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POTENTIAL LEACHING OF METHYL ISOTHIOCYANATE THROUGH NON DISTURBED SOIL COLUMNS

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SUMMARY

The mobility of methyl isothiocyanate (MITC), active metabolite of metam- sodium (MS), was evaluated using undisturbed soil columns. The recovery of MITC in the leachates after two weeks was 14.4 % of the initial applied dose and the total leaching of MITC was estimated at 30 %. One week after application of MS, MITC was recovered in all soil profiles and estimated at 19.8 % of the initial applied amount. The maximum volatilisation of MITC was estimated at 3.75 % after 24 hours and the total volatilisation was about 7 % after three weeks.

KEYWORDS:
Metam-sodium, methyl isothiocyanate, non disturbed soil columns, water leachates, volatilisation, distribution

INTRODUCTION

Methyl isothiocyanate (MITC) is the active metabolite of the soil fumigant, metam-sodium (Sodium, N- methyldithiocarbamate) (MS). MITC is suspected to be a possible contaminant of ground water because of its increased utilisation [1], high leaching characteristics [2], high water solubility [3] and low soil adsorption [4,5]. Soil disinfection with MS is widespread in Morocco and could be an alternative product to methyl bromide. Considering the elimination of methyl bromide, the objective of this research was, to determine the potential leaching of MITC in soil, to anticipate a possible contamination of ground water, the potential of volatilisation of MITC and its distribution in the soil profiles.

MATERIALS AND METHODS

Leaching of MITC: The experimental design used was already described [6] and consisted of polyethylene columns (1m length and 12 cm diameter). Each soil column was first brought to its field capacity and treated with 2 ml of formulated MS (Nemsol: 510 g MS /l). The formulated MS was dissolved in 100 ml of water and applied in each column to simulate an application rate of 1000 l /ha of Nemasol.. Each soil column was irrigated with 200 ml per day dispensed by peristaltic pumps. At the bottom of each column, the leachtes were collected in dark glass bottles. MITC residue analysis in the leacheates were carried out daily during seven weeks. The theoretical amount of MITC applied per column was 833 mg assuming 98 % conversion of MS to MITC as reported in the literature [10]. The physical and chemical characteristics of the soil used are given in Table 1.

TABLE 1 - Selected physical and chemical characteristics of the soil

Soil Depth cm	Sand %	Silt %	Clay %	Organic matter %	pH	Bulk density g cm-3	Field capacity $cm^3\ cm^{-3}$	Vi ml	V total ml
0-20	90,9	5,28	10,41	1,29	7,5	1,57	8,7	309,54	
20-40	92,5	5,71	10,14	1,68	7,6	1,52	6,76	233,03	800,49
40-60	90,9	4,92	8,78	1,065	7,69	1,45	5,86	192,99	
h>60	93,47	4,4	9,07	0,87	7,78	1,503	3,82	64,93	

Distribution of MITC: Four other soil columns (1m length and 12 cm diameter) were used to study the distribution of MITC along the soil profile. A dose of 2 ml of Nemasol (51% MS) was dissolved in 800 ml of water and applied to each column. After 24 hours, one, two and three weeks of application of MS, one each of soil column was cross sectioned into segments on five depths, 0 - 20 cm, 20 - 40 cm , 40-60 cm , 60-80 cm and >80 cm, and analysed for MITC residues.

Volatilization of MITC: MITC volatilization was esteemed in the leaching and distribution experiments. In the first case, each column was linked to a glass tube connected to a 100 ml of ethyl acetate containing flask and charcoal tube. In the second case, each column was connected to an activated charcoal tube fitted to a pump. MITC was pumped daily using an air flow of 1 l/h during 30 min. The activated charcoal was renewed, first, after 24 hours, and after three days for the rest of the experimental period.

Analytical method: The MITC in the leachates was extracted as described in the literature [1]. 500 ml sample was transferred into 2-litre separatory funnel and 100 g of dried sodium chloride was dissolved in the sample. Each sample was extracted twice with 10 ml of methylene chloride. The extract was passed through dried sodium sulfate and transferred into a 15-ml tube. Extraction of MITC residues from soil was accomplished by a method described by Leistra et al [7]. 50 g of soil was transferred into 120 ml glass flasks containing 50 ml ethyl acetate and 25 ml of water. The soil was extracted twice with ethyl acetate (50 ml).The flasks were sealed and shaken for 2 hours. The ethyl acetate layer was separated by centrifugation at 2000 rpm for 2 min. Supernatant organic layer was pipetted out and dried with anhydrous sodium sulfate. Extraction of MITC, volatilized and adsorbed on charcoal, was eluted with 10 ml mixture of methanol: methylene chloride 5: 95. The extracts, from soil, water and charcoal were analysed using a chromatograph 8700 equipped with a flame photometer detector (FPD) in the sulfur mode. The column was a DB-1 (length 25m with inner diameter 0.53mm).The carrier gas was nitrogen at a flow rate of 30ml/min. The temperatures were, respectively, 45 °C in the column, 190 °C in the injector and 350 °C in the detector.

RESULTS AND DISCUSSION

Leaching of MITC: The results of the mobility of MITC in soil columns after application of MS are presented in Fig.1 (A,B). In our study, the first traces of MITC in the leachates were observed during the first week with the highest concentration in the second week, following 2400 ml irrigation water in each column. This highest amount was estimated at 14.4 % of the initial applied quantity of MITC (833 mg). The total amount of MITC lost in the percolation water was observed after seven weeks and estimated at 30 % of the initial applied amount corresponding to 9400 ml irrigation water. Under other conditions, using the similar experimental design, the loss in the leachates reported varied from 15 to 68 % of the initial applied amount of MITC and increased with the frequency of irrigation [8]. However, this loss could be very low (0.2 %) when the soil temperature is around 2°C and low frequency of irrigation [1]. The quantities of MITC leached were low compared to the quantity applied. The major pathway of MITC was lost by degradation. This was may be attributed to the higher temperature above 22°C. At higher temperature the loss of MITC through degradation is increased, and the leaching decreased with the percolation water [4,9].

Distribution of MITC in the soil profile: The distributions of MITC observed in each column after, respectively, 24 hours, one, two and three weeks are shown in Fig. 2. The amounts of MITC in the soil profiles 0-20 cm, 20 - 40 cm, 40-60 cm and 60-80 cm were assessed, respectively, at 6.36 %, 5.65 %, 4.48 % and 3.32 % of the initial amount, 24 hours after application of MS. One week after application of MS, MITC was observed in the soil profile down to 80 cm, these amounts decreased in all soil profiles and were estimated, respectively, at 5.14 %, 2.88 %, 3.60 %, 3.48 % and 4 % of the initial amount. After two weeks, the concentration of MITC remained mainly 2 % in the deeper layer (h > 60 cm). The presence of MITC was in much lower level (< 0.1%) after three weeks. The MITC dissipation from all the soil profiles within the first day after application of MS could be attributed to the high MS and MITC volatility [4,11]. The concentration of MITC remained higher in the middle and the deeper soil layers, where the level of organic matter is low, after two weeks of application of MS [4,5]

Volatilization of MITC: The volatilisation potential of MITC are presented in Fig.3. MITC volatilization was determined using the same experimental design, but with different irrigation regimes. In the first case, when the soil columns were frequently irrigated, the total volatilization loss was estimated at 5 %. However, in the second case, when the soil columns were not irrigated, the maximum of MITC volatilisation was observed on the first day after application of MS and was estimated at 3.75 % of the initial amount. In this case the total loss by volatilization was 7 % after three weeks. The MITC volatilization loss was influenced by the frequency of water applications after MS injection. Under these conditions, volatilization is reduced and MITC degradation and leaching is increased. The total MITC loss by volatilization was measured under two irrigation regimes and varied from 3 to 30 % [8] of the applied MITC. Under field conditions, volatilization of MITC from the soil varied from 4 to 34 % [12], while this loss was computed to be 50 to 64 % from a greenhouse soil over two weeks following application of MS [11.]

FIGURE 1
Amount of methyl isothiocyanate (MITC) leached through soil columns with percolation water (A).
Leaching potential of MITC through the soil columns (B).

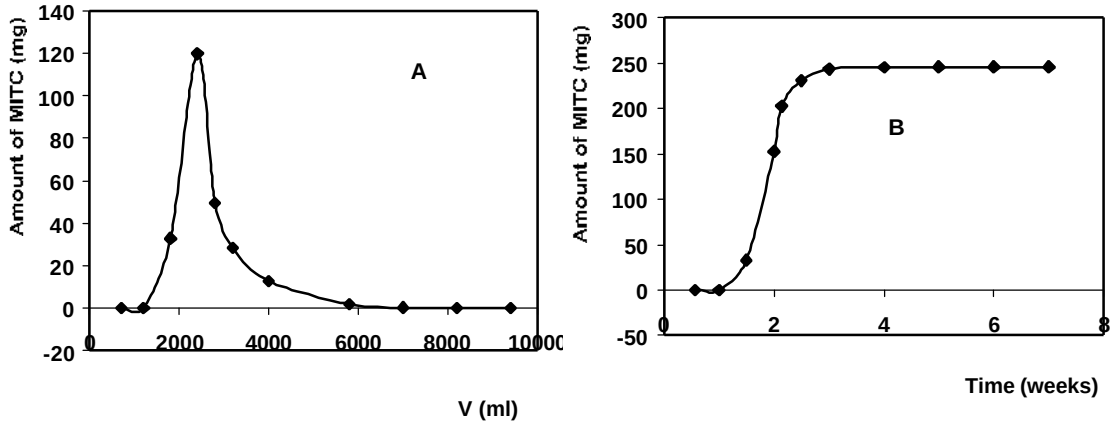


FIGURE 2
Distribution of methyl isothiocyanate (MITC) in soil profiles.

