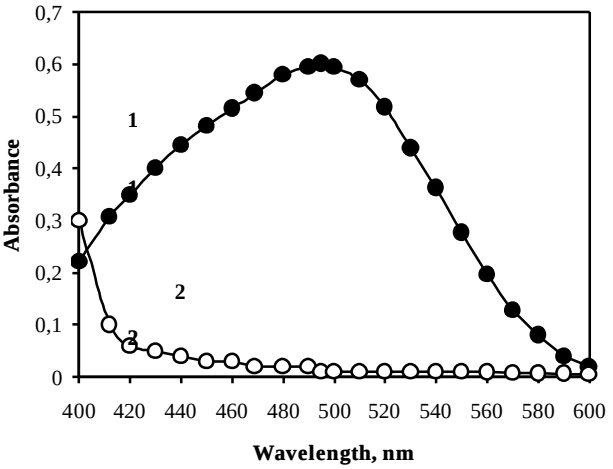
Effect of coupling agent

The effect of varying the concentration of citrazinic acid was studied using the proposed procedure and adding 0.05 – 5.0 ml of 0.1% citrazinic acid to a series of nitrite solutions. It was found that a maximum and stable colour was formed with 2 ml of 0.1% citrazinic acid solution in a final volume of 10 ml.

Effect of sodium hydroxide concentration

The effect of the sodium hydroxide concentration on the absorbance was studied, volumes from 1 to 5ml of 4 M sodium hydroxide solution were examined. The investigation showed that 2 – 4 ml gave constant and maximum absorbance, and 3 ml was selected for the procedure. Other alkaline solutions were tried, but the good results were obtained by using sodium hydroxide as a base.

FIGURE 1
Absorption spectra of (1) Azo dye [1 ppm, NO_2^-] Vs reagent blank, (2) Reagent blank Vs distilled water



Proposed reaction mechanism

In the presence of hydrochloric acid, nitrite is converted to nitrous acid, which reacts with the amino group of p-aminoacetophenone to form a stable diazonium cation. The diazonium salt then couples with citrazinic acid in an alkaline medium to form an azo dye (Scheme 1).

SCHEME 1 – Reaction mechanism

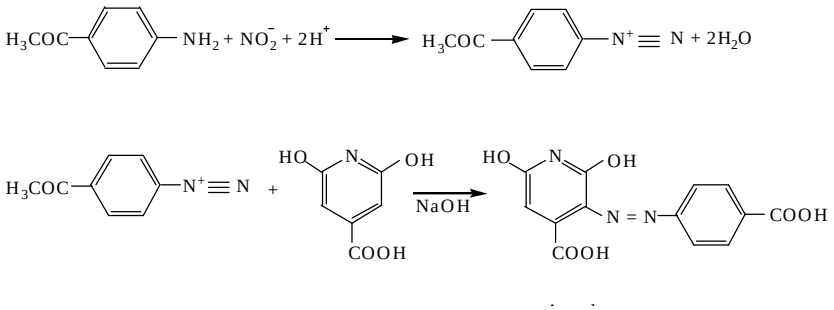


TABLE 1
Effect of diverse ions on the determination of nitrite [1 ppm].

Interferent	Tolerance limit (ppm)	Interferent	Tolerance limit (ppm)
Acetate	3500	Lead	1000
Iodate	3000	Magnesium	500
Citrate	3000	Manganese(II)	150
Fluoride	3500	Aluminum	500
Carbonate	3000	Calcium	20
Tartrate	2500	Iron(III)	10
Sulphite	3500	Sodium	5000
Chloride	4000	Cobalt(II)	20
Nitrate	3500	Nickel(II)	100
Phosphate	1800	Potassium	5000
Sulphate	4000	Copper(II)	20
Sulphide	80	Mercury(II)	100
Barium	500	Chromium(VI)	100
Cadmium	200	Iron(II)	20

