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CONTENTS

ORIGINAL PAPERS

EQUILIBRIUM ADSORPTION STUDIES OF ACTIVATED COKE TOWARDS PHENOL AND 4-NITROPHENOL

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SUMMARY

Equilibrium adsorption of activated coke towards phenol and 4-nitrophenol has been studied. The activated coke was produced by impregnating ground green coke with pulverized KOH (2:3) and then washing the activation product with 15% HCl solution after thermal treatment. The experimental variables investigated in these studies were contact time, initial concentration, temperature and pH of solution, and sorbent dosage. Adsorption dynamics parameters, namely the rate constants for film diffusion (K_{ad}), intraparticle diffusion (K_p) and the diffusion coefficient (D^i) were determined. Isotherm modeling was carried out using both Langmuir and Freundlich equations. From the experimental data thermodynamic parameters including free energy ($\Delta G°$), enthalpy ($\Delta H°$) and entropy ($\Delta S°$) were also calculated.

KEYWORDS: Adsorption, petroleum coke, phenol, 4-nitrophenol, dynamic parameters, Langmuir and Freundlich isotherms, thermodynamic parameters.

INTRODUCTION

Petroleum coke, a solid material derived from crude oil, is recognized as a by-product in petroleum industry. The total world production of petroleum coke today is about 140,000 tons. In view of its large quantity it is interesting to find alternative utilization for this solid material. One of the possible alternatives is its utilization as an activated carbon precursor. For this purpose, petroleum coke is considered to be very potential taking into account its cheap and ready availability. In addition to these advantages it has low ash content and moisture, and generally high yields of active carbon [1]. The carbon contents of petroleum coke and some other carbonaceous precursors are listed in Tab. 1.

TABLE 1
Carbon contents of petroleum coke and some materials commonly used as a precursor for activated carbon production [2].

Materials	%Moisture	%Carbon
Petroleum coke	1.8[a)]	84-97[b)]
Anthracite	2.0	86-92
Bituminous coal	10	78-86[c)]
Lignite	30-70	60-70
Peat	>75	50-60

[a)]reference [3], [b)]reference [4], and [c)]reference [5]

In an earlier publication [6], a modified method of petroleum coke activation technique by the use of KOH as an activator was reported. The active sorbent produced by impregnating raw material with pulverized KOH followed by washing the activation product with a HCl solution is adequately effective to remove phenolic compounds from an aqueous solution.

Continuing that earlier investigation, this work reports some more results of studies on equilibrium adsorption of activated petroleum coke performed to demonstrate the feasibility of activated petroleum coke treatment, especially for phenol and 4-nitrophenol. The reasons for choosing the phenolic compounds as pollutant models in these studies were that these compounds are very common contaminants in water and suspected as toxic and carcinogenic [7]. Therefore, phenol and phenolic compounds were designated as priority pollutants by the US EPA, which take the 11th place under the 129 chemicals [8]. Apart from their toxicity and carcinogenity, phenols can cause bad taste and odor, even at low concentration [9].

The purpose of the present work was to examine the adsorption properties of activated coke towards phenolic compounds. This isothermal study is necessary to determine the feasibility of using the applied sorbent for practical application. The data were fitted to Langmuir and Freundlich models, and the thermodynamic parameters were also evaluated.

MATERIALS AND METHODS

Activated coke preparation

Two green petroleum cokes obtained from Pertamina UP II Dumai, Indonesia (DG-32) and from Ruhr Öl Gelsenkirchen, Germany (GG-64) were utilized in these studies. The pre-dried and ground raw materials having particle diameters of 0.63-2.0 mm were dried in an oven at 110 °C for 2 h prior to activation.

The activation of coke samples was carried out in a horizontal quartz tube 34 mm inner diameter, and an oven furnace Heraeus type ND1-30755. 10 g of raw coke was impregnated with 15 g of pulverized KOH. The mixture was heated at 450 °C for 2 h followed by carbonization at 850 °C for 1.5 h. The activation product was immersed in a 15% HCl solution for 4 h and finally washed with distilled water. The activated coke was dried at 120 °C in an oven for 2 h.

Adsorption studies

Adsorption experiments were performed using a batch technique by stirring 0.02-0.15 g of activated petroleum coke with 100 mL of phenolic solutions of 100 mg/L concentration in 100 mL glass stoppered Erlenmeyer flasks. The flasks were placed in water-baths and stirred continuously with a speed of 500 rpm for 5-180 min at desired temperature. The buffer solutions were required to adjust the pH of the systems. Each glass used was pre-washed with 10% HNO_3 solution, rinsed with distilled water and dried at 110 °C for 2 hours prior to usage. After equilibrium, the reaction mixture was filtered through blue ribbon filter paper (S & S, Germany). The concentrations of the sorbates in the residual solution were determined spectrophotometrically with UV-VIS Model HP-UV 8254A Diode Array Spectrometer at wavelengths 270 and 320 nm for phenol and 4-nitrophenol, respectively.

RESULTS AND DISCUSSION

Coke activation

The results in Tab. 2 show that mass recoveries of activated petroleum cokes obtained from two coke materials, namely, DG and GG are 90% and 87%, respectively.

TABLE 2
Percent recovery of the activated petroleum coke products

Sample	Temperature of Activation , °C	KOH : Coke ratio	R (%)
DG-32	450 (2h); 850 (1.5h)	3 : 2	90
GG-64	450 (2h); 850 (1.5h)	3 : 2	87

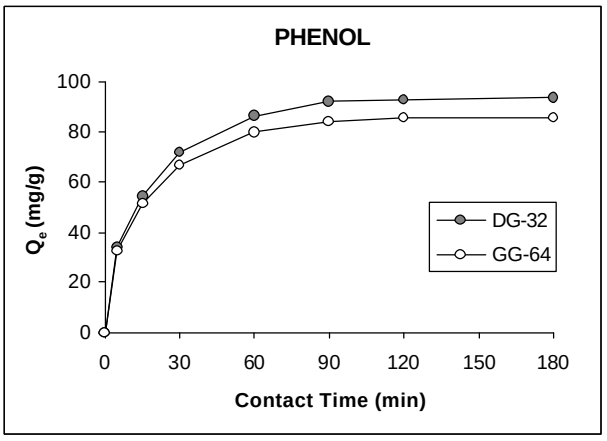
DG and GG are Dumai and Gelsenkirchen petroleum cokes, respectively. R is the recovery of activated coke.

These yields are considerably higher compared with alternative materials, which receive a major concern from recent researchers, especially those who are interested in byproduct utilization and sorbent development.

For example, the percentage of sorbent obtained from coconut shell [10], peach stone [11], apricot stone [12] and contaminated soil [13] are only 45, 32, 55 and 75%, respectively. The relatively high yield of activated carbon is a major advantage of petroleum coke as an alternative precursor.

Contact time studies

The adsorption of phenol and 4-nitrophenol by the prepared coke sorbents is a function of contact time as depicted in Fig. 1.



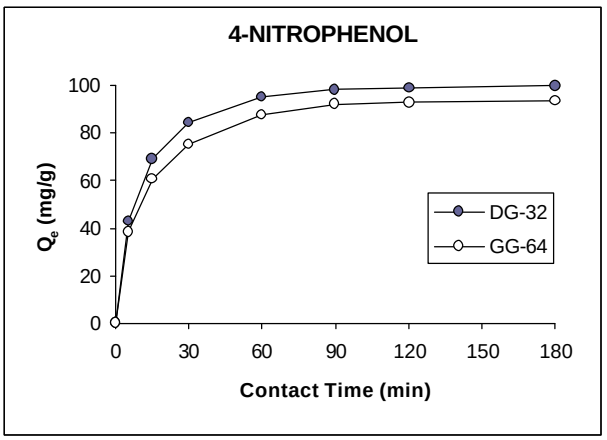


FIGURE 1 - The adsorption kinetics isotherm of phenols is a function of time (Conditions: weight of activated coke: 0.10 g. Volume of solution: 100 mL. Conc.: 100 mg/L. Temp.= 20 °C, pH = 6)

The amount of phenol and 4-nitrophenol adsorbed progressively increased as the contact time increased and then gradually attained equilibrium after 30 min. Fig. 1 shows that the adsorption of phenols by two activated petroleum cokes occurred very rapidly within the first 15 min,

whereas no significant change in percent uptakes was observed after 60 min. This equilibrium was almost completely reached after 90 min.

In order to ensure equilibrium all adsorption experiments in this investigation were done by stirring each reaction mixture for up to 180 min.

Adsorption dynamics

The adsorption dynamics of phenol and 4-nitrophenol was investigated under different conditions by studying two rate mechanisms of adsorption, namely the kinetics of film (external) diffusion and particle (internal) diffusion giving the dynamics parameters K_{ad}, K_p and D^i. The values of these parameters were used both to determine the relative adsorption rates and to distinguish the rate mechanisms of the adsorption processes.

(a) Film diffusion

Boyd et al. [14] derived the equation (1) for an exchange rate controlled by film diffusion,

$$\log(1-F) = -\frac{R}{2.303}t \qquad \textbf{(1)}$$

where F is the fractional attainment of equilibrium, i.e., the total amount adsorbed at any time divided by the total amount at equilibrium (Q_t/Q_e). By substituting Q_t/Q_e for F and K_{ad} for R, and by rearranging equation (1), it will be obtained equation (2) known as Lagergren's equation [15],

$$\log(Q_e - Q_t) = \log Q_e - \frac{K_{ad}}{2.303}\,t \qquad \textbf{(2)}$$

where K_{ad} is equilibrium rate constant of the adsorption. The value of K_{ad} was obtained from the slope of the linear graph of log (Q_e-Q_t) versus t.

(b) Intraparticle diffusion

In a batch system with vigorous stirring, besides adsorption at the outer surface of the sorbent, there is also possibility of sorbates transport from the solution into the pores of sorbent [16]. Such internal diffusion may be the slowest step and hence, it will control the overall rate of the adsorption.

The possibility of the particle diffusion mechanism can be tested by plotting the amount adsorbed of phenols versus the square root of time. The double nature of these plots would be recognized as the results of a film diffusion effect (initial curved portion) and an intraparticle diffusion effect (the second linear portion). The rate constant for intraparticle diffusion, K_p, was calculated from the slope of the second linear portion [17-18].

$$Q_t = K_p\, t^{1/2} \qquad \textbf{(3)}$$

K_p is expressed as a relative rate constant by several investigators because its unit ($mgg^{-1}min^{-1/2}$) is not the usual dimensions for the rate constant. The intercepts of the plot are proportional to the extent of boundary layer thickness. Although it is not the true reaction rate, K_p is useful for comparative purposes [17].

The effective diffusion coefficient, D^i, for the intraparticle transport of phenols was calculated using the following Boyd's equations (4) and (5) [14].

$$F = 1 - \frac{6}{p^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{D^i p^2 n^2 t}{r_0^2}\right) \qquad \textbf{(4)}$$

$$F = \frac{Q_t}{Q_e} = 1 - \frac{6}{p^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-n^2 Bt\right) \qquad \textbf{(5)}$$

where $B = \frac{D^i p^2}{r_0^2}$, r_o is the radius of sorbent particle assumed to be uniformly spherical and n = 1, 2, 3,..etc.

The values of Bt for every observed value of F ranging from 0 to 0.85 and from 0.86 to 1.0 were calculated using the following Reichenberg's equations (6) and (7), respectively [19].

$$Bt = -\log_e\frac{p^2}{6}(1-F) \qquad \textbf{(6)}$$

$$Bt = 2p - \frac{p^2 F}{3} - 2p\left(1 - \frac{pF}{3}\right)^{1/2} \qquad \textbf{(7)}$$

Equilibrium adsorption studies

a) Effect of initial concentration

The initial concentration of sorbate is one of several important parameters to be investigated, since a given mass of sorbent can only adsorb a fixed amount of the sorbate. Thus, the understanding of the concentration dependence of the adsorption process would be a necessary point for practical purposes of the sorbent. Moreover, it is also useful for predicting the rate-limiting mechanism of the adsorption. As previously stated, the kinetics of solutes transport from solution phase to the surface of sorbent is controlled either by boundary or film diffusion and intraparticle diffusion. To predict which one of these effects is predominant, some experiments with a variation of the initial concentrations

(100-400 mg/L) of sorbates were performed. The uptake of phenols is observed to increase as the initial concentration increases as shown in Fig. 2.

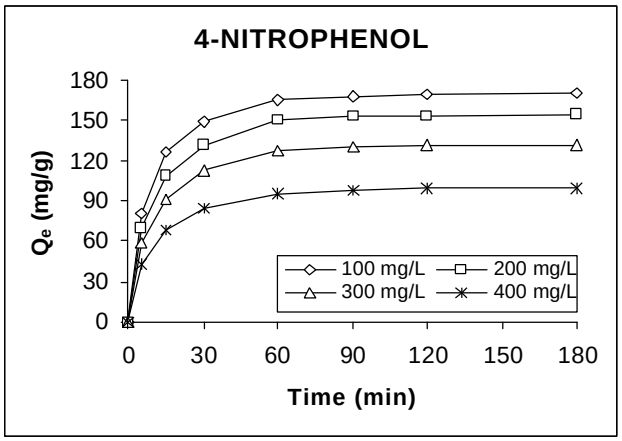


FIGURE 2 - Effect of initial concentration on the adsorption of 4-nitrophenol (Conditions: weight of DG-32: 0.10 g. Volume of solution: 100 mL. Temp: 20 °C)

The graphs of the amount adsorbed of phenols by petroleum cokes versus the square root of time have the same general double nature, i.e., an initial curved portion and a linear part at the final as shown in Fig. 3.

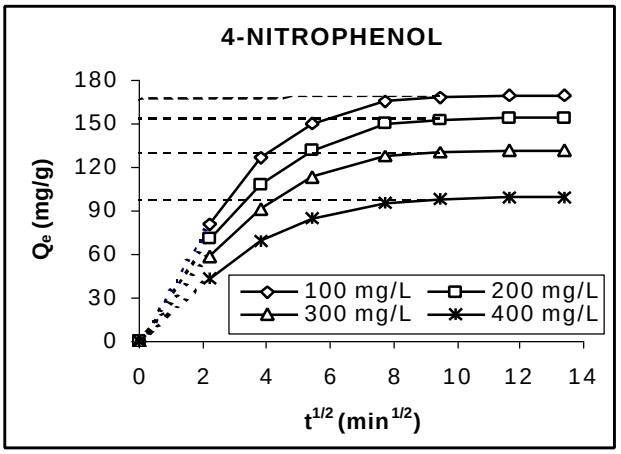


FIGURE 3
Intraparticle diffusion plot for the adsorption of 4-nitrophenol.

From these experimental data the kinetic parameters of the adsorption, K_{ad}, K_p and D^i were determined using equations (2), (3) and (5), and the results are given in Tab. 3. There it can be seen that the internal diffusion parameters, K_p and D^i, of the adsorption are increased with a rise in initial concentration of the sorbates, while the effect of concentration on the rate constant, K_{ad}, seems to be contrary to those internal diffusion parameters, i.e., the values of K_{ad} decreases with increasing initial concentration of phenols. This is an indicative that the adsorption of activated petroleum coke towards phenol and 4-nitrophenol at the experimental concentrations is controlled by internal diffusion. This is supported by the straight graphs of *Bt* versus *t* with zero intercepts (not shown).

There are sufficient attempts which are in agreement with this phenomenon. One of them is the statement of Zogoroski et al. [20] saying that in the adsorption of phenols at lower concentrations (below 82.5 mg/L) the rate-limiting step is external transport and above this, intraparticle transport becomes increasingly important in limiting the transfer of the sorbate to the adsorption sites. According to McKay [21] the decrease of K_{ad} with initial concentration is due to the higher concentration of phenols, the lower fraction adsorbed as summarized in Tab. 3.

b) Effect of pH

The effect of pH of solution on the adsorption is depicted in Fig. 4. Both phenol and 4-nitrophenol seem to be very strongly adsorbed at low pH values. The horizontal plateaus of Fig. 4 between pH values 2 and 10 for phenol and 2 and 8 for 4-nitrophenol indicate no significant influence of hydronium ions within these pH ranges. A rapid decrease in the removal amounts occurs at pH values greater than 10 for phenol and 8 for 4-nitrophenol.

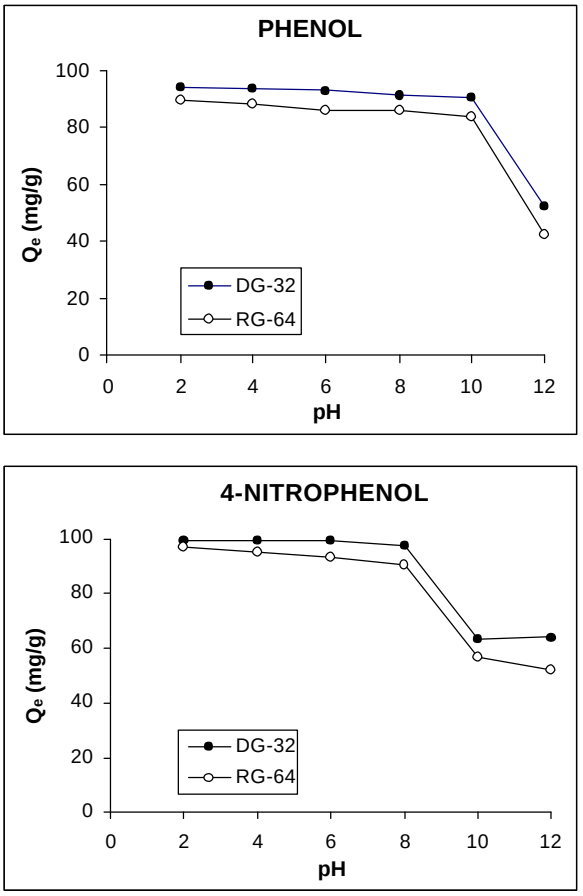


FIGURE 4
Effect of pH on the adsorption of phenol and 4-nitrophenol

TABLE 3
Rate parameters, K_{ad} K_p and D^i for phenol and 4-nitrophenol as a function of initial concentration.

Sorbent sample	Initial Conc. (mg/L)	PHENOL					4-NITROPHENOL				
		Q_e (mg/g)	% Ads.	K_{ad} x10^2 (min^{-1})	K_p ($mg\,g^{-1}\,min^{-0.5}$)	D^i x10^5 ($cm^2\,s^{-1}$)	Q_e (mg/g)	% Ads.	K_{ad} x10^2 (min^{-1})	K_p ($mg\,g^{-1}\,min^{-0.5}$)	D^i x10^5 ($cm^2\,s^{-1}$)
DD-32	100	93.3	93.3	4.72	0.363	10.2	99.7	99.7	4.88	0.366	13.0
	200	118	59.3	4.26	0.373	10.9	132	66.1	4.46	0.377	13.4
	300	138	46.1	4.17	0.425	12.0	154	51.4	4.47	0.435	13.8
	400	160	40.0	4.15	0.458	12.1	170	42.5	4.44	0.463	14.8
GG-64	100	85.8	85.8	4.15	0.349	9.3	93.5	93.5	4.72	0.366	11.7
	200	113	56.5	4.12	0.374	10.6	121	60.5	4.38	0.374	12.0
	300	135	45.1	4.10	0.425	10.8	145	48.4	4.19	0.427	12.1
	400	155	38.6	4.03	0.442	11.5	160	40.2	4.17	0.432	12.4

Adsorption conditions: weight of activated coke: 0.10 g. Volume of solution: 100 mL. pH = 6 and temp. = 20 °C

The decrease of adsorption is due to the dissociation of phenol and 4-nitrophenol as anionic phenolates, which do not adsorb well on the hydrophobic surface of activated coke. All further experiments were performed at pH = 6.0, which was the optimum value for both sorbates.