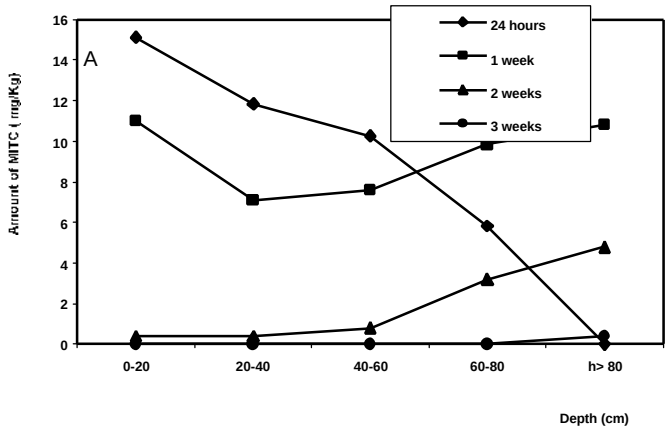
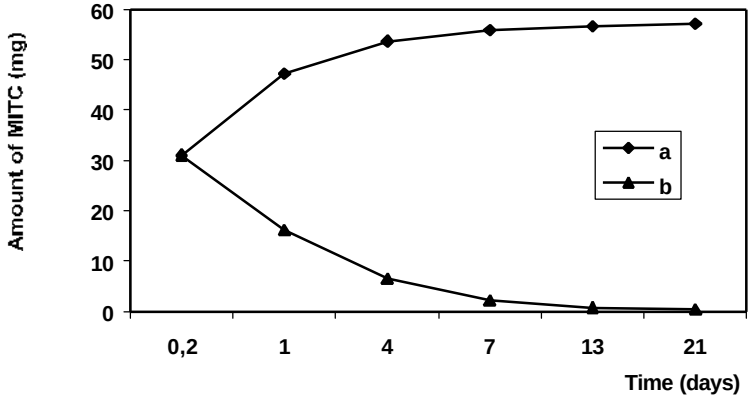


FIGURE 3
Cumulative volatilization of methyl isothiocyanate (MITC) (a). Volatilization of MITC (b).



CONCLUSION

In this preliminary studies, we can conclude that 30 % of the applied MITC may leach through the soil profile in the percolation water. The distribution of MITC through the soil profile was fast after the first day of application of MS. One week after the application of MS, MITC was recovered in all soil profile and was estimated at 19.8% of the initial applied amount. The maximum of volatilization of MITC was observed within the first day and the total volatilsation varied from 5 to 7 %. Soil and climatic factors that control MITC degradation and its volatilization rate affect also its potential for MITC leaching.Under Moroccan conditions, with high soil temperatures (> 25°C) during the application of MS, the risk of ground water contamination by MITC was low due to its, fast degradation and volatilisation.

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THE EFFECTS OF PHENOLIC COMPOUNDS EXTRACTED FROM TWO DIFFERENT FOREST SOILS IN ASPROMONTE (SOUTHERN ITALY) ON GERMINATION OF *PINUS LARICIO* SEEDS

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SUMMARY

The phenolic compounds extracted from two soils under different vegetation, *Fagus sylvatica* (S_1) and *Pinus laricio* (S_2), were tested on germination of *Pinus laricio* seeds. The α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2) and α-glucosidase (EC 3.2.1.20) were assayed. The amount of phenolic acids differed between the sites examined. The data obtained show that the phenols extracted from S_2 site inhibited more the seed germination, α- and β-amylase, and α-glucosidase activities compared to phenolic compounds extracted from S_1 site.

KEYWORDS:
phenolic compounds, amylases, glucosidase, seed germination.

INTRODUCTION

Phenolic compounds which are of widespread occurrence in soils, can positively or negatively affect the growth and the development of vegetation. The phenolic acids in forest soils are derived mainly from the decomposition of plant residues and from synthesis by soil microorganisms (1, 2, 3). Therefore, the plant species were found to exert a marked influence on the amount and the composition of phenolic compounds in soils (4). Many investigations on allelopathic interactions, showed that these compounds influence many physiological processes such as cellular expansion, membrane permeability, nutrient uptake, respiration, enzyme activity and protein synthesis (5, 6). The phenolic compounds have also an important ecological significance, being able to modify or to control processes such as succession or species interaction in nature (7).

In the present study, we have analysed the content of phenolic compounds extracted from two forest soils in Southern Italy (Aspromonte, Calabria) collected under two different tree species (*Fagus sylvatica*, L. and *Pinus laricio*, Poiret, spp. Calabrica) and tested the identified phenolic compounds on germination of *Pinus laricio* seeds, a typical tree of Southern Italy forests, in order to obtain further informations on biological effects of these phenolic compounds, constantly present in forest soils and in direct contact with seeds and roots. The activities of α-amylase, β-amylase and α-glucosidase, enzymes involved in starch degradation, during the early stage of seed germination, were assayed.

MATERIALS AND METHODS

Phenolic compounds were extracted from two experimental sites located in Aspromonte, Southern Italy. The sites were representative of one principal type of vegetation, and they have been designated as S_1 (under *Fagus sylvatica*, L.) and S_2 (under *Pinus laricio*, Poiret, spp. Calabrica). The soils were classified as Haplic Phaeozem and Eutric Cambisol, respectively, according to the FAO UNESCO System Criteria (8).

Soils were collected in May 1999 and the phenolic compounds were extracted with distilled water (1/10 w/v) (9). Soil samples were shaken at 75 r. p. m. for 20 h at room temperature and solutions were filtered through Whatman's n°1 paper (11 µm). Total water-soluble phenols were determined by using the Folin-Ciocalteau reagent, following the Box method (10).

To identify and quantify the monomeric phenolic compounds, the extracts were concentrated in a rotating vacuum evaporator at 30 °C; acidified with 1N HNO_3 to pH 2, followed by extraction (three times) with equal vol of ethyl acetate. The organic fractions were concentrated to 2 ml. The extracted phenolic compounds were analyzed by HPLC, injecting 50 µl of sample. A Perkin-Elmer LC 250, equipped with a 250 x 4.6 mm i. d. Nucleosil C_{18} (5 µm) analytical column, was used. Elution was carried out using a linear gradient between

solvent A (methanol, CH_3OH) and B (40 mM methanoic/ formic acid, HCOOH) increasing from 5% to 60% A in 25 min. Flow rate was 2 ml min^{-1} and detection was performed by using a Perkin- Elmer LC 135 diode array detector tuned at 280 nm.

The total water-soluble phenols extracted from soil under *Fagus sylvatica* (FP) had a concentration of approx. 10^{-4} M and the composition of monomeric phenolic compounds is reported in Table 1. The extract FP was used at the concentration of 10^{-4} M (present in soil) and at two diluitions (10^{-5} and 10^{-6} M). The total water-soluble phenols extracted from soil under *Pinus laricio* (PP) had a concentration of approx. 10^{-3} M and the composition of monomeric phenolic compounds is reported in Table 1.

The extract PP was used at the concentration of 10^{-3} M (present in soil) and at three diluitions (10^{-4}, 10^{-5} and 10^{-6} M). The most common phenolic compounds in the solutions of both soils (vanillic acid, p-coumaric acid, protocatechuic acid and p-hydroxybenzoic acid) were also bioassayed at concentrations of 10^{-3}, 10^{-4} M, 10^{-5} and 10^{-6} M.

The solutions were bioassayed using seeds of *Pinus laricio*, collected in Monte Peripoli, Aspromonte (Southern Italy), 1270 m above sea level, lat 38°03'34" N, long 15°51'05"E with a mean rainfall of about 1250 mm. The seeds were dried to a storage moisture content of 8% (fresh weight basis) and then stored at 4°C in plastic bags for varying periods before the beginning of the experiments. The seeds were surface sterilised with 30% (v/v) sodium hypochlorite for 10 min and rinsed with distilled water. Forty seeds were placed on Whatman 3 MM paper in a 9 cm diameter Petri dish to which 3 ml of solutions were added at the start. An additional ml of each solution was added every 48 hours thereafter. In all the experiments distilled water was used in the control set. Three replicates of each treatment were incubated in a germination chamber with 16 h light and 8 h darkness, 24 °C and a relative humidity of 50%. The effects of phenolic compounds were determined, after nine days incubation, by counting the number of germinated seeds. The seeds were homogenised in a chilled mortar with distilled water 1:4 (w/v) and centrifuged at 14000 g for 30 min. The supernatants were filtered through a single layer of muslin cloth to remove the lipid fraction and were used for α-amylase (**EC 3.2.1.1**) (11), β-amylase (**EC 3.2.1.2**) (12) and α-glucosidase (**EC 3.2.1.20**) (13) estimation.

Statistical analysis was performed with the software SYSTAT v. 8.0 using one-way Anova, followed by LSD test to evaluate significant treatment effects.

RESULTS AND DISCUSSION

Of 12 phenolic compounds present in the standard solution, 10 monomeric water-soluble compounds were identified in each soil (Table 1). Their concentration varied considerably between sites and ranged from 3.40 $*10^{-8}$ to 8.88 $*10^{-5}$ moles/g dry soil for S_1 site and from 1.50 $*10^{-7}$ to 3.63 $*10^{-5}$ moles/g dry soil for S_2 site. The amount of phenolic acids differed considerably between the two sites examined, in fact p-hydroxybenzoic and protocatechuic acid were more concentrated in S_1 soil, while more p-coumaric and vanillic acid were present in S_2 soil (Table 1).