TABLE 2 - Determination of nitrite in natural waters

Sample	Amount taken, ml	Added nitrite, µg/ml	Proposed method			Standard method			F-test[b]	t-test[c]
			Nitrite found, µg/ml[a]	Recovery, %	RSD, %	Nitrite found, µg/ml[a]	Recovery %	RSD, %		
River water	2.0	-	1.09 ± 0.02	-	1.8	1.08 ± 0.01	-	0.9	4.00	1.00
	3.0	-	1.10 ± 0.02	-	1.8	1.11 ± 0.03	-	2.7	2.25	0.63
	4.0	-	1.08 ± 0.04	-	3.7	1.10 ± 0.03	-	2.7	1.78	0.91
			Avg. 1.09			Avg. 1. 096				
	2.0	0.5	1.58± 0.01	99.4	0.6	1.59 ± 0.02	99.6	1.3	4.00	1.00
Lake water	2.0	-	0.89 ± 0.02	-	2.2	0.90 ± 0.03	-	2.2	2.25	0.63
	3.0	-	0.90 ± 0.05	-	3.3	0.90 ± 0.03	-	3.3	2.78	0.00
	4.0	-	0.91 ± 0.01	-	1.1	0.89 ± 0.02	-	2.2	4.00	2.00
			Avg. 0.9			Avg. 0.896				
	2.0	0.6	1.498 ± 0.02	99.8	1.3	1.496±0.01	100.0	0.6	4.00	0.20
Tap water[d]	2.0	0.5	0.49 ± 0.03	98.0		0.50 ± 0.04	100.0		1.78	0.45
	3.0	0.7	0.71 ± 0.05	101.4		0.69 ± 0.04	98.5		1.56	0.71
	4.0	0.8	0.80 ± 0.03	98.8		0.80 ± 0.02	100.0		2.25	0.00

[a] Mean ± standard deviation (n=5). - [b] Tabulated F value for (4,4) degrees of freedom at P(0.95) is 6.39.
[c] Tabulated t-value for 8 degrees of freedom at p(0.95) is 2.306. - [d] Tap water gave no test for nitrite.

Effect of interfering species

As the proposed procedure was applied to the determination of nitrite in natural water samples, the effect of the foreign ions commonly present in water was studied. Metal ions forming hydroxides in alkaline medium were expected to interfere, but a large number of these ions were masked with appropriate amount of EDTA. The tolerance limit of the foreign ions shown in Table 1 is the amount that caused not more than ± 2% changes in the values of absorbance during the determination of a fixed amount of nitrite (1 ppm).

Analytical data

A linear calibration graph was obtained for 0.5 to 12 μg nitrite in a final volume of 10 ml. The detection limit and quantitation limit of nitrite determination was found to be 4 ng/ml and 14 ng/ml, respectively. The calibration graph has a correlation coefficient of 0.999. The molar absorptivity and Sandell's sensitivity of the colour system is 2.9×10^4 l mol^{-1} cm^{-1} and 16 ng cm^{-2}, respectively. The stability of the reagents and also the reliability of the proposed method were tested by determining the concentration of 10 samples containing 10 μg nitrite per 10 ml over a period of 7h, the mean absorbance was found to be 0.6 and the relative standard deviation 2.0%, respectively. The results showed no systematic error in the method and thus indicate its reliability.

Application

The proposed method was applied to the determination of nitrite in natural water samples.

Freshly collected water samples were filtered through Whatman filter paper No. 41 before analysis. As the concentration of common pollutants in the natural water is generally far below the tolerance levels shown in Table 1, the method was applied directly to the determination of nitrite in natural water samples. Table 2 shows the analytical results for polluted river, lake and ground water, obtained following the recommended procedure and also by the standard sulfanilamide – NEDA method. The reliability of the method to analyse real samples was checked by recovery experiments, which gave quantitative results with convenient reproducibility.

Statistical analysis of the results by F and t–test showed no significant difference in accuracy and precision between the proposed and standard method (Table 2).

CONCLUSIONS

The procedure for determining nitrite is facile, rapid, and fairly sensitive (2.9×10^4 l mol^{-1} cm^{-1}). The calibration graph is linear over the range 0 – 12 μg nitrite in a final volume of 10 ml. The detection limit of nitrite is 4 ng /ml. The developed colour is stable for 3 h. The method is useful for the determination of nitrite in natural waters. The method has added advantage over other methods[2-11] owing to its simplicity, freedom from pH effects, temperature independence, less interference and avoidance of a lengthy extraction steps.