(c) Effect of temperature

To study the effect of temperature on the adsorption of phenols on activated petroleum coke in a practically relevant range some experiments were conducted at temperatures of 20, 30 and 40 °C. The results illustrate that the adsorption is an exothermic process. The adsorption of phenols decreases as temperature increases. However, the temperature effect observed is small. For example, raising the temperature from 20 to 30 °C decreased the amount adsorbed by DG-32 from 99.7 to 98.3 mg/g for 4-nitrophenol and from 93.3 to 91.0 mg/g for phenol. At further increase to 40 °C the amount of uptake of 4-nitrophenol and phenol decreased to 89.2 and 83.3 mg/g, respectively. The increase of the percent removal of phenols was caused either by external and internal diffusion of the sorbates as confirmed by the values of dynamic constants in Tab. 4.

TABLE 4
Effect of temperature on dynamics parameters of the adsorption for phenol and 4-nitrophenol.

Coke sample	Temp. (°C)	PHENOL					4-NITROPHENOL				
		Q_e (mg/g)	% Ads.	K_{ad} x10^2 (min^{-1})	K_p ($mg\,g^{-1}\,min^{-0.5}$)	D^i x10^5 ($cm^2\,s^{-1}$)	Q_e (mg/g)	% Ads.	K_{ad} x10^2 (min^{-1})	K_p ($mg\,g^{-1}\,min^{-0.5}$)	D^i x10^5 ($cm^2\,s^{-1}$)
DD-32	20	93.3	93.3	4.72	0.363	10.2	99.7	99.7	4.88	0.366	13.0
	30	91.0	91.0	4.45	0.352	10.0	98.3	98.3	4.52	0.364	12.6
	40	83.3	83.3	4.10	0.349	9.88	89.2	89.2	4.31	0.361	12.5
GG-64	20	85.8	85.8	4.15	0.349	10.3	93.5	93.5	4.72	0.366	11.7
	30	84.2	84.2	4.12	0.345	10.2	91.9	91.9	4.33	0.359	11.2
	40	79.5	79.5	4.03	0.337	9.80	87.8	87.8	4.24	0.332	10.9

Conditions: weight of activated coke: 0.10 g. Volume of solution: 100 mL. pH = 6

TABLE 5
Freundlich and Langmuir parameters of phenol adsorption on the sorbents

Coke Sample	PHENOL				4-NITROPHENOL			
	Freundlich		Langmuir		Freundlich		Langmuir	
	K_F (mg g^{-1})	n (g L^{-1})	$Q°$ (mg g^{-1})	b (L mg^{-1})	K_F (mg g^{-1})	n (g L^{-1})	$Q°$ (mg g^{-1})	b (L mg^{-1})
DG-32	73.9	5.49	158	0.391	122	6.41	227	0.620
GG-64	58.6	4.58	150	0.302	110	4.99	215	0.489

Isotherms analysis

The modeling of adsorption equilibrium was achieved by applying both the Freundlich and the Langmuir isotherms represented mathematically by equations (8) and (9):

$$Q_e = K_F C_e^{1/n} \quad \textbf{(8)}$$

$$\frac{C_e}{Q_e} = \frac{1}{Q^o b} + \frac{Ce}{Q^o} \quad \textbf{(9)}$$

where Q_e is the amount of solute adsorbed per unit weight of sorbent, K_F, 1/n and b are the characteristic constants, C_e is the solute phase and $Q°$, the solid phase concentration corresponding to complete coverage of available adsorption sites.

A linear graph of ln Q_e against ln C_e according to eq. (8) would give the Freundlich parameter values, n from the slope and K_F from the intercept. The Langmuir constants, b and $Q°$, were obtained from the slope and the intercept from a plot of C_e/Q_e against C_e. The calculated results of the isotherm constants are given in Tab. 5. It was found that the adsorptive behavior of both phenol and 4-nitrophenol onto activated cokes agreed with the Langmuir rather than with the Freundlich isotherm, indicating that the adsorption was achieved with the formation of a monolayer.

Thermodynamic parameters

Thermodynamic parameters $DG°$, $DH°$ and $DS°$ were calculated using the following equations.

$$\Delta G^o = -RT \ln K \quad \textbf{(10)}$$

$$\Delta H^o = R\left[\frac{T_2 T_1}{T_2 - T_1}\right] \ln \frac{K_2}{K_1} \quad \textbf{(11)}$$

$$\Delta S^o = \frac{\Delta H^o - \Delta G^o}{T} \quad \textbf{(12)}$$

where R is the gas constant (8.315 J mol^{-1} K^{-1}) K, K_1 and K_2 are the Langmuir equilibrium constants at 20, 30 and 40 °C obtained from the graphs of the adsorption isotherm.

The values of the thermodynamic parameters summarized in Tab. 6 indicated the feasibilty of activated cokes for the adsorption of phenol and 4-nitrophenol. The negative value of free energy of the adsorption is an indicative for the spontaneous nature of the adsorption process. The higher free energy of DG-32 suggest its better quality compared with GG-64. The enthalpy change values obtained were very small, indicating that the adsorption was physical in nature. The negative values of enthalpy suggest the exothermic nature of the adsorption process. The positive values of entropy for the process have also supported the interpretation previously mentioned, i.e., they reflect the affinity of the activated coke towards phenol and 4-nitrophenol.

TABLE 6 - Thermodynamic parameters for the adsorption of phenol (P) and 4-nitrophenol (4-NP)

Coke	Phenols	Temp (°C)	$-\Delta G°$ (kJ/mol)	$-\Delta H°$ (kJ/mol)	$\Delta S°$ (J/Kmol)
DG-32	P	20	25.6	9.4	57.4
		30	26.2		
		40	27.1		
	4-NP	20	26.7	10.1	59.1
		30	27.3		
		40	28.1		
GG-64	P	20	24.9	9.0	56.3
		30	25.1		
		40	26.2		
	4-NP	20	26.2	9.8	57.4
		30	26.7		
		40	27.1		

CONCLUSIONS

The results of the present equilibrium studies show that activated cokes are suited for the removal of phenol and 4-nitrophenol from aqueous solution.

The adsorption occurred very rapidly within the first 15 min and gradually reached equilibrium within the contact time of 90 min. The uptake of phenols increases as the initial concentration increases, even though the adsorbed fraction decreases with increase in concentration. There is no significant influence of pH observed on

the adsorption between pH values 2 and 10 for phenol and 2 and 8 for 4-nitrophenol. No significant difference of the percent removal of the sorbates at 20 °C and 30 °C. Thus, the removal process for practical applications can be performed under a neutral condition and at room temperature. This may be important to be noted especially when viewed both from the ecological and economic perspectives. At concentrations of the experiments the rate-limiting step is intraparticle transport. It was found that the intraparticle diffusion becomes increasingly dominant in limiting the transfer of the sorbate with an increase of concentration.

These equlibrium adsorption data would be meaningful for further experiments of down-flow adsorption technique using a column which is imperative to conduct prior to obtaining design model.

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ELECTROCHEMICAL REDUCTION OF CO_2 ON GRANULE ELECTRODES IN A FIXED-BED REACTOR IN AQUEOUS MEDIUM

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SUMMARY

Electrochemical reduction of CO_2 has been investigated in aqueous carbonate and bicarbonate solutions under ambient conditions (electrode potentials of –1.6 to –2.2 V). An undivided fixed-bed reactor was used as electrochemical cell, where Pb and Sn granules have been placed at the bottom of the cell as electrode material. Only one organic product, formic acid, has been obtained by the reaction with high current efficiencies. The current efficiencies of formic acid produced on Pb granules and in $KHCO_3$ solution were found to be higher than those on Sn granules and in K_2CO_3 solution. The best results, with maximum Faradaic efficiencies up to 95%, have been obtained by using a Pb/$KHCO_3$ system.

KEYWORDS: Electrochemical reduction, carbondioxide, bed-reactor, Pb and Sn granule electrodes.

INTRODUCTION

Energy-related activities, industrial processes, land-use change and waste combustion are the main sources of carbon dioxide increase in the atmosphere causing environmental pollution and enhancement of the greenhouse effect. Conversion of CO_2 to large-scale chemical products or its use as potential energy source are some of the most important topics in electrochemistry. The fundamental research is stimulated by the similarity of CO_2 reduction by photosynthesis. It has been the aim of many chemists [1 - 3] to utilize the naturally occurring process of carbon fixation under laboratory conditions or as a model for manufacturing of synthetic organic materials. Product distribution in CO_2 reduction mainly depends on electrode material and the supporting electrolyte. For example, Hg, In, Pb and Sn electrodes are used for the cathodic reduction of CO_2 and the only product found in aqueous medium is formic acid [1, 4 – 7]. Some metals, such as Pb, Tl and Hg, give oxalic acid as main product in aprotic medium With Au, Ag, In and Zn as electrodic material oxalic acid and CO are mainly produced [1, 5, 7]. Pt, Pd and Ni are selectively used for CO formation.

Additionally, the same metals can behave differently in protic and aprotic medium. Cu provides extraordinary catalyses for the electroreduction of CO_2 in aqueous and non-aqueous electrolytes depending upon varied experimental conditions [8, 10]. Cu electrodes exhibit an interesting high electroactivity for the formation of CH_3OH, CO and hydrocarbons such as CH_4 [9, 10]. At ambient temperature and pressure CO_2 has been reduced photoelectrochemically to hydrocarbons [11, 12], such as CH_4, C_2H_4, C_2H_6 and CO, HCHO, HCOOH and EtOH in aqueous bicarbonate solution. The reduction of CO_2 has also been investigated under high pressure on gas diffusion electrodes [13, 14] with faradaic efficiencies up to 86%.

However, most of the afore-mentioned works have been done in a divided H-cell with metal plate electrodes. The electrochemical reduction of carbonate ions on metal plate electrodes in aqueous 5 M K_2CO_3 solution have been studied in an undivided cell by Osterova *et. al.* [15] and they found methane, ethylene, formaldehyde and other organic compounds as reaction products in low concentrations. In the present study, we have attempted to reduce CO_2 on Pb and Sn granule electrodes in an undivided fixed-bed reactor. The aim was to extend the electrode surface as much as possible within a small electrochemical cell volume to reduce CO_2 selectively with high Faradaic efficiencies to organic compounds without any pollutant.

MATERIALS AND METHODS

Cell construction and electrode preparation

The fixed bed reactor (Fig. 1) used for the electrolyses consists of a glass tube (250 mm in length and 56 mm in diameter, maximum volume 100 mL). A glass frit as gas inlet is placed at the bottom of the cell. The bed electrodes

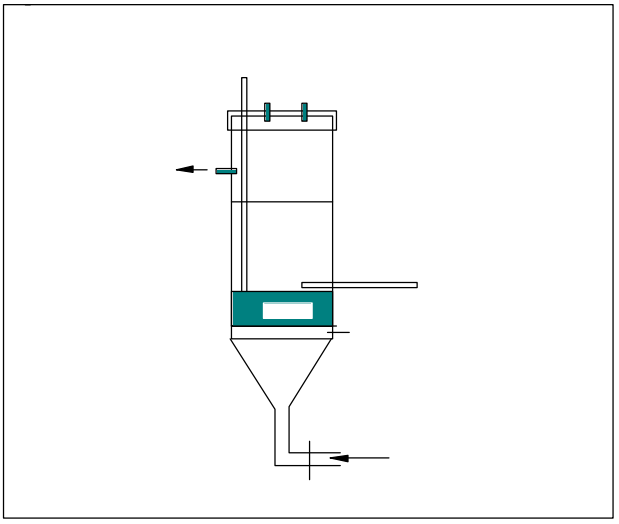

FIGURE 1 - Electrochemical fixed-bed reactor (schematically).

were Pb (1 mm diameter, 38 cm^2 surface area) or Sn- (3 mm diameter and 190 cm^2 surface area) granules, respectively. The bed thickness was approximately 2 cm and a Pt-counter electrode (surface area 6 cm^2) was placed over the granules. Gas outlet, mountings for thermometer and reference-electrode were fixed at the top of the cell and the electrical contact to the bed was facilitated by a Pb or Sn wire. Electrode potentials were measured by a saturated calomel reference electrode (SCE) throughout the experiments.

Pb and Sn granules (Merck, 99.99%) were activated electrochemically for 2 hours at -2.5 V (SCE) before each electrolysis. Following these activation processes, the granules received their characteristic shiny metallic colour.

Experiments

Cyclic voltammetric measurements have been carried out with a PAR model 362 potentioscanner on working Pb and Sn plate electrodes with a surface area of 1 cm^2 in a common H-cell. After preparation of electrodes, the electrolytes have been changed with freshly prepared solutions of 0.1M K_2CO_3 and 0.5 M $KHCO_3$, respectively, and the solutions have been saturated with CO_2 for one hour. All electrolyses have been performed and controlled at five different electrode potentials of -1.6, -1.8, -2.0 and –2.2 V (SCE). During the electrolyses, CO_2 was bubbled through the solution at a constant flow-rate of 6 mL/min. 5 mL of the solution has been sampled in 30 min intervals and formic acid content of the samples has been repeatedly determined by high performance liquid chromatography (Perkin Elmer LC 200, ODS-18 coulumn) and gas chromatography (Hewlett-Packard 6890, TCD, FID, Propac Q and QS coulumns). The results were found to be quite reproducible.

RESULTS AND DISCUSSION

Formic acid electrolyses

Since an undivided cell has been used throughout the experiments, electrolysis control tests to prove the possible oxidation on the Pt-anode (counter electrode) of HCOOH produced, were performed in 0.1 M K_2CO_3 and 0.5 M $KHCO_3$ at various electrode potentials of -1.6, -1.8, -2.0 and -2.2 V (SCE) with an initial HCOOH concentration of 8.7 mmol. The same procedure as described in the electrodes section, has been used for this electrolysis. In 0.1 M K_2CO_3 up to 11% of HCOOH were found to be oxidized, whereas the amount in 0.5 M $KHCO_3$ was considerably low (6-6.5 %).

CO_2 reduction Pb/ 0.1 M K_2CO_3 electrolyte system

Experimental results obtained from CO_2 reduction on Pb in 0.1M K_2CO_3 are presented in Fig. 2. During the CO_2 reduction, HCOOH has been detected as the only hydrocarbon produced at negative potentials between –1.5 to -2.3 V vs SCE in aqueous solution (Fig. 2b). The electrolysis under these conditions has given no detectable gaseous hydrocarbons.

The maximum current density of ca. 5.1 mA/cm^2 for CO_2 reduction on Pb electrode in carbonate solution has been obtained at -2.25 V (Fig. 2a). It has also been observed that competitive H_2 evolution on Pb granules increased as the electrode potential shifts to more negative values. During the electrolysis, significant changes have been observed in current densities on Pb electrode between -1.5-to -2.1 V. The maximum current efficiency of 40 % has been observed at –1.8 V after 30 min. (Fig. 2b).

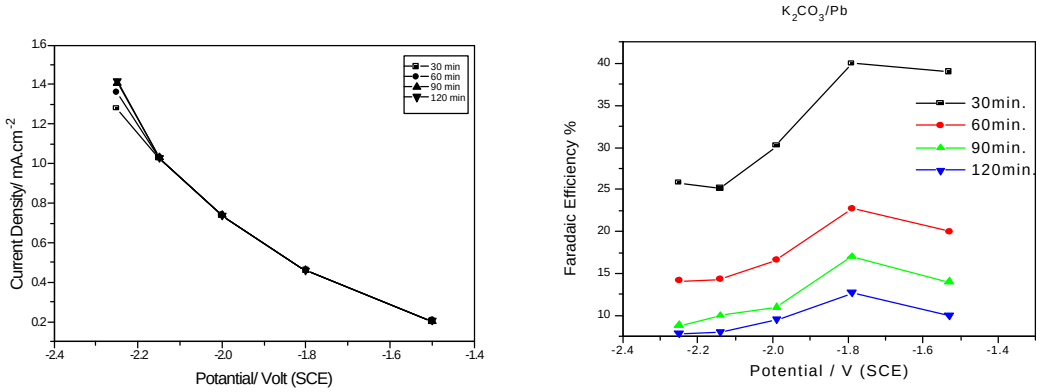

FIGURE 2 a - The current density-potential diagram of CO_2 reduction on Pb electrode at various time periods;
FIGURE 2 b - Faradaic current efficiency-potential diagram for formic acid formation on Pb electrode at different time intervals (0.1 M K_2CO_3, pH: 7.40).

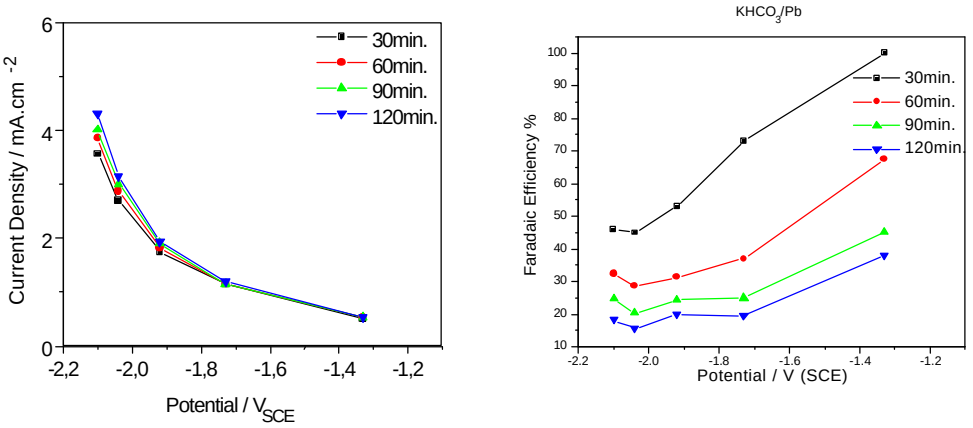

FIGURE 3 a - Current density-potential diagrams for HCOOH formation on Pb granules at different electrolysis time in 0.5 M $KHCO_3$ as supporting electrolyte
FIGURE 3 b - Faradaic efficiency-potential diagrams for HCOOH formation on Pb granules at different times in 0.5 M $KHCO_3$ as supporting electrolyte.

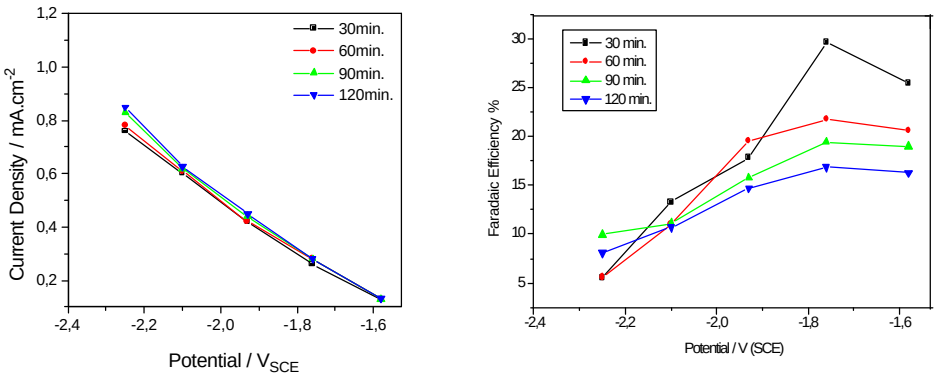

FIGURE 4 a - Current density-potential diagrams for HCOOH formation on Sn-granules at different times in 0.1 M K_2CO_3 as supporting electrolyte.
FIGURE 4 b - Faradaic efficiency-potential diagrams for HCOOH formation on Sn-granules at different electrolysis time in 0.1 M K_2CO_3 as supporting electrolyte.