TABLE 1 - Low molecular weight phenolic content (moles /g dry soil) in two sites with different vegetal cover (*Fagus sylvatica* and *Pinus laricio*). n.d.= below the detection level.

Phenolic compound	*Fagus* (S_1)	*Pinus* (S_2)
Gallic acid	n.d.	n.d.
Protocatechuic acid	$5.00 * 10^{-5}$	$1.11 * 10^{-5}$
Syringic acid	$6.50 * 10^{-6}$	$2.40 * 10^{-6}$
Vanillic acid	$9.40 * 10^{-6}$	$1.58 * 10^{-5}$
p-Hydroxybenzoic acid	$8.88 * 10^{-5}$	$2.64 * 10^{-5}$
Ferulic acid	$1.70 * 10^{-7}$	$7.00 * 10^{-6}$
o-Coumaric acid	$3.40 * 10^{-8}$	n.d.
m-Coumaric acid	n.d.	$2.20 * 10^{-6}$
p-Coumaric acid	$1.50 * 10^{-5}$	$3.63 * 10^{-5}$
Cinnamic acid	$2.7 * 10^{-6}$	$1.70 * 10^{-6}$
Caffeic acid	$5.8 * 10^{-6}$	$2.20 * 10^{-6}$
Gentisic acid	$6.3 * 10^{-6}$	$1.50 * 10^{-7}$

TABLE 2
Effects of different concentrations of single phenolic acids and phenolic compounds extracted from *Fagus sylvatica* (FP) and *Pinus laricio* (PP) on seed germination, and a-amylase, b-amylase and a-glucosidase activities. Different letters indicate significant differences at level p=0.05 between treatments.

Treatments	Seed Germination (%)	a-Amylase µg maltose/g fresh weight	b-Amylase µg maltose/g fresh weigth	a-Glucosidase µg maltose/g fresh weight
Control	100 [a]	23.0 [a]	14.3 [a]	0.270 [a]
FP 10^{-6} M	92 [ab]	21.5 [a]	12.8 [b]	0.194 [b]
FP 10^{-5} M	78 [c]	7.8[c]	6.6 [d]	0.133 [C]
FP 10^{-4} M	70 [c]	7.2 [C]	6.0 [d]	0.098 [d]
PP 10^{-6} M	73 [c]	7.1 [C]	7.0 [d]	0.190 [b]
PP 10^{-5} M	75 [c]	7.2 [C]	6.1 [d]	0.134 [C]
PP 10^{-4} M	73 [c]	7.2 [C]	6.2 [d]	0.094 [d]
PP 10^{-3} M	29 [d]	2.8 [d]	2.3 [e]	0.047 [e]
Vanillic acid				
10^{-3} M	66 [c]	6.80 [C]	5.20 [e]	0.102 [d]
10^{-4} M	75 [c]	9.50 [b]	8.80 [c]	0.210 [a]
10^{-5} M	95[a]	19.0 [a]	13.8 [a]	0.260 [a]
10^{-5} M	98[a]	21.1 [a]	13.5 [a]	0.265 [a]
Protocatechuic acid				
10^{-3} M	89 [b]	21.0 [a]	13.2 [a]	0.287 [a]
10^{-4} M	90 [b]	21.5 [a]	14.0[a]	0.285 [a]
10^{-5} M	97[a]	21.8 [a]	13.9 [a]	0.288 [a]
10^{-5} M	98[a]	22.1 [a]	14.0 [a]	0.280 [a]
p-Coumaric acid				
10^{-3} M	88 [b]	21.0 [a]	12.8 [a]	0.280 [a]
10^{-4} M	89 [b]	20.0 [a]	12.3 [a]	0.270 [a]
10^{-5} M	89[b]	20.5 [a]	12.7 [a]	0.275 [a]
10^{-5} M	95[a]	22.0 [a]	13.8 [a]	0.287 [a]
p-Hydroxybenzoic acid				
10^{-3} M	70 [c]	7.15 [c]	5.85 [c]	0.138 [c]
10^{-4} M	72 [c]	7.25 [c]	6.90 [c]	0.198 [b]
10^{-5} M	90[b]	19.0 [a]	13.2 [a]	0.268 [a]
10^{-5} M	98[a]	21.8 [a]	13.8 [a]	0.275 [a]

The qualitative and quantitative differences found in water soluble phenol concentrations in the soils investigated may be partly explained by differences in litter properties and may be also depended on the physical and chemical properties of soil, on the temperature and on moisture (3, 14). Data obtained on seed germination of *Pinus laricio* treated with different concentrations of FP phenolic compounds, showed that the seed germination was inhibited at all concentrations with respect to control. In particular, the lower concentration (10^{-6} M) induced an inhibition of 8 %, while the highest concentrations (10^{-5} and 10^{-4} M) inhibited more seed germination, 22 and 30 %, respectively, compared to the control (Table 2). The PP phenolic compounds inhibited seed germination more than FP phenolic compounds. In fact, the *Pinus* phenolic compounds tested at concentrations of 10^{-6}, 10^{-5}, 10^{-4} M showed an inhibition of about 25%, with respect to the control; when they were used at a concentration of 10^{-3} M showed an inhibition of about 71% (Table 2).

The vanillic and p-hydroxybenzoic acids used at a concentration of 10^{-3} and 10^{-4} M inhibited seed germination of about 30% with respect to the control, more than the p-coumaric and protocatechuic acids (approx. 10%). The other concentrations of individual phenolics did not affect significantly seed germination. Moreover, there is no correlation between the concentration of the four single phenolic compounds used and the percentage of seed germination.

The activities of α-amylase, β-amylase and α-glucosidase, enzymes involved in seed germination - a period of considerable starch breakdown (15) - were affected by the phenolic treatments at 9 days of germination. In fact, α-amylase activity was lower in seeds treated with both phenolic extracts at all concentrations, with respect to the control. Moreover this activity was strongly correlated to the percentage of germination. In fact, in seeds treated with *Fagus sylvatica* phenolic compounds at concentration of 10^{-6} M, which inhibited less seed germination, the α-amylase activity was higher compared to that tested in seeds treated with phenolic compounds at 10^{-5} and 10^{-4} M (Table 2).

In seeds treated with PP phenolic compounds, in the range of 10^{-6} - 10^{-4} M concentrations, the α-amylase activity was lower with respect to seeds treated with FP phenolic compounds, showing a direct relationship between seed germination and α-amylase activity. The PP phenolic compounds, used at concentration of 10^{-3} M strongly inhibited both seed germination and α-amylase activity. The same behaviour was observed for the β-amylase and α-glucosidase activities. In fact, in seeds treated with both phenolic compounds, these enzymes were inhibited with respect to the control, and the inhibition was strictly correlated to the percentage of germination (Table 2). Of four single phenolic acids utilised, only the vanillic and p-hydroxybenzoic acids at the concentration of 10^{-3} and 10^{-4} M, that most affected the seed germination of *Pinus laricio*, inhibited the α- and β-amylase and the α-glucosidase activities, demostrating a direct correlation between the enzymes involved in starch breakdown and the germination. It is known that the α-amylase, β-amylase and α-glucosidase are enzymes involved in the mobilization of starch, the major storage reserve of the seed. In fact, α-amylase catalyzes the initial steps in depolymerisation of both amylose and amylopectin, while the following hydrolysis of the released dextrins is completed by a combination of β-amylase, limit dextrinase, and α-glucosidase (15). The sugars or oligosaccarides released during starch breakdown are the substrates consumed in respiratory metabolism, a process significantly active during seed germination (16). Thus, the inhibition of amylase activities, related to treatments with soil phenolic extracts, could be the cause of reduced germination of *Pinus laricio* seeds.

The results obtained in the present work show that both phenolic extracts inhibit the germination of *Pinus laricio* seeds and also the α-amylase, β-amylase and α-glucosidase activities. In particular, the phenolic compounds extracted from S_2 site inhibited seed germination and amylases activity more strongly compared to phenol compounds extracted from S_1 site. It is quite difficult to draw conclusions, but we can suggest that the different composition and the diverse concentration of single phenolic acids in the two soil extracts could be responsible for the different effects on seed germination of *Pinus laricio*. This may also be ascribed to the combined actions of phenolic compounds resulting in additive, antagonistic or synergic effects (17, 18, 19).

These data are relevant to explain the problems linked to natural reforestation and regeneration present in *Pinus laricio* forest of Aspromonte (Southern Italy).

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A SIMPLE METHOD FOR AMYLASE DETERMINATION IN SOIL

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SUMMARY

The aim of this study was to suggest a more definite, careful and rapid method than the ones used in the past for amylase determination in soil. The main parameters affecting amylase activity in soil, such as pH, temperature, incubation time, soil:solution ratio, were evaluated by laboratory investigation. A substrate solution of starch (1%) and NaCl (1%) was used. Results indicated that the optimal conditions were: pH 6.5, incubation 24 hours at 50°C, and a soil:solution ratio 1:5. The activity of soil amylase was determined by calculating the glucose produced during enzymatic reaction through glucose test strips.

KEYWORDS : amylase assay; soil; starch; glucose test strips.

INTRODUCTION

The most abundant organic compounds in nature are represented by polysaccharides. A large part of the plant residues and a smaller part of the animal residues that find their way into soil are polysaccharides, which constitute from 5 to 25% of the soil organic matter (1). Polysaccharides contribute to soil quality through their role in the formation and stabilization of soil structure and are likely to be the most readily available source of energy for organisms (2).