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STUDIES OF NATURAL RADIOACTIVITY IN CEMENT PRODUCTS USING GAMMA RAY SPECTROSCOPY

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SUMMARY

In this study we have selected 12 brands of cement and related products commonly used in the Malaysian construction industry. They have been analysed for natural radioactivity (U,Th and K) using the method of gamma ray spectrometry. The gamma energies of interest are 583.1 keV, 609.3 keV and 1460 keV for nuclides ^{208}Tl, ^{214}Bi and ^{40}K, respectively. Results of our analysis have shown a relatively high activity of ^{40}K for all samples, ranging from 33 Bq/kg to as high as 3010 Bq/kg. Uranium activity was found to range from 9 Bq/kg to 672 Bq/kg, while thorium activity lies between 6 - 94 Bq/kg. The radium equivalent activity, Ra_{eq} calculated to range from 24 Bq/kg to 879 Bq/kg. Eight of the 12 samples were found to have radium equivalent greater than 370 Bq/kg, a threshold limit for radiation dose equivalent to 1.5 mSv per annum.

KEYWORDS: Radioactivity, cement

INTRODUCTION

Naturally occurring radionuclides can give rise to external doses when contained in raw materials used to construct buildings. Our previous γ - ray analysis on several types of building materials have shown that cement, a vital component in construction industry, contains a substantial amount of naturally occurring radioactive material [1]. This finding is of concern to us since mankind are continuously confined in buildings, be it at home or workplace, and this situation can give rise to the possibility of radiation exposure.

The concentrations of primordial nuclides, U, Th and K are dependent on the process by which cement is manufactured. Its chemical composition is controlled by the amount (or percentile) content of silica, lime, alumina and iron. These oxides become the characteristic components of clinker minerals. When clinkers are mixed with gypsum they become cement. There are many brands of cement (and related products) in the market, all of which contain different percentage chemical composition. For instance, the composition of Portland cement contains 60 to 67 percent lime, 17 to 25 percent silica and 2 to 8 percent alumina.

In this study we analyse for the activity of gamma ray emitted by naturally occurring nuclides present in several types of cement and related product. Our aim is to quantify the activity of these nuclides using the gamma counting technique.

EXPERIMENTAL

Cement materials were collected from several construction sites in the vicinity of the university campus. We obtained 12 different samples, comprising of six brands of cement and another six brands of cement plaster. These samples are already in the form of fine granules and, therefore, does not require further grinding. Each of the sample brand was then oven-dried and homogenized. They all were then filled into plastic cylindrical containers of approximately 100 ml in volume with their net weight recorded. These samples were then left for approximately four weeks to allow for radium and its short-lived progeny to reach radioactive equilibrium.

The gamma ray spectra of the samples were collected using a high-resolution HPGe detector with 20% efficiency. All of the procedures and calculation adopted are similar to that of our previous work [1].

^{226}Ra (or ^{238}U for samples at radioactive equilibrium) and ^{232}Th activities were estimated from the 609.3 and 583.1 keV γ-ray of ^{214}Bi and ^{208}Tl, respectively, while ^{40}K activity was determined using the 1460 keV γ-ray. Ra equivalent activity (Ra_{eq}) is a common index used to compare the activity concentrations containing U, Th and K, which is defined as a weighted sum of the activity concentrations [2]. It is assumed that 370 Bqkg^{-1} of U, 259 Bqkg^{-1} of Th and 4810 Bqkg^{-1} of K produce the same γ-ray dosage.

RESULTS AND DISCUSSION

Table 1 shows the γ-ray activity concentrations of U, Th and K measured for 6 brands of cement, together with their respective Ra equivalent activities. The world average for U and Th in soil is 25 Bq/kg, while for K is 370 Bq/kg [2]. From Table 1 we can deduce that the activity of U in cement far exceed that of the world average (except for sample CS2). As for Th activity concentration, only samples CS1 and CS6 show values slightly above the average of 25 Bq/kg. The K activities in all samples (except CS2 and CS5) far exceed that of the threshold value 370 Bq/kg. Fifth column of Table 1 shows the radium equivalent activity for the various samples. Values exceeding 370 Bq/kg depict the combined activity concentrations of U, Th and K to be above the minimum permitted value.