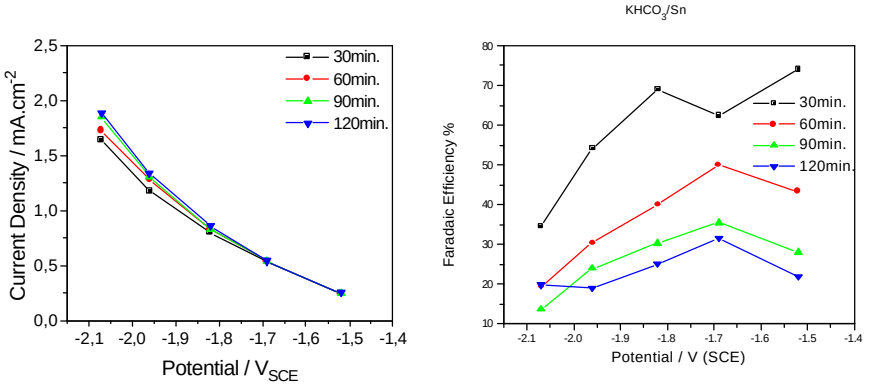

FIGURE 5 a - Current density-potential diagrams for HCOOH formation on Sn-granules at different electrolysis time in 0.5 M $KHCO_3$ as supporting electrolye.

FIGURE 5 b- Faradaic efficiency-potential diagrams for HCOOH formation on Sn-granules at different electrolysis time in 0.5 M $KHCO_3$ as supporting electrolye.

TABLE 1 – Experimental results of HCOOH formation at different electrolyte/ electrode systems at ambient temperature and pressure conditions for all electrolyses after 30 min.

Potential V (SCE)	i (mA/cm^2) **0.1 M K_2CO_3**		i (mA/cm^2) **0.5 M $KHCO_3$**		η (%) **0.1 M K_2CO_3**		η (%) **0.5 M $KHCO_3$**	
	Pb	Sn	Pb	Sn	Pb	Sn	Pb	Sn
-1.50	0.21	-	**0.79**	0.25	39.0	-	**90**	74
-1.60	0.29	0.13	**0.96**	0.35	39.5	27	**80**	66
-1.70	0.38	0.20	**1.13**	0.54	39.7	29.5	**74**	62
-1.80	0.47	0.26	**1.41**	0.80	40.0	23.5	**65**	68
-1.90	0.63	0.42	**1.72**	0.90	35.0	16.5	**53**	60
-2.00	0.75	0.55	**2.71**	1.18	30.2	14.2	**47**	47
-2.10	0.95	0.60	**3.56**	1.64	27.1	11.0	**45**	29
-2.20	1.31	0.76	-	-	26.0	6.0	-	-

Due to the H_2-evolution, the Faradaic efficiencies for HCOOH formation decreased slightly with extending electrolysis time. though, the concentration of HCOOH reached the maximum value of 2.2 mmol (1000 ppm) after 2 hours.

CO_2 reduction in Pb/0.5 M $KHCO_3$ electrolyte system

During CO_2 reduction on Pb in 0.5 M $KHCO_3$, HCOOH was also the only organic product. However, current densities and Faradaic efficiencies were found to be relatively higher than those found in 0.1 M K_2CO_3 electrolyte. Experimental results for this system are presented in Fig. 3.

Maximum current densities on Pb granules under identical conditions were ca. three times higher than in K_2CO_3 (Fig. 3a). Maximum current efficiency for formic acid formation was found to be about 95% after 30 min of electrolysis (Fig. 3b). Azuma *et al* [6]. have found current efficiency values of 28.5 % on Sn and 16.5 % on Pb electrodes for formic acid formation at –2.2 V in bicarbonate solution and Ikeda *et al* [4] 72.9% for Pb and 67.5% for Sn electrodes at –2.0 V in an 0.1 M TEAP/H_2O electrolyte system. The current efficiencies in our experiments are significantly higher and the overpotential applied is smaller.

Hori *et.al* [2] have reported that the product soluble in the electrolyte solution was also only formate ion. Their experiments carried out on metal plate electrodes at –1.62 V and constant current densities of 5 mA/cm^2 electrolyses in a divided cell have given current efficiencies for Pb electrodes between 72.5% and 88.8%. Therefore, the current efficiencies are varying for Sn electrodes between 65 and 79.5% at –1.4 V. Kyriacou *et al* [5] have investigated the influence of CO_2 partial pressure and the supporting electrolyte cation on the product distrubution in CO_2 reduc-

tion and found a current efficiency value of 5 % for formic acid formation on copper electrodes in $KHCO_3$ solution. With decreasing CO_2 pressure current efficiency usually diminishes linearly and the hydrogen evolution increases. These results are corresponding with our high-pressure results, which are not presented in this paper.

Azuma *et al* [7] have investigated the influence of temperature on CO_2 reduction in $KHCO_3$ solution. At about 2 °C and –2.2 V they have found a current efficieny of 3.8 % for HCOOH formation on Pb electrode. At 0 °C and –1.8 V, this value rises to 16.7 % and at –2.0 V to 16.5 %. Also, these low-temperature experiments have given no reasonable results regarding current efficiencies.

CO_2 reduction in Sn/0.1 M K_2CO_3 electrolyte system

With 0.1 M K_2CO_3 as supporting electrolyte and Sn granules as electrode only formic acid was sysnthesized as organic compound. The current densities and current efficiencies were lower than those of the Pb/K_2CO_3 system (Figs. 2a and 2b). Current density potential and current efficiency-potential are presented in Figs. 4a and 4b, respectively.

The maximum current density for the formation of formic acid on Sn granules in K_2CO_3 (1.6 mA/cm^2) is about three times lower than that found for Pb/ K_2CO_3 system (Fig. 4a). The Faradaic efficiencies have a maximum value of ca. 75 % (Fig. 4b).

CO_2 reduction in Sn/0.5 M $KHCO_3$ electrolyte system

Only formic acid was detectable as organic compound by HPLC and GC analyses after the electrochemical reduction of CO_2 on Sn granules in $KHCO_3$ solution. Current density-potential and Faradaic efficiency-potential diagrams of HCOOH formation on Sn granules in $KHCO_3$ are shown in Figs. 5a and 5b, respectively.

In analogy to the results obtained on Pb electrodes, also in bicarbonate solution the current densities and efficiencies are higher than those in Sn/K_2CO_3 system (Fig. 5). As can be easily inferred from this comparison, for CO_2 reduction $KHCO_3$ is a better electrolyte than K_2CO_3 for both of Pb and Sn electrodes. Comparison of the experimental results obtained from the different systems is presented in Table 1.

CONCLUSION

For the first time the electrochemical reduction of CO_2 on Pb and Sn electrodes in aqueous $KHCO_3$ and K_2CO_3 electrolyte in a fixed-bed reactor has been studied. Formic acid as predominant product was observed in the working potential ranges for both electrodes. The highest current efficiencies for formic acid production obtained in bicarbonate solutions after 30 min at –1.5 V [SCE] were found to be 74 and 95 % for Sn and Pb electrodes, respectively. At more negative overpotentials, i.e. –2.0V [SCE], current efficiencies drop to values of 30.2 % and 23.5 % for Pb and Sn electrode, respectively, due to the increase of H_2-evolution in bicarbonate solution.

Compared to the values given in literature, we have obtained appropriate results in respect to the current efficiencies and the overpotential values applied in the bed-reactor. But, the current densities are not high enough for a conventional process in the present system. To increase the solubility of CO_2 in aqueous solution and the current density of the process, electrolysis should be carried out under sub/supercritical conditions.

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SOLID PHASE MICROEXTRACTION (SPME) USED FOR DIRECT FRUIT-BODY SAMPLING IN COMPARISON WITH ASE-GC-MS TO DESCRIBE PAH PARTITIONING IN MUSHROOM CULTURES

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SUMMARY

Partition of fluorene, phenanthrene and pyrene in the cultures of the basidiomycete *Pleurotus ostreatus* (oyster mushroom) was determined by GC-MS analysis coupled with SPME directly within the growing mushroom fruit-body. Headspace (HS) SPME of the corresponding straw/ mycelium culture provided information on the contents of PAHs remaining, thus enabling the transfer ratios of PAHs to be estimated. ASE-GC-MS was applied by way of comparison.

KEYWORDS:
SPME fruit-body sampling, mushrooms, PAH, translocation

INTRODUCTION

Environmental transport pathways involved in the contamination of plants by mainly pesticides, PAHs and chloroorganic compounds are relatively well studied [1, 2]. By contrast, little is known about the behavior of organic pollutants in fungal cultures such as cultivated edible mushrooms. Instead, interest has been primarily focused on fungal ability to accumulate heavy metals and radioactive isotopes [3, 4]. For example, wild mushrooms have been used as bioindicators for the radionuclides Cs-134 and Cs-137, which typically appear in the environment after nuclear weapons tests (and were also released by the accident at Chernobyl). Nevertheless, the contents of pesticides used in mushroom production have been monitored to ensure food quality. The deposition of particles and airborne substances on the mushrooms fruit-bodies is assumed to be the main pollution pathway, with a lesser role being played by the uptake *via* the mycelium. Analytical protocols used to determine the contaminants in the food are based on the extraction of lyophilized and homogenized plants and fruits, several clean-up steps, and appropriate analysis – steps which taken together are both time- and chemical consuming [5, 6]. In food control alone, large series of analyses have to be performed, since fast results are often needed. As recently described for flavor, terpene and pesticide analyses in food, SPME sampling in juicy fruit material is a useful technique [7 - 11] but is mostly geared to determine polar and volatile compounds at fairly high concentrations.

This study examines the suitability of direct SPME sampling of less volatile and lipophilic compounds, such as PAHs, from solid materials such as mushrooms.

MATERIALS AND METHODS

Fluorene (FLO), phenanthrene (PHE), and pyrene (PYR) of research grade purity were supplied by SUPELCO. Methanol used for the preparation of spike solutions, acetone and n-hexane used for Accelerated Solvent Extraction (ASE) were of UV grade purity and purchased from Merck, Darmstadt.

Preparation of contaminated mycelium/ straw cultures

Dried, chopped, wheat straw (100 g) was moistened with distilled water (300 ml) and sterilized in plastic bags at 120 °C for 45 min. The bags were inoculated with mycelium of *Pleurotus ostreatus* (Jacq.:Fr.) Kumm., strain 670/93 and incubated at 26 °C for 4 weeks. Two bags were used for biotic and analytical control. Two other bags were mixed with 200 g of wet, sterilized straw spiked (sprayed) with a methanolic solution of analytes to reach a final concentrations of 11 µg of FLO, PHE, PYR per gram of dry straw. All the bags were incubated for 2 more weeks at 26 °C and after that subjected to a cold shock (3 days, 5 °C) to induce fructification. The

fruit-bodies developed under day light at 20 °C during the next two weeks. When primordia of fruit-bodies appeared on the mycelium colonized straw, few cuts in the plastic bags allowed the fruit-bodies to be formed outside the bag.

SPME conditions

SPME was carried out with polyacrylate (PA, Supelco) coated fibers (film thickness 85 µm). Mushrooms samples were prepared by direct fiber exposure within the fruit-bodies. While growing (fruit-body size about 3 cm), the fiber was positioned in the upper part of the stem by piercing through the cap of the oyster mushroom. The sampling procedure was continued for two days until the mushroom reached about 7 cm in diameter. Subsequently, GC-MS analysis of the PAHs extracted was carried out. After the mushrooms had been harvested, the remaining straw with mycelium was lyophilized and homogenized. For HS-SPME, 1 g of this material was thermostated for 10 min in a 10 ml vial, then sampled by SPME and analyzed by GC-MS. Standard addition was used to quantify PAHs. Preliminary HS-SPME experiments with spiked straw/mycelium material (50 ng/g straw of each PAH) guaranteed optimum microextraction efficiencies as a function of extraction time and temperature.

ASE conditions

The mushroom fruit-bodies as well as the straw grown with mycelium were lyophilized, homogenized, spiked with 100 ng tetrachloronaphthalene as internal standard, and ASE-extracted twice with n-hexane/acetone (50:50, v/v) at 100 °C and 100 bar for 5 min. The combined extracts were dried with sodium sulfate and concentrated to a final volume of 1 ml. 1 µl was injected for GC-MS analysis. Aliquots of control straw/mycelium and the spiked fungal cultures (4 g of each after mushroom harvest) were lyophilized, homogenized, spiked with an internal standard and extracted by ASE as used for the mushrooms. Additionally, after the evaporation of the combined extracts, silica gel clean-up was performed. Even then, parts of the matrix remained and strongly interfered with the PAH signals, making it difficult to interpret the data.

GC-MS analyses

GC-MSD system "6890" (Agilent Technologies, San José, USA) equipped with a 30 m HP-5 MS capillary column (0.25 mm I.D., 0.25 µm film thickness) was used. An injector temperature of 280°C was used for both syringe injection and SPME fiber desorption (for 2 minutes) in splitless mode. The overall analysis program used was: 110 °C (1 min.), 7 °C/min to 280 °C (10 min.). Helium was applied as carrier gas at constant flow conditions of 40 ml/min.

RESULTS AND DISCUSSION

PAHs in mushrooms

Direct fruit-body SPME sampling and HS-SPME/GC-MS of straw/mycelium homogenized were performed to determine the partition of PAHs in the whole fungal culture. The advantage of using mushroom culture in this study is that the respective pollutants (PAHs) are translocated *via* the fungal mycelium from the contaminated straw inside the plastic bag into the fruit-bodies that are formed outside the bag. In the case of using white rot fungi (group of the oyster mushroom) their ability to degrade different organopollutants (including PAHs) has to be taken into account when the total sum of PAHs is calculated at the end of the experiment. The PAH amounts directly sampled in the mushrooms and analyzed by GC-MS are shown in Fig. 1.

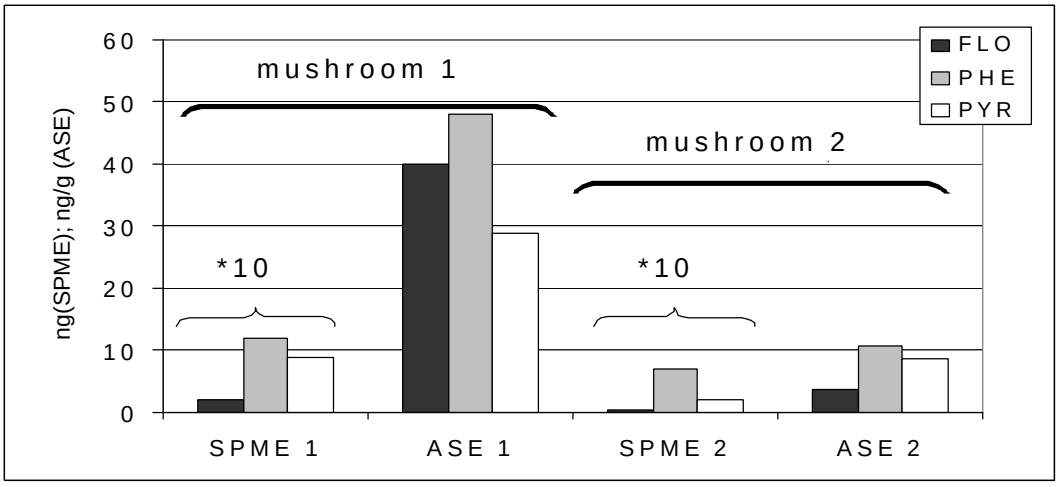

FIGURE 1 - Comparison of directly SPME-sampled mushrooms with ASE-GC-MS results.

SPME results obtained from a parallel mushroom culture bag (Fig. 1) were in a comparable ng range confirming the PAH ratio. Deriving a relationship from the PAHs content present in the whole fruit-bodies is difficult because the volume or distance from which the PAHs were extracted is not yet clear. Therefore, the values are only given in ng, as opposed to the PAH concentrations in ng/g mushroom obtained by ASE-GC-MS (Fig. 1). Nevertheless, SPME analyses reflect the same partition of individual PAHs as found by ASE. Both methods of analysis showed the predominance of PHE followed by lower levels of PYR and FLO.

All mushroom fruit-bodies harvested were analyzed by ASE-GC-MS and quantified with external calibration. The discrepancy in the ASE results of the two mushroom cultures may have been caused by the differences in degradation rates and amounts of mushroom fruit-bodies collected or by a delay in the extraction of the second mushrooms stored in the refrigerator for 4 days prior to analysis. Further experiments are needed to clarify these effects and also to investigate the actual distribution of PAHs within the mushroom culture.

PAH analyses of straw/mycelium

A series of HS-SPME experiments with spiked, lyophilized and homogenized straw/mycelium material provided optimum extraction conditions for polyacrylate coated fibers at 60 °C for 1 h extraction time including a precondition phase of 10 min at the same temperature (Fig. 2).

Higher temperatures result in lower extraction yields of the analytes from SPME fiber. However, extending the extraction time by 15 min improved the extraction yield, at least in the case of PHE and PYR (Fig. 2, bottom).

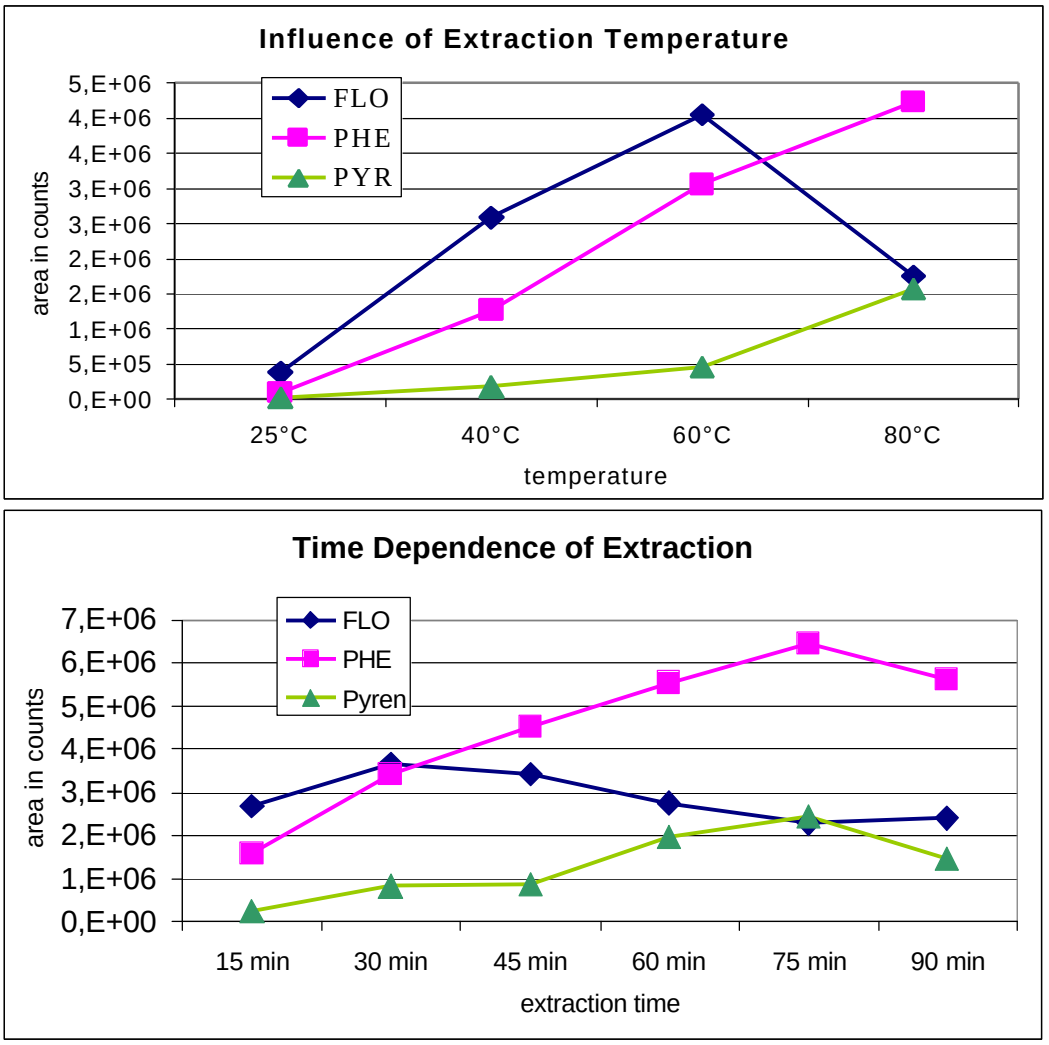

FIGURE 2 - Efficiency of HS-SPME extraction from straw/ mycelium spiked with PAHs depending on the extraction temperature and sampling time.