Starch is a homopolysaccharide containing only a single type of monosaccharide unit: D-glucose. Amylase catalyses the hydrolytic depolymerization of starch in soil. Soil enzymes can be used as indicators of soil quality, because their activities are affected by environmental variables and farming practices (3). Concerning soil quality, amylase activity may be regarded as an index of the self-purification capacity of soil (4) and is represented in the Enzyme Activity Number (5, 6), a biological index of soil fertility. As a result of its role in both the carbon cycle and soil aggregation, amylase is of great agricultural importance.

Because soil enzyme assays are done in vitro under optimal controlled conditions of temperature, buffer pH and incubation time, each of which can affect activity rates, enzyme activity must be operationally defined. Because of these optimal conditions and because assay mixtures are saturated with substrate, these assays measure potential activity, and not the in situ soil enzyme rates (7). The activity of an enzyme is determined by measuring product formation or substrate remaining during incubation of soil samples (8). Glucose is the common major product of all soils incubated with starch, and all reducing sugars may, therefore, be included together and reported as "glucose" (9). Consequently, the activity of amylase may be determined by calculating the glucose produced in a soil incubated with a starch solution.

A few authors have studied amylase in soil, but no standard methodology considering all the variables influencing enzyme activity has been evolved. Ross (9, 10) used an incubation period of 24 h at 37 °C, with toluene, an acetate-phosphate buffer, pH 5.5, and a determination of reducing sugars that involved Shaffer-Hartmann reagent (11). Pancholy and Rice (12) used an incubation time of 24 h at 30 °C with toluene, an acetate buffer, pH 5.9, and the final determination of glucose content by Nelson's method (13). Sparling (14) used sodium azide without buffer at 35 °C for 18 h, and analysed for reducing sugars using alkaline ferricyanide (15). Beck (5) developed a turbidimetric method to measure the unreacted starch after an incubation of 16 h at 37 °C with toluene and a phosphate buffer, pH 7.5.

The aim of this study was to evaluate by laboratory investigation the main parameters, one by one, affecting amylase activity in soil, such as pH, temperature, incubation time, soil:solution ratio, and to find an improved, easy, quick, relevant and correct procedure to determine amylase activity in soil through glucose test strips.

MATERIALS AND METHODS

First of all, a number of tests were carried out in order to correlate the various parameters. Quantity of soil (from 0.5 to 15 g), type and pH of buffer, concentration of starch and NaCl solutions (from 0.3 to 2 %), temperature (from 15 to 57 °C) and time of incubation (from 24 to 96 h), with or without agitation during incubation time, were investigated to identify optimal conditions in which soil amylase shows maximal activity.

One soil with good general features was chosen (sand 47%, silt 27%, clay 26 %, pH 7.3, $CaCO_3$ 2.4 %, C.E.C. 30.4 meq/100 g, organic C 3.01 %, total N 2.38 ‰), air-dried and passed through a 2- mm sieve. 5 g of soil were used for the definitive test.

Toluene is the most widely used antiseptic for soil enzyme assays. Ross (9) reported that in the presence of toluene the microbial population remained the same during the enzyme assay and that glucose was released at a constant rate up to 48h. Toluene increases the permeability of cell walls allowing the passage of substrates and products through microbial cells (16). 1.5 ml of toluene was added to soil in 250 ml flasks.

Typically, amylase activity can be increased by the addition of sodium chloride (17). Sodium, as dispersing agent, promotes enzyme and substrate combination by intermediate complexation. 25 ml of phosphate buffer, NaCl and soluble starch (Sigma S-9765) solution were added to the soil. Phosphate buffer 0.1 M was prepared at different pH in the various tests. Afterwards, NaCl was dissolved in phosphate buffer at variable quantities, always maintaining the 1:1 ratio between NaCl and starch, and shaking and heating continuously. Starch 1 % was chosen in the definitive test.

Controls were prepared with soil, toluene, buffer-NaCl solution, but did not include starch.

The different temperatures investigated were maintained constant during the whole period of incubation.

At the end of the different incubation periods, 23.5 ml of water were added to the flasks; samples were vigorously shaken, centrifuged for 10 minutes at 6000 rpm and finally filtered with fast filter paper (Whatman n° 41). The clear solution was immediately analysed for glucose.

Chemical description of the test used :

To measure the glucose quantity in a solution rich in many other carbohydrates, glucose test strips of Reflectoquant® (Merck)* were used.

Due to the catalytic effect of glucose oxidase, Reflectoquant® strips convert glucose to gluconic acid lactone. The resultant hydrogen peroxide reacts with an organic redox indicator in the presence of peroxidase to form a blue-green dye, the concentration of which is determined reflectometrically.

Since glucose oxidase is very sensitive, it has been especially useful for detecting small amounts of D-glucose that may be produced from starch and glycogen by various amylases (18, 19). This method, tested also with standard solutions of glucose and starch, showed excellent results for rapidity, repeatability and linearity of regression between glucose concentration and reflectometry values.

All the results reported are the means of determinations made on three replicates. The mean values were compared by using ANOVA (DMS, P=0.05)(20).

* No endorsement of the trade name mentioned in this paper by the Authors is intended, nor is any criticism implied of similar products not mentioned.

RESULTS AND DISCUSSION

Buffer

As regards the type of buffer, the choice between acetate, acetate-phosphate and phosphate fell on phosphate buffer comparable, at the same pH, in acetate, acetate-phosphate and phosphate buffer (data not shown).

pH values

As is well known, enzymes have an optimal pH : if the pH is too low or too high activity falls. pH sensitivity is due to acidic and basic side groups of amino acids; moreover enzymes adapt to their environment. The effect of pH on amylase activity is shown in Fig. 1. Amylase showed good activity in the interval from pH 5 to pH 7, while the glucose produced decreased at subalkaline pHs. This confirms that verified by previous authors (9, 12, 17). Optimal pH was at 6.5, significantly different from the others.

Substrate concentration

Enzyme reaction velocity is a function of substrate concentration. Starch-NaCl solutions, at several concentrations (0.3, 0.6, 1, 1.5, 2 %), were investigated (data not shown); amylase activity reached greater velocity when the substrate concentration was raised, following the Michaelis-Menten equation, which is the mathematical model describing activity of many different enzymes. The positive influence of NaCl, with its dispersing action that promotes the diffusion of substrate into the active site of amylase, was evident only at low concentrations of starch (0.3 %), while its favourable action decreased to zero at higher concentrations of starch (2%). However, the difficulty in dissolving starch, even in hot water, was very considerable. Therefore, in order to take advantage of the influence of NaCl and, at the same time, to allow substrate solubilization, a solution of starch 1% and NaCl 1 % was chosen.

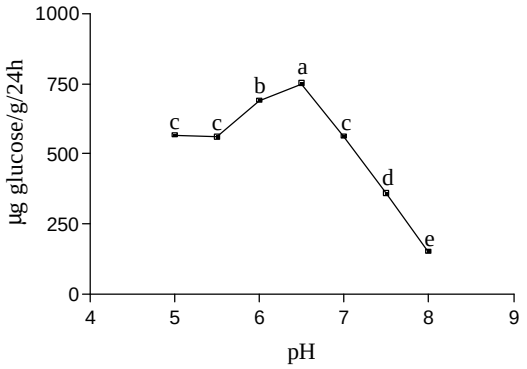

FIGURE 1 – Effect of buffer pH on amylase activity.
Values that do not contain the same letter are significantly different at P=0.05.

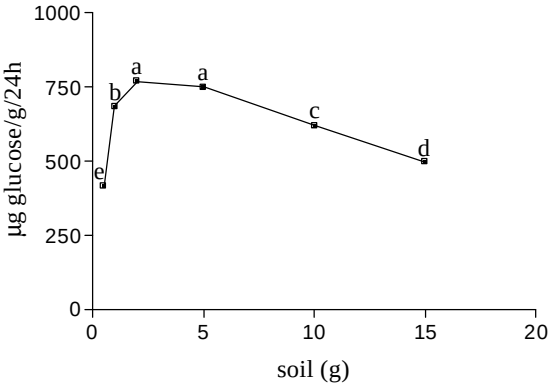

FIGURE 2 – Influence of soil quantityon amylase activity.
Values that do not contain the same letter are significantly different at P=0.05.

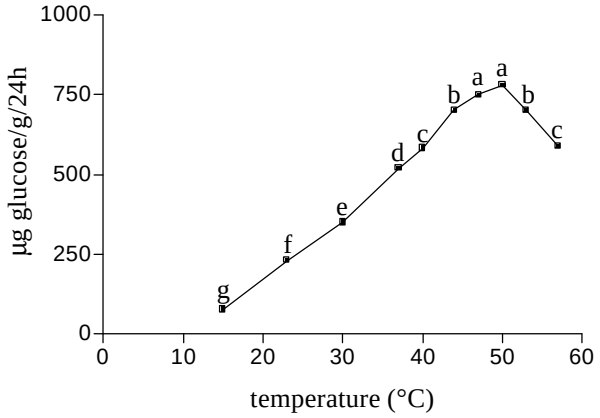

FIGURE 3 – Efect of temperature on amylase activity
Values that do not contain the same letter are significantly different at P=0.05.

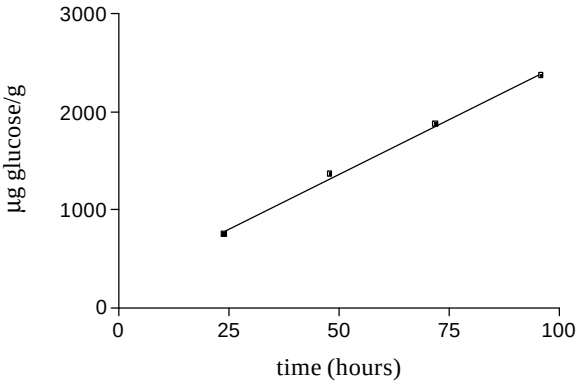

FIGURE 4 – Progress of amylase activity expresses as cumulative glucose production.