TABLE 1 - Activity concentrations of natural radionuclides in various brands of cement under study (Bq kg^{-1})

Cement Samples	^{238}U	^{232}Th	^{40}K	$^{226}Ra_{eq}$
CS1	592	28	2445	820
CS2	9	6	83	24
CS3	380	23	1392	520
CS4	450	12	3099	706
CS5	78	9	98	99
CS6	634	31	2612	880
Average	372	18	1771	508

TABLE 2 - Activity concentrations of natural radionuclides in various brands of cement plaster under study (Bq kg^{-1})

Cement Plaster	^{238}U	^{232}Th	^{40}K	$^{226}Ra_{eq}$
CP1	672	31	936	788
CP2	42	81	33	152
CP3	333	94	651	517
CP4	336	94	347	498
CP5	123	58	380	389
CP6	135	83	439	287
Average	264	73	464	439

Table 2 shows the measured activity concentrations for six brands of cement plaster. Our findings have shown that four brands contain radium equivalent activity greater than 370. Brand CP1 shows the highest radium equivalent of 788 Bq kg^{-1} followed by CP3 of 517 Bq kg^{-1} and CP4 with 498 Bq kg^{-1}.

When we compare the activities of U, Th and K in cement and cement plaster, we find that, on average, the activity concentrations of U and K in cement samples are higher than that measured in cement plaster. For Th, the average value in cement is 18 Bq kg^{-1}, whereas in cement plaster the value is almost five times higher at 73 Bq kg^{-1}.

Generally, the concentration of each radionuclides in cement samples is quite consistent between brands except for CS2 and CS5. Similarly for cement plaster, only CP2 display activity not of the same order as the others.

CONCLUSIONS

Our analyses have shown that the activities of natural radionuclides found in cement and cement plaster are significantly high with an average radium equivalent concentration of 508 Bq/kg and 439 Bq/kg respectively. The main contributor for the high activity is potassium-40.

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FEB PRESS RELEASES

8th International Working Conference on Stored Product Protection (IWCSPP) – Technology into Action, 22-26 July 2002, York, UK

The "International Working Conference on Stored Product Protection (IWCSPP)" was first held in 1970 and has since been held every four years. The UK will host the next conference to be held in 2002 in the historic city of the historic city of York, England.

The meeting will showcase work on the pests and diseases which may cause spoilage, adverse health effects and loss of the crop and discuss new techniques aimed at safe, effective and environmentally friendly management of stored commodities. In the field of stored product protection it is important that scientific advances are translated into practical solutions, hence the theme of the 2002 conference – "Technology into Action".

SCIENTIFIC PROGRAMME

Monday, 22 July

Biology, Detection and Biological Control: *Biology of invertebrate and vertebrate pests and fungi; Sampling and trapping; Biological control; Relevance to IPM*
(Convenors: Peter Credland and Bhadiraju Subramanyam)

Tuesday, 23 July

Food Safety: *Pesticide residues ; Mycotoxins and other contaminants ; Product quality determination ; Quarantine and regulatory issues ; Risk assessment*
(Convenors: Zofia Kozakiewicz and Frank Arthur)

Wednesday, 24 July

Chemical and Physical Control: *Fumigation and control atmospheres; Conventional pesticides; Natural pesticides including plant derivatives; Inert dusts; Physical control*
(Convenors: Christoph Reichmuth and Paul Fields)

Thursday, 25 July

Processing and Applications: *Food processing and added value; Storage systems (including engineering); Technology transfer and extension systems; Modelling of storage systems* (Convenors: Dirk Maier and George Srzednicki)

Friday, 26 July

The Future of Stored Product Protection - Impacts of Global Issues: *Trade liberalisation; International standards; Food supply and demand; Co-ordination of research; GMOs; Impacts in different economic regions*

KEY DATES

- Closing date for receipt of abstracts for poster/papers: 31 December 2001
- Notification of acceptance of papers: February 2002
- Final Communication to delegates: May 2002

SOCIAL PROGRAMME

Social events have been arranged for the afternoon of Wednesday 24 July 2002. Delegates and partners can choose one of the following:

- A visit to Castle Howard and a tour of the 8th Century palace surrounded by spectacular gardens.
- A return journey on the North York Moors´ Steam Railway at Pickering, one of the world´s oldest railway lines.
- A visit to the Royal Society for the Protection of Birds centre on the East Coast at Bempton which has the largest concentration of breeding seabirds in England.
- Social programme for accompanying partners

REGISTRATION DETAILS

The registration fee includes attendance at the Scientific Programme, Trade Exhibition, teas and coffees and a copy of the Conference Proccedings. The registration fee is £260 received by 31 December 2001, rising to £320 for those received after this date. Students can apply for a reduced fee - £180 for registration before 31 December 2001 rising to £260 for registrations received after this date. *Bona fide* students must enclose a confirmation letter from their institute with their registration form (Cheques made payable to Central Science Laboratory; UK pounds sterling – International Bank Transfers: bank charges paid in addition to the total amount due – Credit card payments are the preferred option).

Cancellations: Refunds will be given before 31-03-2002 subject to a £75 cancellation charge to cover a lost deposit on reserved acommodation.

ADDITIONAL INFORMATION

Conference Banquet: The Conference Banquet will be held on Wednesday 24 July 2002 at the National Railway Museum located in the City of York. The banquet will take place in the Great Hall, surrounded by

a stunning display of the finest locomotives in the collection. Price includes a 3-course meal, wine, waitress service and live music.

Catering: Lunch will be available to delegates at the University. Please note that there are few alternative options for 1unch within easy reach of the University. Evening meals will consist of a self service facility offering a variety of hot and cold food. Special dietary requirements can be catered for.

FURTHER INFORMATION

All enquiries should be directed through the conference secretariat at the following address:

8th IWCSPP,
c/o Central Science Laboratory
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Email: iwcspp@icscs.co.uk

For the latest information on the conference consult the meeting web site at: www.icscs.co.uk/iwcspp2002.

THE SECOND PCB WORKSHOP -
Recent Advances in the Environmental Toxicology and Health Effects of PCBs (Emphasis on the latest advances and the perspective of Central and Eastern Europe),
May 07-11, 2002, Brno, CZECH REPUBLIC

WORKSHOP OBJECTIVES

Polychlorinated biphenyls (PCBS) are chemical substances which are persistent, bioaccumulate, und pose a risk of causing adverse effects to human health and the environment. They can be transported long distances, and have been detected in the furthest sites of the globe, including places where they were manufactured or used. While manufacture of PCBs has reportedly ceased, the potential or actual release of PCBs into the environment has not, since significant quantities of existing PCBs continue in use or storage.

The likely extended period of these continuing uses, and the persistence of PCBs once released into environment together mean that PCBs could pose a threat for decades to come. Accordingly, UNEP's Governing Council included PCBs in the Stockholm Convention among the 12 persistent organic pollutants (POPs) identified for international action.

PCBs are one from the most studied chemicals round the globe. But many years of very intensive scientific work still did not solve all open problems concerning the environmental fate of PCBs and their toxicological and ecotoxicological effects.

The 1st PCB Workshop was held in Lexington, KY, April 2000. The 2nd Brno PCB Workshop 2002 will provide opportunities for researchers of varied scientific backgrounds and from many countries of the world to discuss the various aspects of these problems and to contribute to the answering of many open questions of PCBs environmental fate.

MAIN THEMES

- **Origin und characteristics of PCB contamination with emphasis on Eastern Europe:** Global and regional distribution of PCBs; Regional sources, distribution and levels; Contaminated sites; Hot spots
  (Chairs: K. C. Jones, L. L. Needham)
- **Human exposures - Characteristic PCB mixtures as related to human health effect:** Profiles in multiple pathway exposures; Occupational, accidental, environmental and dietary exposures; Health Effects; Residue Profiles
  (Chairs: L. G. Hansen, R. Sram)
- **Advances in modes of action of PCBs:** Oxidative stress; Endocrine disruption; Cancer
  (Chairs: D. Schrenk, L. J. Fisher)
- **Biomarkers:** In vivo, in vitro and epidemiological models for detection of PCB effects in humans
  (Chairs: T. Zacharewski, M. van den Berg)
- **Risk Issues:** Risk Assessment, Summary of needs
  (Chairs: P. Grevatt, A. Brouwer)
- **Remediation Issues:** Remediation issues, Microbial Degradation, European Union PCB Regulations and Recommendations: Current and Future Directions
  (Chairs: G. S. Sayler, K. Demnerová)