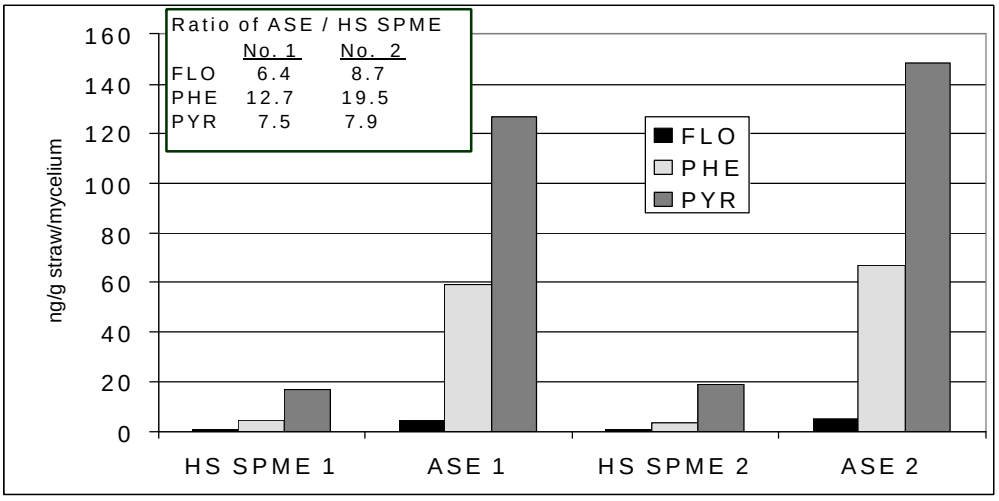

FIGURE 3 - Comparison of PAH contents in straw/mycelium determined by HS-SPME and ASE both coupled to GC-MS (1 and 2 indicate two parallel experiments).

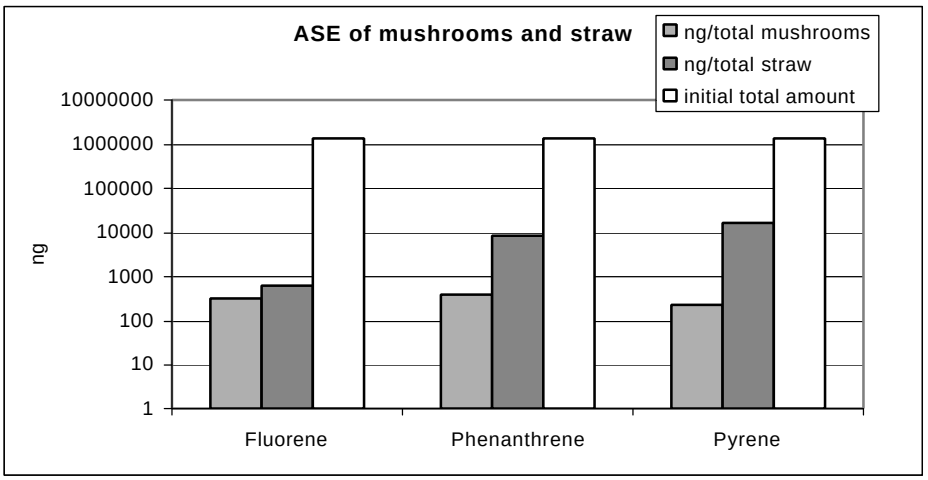

FIGURE 4 - Concentration of FLO, PHE and PYR analyzed of two parallel Pleurotus ostreatus straw cultures by ASE and GC-MS and compared with initially applied PAHs amounts.

Of course, ASE extracts PAHs more exhaustively than HS-SPME, which is based on the complex adjustment of partition equilibria between several phases of headspace extracted sample. Both extraction methods showed fairly reproducible results, bearing in mind the fact that the experiments were prepared and performed separately. Moreover, the ratio between ASE and HS-SPME results is also constant (Fig. 3). Despite including the blanks in the calculation, the difference in PHE extractions is one magnitude higher than that found for FLO and PYR. Some more experiments are needed to establish whether there exists a constant factor between ASE and HS SPME for PAHs, volatile enough for headspace analysis.

As the higher volatile FLO showed optimum extraction yield at 30 min, the above-mentioned conditions are a compromise. The corresponding concentration of PAHs remaining in the straw/mycelium was also determined by ASE. Standard addition was used for both methods to calculate PAH concentration. Although these two very different extraction principles can scarcely be compared, doing so allows the HS-SPME results to be ranked (Fig. 3).

In order to consider the partition of FLO, PHE and PYR in the whole fungi cultures, the translocation behavior of the analytes was estimated using the ASE results. Fig. 4 shows the concentrations determined in mushrooms and straw/mycelium.

Originally the target analytes were applied in the same amounts of 1.3 mg of each to spike the culture bags. After the experimental run-time, the PAHs were detected in very different amounts. PYR mostly remained in the straw compared to PHE and FLO. Several mechanisms may cause the reduction of the target PAHs. In addition to degradation, physical processes such as ad-/absorption and losses, might be involved, while more volatile PAHs (FLO) might be particularly susceptible to the evaporation of extracts. In order to avoid such influences, the controls were treated in the same way and so the main reducing effect ought to be related to transformation and degradation as well as translocation.

The results indicate the different degradation efficiency *of Pleurotus ostreatus vis-à-vis* the target analytes. Only 1.2% of PYR, 0.6% of PHE and 0.04% of FLO remained in the whole cultures. Martens and Zadrazil [12] during their screening of a set of white rot fungi on the ability to mineralize selected PAHs found the strains belonging to *Pleurotus* genus to be most effective; *e.g.* mycelium of a strain of *Pleurotus* sp. growing on straw contaminated with ^{14}C-pyrene was able to mineralize over 40% of the added radioactivity to $^{14}CO_2$. Also our previous investigations using liquid cultures of ligninolytic fungus *Irpex lacteus* confirmed these degradation results for PHE and PYR [13].

The portions of PAHs translocated from contaminated straw to mushroom fruit-bodies are not negligible if they are related to the contents in the straw substrate. Fig. 4 indicates that PHE is the major compound in the mushroom fruit-bodies, however, lower concentrations of FLO and PYR are also present there. Related to the contents in the straw, the analytes seem to be accumulated in fruit-bodies in the order FLO>PHE>PYR, which is probably caused by their descending water solubility [14].

The analytical investigations presented prove the incorporation of even lipophilic compounds, such as PAHs, *via* the following route: culture substrate (straw) – mycelium – fruit-body.

Direct SPME in fruit-bodies is a useful, simple sampling procedure that, when combined with GC-MS, delivers fast screening results in, for instance, food control.

ACKNOWLEDGEMENT

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ANALYTICAL METHODS FOR THE DETERMINATION OF PRETILACHLOR AND FENCLORIM IN SOIL AND RICE PLANTS

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SUMMARY

HPLC method to determine simultaneously pretilachlor and fenclorim in soil and in rice plants is reported. The determination is also carried out by GC and GC-MS technique for the identification of the analytes. The method allows for a rapid and quantitative detection of the two compounds both in soil and rice plants. The limit of detection was 5 ng for HPLC and 10 ng for GC. Recoveries were satisfactory and varied from 79.9% to 93.0% in soil and 77.1% to 95.8% in rice plants. Their reproducibility and accuracy make these methods a selective and sensitive tool for routine analyses.

KEYWORDS:
Pretilachlor, fenclorim, analytical methods, soil, rice.

INTRODUCTION

Weed control in rice fields is difficult owing to the fact that some weeds such as *Oyiza sativa* var. *sylvatica* belonging to the same family or species as rice [1].

Pretilachlor [2-chloro-2', 6'-diethyl-*N*-(2-pro-poxyethyl) acetanilide = P] is a chloroacetamide herbicide commonly used in transplanted rice fields to control certain broad-leaved weeds and annual grasses including weeds similar to rice [2].

As the tolerance of rice to chloroacetamide herbicides is marginal, herbicide antidotes or safeners have been developed in order to avoid significant crop injury [3].

The development and the commercialization of the safener fenclorim [4,6-dichloro-2-phenylpyrimidine = F] have facilitated the selective use of P in rice fields to the extent that P and F are now generally coformulated [4 - 6).

Available databases [7] show a relatively strong adsorption on soils for P and F, and medium half-life values in soil (17-35 days for F and 20-50 days for P). The above chemodynamic properties suggest that the two compounds be classified as non-leachers and are thus not hazardous from a potential groundwater pollution point of view [8]. Since the rice field is an artificial environment, in which the equilibrium is somewhat weak and could be easily altered, an accurate and continuous control of the level of xenobiotic pollution is of interest.

A multiresidue method is reported in literature for GC detection of some herbicides, with a P detection limit of 0.01 µg L^{-1} in water and 0.05 µg g^{-1} in suspended substances [9].

The present paper describes an analytical method for a sensitive simultaneous determination of P and F in soil and rice plants. The procedure was standardized for HPLC analysis of the two analytes. However, it can be also performed by GC analysis, while GC-MS may be employed for the identification of the two compounds.

MATERIALS AND METHODS

Chemicals and Apparatus

Standard pretilachlor (96.0%) and fenclorim (99.5%) were supplied by Dr. Ehrenstorfer GmbH, D-86199 Augsburg.

A Perkin-Elmer Series 410 HPLC, equipped with an LC 95 UV detector at 210 nm wavelengths and with a C-18 column (4.6 mm i. d.; 25 cm length), was used for residue determinations. A CH_3CN/ H_2O (70/30) mixture was used as mobile phase with a flow rate of 1 ml min^{-1}. Under these conditions retention time was 11.5 min for P and 13.0 min for F.

A Varian Star 3400 gas chromatograph equipped with an OV17 3% capillary column (0.25 mm i.d. x 25 m length) and a Varian Saturn II mass spectrometer, electron impact ionization at 70 eV, were employed. Injector temperature was 150 °C and column temperature 60 °C for 1 min and then raised by 20 °C min^{-1} to 260 °C; cleanup was at 260 °C for 10 min and gas carrier flow (helium) was 2 mL min^{-1}. Mass spectrometer conditions were as follow: transfer line, 260 °C; manifold, 270 °C; automatic gain control; electric current emission, 10 mA. Under these conditions the retention time was 10.6 min for F and 13.3 min for P.

Sample preparation and analysis

Soil

One soil type taken from the 0-20 cm layer at one site in Central Italy (Papiano, Perugia) was used. Determination of pH, cation exchange capacity, organic carbon content and particle size distribution was performed according to ASA-SSSA methods [10, 11] (Table 1).

TABLE 1 - Physico-chemical properties of soil used.

pH (H_2O)	8.10
Organic carbon (%)	1.22
C.E.C.* (meq $100g^{-1}$)	16.5
Sand (%)	23.5
Silt (%)	47.5
Clay (%)	29.0

For the soil sample, a suitable amount of moist soil was air-dried and sieved (<2 mm) to remove plant materials, soil macrofauna and stones. After sieving, the soil was homogenized for 3 h in a rotary cylinder and stored at 20 °C in the dark for 3 days (pre-incubation).

For P and F recovery studies, triplicate soil samples (100 g) were contaminated with methanolic solutions of them to obtain concentrations of 0.1, 0.5, 1.0, 2.0 and 5.0 mg kg^{-1}. Each sample was immediately extracted twice with 100 ml methanol-water solution (4:1). After centrifugation at 5000 rpm for 10 min, the residues were filtered under vacuum and the extracts were combined and partitioned in $CHCl_3$ (40 ml x 3 times). The $CHCl_3$ phase was evaporated to dryness, rinsed with 1 ml of the mobile phase and subjected to HPLC analysis.

Plant material

Rice (hybrid Pegaso) seeds were used. The seeds (45 g) were placed on filter papers in growth dishes (750 cm^2) moistened with water and kept in a growth chamber in the dark at 25 °C and relative humidity, 75%. After 4 days the seedlings were subjected to day-night conditions (12 h of light, 5000 lux and 12 h of darkness) at 25 °C. 10 days after seeding the seedlings were collected and P and F were added to the samples so as to obtain concentrations of 0.1, 0.5, 1.0, 2.0 and 5.0 mg kg^{-1} of each of them. 2 g of samples in triplicate, for each concentration, were homogenized for 3 min, each with 20 ml of methanol-water solution (4:1); after sonification for 3 min the samples were centrifuged for 15 min at 5000 rpm and filtered under vacuum. After the addition of 20 ml of water, the residues were partitioned in $CHCl_3$ (2 x 30 ml), evaporated to dryness, rinsed with 1 ml of mobile phase and analyzed by HPLC.

Tests were also performed in which the residues from evaporated $CHCl_3$ solutions were rinsed with 1 ml of *n*-hexane and subjected to GC-MS analysis.

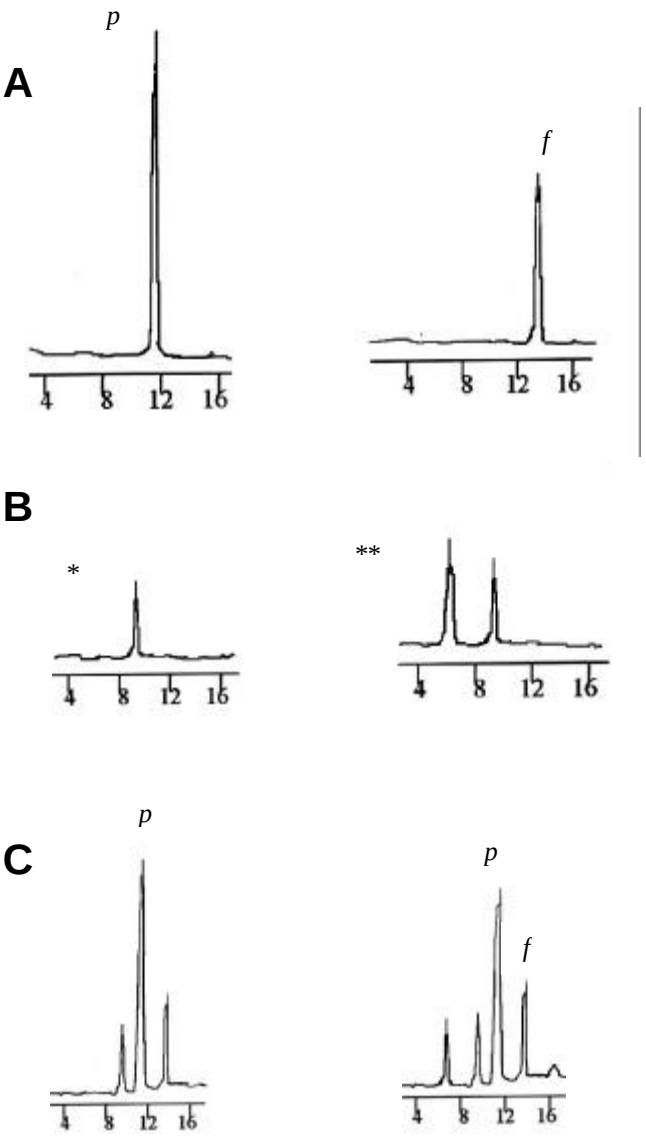

FIGURE 1 – HPLC chromatograms of standard of pretilachlor (*p*) and fenclorim (*f*) (A) and extracts from untreated (B) and treated (C) soil and rice plants (*=soil; **=plant).

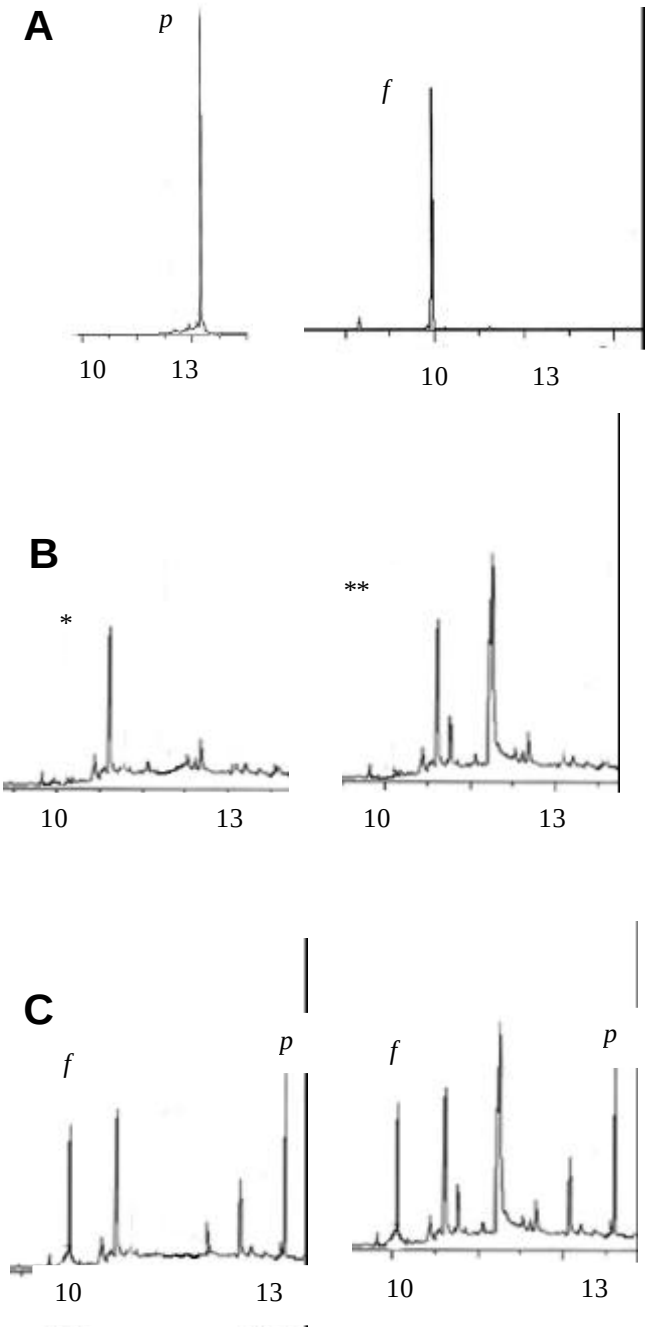

FIGURE 2 – GC chromatograms of standard of pretilachlor (*p*) and fenclorim (*f*) (A) and extracts from untreated (B) and treated (C) soil and rice plants (*=soil; **=plant).

RESULTS AND DISCUSSION

Figure 1 shows the HPLC chromatograms of the standards of P and F (A) of extracts from untreated (B) and treated (C) soil and rice plants. The GC chromatograms of the same extracts are shown in Figure 2.

As can be seen, the peaks of the two compounds are well-separated from the peaks of soil and plant matrices and there is no interference between them in either the HPLC or GC analysis. The detection limit of the method, determined at a signal to noise ratio of two (12), was 5 ng of P and 8 ng of F for HPLC and 8 ng of P and 10 ng of F for GC analysis.

Figures 3 and 4 show the mass spectra of the standards P and F, and of the relative extracts from treated soil and rice plants. As can be seen, the identification of the two compounds in both soil and rice extracts was possible due to the complete overlapping of the relative spectra with those of the standards whether in terms of molecular ions or relative abundance of the peaks.

Due to the higher sensitivity shown by HPLC analysis, the subsequent recovery, reproducibility and accuracy tests were performed for the HPLC determinations of P and F.

Recoveries of the two compounds from soil and rice samples at different initial concentrations are reported in Table 2. They are satisfactory and varied from 79.9% to 93.0% in soil and from 77.1% to 95.8% in rice, depending on initial concentration.

In Table 3 the data obtained for "within day" analyses are reported in order to ascertain the reproducibility and accuracy of the method (Rustum, 1991). Reproducibility was quantified as the Relative Standard Deviation (RSD), being the percent ratio between the standard deviation of the data and the mean concentration determined. Soil RSD values ranged from 3.2% to 4.4% at different initial concentrations. Plant RSD values varied from 1.2% to 3.7%, thus indicating a good reproducibility of the method developed. Accuracy is the percent ratio between the mean concentration determined and the expected concentration that is the same as the recovery percentage in Table 2. The values found in the present experiment for soil and plant varied from 77.1% to 95.8%, indicating a good degree of constancy in recoveries.