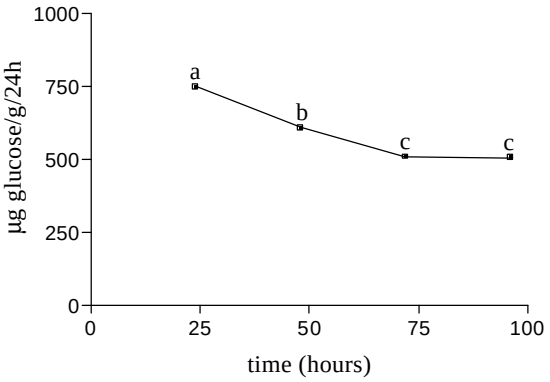

FIGURE 5 – Daily amount of glucose production in amylase activity.
Values that do not contain the same letter are significantly different at P=0.05.

Amount of soil sample

Another very important factor in the amylase test was the quantity of soil, corresponding to different levels of enzyme. In Fig. 2, the production of glucose from 0.5, 1, 2, 5, 10 and 15 g of soil in a fixed amount of 25 ml of substrate solution is represented. Maximal activity occurred with 2 or 5 g of soil, evidently representing the range in which amylase operated better because of the easier formation of the enzyme-substrate complex. In order to obtain a more representative sample, which is very important in a heterogeneous material, such as soil, an optimal quantity of soil was established at 5 grams.

Shaking of sample

The possible influence of the shaking of samples during incubation was investigated. No significant differences between stirred and not-stirred samples were observed (data not shown), and so in the methodology reported no shaking was applied during the incubation period.

Incubation temperature

Amylase, like all enzymes, was very sensitive to temperature. As expected, increasing temperature caused an increase in activity (Fig. 3) because of the greater kinetic energy of the molecules in solution. A decline in activity was observed with temperature over 50 °C because of thermal denaturation of the enzyme protein. For each enzyme there is an optimal temperature. Ross (9, 10) and Beck (5) choose 37 °C for the amylase determination because of the common misconception about enzyme activity that the optimum temperature is 37 °C, which is human body temperature. In this study, amylase activity reached its maximum rate at 50 °C (Fig. 3), with 780 µg of glucose produced.

Incubation time

Standard analyses of enzyme kinetics assume that during the first period of incubation the rate of product accumulation increases over time, after a very short lag-phase corresponding to an adjustment phase. In our study, for an extended period of incubation of some days, the glucose concentration increased linearly with time showing a constant rate of product formation (Fig. 4).

The trend of amylase activity is shown in Fig. 5, where the production of glucose from 0 to 24 hours appears significantly higher than the others at longer times. This suggests that amylase, under optimal conditions, immediately begins converting starch into glucose, without an initial phase of adjustment. Subsequently, amylase activity appears constant with time for some days. Therefore, and also for saving on test duration, an incubation period of 24h was chosen in the methodology reported here. A shorter period was not chosen so as to allow an adequate production of glucose, permitting improved sensitivity of the strips, which have a measuring range from 1 to 100 mg/l.

In controls, tiny quantities of glucose were found, arising from the natural soil content. These quantities were subtracted from the results of the sample tests, and anyway demonstrate that there was very negligible spontaneous formation of product without added substrate.

CONCLUSIONS

To determine soil amylase activity this study suggests a method which is more definite, accurate and rapid than the ones used in the past (5, 9, 10, 12, 14). All the principles of enzyme kinetics were taken into consideration. The best results were selected fixing all the other parameters at optimal conditions. Obviously, the method should be tested on soils differing in texture, amount and type of colloids, and biological activity. However, the suggested method could be extended to every type of soil because the fixed parameters, found optimal for amylase, do not affect biological, chemical and physical soil characteristics.

In conclusion determination of amylase activity in soil can be summarized as follows:

- Prepare phosphate buffer 0.1 M at pH 6.5, dissolve 1% NaCl and then 1 % soluble starch (Sigma S-9765), heating and stirring vigorously.
- Place 5 g of soil in a 250 ml flask, add 1.5 ml of toluene and 25 ml of substrate solution. Shake the soil suspension for a few seconds.
- Stopper the flask and place it in an incubator at 50 °C.
- Remove the stopper after 24 h, add 23.5 ml of water, shake vigorously for a few seconds and centrifuge at 6000 rpm for 10 minutes. Filter the soil suspension through a Whatman n°41 folded filter paper.
- Determine the glucose content of the filtrate with the glucose test (cod. 16720) of Reflectoquant® by Merck.
- To perform controls, follow the procedure described above, but add to the soil a phosphate buffer – NaCl solution without starch.
- The filtrate can be diluted with water if glucose concentration is too high.

Methods used to measure the activity of soil enzymes must be simple, rapid, accurate, and reproducible (8). The method reported here has all these characteristics.

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CHARACTERIZATION OF BOTTOM ASH PRODUCED FROM COMBUSTION OF FERMENTABLE MATTER OF MUNICIPAL SOLID WASTES FROM NORTHERN GREECE

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SUMMARY

The bottom ash from combustion of fermentable matter of municipal solid wastes produced at the greater Thessaloniki area, N. Greece, was evaluated according to environmental criteria, as part of an integrated waste management practice. The concentrations of As, Cd, Cr, Cu, Mn, Pb and Zn in bottom ash were low. Bottom ash showed a strong alkaline character with relatively high neutralizing capacity. The leaching behavior was examined according to TCLP method. The concentrations of toxic elements in leachates were below the maximum allowable standards.

KEYWORDS:
bottom ash, fermentable matter, heavy metals, incineration, leaching, municipal solid wastes, neutralizing capacity

INTRODUCTION

The incineration of municipal solid wastes is an alternative management practice that offers reduction of both mass and volume of waste. The generation of large quantities of residues such as bottom or fly ash could result in potential environmental hazards if they are not appropriately handled. These residues are mainly landfilled or used as construction related materials ranging from roadbase to masonry bricks and ceramic products. For a proper assessment of the potential environmental impact associated with landfilling or use in construction of combustion residues, chemical and leachability properties of them must be carefully examined.

Bottom ash is a very porous lightweight aggregate material with high specific areas (1). The composition of bottom ash is dependent on the waste composition, the type of incinerator and the combustion conditions (2,3). Bottom ash is primarily composed of silica, metal oxides, silicates, chlorides and sulphates, but also contains relatively high concentrations of potentially toxic elements and small amounts of unburnt organic matter (2). It is an unstable material under atmospheric conditions, since it is produced by incineration at high temperatures and cooled fairly rapidly. During landfilling of bottom ash fast or slow acid/base reactions can occur and be continued for a long term (4). Moreover, weathering reactions, such as carbonation, hydration, hydrolysis and microbiologically mediated oxidation/ reduction reactions, usually occur in landfills and control the element mobility (5,6).

Landfilling, composting, incineration, recycling and a combination of them are the most common integrated waste management practices. Contrary to other European countries, the municipal waste incineration is not applied in Greece and the main waste management practice is landfilling. A project concerning the comparison of possible management scenarios of municipal solid wastes in the municipality of Pilea, at the greater area of Thessaloniki, N.Greece was conducted (7). Different integrated management scenarios (including collection, transportation, recycling, composting, landfilling, incineration, disposal options and choice of suitable disposal areas) were proposed and evaluated according to hydrogeological, social, economical and environmental criteria.

In this paper the evaluation of bottom ash produced from the combustion of fermentable matter, which is the major component of municipal solid wastes, is presented according to environmental criteria. For this purpose the concentrations of toxic elements in bottom ash were determined, their leachability under landfill conditions as well as their neutralizing capacity was evaluated.

MATERIALS AND METHODS

Collection of municipal solid wastes

Municipal solid wastes (MSW) were collected from five locations (S_1-S_5) in Pilea municipality at the greater area of Thessaloniki, N. Greece, which are characterized by different social-economic level. In a total population of 28000 inhabitants, 10% have low income (site S_1), 60% middle income (site S_2) and 30% high income (sites S_3 and S_4). The location S_5 is in the border of residential area and has many handicrafts and storages. About 30 m^3 day^{-1} of MSW were collected from representative buckets with a truck. A representative subsample (3 m^3 day^{-1}) was prepared by quartering method and separated into six components: fermentable matter, paper, plastics, glass, combustibles (wood, leather, textiles, rubber) and miscellaneous inert materials. Four sampling campaigns were conducted: April 1998, June 1998, October 1998 and December 1998. During the sampling campaign municipal solid wastes were collected in five different days over a period of two weeks.

Sample preparation

Upon receipt at the laboratory municipal waste samples were homogenized and oven dried at 105 ^oC. A laboratory scale combustion system was employed for incineration of fermentable matter by using an SVR/E type incinerator at 780-950 ^oC for 40-60 min. The bottom ash was collected and analyzed.

Chemical analysis of bottom ash

Subsamples of bottom ash were crushed, grounded, homogenized and treated in low temperature by cool plasma asher. Then the samples were digested with aqua regia according to AFNOR NF X31-151 reflux mineralization method (8). Sample digests were analyzed for As, Cd, Cr, Cu, Pb, Mn and Zn.

Leaching test of bottom ash

The leaching behavior of bottom ash was evaluated by employing the US-EPA Toxicity Characteristic Leaching Procedure (TCLP) (9). According to this test 100 g of a representative sample was placed in an extractor with 2000 ml of acetic acid solution (pH=2.88$\pm$0.05). The mixture was agitated (30$\pm$2 rpm) for 18$\pm$2 h at 22$\pm$3 ^oC. Then, the two phases were separated by filtration and the filtrates were analyzed for As, Cd, Cr, Cu, Pb, Mn and Zn.