WORKSHOP PROGRAMME

The PCB Workshop 2002 will take place in the Hotel Voronež, Brno, Czech Republic, May 07-11, 2002. Platform and poster sessions will provide information on key topics of the PCB problems. Workshop exhibition will be suitable platform for contacts with industry, publishers, consultants and will inform about new publications, software, products, services in the field of environmental chemistry, toxicology, ecotoxicology and risk analysis of PCBs. The abstracts of all platform and poster presentations will be published in Symposium Proceedings.

The special mini-symposium TOCOEN 2002 will be organised as a special part of the PCB Workshop 2002 (a meeting concerning the trends of environmental pollution by PCBs and other POPs in Europe with special attention to Central and Eastern European region).

SYMPOSIUM VENUE

The Symposium will be held in Brno, metropolis of South Moravia. Brno is the second largest city in the Czech Republic with population of more than 400 000. Brno is the administrative, cultural and economic capital of the region. The Symposium venue is located in the Interhotel Voronež, Brno (http://www.voronez.com).

TRAVEL TO BRNO

Brno is located between Prague und Vienna (Austria) and has convenient connections. By air, daily to Prague or Vienna und from there by bus or rail to Brno (approximately two and half-hours). By road, Brno is linked by motorway to Prague and Vienna (about 200 km from each). Limited connection by air also exists.

NOTE: Bus services will be provided from Prague and Vienna airports on Tuesday, May 07, 2001 and back to the airports on Saturday, May 11th. Foreign guests, planning to participate in SETAC Europe in Vienna, will also be provided transportation, on May 11th, to their hotels in that city.

REGISTRATION FEES

Registration fees are $175 and $50 for students, if registered and paid before March 01, 2002. The registration fees after March 01, 2002 will be $250 and $75 (students), respectively. The registration fee covers published Workshop document, coffee breaks, lunches and invitation to the Welcome party.

Registration fee for participants from the countries with economy in transition and in some special cases can be negotiated.

Full payment or purchase order must be enclosed in order to guarantee registration.

REFUNDS

Requests for refunds must be made in writing and received by the Local Organizing Committee: Before March 01, 2001 refund in full less administrative fees of $25; From March 01 to April 08, 2002 50% of registration fees refunded; After April 08, 2002 fees will not be refunded.

All refunds will be made at the end of the Workshop. Participants who have already paid their registration fees and who wish to be replaced by a representative of the same organization must forward written notification to this effect to the Local Organizing Committee. All such requests will be accepted up until the start of the Workshop.

SOCIAL EVENTS

A complete Social Programme is also being arranged. The welcome reception on Tuesday, May 07 and the banquet on Thursday, May 09 in typical south Moravian style are prepared. Accompanying persons will be offered programmes in parallel with the conference focusing on Czech art, culture and history.

ACCOMMODATION

Prices for accommodation in the Interhotel Voronež (four stars) (http://www.voronez.com) is for Superior Class: $70 (double room), $55 (single room); Standard Class: $60 (double room), $50 (single room); Economy Class: $45 (double room), $40 (single room). The price is including breakfast und taxes. The prices are approximately (in USD), we are not responsible for currency fluctuations - see more details in Accommodation Form. Lower cost hotel and low cost student housing will be available. To reserve, please fill in hotel reservation form and return directly to the Hotel Voronež.

KEYNOTE SPEAKERS

Selected keynote speakers will be invited frorn various countries to present current overview in rapidly emerging/changing areas of PCB research.

CONFERENCE INFORMATION AND REGISTRATION

Registration form and abstract please mail to the following address:

Before December 31, 2001: TOCOEN, s.r.o,
Veslarska 230 B, 637 00 Brno, Czech Republic
After January 01, 2002: TOCOEN, s.r.o,
Kamenice 3, 625 00 Brno, Czech Republic

For any additional information contact:
holoubek@chemi.muni.cz or kuba@chemi.muni.cz or you write the Chair Persons directly.

All information you can also find on: http://recetox.chemi./muni.cz/

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