In conclusion, the method described in this paper allows a rapid and quantitative detection of P and F in soils and rice plants. The reproducibility and accuracy, together with the good recoveries observed, make the method selective, reproducible and sufficiently sensitive. Furthermore, the absence of interference caused by solvents, reagents or soil and plant matrices as well as the good peak resolution make this procedure particularly suitable for routine experiments.

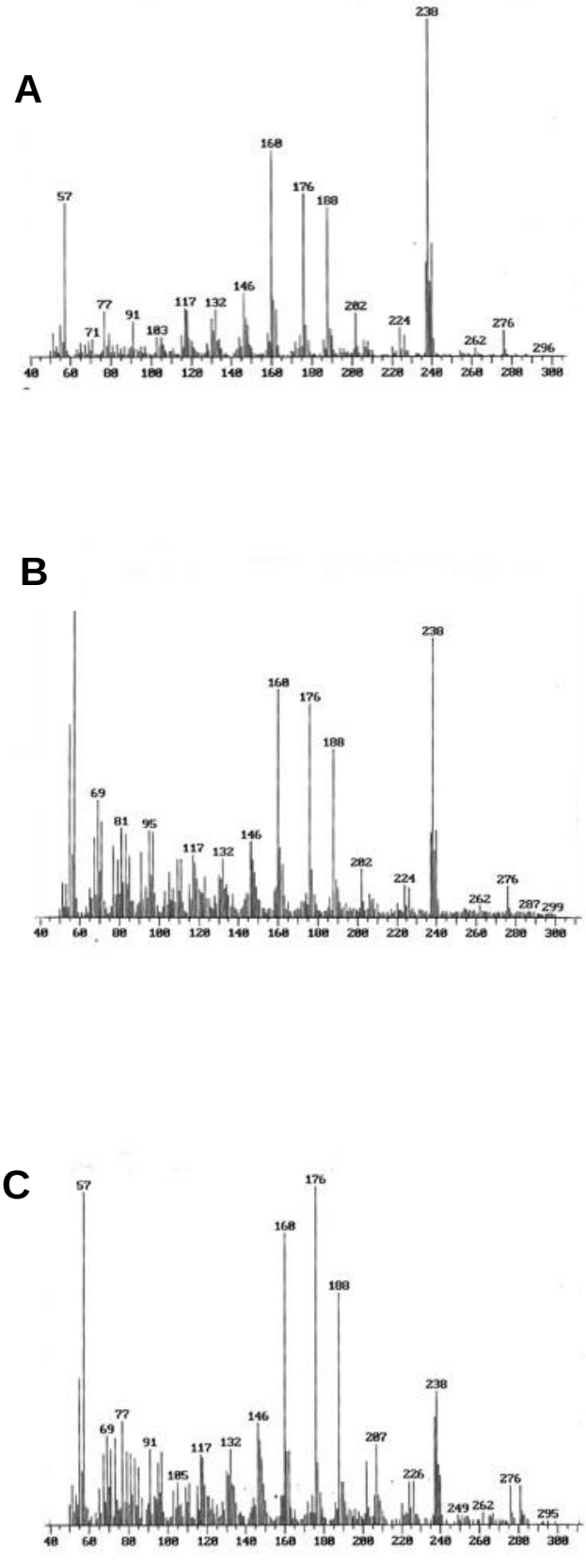

FIGURE 3 – Mass Spectra of pretilachlor: A standard, B soil extracts and C plant extracts.

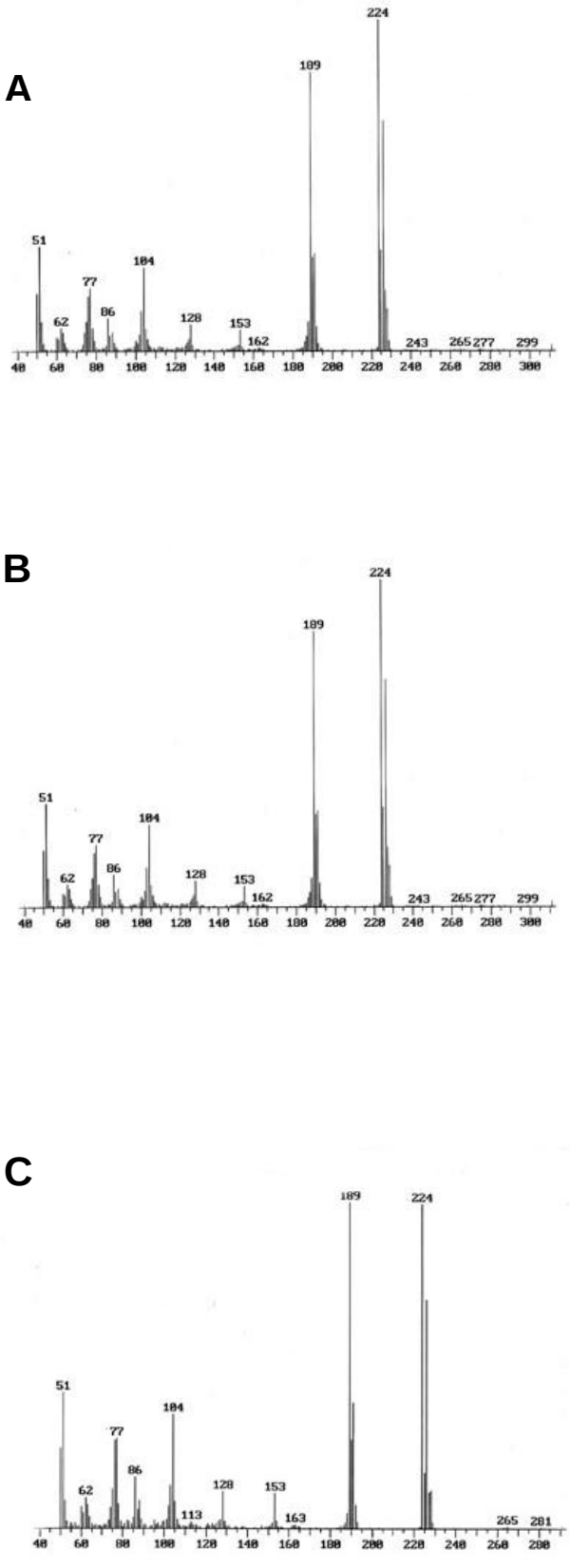

FIGURE 4 – Mass Spectra of fenclorim: A standard; B soil extracts and C plant extracts.

TABLE 2 - Recoveries (%) of pretilachlor (P) and fenclorim (F) from soil and rice plants at the three initial concentrations (standard deviation in brackets).

Initial concentration µg kg^{-1}	Soil		Rice	
	P	F	P	F
100	79.9(3.9)	85.3(4.1)	80.7(3.1)	82.1(3.3)
500	84.6(3.2)	82.4(3.8)	78.4(3.7)	77.1(2.6)
1000	89.0(4.0)	88.7(3.8)	95.8(1.2)	80.5(3.4)
2000	89.5(4.1)	90.5(3.6)	92.2(2.9)	83.7(3.0)
5000	93.0(4.4)	90.2(3.4)	90.6(3.6)	85.7(3.3)

TABLE 3
"Within day" assay reproducibility and accuracy of pretilachlor (P) and fenclorim (F) in soil and in rice plants.

Soil						
µg kg^{-1}	c.d. (µg kg^{-1}) mean (S.D.)		R.S.D. (%)		Accuracy (%)	
	P	F	P	F	P	F
100	159.8 (9.8)	85.3 (4.1)	3.9	4.1	79.9	85.3
500	423 (16)	412 (19)	3.2	3.8	84.6	82.4
1000	890 (40)	887 (38)	4.0	3.8	89.0	88.7
2000	1790 (82)	1810 (72)	4.1	3.6	89.5	90.5
5000	4650 (350)	4510 (170)	4.4	3.4	93.0	90.2
Rice						
100	80.7 (3.1)	82.1 (3.3)	3.1	3.3	80.7	82.1
500	392 (18)	385 (13)	3.7	2.6	78.4	77.1
1000	958 (12)	805 (34)	1.2	3.4	95.8	80.5
2000	1844 (58)	1675 (60)	2.9	3.0	92.2	83.7
5000	4530 (180)	4285 (165)	3.6	3.3	90.6	85.7

a.c. = actual concentration; c.d. = concentration determined; S.D. = Standard Deviation; R.S.D. = Relative Standard Deviation

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EFFECTS OF DIFFERENT SALT CONCENTRATIONS AND pH CONDITIONS ON GROWTH OF *Pennisetum clandestinum* HOCHST. (KIKUYU GRASS)

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SUMMARY

The effects of different salt concentrations and acidity on the growth of *Pennisetum clandestinum*, a perennial wild grass native to Kenya were tested to verify its adaptability to stress conditions and its possible use on Southern Italian soils. The root growth, the biomass and the activities of glutamine synthetase (GS), glutamate synthetase (GOGAT), glutamate dehydrogenase (GDH), phosphoenolpyruvate carboxylase (PEPC), and malate dehydrogenase (MDH), the key enzymes involved in ammonium and carbon metabolism, were tested. The data obtained showed that Kikuyu grass grew well in nutrient solutions with 50 and 100 mM NaCl or nutrient solutions with pH values of 4 and 5. But very high salt concentration and very low acidity reduced the plant growth. The enzyme activities of GS, GOGAT and PEPC were decreased when the grasses were treated with a salt concentration of 200 mM or grown in a solution with pH 3. The GDH activities increased in leaves of Kikuyu grass grown with a salt concentration of 200 mM, but decreased in leaves of those grown at very low pH. The MDH activities increased in leaves and roots of Kikuyu grass grown at pH 3, but decreased in leaves of those treated with a 200 mM salt solution. In conclusion, Kikuyu grass has a wide adaptability to stress conditions, therefore, it is possible to use it to prevent soil erosion in Southern Italy or in other similar soils with unfavourable environmental conditions for other plant species.

KEYWORDS:
Kikuyu grass, salinity, pH, grass growth.

INTRODUCTION

Kikuyu (*Pennisetum clandestinum*, Hochst.) is a perennial wild grass native to Kenya, which spreads by runners growing both on and below the surface of the soil. The Kikuyu grass grows, in general, in a tropical area (Australia, South Africa, Tanzania) from sea level to 3500 m above sea-level, making a solid lawn right for pasture [1]. The attention of researchers for this grass is due to its easy runner growth and, in particular, its radical development that can be used in the environment for soil stabilization. Moreover, the research shows that Kikuyu is a grass with high nutritive properties. In fact, biochemical parameters evidence that the leaves of this grass are rich in digestible crude protein and organic matter [2]. Its natural occurrence is mainly on deep latosolic soils of good fertility, but it has quickly adapted to similar soils elsewhere, and also thrives on alluvial, moist and sandy soils. In the last few years some trials of Kikuyu acclimatization in Southern Italy soils have been successful [3].

In this study we have tested the Kikuyu grass growth with different salt concentrations to define its range of compatibility, to verify the possibility of employing this grass on Southern Italian coastal soils that have high salt concentrations caused by the closeness to the sea and a dry climate. Moreover, we have tested the growth of *Pennisetum clandestinum* under varied acid pH values to verify its adaptability for possible use to prevent soil erosion on Southern Italian mountain soils with acidic pH.

MATERIALS AND METHODS

Pennisetum clandestinum Hochst. seedlings were obtained by cutting and grown on a water culture in a plexiglas chamber under white light (80W m^{-2}, Osram HQI halogen vapour W lamp), in a 16/8 h photoperiod and 70% relative humidity at 25 °C.

After 20 days of rooting, seedlings were adapted to an aerated nutrient solution, pH 6.0, with 100 μM NH_4NO_3 [4]. The different conditions of acidity in hydroponic cultures (pH 3, 4 and 5) were obtained by adding 1 N HCl to the nutritive solution. The different salt conditions were obtained by dissolving NaCl in the Hoagland solution to reach final concentrations of 50, 100 and 200 mM. The nutrient solutions were replaced every second days.

After 15 days of incubation, the seedlings grown in the different conditions were collected, biomass (as fresh weight) and root growth (as length) were estimated. Roots and leaves were employed to determine crude protein; crude fibre; ashes and moisture [5, 6]. For enzymatic analysis roots and leaves were cold-homogenized in 0.1 M Tris-HCl (pH 8.2) containing 5 mM β-mercaptoethanol, 1 mM Na-ethylene-diaminetetraacetic acid and 10% glycerol. The extracts were centrifuged at 12000 g for 20 min at 4 °C. The supernatants were analyzed for glutamine synthetase (GS; **EC 6.3.1.2**) [7], glutamate dehydrogenase (GDH; **EC 1.4.1.3**) [8], phosphoenolpyruvate carboxylase (PEPC; **EC 4.1.1.31**) [9], glutamate synthase (GOGAT; **EC 1.4.1.14**) [10], and malate dehydrogenase (MDH; **EC 1.1.137**) [11].

RESULTS AND DISCUSSION

Table 1 describes the general plant responses to the different salinity ranges. The results obtained showed that *Pennisetum clandestinum* cultures treated for 15 days with the salt concentrations of 50 and 100 mM had a root and leaf length similar to that of control (plants grown with Hoagland solution). Plants treated with a salt concentration of 200 mM showed a root and leaf length reduction of about 50 and 23 %, respectively, compared to control.

TABLE 1 - Nutritive and chemical properties of Kikuyu grass leaves and roots grown in different salt conditions. The values are the mean ± S.D. of three experiments.

Treatment	Length (cm)	Biomass (g fresh weight plant^{-1})	Moisture (%)	Crude protein (%)	Crude fibre (%)	Ashes (%)
Leaves						
Control	30 ± 0.9	4.00 ± 0.2	78.9	12.1	23.0	12.7
NaCl 50 mM	31 ± 0.4	3.97 ± 0.1	77.9	11.9	22.1	12.0
NaCl 100mM	30 ± 0.2	3.50 ± 0.2	76.9	10.8	21.3	11.6
NaCl 200mM	23 ± 0.3	2.00 ± 0.3	40.8	4.0	13.5	5.5
Roots						
Control	23 ± 0.4	3.42 ± 0.5	70.7	4.5	22.6	6.0
NaCl 50 mM	22 ± 0.5	3.00 ± 0.8	70.3	4.3	22.0	6.3
NaCl 100mM	21 ± 0.8	2.85 ± 0.5	68.7	4.0	20.0	5.8
NaCl 200mM	12 ± 0.2	1.25 ± 0.4	37.9	2.5	8.5	3.2

TABLE 2 - Glutamine Synthetase (GS), Glutamate Synthase (GOGAT), Glutamate Dehydrogenase (GDH), Malate Dehydrogenase (MDH) and Phosphoenolpyruvate Carboxylase (PEPC) activities in leaves and roots of Kikuyu grass treated with different salt (NaCl) concentrations. The values are the mean ± S.D. of three experiments.

Treatments	GS (μmol/min/ g fresh weight)	GOGAT (nmol/min/ g fresh weight)	GDH (nmol/min/ g fresh weight)	MDH (nmol/min/ g fresh weight)	PEPC (nmol/min/ g fresh weight)
Leaves					
Control	34.06 ± 0.50	25.64 ± 0.09	30.79 ± 0.90	0.95 ± 0.02	1274.68 ± 2.0
NaCl 50 mM	30.05 ± 0.50	26.85 ± 0.20	20.66 ± 0.50	1.62 ± 0.02	795.70 ± 0.90
NaCl 100 mM	27.90 ± 0.60	23.46 ± 0.30	48.34 ± 0.50	2.14 ± 0.20	611.75 ± 0.50
NaCl 200 mM	25.15 ± 0.50	19.50 ± 0.50	61.83 ± 0.50	0.47 ± 0.02	197.12 ± 0.30
Roots					
Control	51.02 ± 0.10	39.19 ± 0.40	414.84 ± 0.60	1.12 ± 0.02	325.16 ± 0.30
NaCl 50 mM	53.69 ± 0.10	35.56± 0.60	417.26 ± 0.90	1.17± 0.01	345.62 ± 0.20
NaCl 100 mM	41.42 ± 0.80	33.78± 0.60	330.87 ± 0.50	1.12± 0.04	315.52 ± 0.50
NaCl 200 mM	28.19 ± 0.90	12.60± 0.10	252.52 ± 0.50	0.87± 0.02	286.14 ± 0.30

The weight of Kikuyu leaves grown in the nutrient solution with 50 mM NaCl was similar to that of control (Tab. 1). In the presence of higher salt concentrations), the biomass decreased, reaching at 200 mM NaCl a value of 2 g fresh weight. The root weight was decreased by salt in a concentration-dependent manner, reaching a final value of 1.25 g fresh weight.

These results showed that a salinity up to 100 mM did not affect plant survival, but decreased the plant growth, reducing the fresh weight of all plant components. Up to a threshold of 50 mM, salt did not affect the crude proteins and fibre in leaves and roots of Kikuyu, whereas these values decreased slightly at 100 mM and strongly at 200 mM NaCl concentrations (Tab. 1).

Table 2 shows the specific activities of the tested enzymes after 15 days of salt treatments. The GS was mainly present in roots, in comparison to leaves, in all treatments indicating that the ammonium assimilation takes place chiefly in roots. The GS activity in leaves of *Pennisetum clandestinum* decreased with increasing salt concentrations. In roots of plants treated with 50 mM NaCl, the GS activity was slightly increased compared to control, while increasing the salt concentration (100-200 mM) caused a strong decrease of activity, showing a negative correlation between salinity and GS activity.

The GOGAT activity, an enzyme strictly related to GS in the pathway of ammonium assimilation [12, 13], showed the same behaviour as glutamine synthetase, both in roots and leaves of Kikuyu grass treated with different salt conditions (Tab. 2). The GDH activity in leaves was increased by salt in a concentration-dependent manner, indicating a request of carbon skeletons under stress conditions (Tab. 2). In fact, the reduction of biomass observed when the plants were grown with increasing salt concentrations, indicate a reduced photosynthetic activity with low availability of carbon skeletons explaining the increase of GDH activity in leaves. This supports the notion that GDH is induced under conditions of carbon starvation because of its anaplerotic function in maintaining the supply of carbon to the tricarboxylic acids (TCA) cycle in plants [14]. NaCl used at concentrations of 50 and 100 mM increased MDH activity in leaves with respect to control by 70 and 125 %, respectively, but it did not have significant effect on roots (Tab. 2). MDH activity was inhibited in a 200 mM NaCl solution both in leaves (50%) and roots (22%) (Tab. 2).

The PEPC, an enzyme providing malate from oxalic acid via dark CO_2 fixation [15, 16], was inhibited by all treatments compared to control, particularly in leaves (Tab. 2). The results obtained showed that the treatments with 50 and 100 mM NaCl did not affect significantly the growth and the metabolism of *Pennisetum clandestinum.* In fact, in the presence of 100 mM NaCl it was possible to observe an increase of MDH, an enzyme providing oxalacetic acid oxidizing malic acid [17, 18], in response to a decrease of other enzyme activities involved in carbon-ammonium metabolism.

The *Pennisetum clandestinum* has a good tolerance to the different pH conditions used. The length and the weight of roots and leaves grown in Hoagland solutions at pH 5 and 4 were similar to that of control (Hoagland solution pH 6.0) (Tab. 3).

Instead of this a length reduction of roots (about 40%) and leaves (about 17 %), and mostly of biomass (about 60 % in roots and about 53 % in leaves) in *Pennisetum clandestinum* grown in Hoagland solution at pH 3 were observed.

TABLE 3 - Nutritive and chemical properties of Kikuyu grass leaves and roots grown under different pH conditions. The values are the mean ± S.D. of three experiments.