Element analyses

The determination of toxic elements in sample digests and leachates was performed by employing standard methods of analysis (10). The elements, Cd, Cr, Cu, Pb, Mn and Zn were determined by FAAS or GF-AAS. Arsenic was determined by AAS coupled with hydride generation system.

Quality control of analytical data was assured by duplicate samples, blanks and the method of standard additions. Analytical variation of instrumental measurements was <10%.

Neutralizing capacity of bottom ash

The neutralizing capacity of bottom ash was evaluated by titration of suspension of 1 g of sample in 20 ml deionized water with 1N HCl. The pH of this suspension was recorded after addition of 0.1 ml of acid solution.

RESULTS AND DISCUSSION

Composition of MSW

The average generation rate of MSW is 1.26 kg per capita day^{-1}. This value is in the middle of the range of MSW generation rates reported for other countries (from 0.41 kg per capita day^{-1} in India to 1.98 kg per capita day^{-1} in USA) and close to German rate (1.15 kg per capita day^{-1}) (11). The composition of MSW is presented in Table 1.

The major components of MSW are: fermentable matter (46.7 % ww), paper (21.7 %ww), plastics (12.8 %ww), combustibles (3.7 %ww) and metals (3.5 %ww) (7). These results are comparable to those reported for the Greek cities, Thessaloniki and Athens, although a small decrease of fermentable matter and an increase in paper fraction are observed and could be attributed to the high standards of living in the studied area as well as to different consumption habits of inhabitants in regard to fast food and packing/wrapping materials during the last decade. Generally, the composition of MSW was in the range reported for middle income countries.

Spatial variations in MSW composition were observed probably due to different socio-economic level of population in the examined locations (Fig. 1). A distinct difference in MSW composition was observed in location S_5, where paper and plastics were found at higher percentages, due to the presence of handicrafts and storages in the area. The data from S_5 were excluded from the calculation of the average MSW composition (Table 1).

Chemical analysis of bottom ash

The mean value of ash produced from combustion of fermentable matter of MSW was 19.1 %. The mean concentrations and the range of toxic elements (As, Cd, Cr, Cu, Pb, Mn and Zn) determined in bottom ash are presented in Table 2. In the same table, the concentrations of toxic elements in MSW, organic component of MSW and bottom ash from MSW incineration reported in the literature, are also presented.

TABLE 1 - Composition of municipal solid wastes

Components (% ww)	Greece			France	USA	Jordan	Quatar
	Pilea[a] (this study)	Thessa-loniki (7)	Athens (7)	(2,8)	(11)	(12)	(11)
Fermentable	46.7	51.7	59.8	25	27	63	53.3
Paper	21.7	17.7	19.5	32	44	11	17.7
Glass	3.3	4.1	2.6	12	8	2	3.1
Plastics	12.8	7.2	7.0	10	3	16	15
Metals	3.5	5.9	3.8	6	9	2	4.3
Combustibles[b]	3.7	9.4	3.5	6			
Miscellaneous	8.3	4.0	3.9	9	9	6	6.6

[a]overall weighted average composition (7)

[b]textiles, leather, wood, rubber

FIGURE 1 - Average composition of MSW produced in studied locations

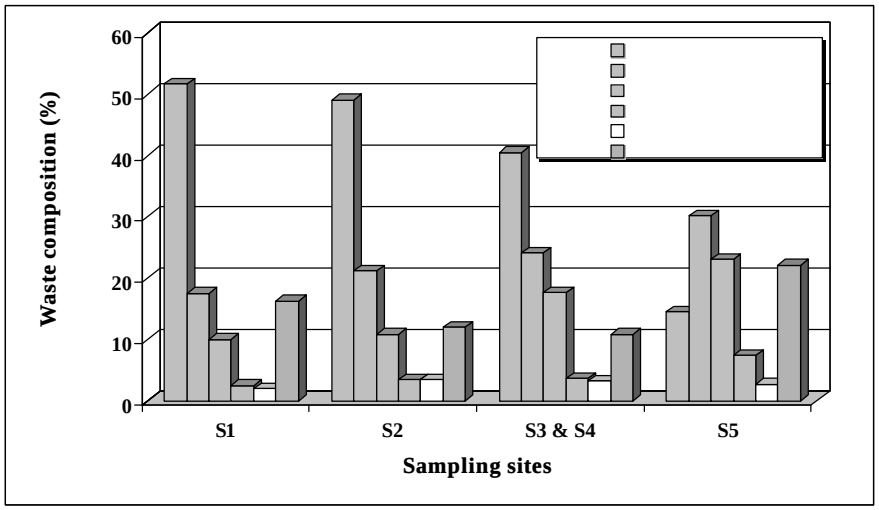

TABLE 2 - Concentrations of toxic elements (mg kg^{-1} dry wt) in different groups of solid wastes

Toxic elements	Bottom ash from fermentable matter (this study)		MSW (13)	MSW (8)	Organic fraction from MSW (14)	Compost from MSW (13, 15)	Bottom ash from MSW (1)
As	3.8	(0.4-17.4)					0.12-190
Cd	0.8	(0.4-1.1)	6	4		1.5-8	0.3-71
Cr	96	(25-419)	323	350	118	45-407	23-3200
Cu	60	(20-430)	270	77	130	149-230	190-8200
Pb	27	(2-121)	438	230	113	101-430	98-14000
Mn	141	(34-315)	266		106	274-454	83-240
Zn	448	(94-2167)	536	380	263	448-671	610-7800

FIGURE 2 - Titration curves of bottom ash from incineration of fermentable matter of MSW

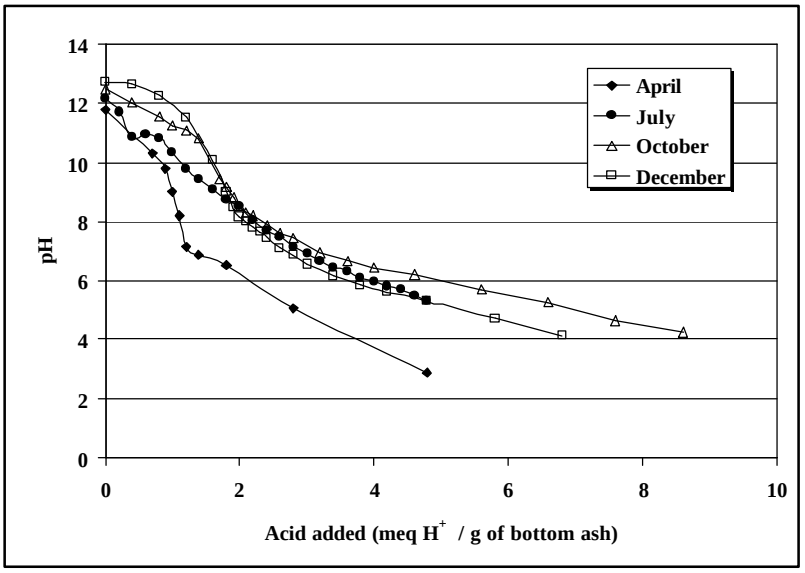

TABLE 3 - Concentrations of toxic elements in leachates from US-EPA TCLP method

Element	Bottom ash (mg L^{-1})	EC/GR DWS x100	Leachability (%)
As	0.002-0.025	1	0.7-65
Cd	0.001-0.023	0.5	5.7-90
Cr	0.002-0.287	5	0.1-7.3
Cu	0.010-0.042	300	0.1-2.7
Mn	0.052-0.700	5	0.4-12
Pb	0.005-0.085	5	0.7-18
Zn	0.050-0.900	500	0.1-7.0
pH	7.5-11.0		

TCLP: Toxicity characteristic leaching procedure
EC/GR DWS: Drinking water standards in European Community and Greece

The concentrations of toxic elements in ash varied considerably, although only Pb showed significant spatial variation at 95% confidence level by using one–way ANOVA procedure. The concentrations of toxic elements in bottom ash from fermentable matter were lower than the reported values for bottom ash from MSW incineration. This can be attributed to the different contributions of individual components to toxic content of MSW. For example, plastics contribute highly (50%) to total cadmium load of MSW, while leather represents the 25% of total chromium load. Copper is found as non ferrous metal, but is also associated to paper and cardboard, nickel is mostly associated to scrap metal and to a lesser extent to glass, and zinc is found in rubber. Fine particles, usually loaded with copper, lead and zinc, are linked to organic particles thus increasing the elemental load of fermentation matter (8).

Neutralizing capacity of bottom ash

The titration curves of bottom ash with 1N HCl are shown in Figure 2. The suspensions of bottom ash in water were highly alkaline (pH 11-13). The alkaline character of the suspensions is usually due to the presence of calcium salts (e.g. $CaCO_3$, CaO, $CaCl_2$) as well as to calcium and alkali metal hydroxides (16, 17). The acid neutralizing capacity of ash equivalent to the titration end point at pH 7.5 ($ANC_{7.5}$), was 2.0-2.5 meq g^{-1} bottom ash. At this pH value hydroxides, soluble basic silicate hydrates and carbonates of bottom ash have been consumed (16). The $ANC_{7.5}$ for the examined ashes was relatively high, thus ash can act as a natural buffer. Until this buffer capacity is consumed, the pH values will remain in the neutral area and the mobility of many heavy metals will remain low. The consumption of the buffer capacity is

dependent on physical and chemical parameters, such as dimensions of the ash deposit, hydrological conditions and leachate composition (16, 17).

Leaching behavior of bottom ash

The chemical composition of leachates obtained through USEPA TCLP method is presented in Table 3. The concentrations of toxic elements were low and well below the TCLP maximum allowable limits (the criterion of 100-fold the value for drinking water standards). The final pH of the leachates varied from slightly to strongly alkaline (7.5-11) probably depending on calcium content of the ash. Thus, the comparison between leachates is not strictly accurate and the final pH should always be included in the leaching results. At this pH most elements show limited solubility except the elements with amphoteric behavior, such as Pb and Zn, that are leached in both acidic and alkaline conditions (18).

The metal leachability rates (i.e. percentage of total metal in bottom ash that is leached) are also given in Table 3. Cd and As showed high range in leachability (5.7-90 % and 0.7-65 %, respectively), while Cu, Zn and Cr showed lower range. Generally, leachability and mobility of trace elements is largely dependent on their chemical form, the chemical composition of the leachate, the phases in which the metals are bound and the surfaces available for binding (16).

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SOIL PHASE IRON IN HIGH SALT MARSH SOILS IN RELATION TO REDOX POTENTIAL

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SUMMARY

A total of 6 soils from different physiographical positions in a high salt marsh were studied. The Fe species considered were: amorphous-Fe (III), crystalline-Fe (III), pyrite-Fe and acid volatile sulfide of Fe (AVS-Fe). The results showed significant changes in the concentrations of the Fe species with position and depth. In soils with oxic (Eh>300mV) and suboxic conditions (Eh =100-300mV) the dominant forms of Fe were amorphous and crystalline oxyhydroxides of Fe. In contrast, in strongly reduced soils or horizons (Eh< -100 mV; soil 6) pyrite-Fe was the most abundant form. Where conditions were oxic or slightly reduced at the surface and anoxic at depth, high concentrations of amorphous and crystalline oxyhydroxides of Fe were found in the top 10 cm. In these soils, the amorphous oxyhydroxides of Fe quickly disappeared with depth, while pyrite-Fe levels increased significantly. The amount of crystalline oxyhydroxides of Fe also decreased with depth, but more gradually.

KEYWORDS:
Salt marsh soils, redox potential, iron oxyhydroxides, pyrite

INTRODUCTION

The soils of salt marshes can show large spatial variations in their geochemical conditions (e.g. redox conditions) due to differences in physical factors (influence of tides, drainage, physiographical changes, etc.) and/or biotic factors (bioturbation, microbial activity, etc.) (1). These changes can affect the speciation of Fe in the soils, as well the bioavailability of trace metals that are are usually found in association with oxidized and reduced (e.g. pyrite) forms of iron (2). Under reducing conditions, oxyhydroxides of Fe(III) are reduced and solubilized to form Fe(II), which can diffuse towards the surface, be oxidized and precipitate once again as an oxyhydroxide, or it can diffuse downwards and precipitate as a sulfide (2).

The aim of the present study was to establish the spatial variation and changes with depth in amorphous-Fe, crystalline-Fe, pyrite-Fe and acid volatile sulfide of Fe (AVS-Fe). Six soils were selected from different physiographical positions of a high salt marsh in the NW Iberian Peninsula.

MATERIALS AND METHODS

The soils under study were collected from the Ría de Ortigueira salt marshes (Esteiro, Mera and Ladrido; NW Iberian Peninsula), the main characteristics of which have been described in previous studies (3, 4, 5). The sampling sites were chosen in order to represent the different geochemical environments present, therefore, the physiographical position, presence/absence and type of vegetation were taken into account (Fig. 1 A, B). On the basis of these variables a total of 6 sampling sites were selected, the main characteristics of which are shown in Table 1.

Core samples were collected at low tide using PVC tubes, (diameter, 11cm length, 35cm). Tubes were hermetically sealed under pressure and transported in vertical position to the laboratory, where they were frozen at –18 ºC, cut into 5cm sections with a carbon fiber saw and placed again in the freezer until use. Redox potential (Eh) was determined *in situ* with a Solomat 2000 instrument previously calibrated using a redox solution (Crison, Eh:468±5 mV at 25ºC). The value obtained was then corrected by adding the potential of a calomel reference electrode (244 mV). The Fe fractions considered were: (1) AVS-Fe (mainly mackinawite, FeS), (2) amorphous Fe(III), (3) crystalline Fe(III) and (4) pyrite-Fe. The concentration of AVS-Fe was calculated from the concentration of AVS-S assuming a ratio of S:Fe of 1:1 (7). The acid volatile sulfide fraction (AVS) was determined in triplicate using the method of Kostka & Luther (1994). Sulfide was released from AVS in the wet sample (0.5–1 g) by adding 20 ml of previously deaerated 6N HCl. The sample was digested in an air-tight reaction flask for 40-50 min under a continuous flow of nitrogen, which was bubbled through the flask as slowly as possible.

TABLE 1 - General characteristics of the different sampling sites (after Otero 2000)

Soil	Physiographical position	Dominant plant species	Characteristic Soil (6)
Soil 1	Back marsh	*Scirpus maritimus*	Histic Sulfaquents
Soil 2	Hollow	*Spartina maritima*	Typic Sulfaquents
Soil 3	Creek edge	*Halimione portulacoides*	Sodic Hydraquents
Soil 4	Marsh flat	*Juncus maritimus*	Sodic Hydraquents
Soil 5	Hollow	*Spartina marittima*	Typic Sulfaquents
Soil 6	Creek bottom	No vegetation	Typic Sulfaquents

The H_2S released was transferred by the flow of nitrogen to another reaction flask containing 25 ml of 3% Zn acetate, to which 1 ml of concentrated H_2SO_4 and 4 ml of diamine B reagent were added (7). The concentration of sulfide was then determined colorimetrically with a UV-VIS spectrophotometer (Vitatron model MCP) at 670 nm, using the methylene blue method of Cline (8). Test solutions ($Na_2S{\cdot}9H_2O$ in deaerated water) were prepared daily, and the percentage recovery was greater than 92 %. The detection limit was 0.02 µmol g^{-1}, established as 2.5 times the standard error of the blank.

Amorphous or poorly crystalline Fe was extracted from 0.5 g of wet sample to which was added 10 mL of a solution of 0.11 M ascorbic acid/0.20 M sodium citrate, buffered to pH 8 with 0.60 M sodium bicarbonate (7).

Crystalline Fe was obtained from the dithionitecitrate extracted Fe, minus the amorphous and AVS-Fe (7). Extraction was carried out using 0.5 g of wet sample, to which was added 0.5 g sodium dithionite and 10 mL of a solution of 0.35M acetate/ 0.2M sodium citrate at pH 4.8. Samples were placed in a water bath at 60 ºC with constant shaking for 4 h (7).

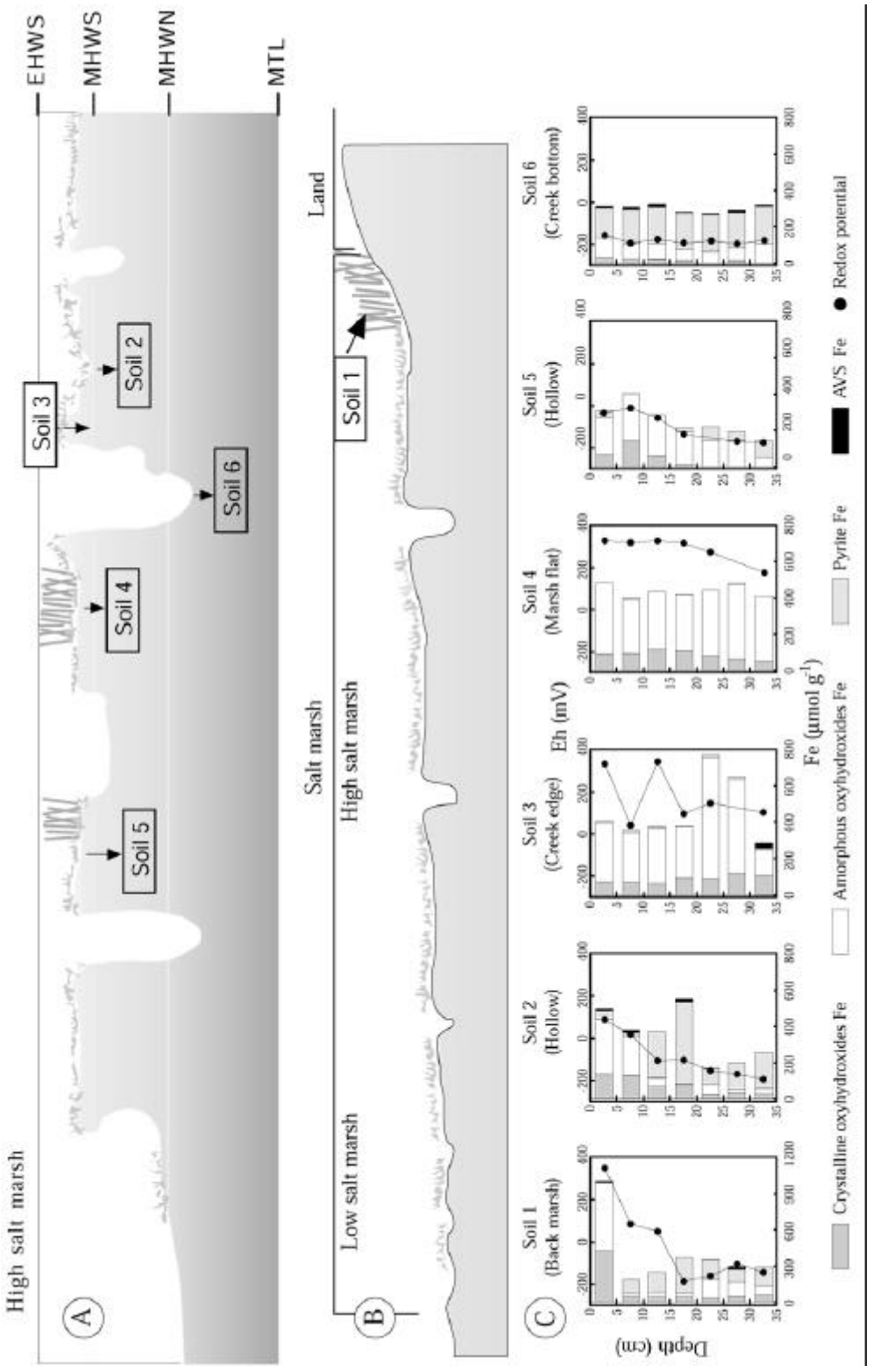
Pyrite-Fe was extracted using the sequential extraction method of Huerta-Díaz & Morse (2). The sample was first placed in 1N HCl for 16 h with constant shaking to extract the metals associated with the AVS fraction, amorphous and crystalline oxyhydroxides of Fe and Mn, and carbonates (2). The residual Fe was then extracted by incubating the sample with 30 mL of 10M HF for 16 h with constant shaking. The sample was then treated with 10 mL of concentrated H_2SO_4 for 2 h to extract the Fe associated with organic matter, which was discarded. Finally, the metals associated with the pyrite fraction were extracted by incubating the sample with 10 mL of concentrated HNO_3 for 2h with constant shaking. All analyses were carried out in triplicate and the concentration of Fe was determined by atomic absorption spectrophotometry (Perkin-Elmer 1100B).