Treatment	Length (cm)	Biomass (g fresh weight plant^{-1})	Moisture (%)	Crude protein (%)	Crude fibre (%)	Ashes (%)
Leaves						
Control	30.0 ± 0.5	4.00 ± 0.2	78.9	12.1	23.0	12.7
pH 5	32.0 ± 0.2	4.17 ± 0.4	79.5	12.9	23.1	13.0
pH 4	30.0 ± 0.5	3.88 ± 0.2	76.9	10.3	20.1	10.7
pH 3	25.0 ± 0.8	1.85 ± 0.3	39.8	5.9	10.5	5.1
Roots						
Control	23.0 ± 0.2	3.42 ± 0.4	70.7	4.5	22.6	6.0
pH 5	24.0 ± 0.8	3.27 ± 0.6	72.3	4.5	22.9	6.6
pH 4	22.5 ± 0.4	3.17 ± 0.2	65.7	4.3	19.3	5.3
pH 3	13.0 ± 0.5	1.33 ± 0.2	33.9	2.4	6.5	2.5

TABLE 4 - Glutamine Synthetase (GS), Glutamate Synthase (GOGAT), Glutamate Dehydrogenase (GDH), Malate Dehydrogenase (MDH) and Phosphoenolpyruvate Carboxylase (PEPC) activities in leaves and roots of Kikuyu grass treated at different pH values. The values are the mean ± S.D. of three experiments.

Treatment	GS (µmol/min/ g fresh weight)	GOGAT (nmol/min/ g fresh weight)	GDH (nmol/min/ g fresh weight)	MDH (nmol/min/ g fresh weight)	PEPC (nmol/min/ g fresh weight)
Leaves					
Control	34.06 ± 0.80	25.64 ± 0.20	30.79 ± 0.90	0.95 ± 0.03	1274.68 ± 2.00
pH 5	41.79 ± 0.90	28.39 ± 0.30	34.45 ± 1.50	1.21 ± 0.05	1227.27 ± 3.00
pH 4	37.89 ± 0.50	27.77 ± 0.15	20.05 ± 0.80	2.18 ± 0.07	341.25 ± 1.50
pH 3	23.84 ± 0.50	22.76 ± 0.30	0.90 ± 0.02	2.43 ± 0.90	193.54 ± 0.90
Roots					
Control	51.02 ± 0.30	39.19 ± 0.50	414.84 ± 1.50	1.12 ± 0.02	325.16 ± 1.50
pH 5	49.97 ± 0.28	39.47 ± 0.50	392.21 ± 1.80	1.10 ± 0.02	346.91 ± 0.90
pH 4	51.63 ± 0.15	38.90 ± 0.60	390.32 ± 1.00	1.28 ± 0.03	303.22 ± 2.00
pH 3	42.04 ± 0.60	27.09 ± 0.90	357.26 ± 0.90	0.45 ± 0.02	193.54 ± 3.50

The crude proteins and fibre extracted from leaves of Kikuyu treated at pH 5 were similar to that of the plant control, decreased slightly at pH 4 and strongly at pH 3 (Tab 3). GS and GOGAT activities increased at pH 4 and 5 in leaves of Kikuyu grass with respect to control, whereas this value decreased at pH 3 (Tab. 4). In roots grown at pH values 5 and 4, these activities were similar to that of control, while at pH 3 a slight decrease was observed (Tab 4). The GDH activity was mainly present in roots with respect to leaves. The GDH activity in leaves of Kikuyu treated with a Hoagland solution at pH 5 was similar to that tested in control leaves, while this activity was totally inhibited in the leaves of plants grown at pH 3. The GDH activities in roots of Kikuyu grown at pH 5 and 4, were similar to that of control, while in the roots of plants grown at pH 3 it was slightly lower (14%) than in control (Tab. 4).

The MDH played a role of replenishment of carbon skeletons for metabolic processes under stress conditions. Its activity in leaves of *Pennisetum clandestinum* grown at different pH conditions increased when the pH of the nutritive solution decreased (Tab. 4). The same behaviour was observed in roots until pH 4.

The results obtained showed that PEPC is an enzyme sensitive to low pH levels because its activity decreased in both roots and leaves (Tab. 4).

In conclusion, Kikuyu grass has a wide adaptability to stress conditions, growing well at pH levels of 5 and 4 and salt concentrations of 50 and 100 mM. In accordance with Sherman and Riveros (19) we confirm that Kikuyu is highly tolerant of soil acidity and able to tolerate high salinity levels. Therefore, it is possible to use this grass to prevent soil erosion in lands of tropical and semitropical areas, where the problem of salinity is intensifying [20], and also in some dryland areas of Southern Italy with unfavourable environmental conditions for other plant species. Moreover, this grass can be used at the same time as pasture, since its nutritive properties were not significantly altered under these experimental conditions (Tables 1 and 3).

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USE OF MICELLAR-ENHANCED CROSSFLOW FILTRATION TO REMOVE CHROMATE FROM AQUEOUS STREAMS

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SUMMARY

Removal of chromate from wastewater was investigated using the micellar-enhanced crossflow filtration technique, in which the cationic surfactant, didecyldimethylammonium bromide (DDAB), was the carrier for the metal ions. The variation of chromate and surfactant rejections and permeate flux with time were measured as a function of DDAB/chromate concentration ratio, while maintaining a constant transmembrane pressure drop(ΔP), crossflow velocity, membrane pore size and pH of the feed solution. The method was found to be effective in removing chromate from water. It was observed that the efficiency of chromate removal increased with increasing DDAB/chromate ratio. In the presence of chromate, permeate flux increased at the same as DDAB concentration, although the surfactant and chromate rejections decreased.

KEYWORDS: crossflow, ultrafiltration, micellar enhanced ultrafiltration, chromate removal, wastewater treatment.

INTRODUCTION

The term "ultrafiltration (UF)" is usually applied to a membrane separation process, where the solute dimensions are significantly larger than the solvent dimension. Ultrafiltration has been used extensively for product recovery and pollution control in the chemical, electronic, electrocoating, as well as in the food, pharmaceutical and biotechnical industries [1]. It was reported that the heavy metal ions and the dissolved organic and other ions might be effectively and economically removed from aqueous solution by Micellar enhanced ultrafiltration (MEUF) [2-7]. MEUF is a separation technique which involves adding surfactant to a polluted water stream. The surfactant forms roughly spherical aggregates called micelles which contain about 50 to 100 molecules. The interior of the micelle contains the hydrocarbon chain of the surfactant and forms a hydrophobic environment. Organic pollutants in the water dissolve or solubilize in micelles primarily through hydrophobic association and interaction with the surfactant head groups. If an anionic surfactant is used, the micelle has a high negative electrical potential on the surface where the charged hydrophilic groups are located. If a cationic surfactant is used, multivalent anionic species in solution will bind to the micelle instead. The solution is then treated in an ultrafiltration device with membrane pore size small enough to block the passage of micelles [3].

In our previous study the removal of chromate ions using surfactant enhanced crossflow filtration was reported (6). In this study we consider the removal of chromium ions from water using the same technique and different surfactant. Chromium, which is very toxic in the industrial wastewater, is usually found in the forms of chromate ions (CrO_4^{2-}) and dichromate ions ($Cr_2O_7^{2-}$) depending on the pH value. However, chromate ions exist as a stable anionic species throughout relatively wide pH ranges. Removal of chromate (or indeed other heavy metal ions) from water can also be achieved using reverse osmosis, electrodialysis or electrodeposition techniques, which require high operating pressures and/or consume large amounts of energy. Therefore, the current technique, like MEUF (2), may be considered as an energy-efficient separation technique, especially if the high surfactant concentration levels could be tolerated. The purpose of this study was to understand the mechanism of crossflow filtration when surfactants are used as carriers for the removal of heavy metal ions. In this study, DDAB was chosen.

MATERIALS AND METHODS

Materials

Potassium chromate (Merck, certified analitical grade), cationic surfactant, didecyldimethylammonium bromide (DDAB) (Aldrich, certified analytical grade) were

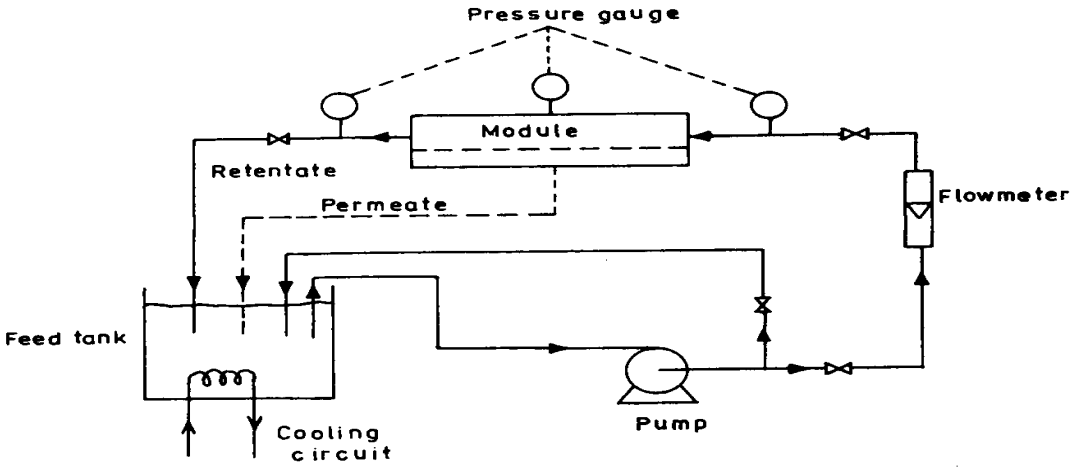

FIGURE 1 - Schematic diagram of the crossflow filtration apparatus.

used as received. Distilled and deionized water was used for preparation of all the solutions. The ultrafiltration membrane (schleicher and schuell) was anisotropic cellulose acetate membrane with 0.2 μm pore diameter size.

Apparatus and Techniques

The experimental apparatus, shown in Figure 1, consisted of a flow circuit, in which 20 litres of distilled water containing a known amount of surfactant and chromate ions (feed solution) was pumped continuously through a crossflow filtration cell at a predetermined crossflow velocity and transmembrane pressure drop. The desired filtration conditions were maintained by two manually operated valves. The temperature of the process solution was kept constant at 30 ºC by using a plate type heat exchanger placed in the feed tank, which had its own cooling circuit. The filtration equipment consisted of a filtration cell, which was constructed from plastic and stainless steel. Flat sheet membranes of 28 cm^2 effective surface area were placed into the cell to form a one sided rectangular filtration channel of length 70 mm, width 40 mm and 1.975 mm thickness. The filtrate produced was returned to the feed tank so that the feed surfactant concentration remained constant.

The feed solution was prepared at 30 ºC in the feed tank containing 20 litres distilled water while recirculating the by-pass line with the filter line shut. The desired concentration in the process solution was obtained by adding a certain amount of active surfactant slowly into the feed tank. Then, at the end of a 60 minutes recirculation period a certain amount of chromate was slowly added into the feed tank in order to obtain a known chromate concentration in the process solution. The recirculation was continued for another 60 minutes prior to the start of the filtration process. During filtration, permeate flow rate, feed flow rate, temperature and transmembrane pressure drop values were recorded. The permeate and feed conductivities were monitored. There were no significant variations in pH (pH=7,10) and in the conductivities of the feed solution and permeate. The permeate samples were collected at predetermined time intervals and later analysed for their surfactant and chromate concentrations. The permeate flux was determined gravimetrically.

Surfactant and Chromate Concentration Determination

Chromate concentrations were determined at 540 nm with an UV-Visible recording spectrophotometer (Shimadzu UV160A) [8] Surfactant concentration was determined by Organic Carbon Analyser (Beckman 915A) with UNICAM 4815 computing integrator.

Calculation of the Chromate and Surfactant Rejections

The efficiency of the ultrafiltration process is defined by the conventional rejection coefficients, R_S and R_C for DDAB and chromate rejections, respectively.

For the surfactant rejection

$$R_S = 1 - \frac{C_{SP}}{C_{SF}} \quad \textbf{(1)}$$

where C_{SP} and C_{SF} are the surfactant concentrations in the permeate and feed streams, respectively, and for the chromate rejection:

$$R_C = 1 - \frac{C_{CP}}{C_{CF}} \quad \textbf{(2)}$$

where C_{CP} and C_{CF} are the chromate concentrations in the permeate and feed streams, respectively.

RESULT AND DISCUSSION

The effect of Surfactant/ Chromate ratio on the rejections and permeate flux

The effect of varying DDAB/ chromate concentration ratios on the DDAB and chromate rejections was investigated in the case of two different series of experiments. In the first experiments, different DDAB/ chromate concentration ratio feed solutions were prepared by maintaining the feed chromate concentration C_{CF} constant, while changing the DDAB concentration. The variations with time of the rejections of DDAB and the chromate for these series of experiments are shown in Figs. 2 and 3, respectively. As seen from these figures, the rejections of the DDAB and chromate increase with increasing DDAB/ chromate ratio. When the concentration of the surfactant in the feed is increased, surfactant deposition rate also increases, and it causes the reduction of time to reach the steady state. The result of this effect, an increasing in the feed DDAB concentration causes an increase in the concentration of chromate bound to the DDAB micelles and a reduction in the free chromate ions in the medium. The experimental data have shown that the rejection of chromate is not further increased, as the DDAB/ chromate ratio increases from 75 to 150 (Fig. 3). The reason for the low value of increase depends on the growth of micelle with the increasing DDAB concentration in the feed. This case is the result of total charge of micelle, which does not increase at the same ratio. It has been reported in the literature that an increase in the micelle size occurs with the increasing surfactant concentration and the shape of micelle is transformed from spherical to lamellar shape, and even the active charge of micelle decreases [9].

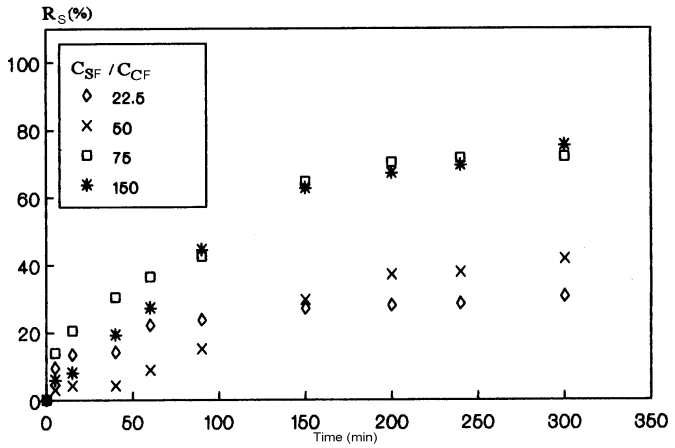

FIGURE 2 - The variation of transient surfactant (DDAB) rejection, R_S, with time as a function of feed DDAB/ chromate concentration ratio (C_{SF}/C_{CF}) when the feed chromate concentration is kept at C_{CF} = 0.2 mM during crossflow filtration.

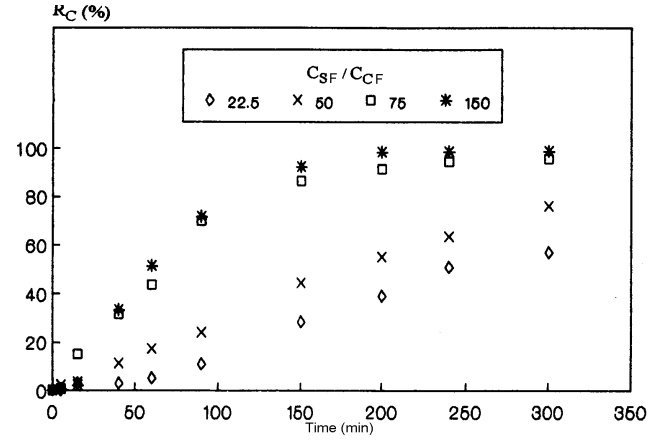

FIGURE 3 - The variation of transient chromate rejection, R_C, with time as a function of feed DDAB/chromate concentration ratio (C_{SF}/C_{CF}) when the feed chromate concentration is kept at C_{CF} = 0.2 mM, during crossflow filtration.

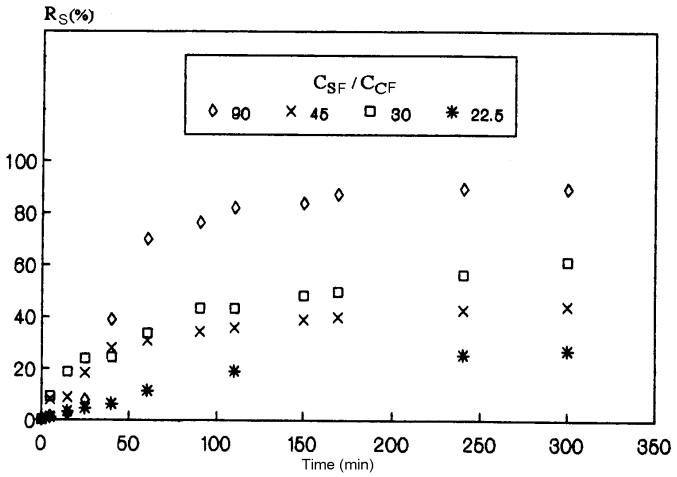

FIGURE 4 - The variation of transient surfactant rejection, R_S, with time as a function of feed DDAB/chromate concentration ratio (C_{SF}/C_{CF}) when the feed DDAB concentration is kept at C_{SF} = 4.5 mM, during crossflow filtration.

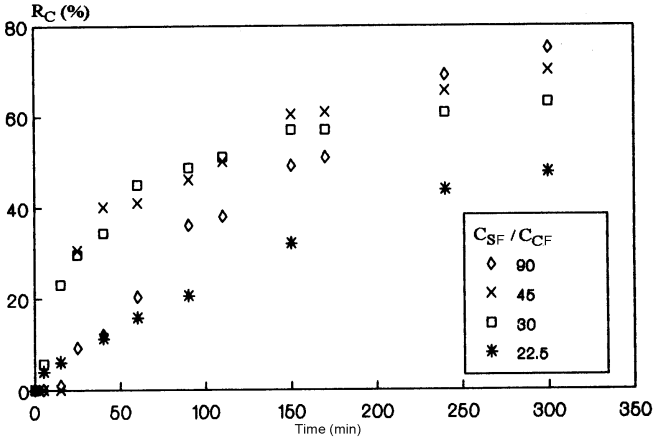

FIGURE 5 - The variation of transient chromate rejection, R_C, with time as a function of feed DDAB/chromate concentration ratio (C_{SF}/C_{CF}) when the feed DDAB concentration is kept at C_{SF} = 4.5 mM, during crossflow filtration.

The rejection of chromate decreases with decreasing DDAB/chromate ratio (e.g. low feed surfactant concentration). This effect may be explained by the lower chromate ions bound onto the DDAB micellar surface. In the second series of experiments, DDAB concentrations were kept constant at 4.5 mM, while changing chromate concentrations. The results obtained for different DDAB/chromate ratios are shown in Figs. 4 and 5, respectively. As seen from these figures, the rejections of the DDAB and chromate increase with increasing DDAB/chromate ratio. When the feed DDAB concentration is kept constant at C_{SF} = 4.5 mM, the being differents at the rejections of DDAB shown in Fig. 4 may be explained with the effect of the chromate ions on the DDAB micelles and the formation of the secondary membrane. As a result, the system comes to steady-state more slowly as the chromate concentration increases (e.g. with decreasing DDAB/chromate ratios). In our previous study, it was observed that the efficiency of chromate removal increased with increasing cetyl trimethyl ammonium bromide (CTAB)/chromate ratio. Furthermore, it was also found that the chromate concentration has a significant effect on the CTAB concentration in the permeate and on the formation time of the secondary membrane [6]. Fig. 6 shows the variation limit of chromate and DDAB rejections for different DDAB/chromate ratios in the first series of experiments. This figure also indicates the variation of the permeate flux under the same DDAB/chromate ratios. As seen from Fig. 6, the limit of chromate and DDAB rejections are a function of DDAB/chromate ratio. The limit of

rejection of DDAB and chromate increases with increasing DDAB/chromate ratios. The limit of flux for the first series of experiments decreases with increasing DDAB/ chromate ratios, as indicated in Fig. 6. This is expected because an increase in DDAB/ chromate ratios causes the DDAB concentration to increase.

The variation limit of chromate and DDAB rejections and permeate flux for different DDAB/chromate ratios for the second series of experiments is shown in Fig. 7. As seen from Fig. 7, the rejection limit of DDAB and chromate increases with increasing DDAB/chromate ratios. In the similar case, it can be seen from this figure that the increasing chromate concentration causes the steady-state flux to increase. However, the time taken to establish steady-state also increases with increasing chromate concentration. It is well known that solution environment affects flux and rejections in the MEUF [5, 6]. Addition of ions reduces the electrical double layer of DDAB micelles and aggregates.

This causes decrease in the repulsive forces of surfactant headgroups and, therefore, a decrease in critical micellar concentration (CMC). A decrease in CMC causes the growth of micelle sizes. As a result of the decrease in CMC, the permeation rate of the secondary membrane and permeate flux increase. Cellulose acetate has polar groups, but the pores are too large to ensure chromate ion repulsion. At the beginning of the filtration, a monomer layer is probably adsorbed onto and within the pores of cellulose acetate membrane through the hydrophilic headgroup. It may be expected that the chromate concentration reduces the potential of the charged micelles and aggregates (e.g. monomer, dimer). Thus, it may be accepted that the number of aggregates adsorbed onto and within the pores of cellulose acetate at a time is reduced.

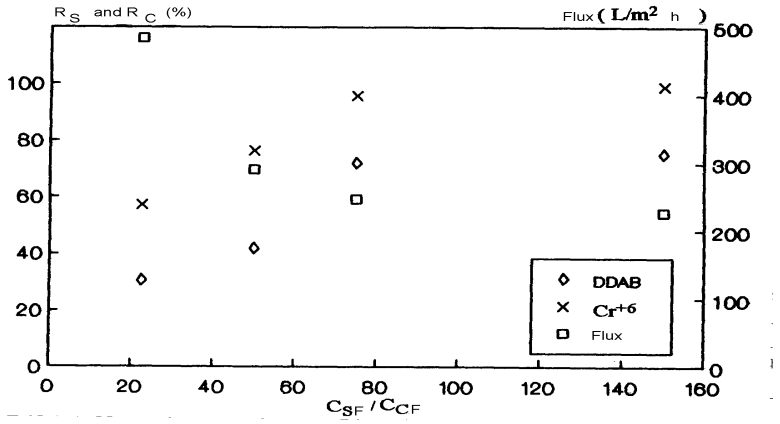

FIGURE 6 - The variation of the steady-state chromate, R_C, surfactant(DDAB) rejection, R_S and permeate flux as a function of feed DDAB/chromate concentration ratio (C_{SF}/C_{CF}) when either chromate concentration is kept constant at C_{CF} = 0.2 mM.

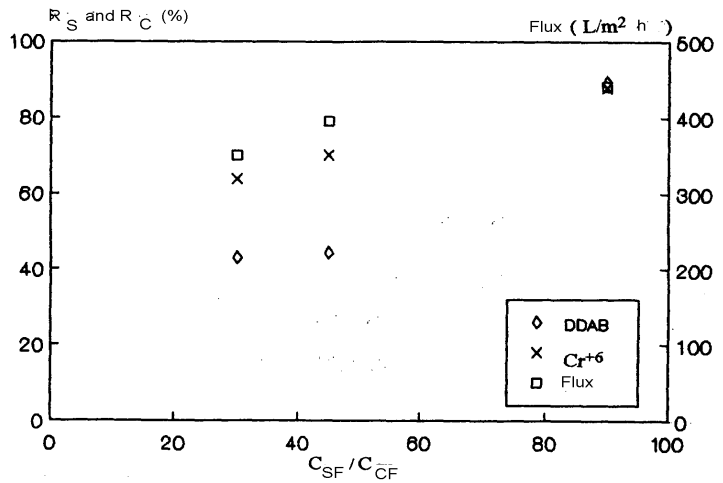

FIGURE 7 - The variation of the steady-state chromate, R_C, surfactant(DDAB) rejection, R_S and permeate flux as a function of feed DDAB/chromate concentration ratio (C_{SF}/C_{CF}) when either chromate concentration is kept constant at C_{SF} = 4.5 mM.

CONCLUSION

Removal of chromate ions from water using a cationic surfactant, didecyldimethylammonium bromide, DDAB, by crossflow microfiltration was investigated. The main emphasis of the investigations was to evaluate the effects of surfactant and chromate concentrations on the transient and steady-state behaviour of permeate flux and chromate and surfactant rejections.

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SEASONAL VARIATIONS OF EPIPELIC DIATOMS IN GÖLBASI LAKE WITH RELATION TO PHYSICAL-CHEMICAL VARIABLES

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SUMMARY

Epipelic diatoms were collected monthly for a year from five sampling stations in the Gölbasi Lake to investigate the relationship between seasonal variations of epipelic diatoms and environmental factors. A total of seventy diatom taxa were determined. *Cyclotella comta*, *C. meneghiniana*, *C. ocellata*, *Navicula cryptocephala* and *N. reinhardii* were the most conspicuous diatoms in terms of frequency of occurrence and relative abundance in the epipelic community. Some of the epipelic diatoms were noticeable for their occurrence only at certain sampling stations. Water temperature was found to be a more important factor in steering the growth of epipelic diatoms in this study.

KEYWORDS:
epipelic, diatom, freshwater, Gölbasi Lake, Turkey

INTRODUCTION

Diatoms are recognized as an important component of benthic algae in both marine and freshwater bodies. Diatoms are also widely used as biomonitors, which is important to accurately define their autoecological characteristic from different geographical areas, in order to find their most accurate preferences [1]. Although mechanisms controlling the abundance of phytoplankton are now well documented, the regulation of benthic algae has been studied less in all details.

There have been few studies carried out on Gölbasi Lake. The limnological features such as zooplankton, zoobenthos and physical and chemical properties of the lake were investigated by Ekingen *et al*. [2]. The phytoplanktons of Gölbasi Lake and their seasonal variations were studied by Cetin [3].

This study is aimed to investigate the seasonal variations in epipelic diatom community in Gölbasi Lake with relation to environmental factors.

STUDY AREA

Gölbasi Lake is located in the north-east of Gölbasi vicinity and south-east Turkey (37^0, 34?E, 37^0, 48?N) (Fig. 1). Its surface area is approximately 2.250 km^2, the maximum volume of lake is 27.106 m^3, the altitude 885 m. The average depth of the lake is 22.5 m. The shore of the lake is mostly covered by *Phragmites* and *Carex*. Additionally, *Ranunculus*, *Myriophyllum* and *Graminae* are found on the shore [2]. However, *Nymphaea* sp. also occasionally occurs.

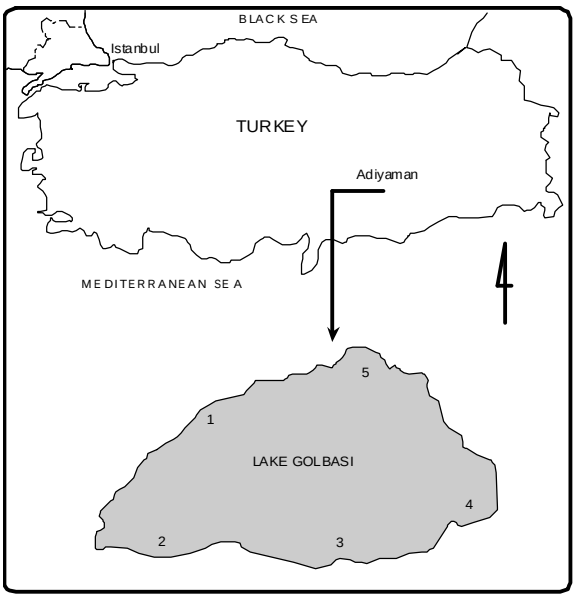

FIGURE 1
Map of Gölbasi Lake and the Position of the Sampling Stations

MATERIALS AND METHODS

Water and epipelic diatom samples were collected monthly from five stations between 28 April 1997 and 18 March 1998. Epipelic diatoms samples were collected from sediment using a core 3 cm in diameter. Individual numbers were obtained by counting at least two hundred valves on each slide and results are expressed as (%) relative abundance. Diatoms were identified mainly referring to Germain [4], Patrick & Reimer [5,6] and Krammer & Lange-Bertalot [7]. Surface water temperature, dissolved oxygen concentration, transparency, pH and conductivity were measured *in situ* with portable measuring instruments. On return to the laboratory, concentrations of Ca^{++}, Mg^{++} and total hardness were measured by titration methods [8].

RESULTS

During the sampling period, dissolved oxygen concentrations were measured between 7.95-12.40 mg L^{-1} (Fig. 2 D). Water temperature showed large seasonal fluctuations. The mean surface water temperature was 14.83 ºC. Maximum water temperature (22.8 ºC) was measured in August, and minimum (5.2 ºC) in January (Fig. 2 A). The pH of Gölbasi Lake was consistent, ranging from 7.00 to 7.60 (Figure 2 C). Transparency was observed to be greatest in August (2.72 m), while the minimum transparency was observed in December (1.86 m) (Fig. 2 D). Concentration of Ca^{++} showed seasonal variations between 28-39 mg L^{-1} (Fig. 2 E). Maximum Mg^{++} concentration was observed in July (17.01 mg L^{-1}) and minimum (10.93 mg L^{-1}) in September. Total hardness changed between 140 and 160 mg $CaCO_3$ L^{-1} (Fig. 2 B).

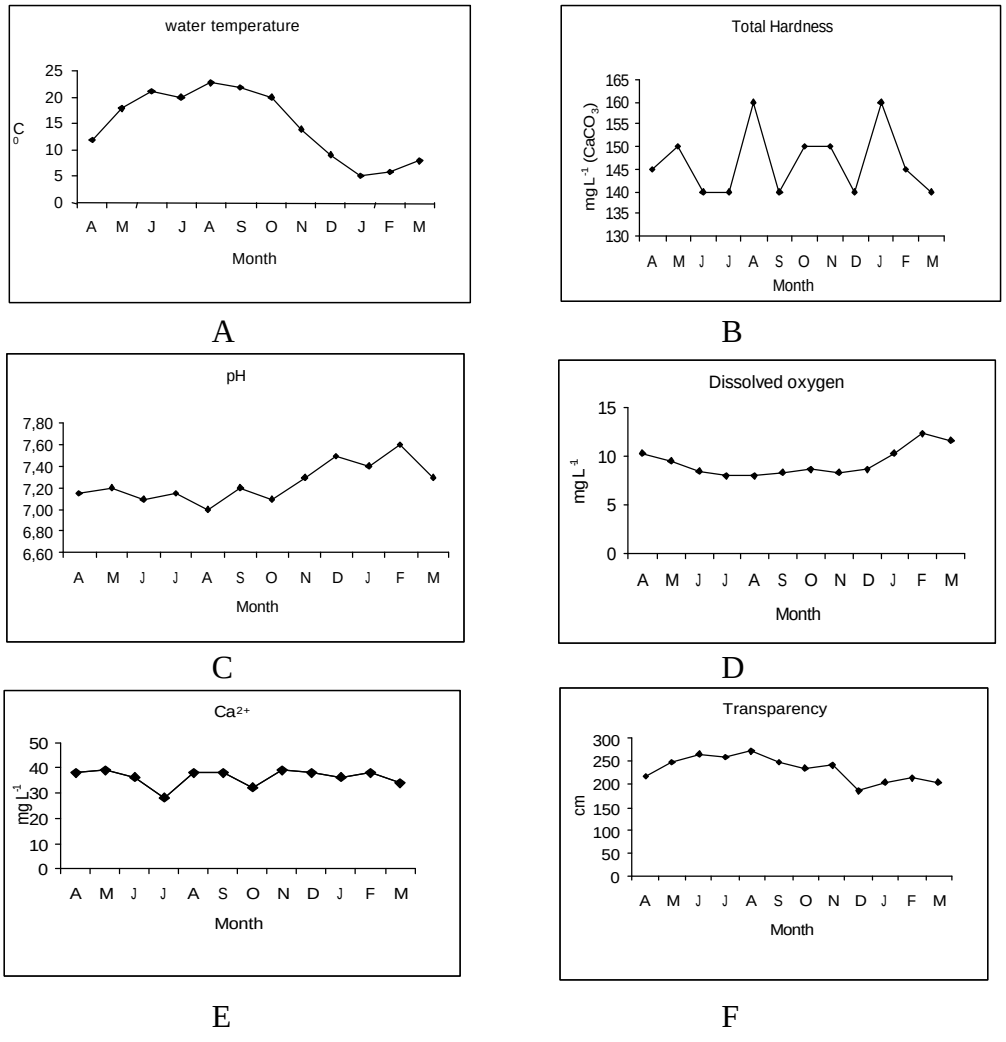

FIGURE 2 - Seasonal variations of physical and chemical features of Gölbasi Lake
A; Water Temperature - B; Total Hardness - C; pH - D; Dissolved Oxygen - E; Ca^{++} - F; Transparency

TABLE 1
List of epipelic diatom taxa in the Gölbasi Lake and their mean relative abundance (%) at sampling stations.

Taxa	Mean relative abundance				
	Station 1	Station 2	Station 3	Station 4	Station 5
Cyclotella comta (Ehr.) Kütz.	7.35	6.25	7.75	8.00	7.75
C. meneghiniana Kütz.	4.91	4.33	4.58	4.54	6.08
C. ocellata Panto.	6.20	6.50	5.90	5.95	6.04
Achnanthes flexella Kütz.	0.51	0.50	0.45	0.50	0.33
A. gibberula Grun.	0.00	0.00	0.04	0.08	0.12
A. lanceolata de Brebisson	0.00	0.00	0.08	0.16	0.16
A. minuttisima Kütz.	0.04	0.04	0.12	0.04	0.08
Amphora ovalis Kütz.	2.83	2.91	2.66	2.70	2.95
Cocconeis placentula Ehr.	6.13	3.77	1.85	2.87	2.16
C. pediculus Ehr.	0.04	0.04	0.08	0.04	0.00
Cymatopleura elliptica (de Brebisson) W. Smith	0.20	0.20	0.25	0.12	0.20
C. solea (de Brebisson) W.Smith	0.70	0.37	0.50	0.50	0.50
Cymbella affinis Kütz.	5.08	5.56	4.75	4.88	5.20
C. amphicephala Naegeli	1.25	1.04	0.79	1.00	1.08
C. angustata (W.Smith) Cleve	0.00	0.08	0.04	0.00	0.04
C. aspera (Ehr.) Cleve	0.04	0.00	0.04	0.04	0.04
C. cistula (Hemprich) Grun.	0.70	0.75	0.45	1.04	0.70
C. cymbiformis (Agardh Kütz.) Van Heurck	0.50	0.58	0.90	1.04	1.12
C. leptoceros (Ehr.) Grun.	0.04	0.12	0.12	0.16	0.08
C. obtisiuscula (Kütz.) Grun.	0.75	0.41	0.70	0.95	1.04
C. tumida (de Brebisson) Van Heurck	0.04	0.00	0.00	0.04	0.04
Diatoma hiemale (Lyngbye) Heiberg	0.12	0.04	0.00	0.12	0.08
D. vulgare Bory	0.00	0.00	0.00	0.00	0.04
Diploneis ovalis (Hilse) Cleve	0.08	0.04	0.04	0.04	0.00
Epithemia adnata (Kütz.) de Brebbisson	0.00	0.00	0.00	0.04	0.00
E. argus Kütz.	0.04	0.04	0.00	0.00	0.00
E. zebra (Ehr.) Kütz.	0.04	0.00	0.00	0.00	0.04
Eunotia pectinalis (Kütz.) Rabh.	0.04	0.00	0.00	0.04	0.04
Fragilaria construens (Ehr.) Grun.	0.04	0.00	0.00	0.00	0.00
F. intermedia Grun.	0.04	0.00	0.00	0.00	0.00
Gomphonema acuminatum Ehr.	0.00	0.08	0.12	0.04	0.08
G. angustatum (Kütz.) Rabh.	0.29	0.33	0.62	0.45	0.58
G. constrictum Ehr.	0.95	1.08	1.08	1.16	1.20
G. dicotomum Kütz.	0.66	0.35	0.66	0.75	0.50
G. intricatum Kütz.	0.04	0.08	0.00	0.12	0.12
G. intricatum var. *vibrio* (Ehr.) Cleve	0.08	0.04	0.04	0.00	0.00
G. lanceolatum Ehr.	5.70	2.70	6.70	6.33	6.00
G. olivaceum Lyngbye	1.16	1.83	2.20	2.16	1.91
G. parvulum (Kütz.) Grun.	0.41	0.33	0.33	0.41	0.25
Gyrosigma attenuatum (Kütz.) Rabh.	0.12	0.12	0.08	0.16	0.12
Hantzschia amphioxys (Ehr.) Grun.	0.41	0.25	0.41	0.37	0.41
H. amphioxys var. *maior* Grun.	0.04	0.04	0.04	0.00	0.00
Navicula crptocephala Kütz.	6.75	7.66	7.41	7.16	7.54
N. cryptocephala var. *veneta* (Kütz.) Grun.	3.75	3.33	3.54	4.54	4.70
N. cuspidata Kütz.	0.04	0.00	0.00	0.00	0.00
N. gracilis Ehr.	0.12	0.00	0.00	0.04	0.04
N. menisculus Schumann	1.54	1.29	1.41	1.12	1.04

TABLE 1 continued

N. mutica Kütz.	0.04	0.04	0.00	0.00	0.00
N. peregrina Kütz.	0.04	0.00	0.00	0.00	0.00
N. reinhardii Grun.	35.58	42.58	39.36	36.36	35.23
N. reinhardii var. *elliptica* Herib.	0.12	0.12	0.04	0.12	0.08
N. rhyncocephala Kütz.	0.00	0.57	0.33	0.16	0.00
N. amphibia Grun.	0.45	0.20	0.20	0.45	0.29
N. apiculata (Gregory) Grun.	0.29	0.00	0.00	0.04	0.08
N. gracilis Hantzsch.	0.12	0.37	0.37	0.29	0.41
N. linearis W.Smith	0.45	0.51	0.45	0.29	0.33
N. palea (Kütz.) W.Smith	0.20	0.04	0.08	0.08	0.20
N. sigmoidea (Ehr.) W.Smith	0.25	0.25	0.16	0.16	0.29
N. stagnorum Rabh.	0.04	0.00	0.00	0.04	0.04
Pinnularia brebissonii (Kütz.) Rabh.	0.25	0.04	0.00	0.00	0.04
Rhoicosphenia curvata (Kütz.) Grun.	0.08	0.00	0.00	0.12	0.08
Rhopalodia gibberula (Ehr.) O.Mull.	0.08	0.00	0.04	0.00	0.04
Surirella angustata Kütz.	0.04	0.00	0.00	0.00	0.00
S. biseriata de Brebisson	0.08	0.00	0.00	0.04	0.00
S. linearis W.Smith	1.50	1.58	1.66	1.12	1.25
S. ovalis de Brebisson	0.04	0.00	0.00	0.00	0.00
S. ovata var. *pinnata* W.Smith	0.08	0.04	0.04	0.08	0.08
S. ovulum Hustedt	0.04	0.04	0.04	0.00	0.00
Synedra ulna (Nitzsh) Ehr.	0.46	0.54	0.50	0.95	1.16

A total of 70 epipelic taxa were recorded in the epipelic community during the study. The species composition and changes in relative abundance of the individual diatom species were almost similar at all stations. *Cyclotella comta, C. meneghiniana, C. ocellata, Navicula cryptocephala, N. cryptocephala* var. *veneta* and *N. reinhardii* were the most common and abundant diatom species at all stations (Table 1).

The epipelic diatom composition in spring was constituted mainly by *Cyclotella comta, C. meneghiniana, Navicula cryptocephala* and *N. reinhardii.* Centric diatoms were constituted 42 % of the total relative abundance in the community. By early summer the species composition of epipelic community was different from those in spring. Takson diversity of epipelic diatoms was the richest in June. *Cyclotella comta, C. meneghiniana, C. ocellata, Navicula cryptocephala, N. cryptocephala* var. *veneta, N.reinhardii* were most common and abundant species in the epipelic community during summer. *Achnanthes flexella, A. lanceolata, C. amphicephala, C. cistula, C. cymbiformis* and *C. obtisiuscula* were typically summer epipelic diatoms in the lake. These diatoms were quite conspicuous with their specific occurrence only in summer.

In late summer and early autumn, epipelic community was dominated by pennate forms, particularly, by *Cymbella affinis, C. amphicephala, C. angustata, C. cistula, C. cymbiformis, C. obtisiuscula, Gomphonema olivaceum, Navicula cryptocephala, N. menisculus, N. reinhardii* and *Amphora ovalis.* In contrast, contribution of centric forms to the epipelic community was less than 10%. Pennate forms reached higher relative abundance in October and became richer in species composition in November. However, a decrease both in the individual number of pennate forms and number of species in the community was recorded in the next month.

In winter, epipelic diatom community was poor in species composition. *Navicula reinhardii* was the most abundant diatom in December constituting 86.1 % of the epipelic community. Relative abundance of centric forms decreased noticeably by late winter whereas an increase in the relative abundance of pennate forms was recorded in the same period. *Cyclotella comta, C. ocellata, Amphora ovalis, C. affinis, G. constrictum, G. dicotomum, G. lanceolatum, G. olivaceum, G. parvulum, Navicula cryptocephala, N. cryptocephala* var. *veneta* and *N. reinhardii* were the most common and abundant diatoms in February and March. However, *G. lanceolatum* was the most noticeable, since highest relative abundance in this period was recorded for this diatom (Table 1).

In general, the growth of epipelic diatoms was found to be positively correlated with water temperature. However, several significant correlations were observed be-

tween the occurrence of most abundant diatom species and environmental factors. Growths of *Cyclotella* species were negatively correlated with pH, dissolved oxygen and concentrations of Ca^{++}. However, abundance of *C. meneghiniana* showed positive correlation with water transparency. *Navicula reinhardii* was positively correlated with concentrations of Ca^{++} and negatively with transparency. Growth of *Navicula cryptocephala* displayed positive correlation with water temperature, whilst negative correlation was found between the growth of the diatom and pH, dissolved oxygen, Mg^{++} and total hardness.

DISCUSSION

The epipelic diatom flora contains many taxa recently reported from Turkish lakes [9, 10, 11, 12]. The species composition of benthic diatoms in the Gölbasi Lake was generally similar to that of phytoplankton of the same lake [3]. The distribution of the epipelic diatoms showed similarities at sampling stations. In addition, there were no great differences in the relative abundance of the individual diatom species between stations. The reasons for this can be due to similar environmental conditions at stations.

Cyclotela comta, C. meneghiniana, C. ocellata, Navicula cryptocephala and *N. reinhardii* were the most abundant and common diatoms in the community at all stations. Of all, *Navicula reinhardii* was the most significant species since it occurred usually by far with higher relative abundance than other diatoms at all stations during the study. However this diatom was particularly abundant in spring and summer. Although *N. reinhardii* was observed to be most significant both with respect to frequency of occurrence and relative abundance in the epipelic community, it was rarely recorded in the phytoplankton of Gölbasi Lake [3]. This may show that *N. reinhardii* is essentially a benthic form in the lake and only occasionally it raises to the phytoplankton.

Generally, *Cyclotella* species are considered to be planktonic [13, 14]. However, it is possible that *Cyclotella comta, C. meneghiniana and C. ocellata* may be thycoplanktonic or even benthic in Gölbasi Lake considering their existence both in the epipelon and phytoplankton. *Navicula cryptocephala* was also another common and abundant member of both phytoplankton and epipelon in the lake. All these findings may show the wide range of ecological tolerance of these taxa.

Some diatoms showed specific distribution in the lake. *Cocconeis placentula* was significantly abundant only at Station 1 although it occurred at other stations. *Fragilaria construens, F. intermedia, Navicula cuspidata, N. peregrina, Surirella angustata* and *S. ovalis* were quite spesific in their occurrence only at Station 1. On the contrary, *Epithemia adnata* and *Diatoma vulgare* were recorded only at Station 4 and 5, respectively (Table 1). This may show that some diatom species occur with narrow ecological tolerance.

Simon [15] reported that growth of algae was limited chiefly by physical factors, such as light and temperature. The present study supported this finding, since high water temperatures supported the growth of diatoms, whilst low temperature inhibited their growth.

There was a negative correlation between relative abundance of *Cyclotella comta, C. meneghiniana, C. ocellata, Navicula crypytocephala* and dissolved oxygen concentration in the lake. On the contrary, a significant positive correlation was found between light transparency and abundance of *Cyclotella comta, C. meneghiniana, C. ocellata, Navicula crypytocephala.* This study would suggest that pH level did not appear to affect the growth of these diatoms, since the changes in pH level in Gölbasi Lake were insignificant (ranged between 7.00 and 7.60). A negative correlation was observed between growth of the most diatoms and Ca^{++} concentrations in the lake, although some species of diatoms were reported to prefer calcium [14]. However, the growth of some abundant diatom species such as *Navicula reinhardii* and *Cymbella affinis* supported this finding, since a positive correlation was found between the growth of these diatoms and calcium concentration in Gölbasi Lake.

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PHOTOCHEMICAL EPISODES AND VOLATILE HYDROCARBONS IN ATHENS GREECE

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SUMMARY

During a monitoring study of 15 selected volatile hydrocarbons (VHCs) for a one-year period in Athens basin (1995-1996), three photochemical episodes were observed in different seasons. In all cases ozone values exceeded the first air quality limit of 180 μg m^{-3} proposed by EU. These episodes were observed with prevailing SW wind and wind speed values less than 2.2 m s^{-1}, which favour the accumulation of pollutants in Athens basin. The maximum VHC concentrations of these three episode days were found to be 415%, 184% and 205% of the annual geometric mean value respectively. Elevated values for benzene up to 228 μg m^{-3} were measured. For toluene, ethyl benzene, m+p-xylenes, and o-xylene the corresponding values were 616 μg m^{-3}, 194 μg m^{-3}, 437 μg m^{-3} and 148 μg m^{-3} respectively. The air quality concerning volatile hydrocarbons and criteria pollutants during the episodes is discussed.

KEYWORDS:
air quality, benzene, BTEX fraction, ozone, meteorological data, factor analysis.

INTRODUCTION

Photochemical pollution is one of the main current air pollution problems. High levels of ozone have been measured in several cities of the world, pointing to a clear problem with respect to urban and regional air quality. Recently, European Environmental Agency [1] reported a decreasing trend in the peak values and an increasing trend in the median concentrations of ozone in the period 1994-1999, indicating that the long-term exposure of the population to ozone has been increased.

Atmospheric hydrocarbons, additionally to their important role in the ozone formation, are now the concern for their effects on population health [2]. Recent European and worldwide legislation proposes limit values for ambient concentrations of these compounds [3,4]. Exposure to volatile hydrocarbons (VHCs) and, in particular, to benzene, in European capitals, has been the subject of recent European research activities such as MACBETH and EXPOLIS programs [5-8].

In Athens basin primary and secondary pollutants frequently exceed the air quality standards of the EU and the WHO [9]. Residents of Athens suffered from frequent air pollution episodes with a “spot” during July 1987, which caused, in conjunction with a heat wave, an increase in the mortality [10].

Although processes leading to episodes of high pollution levels in Athens have been studied in a number of publications [11-22], little information about VHC levels is available. To the best of our knowledge, only during a summer pollution episode VHC levels have been determined, thus, many important questions are still unanswered [23]. The approach of the present work is different. During a one-year monitoring program of 15 selected VHCs [24], including the BTEX fraction, we have studied VHCs levels during three episodes in different seasons. In all three cases the ozone values have exceeded the limit of 180 μg m^{-3}.

MATERIALS AND METHODS

Sampling

The collection of the air samples for the determination of volatile hydrocarbons during the occurence of the photochemical episodes was carried out at Patission street. Patission street is located at the center of the city and it is a commercial area with very heavy traffic (about 62 000 vehicles passing per day); the sampler was located 8 m above street level. For the collection of the 15 selected volatile organic compounds TENAX-TA was used as adsorption media. The sampling time was 30 min with a flow rate of 60 ml min^{-1}. During the episodes one sample per hour was collected for the whole day.

The data for O_3, NO_x and the meteorological parameters used in this work were provided by Directory of Air and Noise Pollution Control, Ministry of Environment, which runs a monitoring network program for the criteria pollutants: CO, SO_2, NO_x , O_3, and smoke. Ozone was also measured in Marousi, a peripheral area of Athens, located S-SE of the city centre, where the maximum values of ozone are observed all over the year [9].

Instrumentation

VHCs determination

Basic considerations concerning the two columns GC. technique (SCOTCH system) have been described elsewhere [25, 26]. The determination of the 15 selected volatile hydrocarbons in the collected air samples was performed using a thermal desorption - gas chromatographic system (3600 CX, Varian Co.) equipped with two columns coupled in parallel and a dual FID. The columns used were a BP1 capillary column (50 m × 0.22 mm × 1.0 μm) and a Cp-Sil 8 capillary column (50 m × 0.22 mm × 1.0 μm). The compounds were injected via a thermal desorption unit (Aerotrap 6000, Tekmar Co.). Star Chromatography Software Version 4.0 (Varian Co) was used for the control of the GC system.

NO_x and O_3 determination.

Both NO_x and O_3 measurements were performed using automatic analysers. For the ozone measurements a UV photometric O_3 analyser (Model 49, Thermo Environment Instruments Inc.) and for the NO_x measurements a chemiluminescence NO_x analyser (Model 14B/E, Thermo Environment Instruments Inc.) were used.

TABLE 1
Pollution and meteorological data during the photochemical episode days in the Patission and Marousi (ozone) sites.

	Case study	31.10.95	24.04.96	04.07.96
METEOROLOGICAL PARAMETERS Daily mean values	Wind speed, m s^{-1}	2.2	1.2	1.7
	Wind direction	SW	SSW	SW
	Temperature, ^oC	15.7	20.1	28.9
CRITERIA POLLUTANTS Hourly maximum values μg m^{-3}	**Patission street**			
	NO	660	510	616
	NO_2	302	472	259
	S[VHC]	670	313	396
	O3	20	93	30
	Marousi			
	O_3	218	181	202

TABLE 2
Geometric mean concentration (μg m^{-3}) of the volatile hydrocarbons at the Patission station during the photochemical episodes.

Compound	31.10.95		24.04.96		04.07.96	
	$X_g \pm S_g$	C_{max}	$X_g \pm S_g$	C_{max}	$X_g \pm S_g$	C_{max}
Hexane	4.54 ± 2.17	16.3	1.12 ± 1.18	12.6	3.98 ± 2.49	22.6
Benzene	62.9 ± 1.82	168	20.0 ± 19.5	173	14.6 ± 15.3	228
Cyclohexane	20.7 ± 1.79	54.3	7.09 ± 10.7	74.1	8.76 ± 7.89	97.7
2,2,4-trimethyl pentane	6.06 ± 1.82	18.2	1.02 ± 1.25	15.3	2.96 ± 2.51	10.9
Dimethylhexane	2.88 ± 1.26	5.00	1.51 ± 1.48	8.40	4.06 ± 3.98	23.1
2,3,4- trimethyl pentane	3.51 ± 1.79	9.12	1.66 ± 1.37	11.7	3.10 ± 3.07	9.23
Toluene	204 ± 1.93	616	59.9 ± 61.2	523	40.6 ± 26.5	503
Methylheptane	2.75 ± 1.44	6.51	1.12 ± 1.17	5.86	3.90 ± 3.75	14.9
2,2,5-trimethyl-hexane	6.67 ± 1.79	17.3	1.09 ± 1.02	27.5	2.53 ± 2.41	15.8
Ethylbenzene	34.0 ± 1.79	85.0	11.7 ± 9.87	106	31.4 ± 12.1	194
m+p-xylene	140 ± 1.78	353	43.1 ± 40.2	369	31.8 ± 28.4	437
o-xylene	52.5 ± 1.80	132	15.4 ± 18.5	148	12.7 ± 13.4	93.7
Propylbenzene	7.44 ± 1.79	17.8	3.14 ± 4.12	35.3	7.69 ± 7.54	61.7
Butylbenzene	11.9 ± 1.81	29.9	5.65 ± 3.67	31.2	3.97 ± 3.84	27.9

X_g: mean geometric value, S_g: standard deviation

RESULTS AND DISCUSSION

A monitoring program of 15 selected volatile hydrocarbons at three different sampling sites was carried out for first time in Athens basin in the period 1995 - 1996 [24]. During this program three photochemical episodes with elevated ozone values were observed in different seasons: autumn 1995, spring and summer 1996. The first episode in October 1995 lasted for four days with peak day on the 31th of the month. The other two episodes were in April and July of 1996, lasted six and five days each and the peak days were on the 24th of April and 4th of July, respectively. In all cases the meteorological conditions favoured the accumulation of pollutants in Athens basin and the ozone values exceeded the limit of 180 μg m^{-3} set by the 92/72/EEC Directive as the threshold value for providing information to the population. Pollution and meteorological data during those days are given in Table 1.

During the episodes the prevailing wind direction was south-west and the mean speed was less than 2.2 m s^{-1}. Under these meteorological conditions the air masses are transported from the city centre to the mountains at the north of the basin, where the dispersion and diffusion of the pollutants are impeded leading to their accumulation. Practically the air masses stagnated over Athens basin [23].

At those days hourly measurements of volatile hydrocarbons were carried out. The results are given in Table 2.

The above results agree with the study by Kassomenos et al. [27], who showed that most photochemical episodes in the Greater Athens Area are observed during the transient period and in the winter, with April, October and December being the months with the greater number of pollution episodes in the past 20 years. This can be explained by the fact that summer is the vacation period, and that during the summer the prevailing winds (subregional and mesoscale), which are due to thermal circulation, are stronger and the mixing height of pollution layer is deeper [27]. Using the t-test at 95% confidence levels to compare the VHC concentrations values it was found that the higher values were measured during October following by those measured in April and July. Comparing the values of October with those in April at the 95% C.L. it was found that the t-value was 2.43 with the critical value to be 1.77. Comparing the concentration values between April and July the calculated t-value was 0.04 with the critical one to be 1.77 showing that there is no significant difference between them.

Attention should be given to the BTEX fraction values, which were noticed to be extremely high. Cocheo at al. [5] have concluded that the annual average concentrations of benzene in the atmosphere of European cities are between a few μg m^{-3} up to about 50 μg m^{-3} at hot spots e.g. in busy streets with high traffic density. Despite those conclusions we can clearly see that in Athens center during episode days the benzene concentrations in street canyons were extremely high compared to the threshold value set by the 2000/69/EC Directive for benzene in outdoor air. The rest of the other compounds of the BTEX fraction also exhibited high hourly concentrations. The maximum observed hourly concentrations for toluene, m+p–xylenes, o-xylene and ethylbenzene were 616 μg m^{-3}, 437 μg m^{-3}, 148 μg m^{-3} and 194 μg m^{-3}, respectively. The increase in BTEX concentration during the episode days compared to the annual geometric mean values can be seen in Table 3.

Taking into consideration that 50% of the measured benzene concentration during these episode days were higher than 22.4 μg m^{-3} and that the sampling height was 8 m above the street level it can be concluded that commuting in Athens center during episode days should be unfavorable for the residents. This situation affects negatively not only the outdoor air quality for Athens residents, but also the indoor air in offices and homes.

The elevated values of VHCs and especially those of BTEX fraction, show the impact of car exhausts as emission source. The caution of photochemical pollution episodes is not only meteorology but also the elevated hydrocarbons emissions. The same was concluded by Lalas et al. [28], studying the sea breeze circulation and the photochemical pollution in Athens, who showed that the daily variation of NO_x, HCs and CO production closely matched the daily traffic variation. The elevated values of benzene, which exceed the limit value set by European Union and the measured ozone values at the peripheral areas of Athens (Marousi), indicate the need for the reduction of volatile hydrocarbons in Athens basin. Bakeas et al. [24] and Ziomas et al. [29] have concluded that ozone abatement strategy should focus mostly on VOC emissions controls rather than controlling NO_x.

The introduction of catalytic cars for the last ten years and the differentiation of traffic conditions in Athens basin slightly changed the occurrence of such strong episodes, but the problem of exceeding the limit values for ozone and benzene still remains unsolved [9].

Factor analysis of the data collected during the episode days was carried out using the principal component analysis as the extraction method and the varimax with Kaiser-Nelson normalization as the rotation method.. The results are given in Table 4.

From the results of factorial analysis it can be seen that during the episodes there is a change on the impact of different sources on pollutants levels. In October it is obvious that the car exhausts (Factor 1) play an important role contributing to the increase of VHC, NO and

TABLE 3 - Daily geometric mean values of BTEX fraction for the three episodes, annual geometric values (μg m^{-3}) for the monitoring period October 1995 - September 1996 and percentage increase.

Case study	31.10.95	24.04.96	04.07.96	Annual geom. mean
[Benzene], μg m^{-3}	62.9	20.0	14.6	7.85
percentage increase	801	255	186	
[Toluene], μg m^{-3}	204	59.9	40.6	19.2
percentage increase	1063	312	211	
[Ethylbenzene], μg m^{-3}	34.0	11.7	31.4	5.37
percentage increase	633	218	585	
[m+p-xylene], μg m^{-3}	140	43.1	31.8	15.1
percentage increase	927	285	211	
[o-xylene], μg m^{-3}	52.5	15.4	12.7	6.78
percentage increase	774	227	187	

TABLE 4 - Factor analysis of pollution data during the episode days.

	October 1995		April 1996		July 1996		
	Component		Component		Component		
	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 3
S[VHC]	0.81	-0.19	0.18	0.90	0.75	-0.15	-0.18
O_3	-0.87	-0.25	0.25	-0.71	0.87	0.38	0.03
NO	0.98	-0.01	0.17	0.91	-0.26	0.93	0.11
NO_2	-0.18	0.96	0.81	-0.51	0.34	0.93	0.01
SO_2	0.38	0.89	0.98	0.05	-0.78	0.06	-0.52
CO	0.90	0.25	0.89	0.34	-0.04	0.09	0.96

CO concentration levels and the reduction of ozone. The compounds associated with Factor 2 (NO_2 and SO_2) are identified commonly with combustion emissions. Such emissions may be automobiles, central heating and industry. Increasing air temperature and solar radiation from October to April and July the results are slightly different. During April we also observed that VHC, NO concentration values are affected by the same source, which in this case is not only the car exhausts, because CO is not affected in the same way. Probably the elevated air temperature makes the contribution from evaporation losses more significant to the total hydrocarbons burden. For the other pollutants the same pattern, as that during October episode, exists. During July episode when the photochemical reactivity is higher (extensive solar radiation) we can see that the major controlling factor is meteorology instead of emission sources.

CONCLUSIONS

In Athens basin under favourable meteorological conditions that lead to strong pollution episodes, elevated volatile hydrocarbon concentration levels were observed. During those days the pollutants levels exceeded the air quality standards making uncomfortable the residents living. Although in the last years the number of strong episodes that occur at Athens basin have been reduced, due to the introduction of the catalytic technology in cars, the set up and operation of a new underground railway and the improvement of the quality of fuels (Auto Oil II), the need for controlling hydrocarbon emissions still remains in order to reach the limit value for benzene and for a better air quality. The abatement of photochemical pollution in Athens basin is now becoming imperative because of the Olympic Games which will take place at the city in 2004.

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