RESULTS

High concentrations of both amorphous and crystalline iron oxyhydroxides were found in the surface layers of the soils with oxic (Eh>300 mV) or suboxic conditions (Eh 100-300 mV) (Fig. 1C). Thus, there was a sharp contrast between the high levels found in soil 1 (amorphous Fe: 425±55.5 µmol g^{-1}; crystalline Fe: 570±37µmol g^{-1}) and those found in the surface layers of soil 6, in which conditions were strongly reduced throughout the profile (Eh< -100) (amorphous Fe: 27.7±1.6 µmol g^{-1}; crystalline Fe: 146±10.0 µmol g^{-1}). There was a sharp decrease in levels of amorphous Fe at depth, where conditions became suboxic or anoxic (Eh < 100 mV), and the lowest concentration was found in the deepest part of soil 6, (average 1.34±0.05 µmol g^{-1}). However, the decrease in crystalline Fe with depth was more gradual than the decrease in amorphous Fe, with high concentrations prevailing even under strongly reduced conditions (e.g. soil 6 at 30-35 cm depth: 113.4±23.1 µmol g^{-1}).

The pattern in the changes in concentration of pyrite Fe was the inverse of that found for the iron oxyhydroxides, i.e. the maximum concentrations occurred at depth, particularly where Eh values of less than -150 mV were reached (Fig. 1C). The highest concentration of pyrite-Fe was found at 17.5 cm depth in soil 2 (471±30 µmol g^{-1}), however, high concentrations were found in soil 6 throughout the profile and were always higher than the concentrations of iron oxyhydroxides, which ranged between 107±8.0 µmol g^{-1} and 402±28 µmol g^{-1}. A clearly different situation was found in the soil from the marsh flat, colonized by a *Limonio-Juncetum maritimi* community, in which the concentrations of pyrite-Fe were never greater than 1 µmol g^{-1}. The levels of Fe associated with AVS were low in all soils, with the highest concentrations being found in the superficial layers of soils with anoxic conditions at the surface (e.g. soil 6: 7.3±2.8 µmol g^{-1}).

FIGURE 1 – Topografical position of the sampling sites. -A: Transverse section of a high salt marsh. – B: Longitudinal section. – C: Partitioning of soil phases of iron and redox potential in salt marsh soils.



EHWS. extreme high water springs. MHWS. Mean high water springs. MHWN. Mean high water neaps. MTL. Mean tide level

DISCUSSION

The values of the redox potentials measured demonstrate the existence of different geochemical conditions, which vary with depth and position in the soils studied (Fig. 1) and which are consistent with the different periods of flooding to which these soils are subjected depending on their position in the salt marsh (an aspect which has previously been studied in this salt marsh by Sánchez (9).

In the present study, we found oxic conditions (Eh> 300 mV) in the soils at the channel edges (soil 3) and in the salt marsh flat (soil 4). The channel edge soils were well drained and aerated because of the fast water flow at this point, which aids drainage, and the presence of channels made by crabs, which significantly increases aeration (10, 11). The oxic conditions in the salt marsh flat soil occur, despite it being frequently flooded by the tide, because of rapid drainage due to the convex profile at this position, as well as to the high density of roots and rhizomes present and to a relatively high water table at low tide (15.9±2.8 cm) (9). In contrast, the soils from the hollows remain flooded for long periods because they are poorly drained and thus conditions are suboxic at the surface, becoming anoxic at depth. Finally the soils from the channel bottom are permanently anoxic because they are always saturated with water.

There were notable changes in the concentrations of the different forms of Fe studied in each soil, consistent with the redox conditions of the soils. Thus, the highest concentrations of pyrite-Fe were found in anoxic soils or horizons (e.g. soil 6 or the lower layers of soils 2 and 5 (Fig. 1C); there was a significant increase in pyrite-Fe at Eh values less than 0 mV, the point at which some authors have established to be the start of sulfate reduction processes in soil (12). On the other hand, the low concentration of Fe monosulfide (AVS fraction) relative to pyrite concentrations, appears to support the findings of a previous study, in which it was established that the main components of the AVS fraction (mackinawite, FeS) are metastable forms which are transformed into pyrite, according to reaction (1) and, therefore, pyrite is the most thermodynamically stable product of sulfate reduction (13).

As recently demonstrated (14), this process requires strictly anoxic conditions, therefore, it could take place in soil 6 (channel bottom) and in the deepest layers of soils 2 and 5. However, high concentrations of pyrite were also found in the upper layers of soils with suboxic conditions (Eh: 100-300 mV) (e.g. soil 5 at 0-5 cm). Previous studies have indicated that these soils are slightly acidic (pH 6-6.5) and contain low concentrations of H_2S (4, 5). Under these conditions, pyrite may be formed directly from interstitial water, according to reaction (2), without Fe monosulfide being required as a precursor (15).

$$FeS_{(s)} + H_2S_{(aq)} \rightarrow FeS_2 + H_{2(g)} \quad \textbf{(1)}$$

$$Fe^{2+} + S_5^{2-} + HS^- \rightarrow FeS_2 + S_4^{2-} + H^+ \quad \textbf{(2)}$$

There were also large differences in the distribution of iron oxyhydroxides with depth and position in the soils studied. The highest concentrations of both amorphous and crystalline iron oxyhydroxides were found in soils, which were colonized by plants and in which the surface layers (0-10 cm) were oxic or suboxic and the deeper layers were anoxic and contained high levels of pyrite-Fe (soils 1, 2 and 5). The levels of amorphous iron oxyhydroxides quickly decrease with depth in these soils at the same time as the pyrite-Fe increases. These results appear to indicate that under anoxic conditions, pyrite is formed mainly at the expense of amorphous iron oxyhydroxides (16), whereas pyritization of the crystalline forms occurs more gradually, indicating greater stability and lower bioavailability to microorganisms (16).

On the other hand, the enrichment of oxyhydroxides found in the surface layer of these soils may occur as a consequence of the oxidation of pyrite present in deep layers, which is favoured by plants. Previous studies have shown the oxidizing effect of vegetation on salt marsh soils, where there was a significant increase in the redox potential of the soil as a result of the effects of roots, which facilitate both the active transport of oxygen (via the arenchyma) as well as passive transport of oxygen downwards into the soil (17, 18) as has recently been demonstrated (14). Under these conditions pyrite can be oxidized by molecular oxygen or by Fe(III), which can then form organic complexes and react with pyrite more efficiently than oxygen (19) as shown in reactions (3) and (4).

$$FeS_2 + 7/2\ O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad \textbf{(3)}$$

$$FeS_2 + 14Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2SO_4^{2-} + 16\ H^+ \quad \textbf{(4)}$$

This process causes acidification of the interstitial water, thus facilitating the dissolution of Fe^{2+}, so that part of this can diffuse towards the surface of the soil, where it is oxidized and precipitated. This generates large amounts of iron oxyhydroxides at the surface and impoverishment in subsurface layers, where the highest density of live roots are found (3). This idea is also supported by the results from soils which do not show changes in redox potential throughout their profile, i.e. oxic (soil 4) or anoxic (soils 6) soils, in which the oxidized or reduced forms of Fe remain stable and do not move throughout the profile.

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REMOVAL OF CHROMIUM POLLUTION IN WATERS BY ADSORPTION ON NATURAL SEPIOLITE IN PACKED BEDS

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SUMMARY

Adsorption of Cr(VI) from aqueous solution by natural sepiolite was studied. Experiments were carried out at continuously operating packed beds. Adsorption yields were determined by distribution curves plotted as chromium removal concentrations versus time. Effects of factors, such as pH, temperature, concentration and flow rate on adsorption yield were investigated. It was conclusively observed that the yield decreased with increasing liquid flow rate. Effects of pH, temperature and concentration on yield did not show a steady tendency.

KEYWORDS:
Oxidation, sulphide, packed column, water pollution

INTRODUCTION

The presence of chromium in the environment has led to an increasing awareness and concern of its detrimental effect to nature and human beings. Chromium is of particular concern due to its widespread industrial use and its chemical complexity. Dyeing and plating are major sources of dispersion of chromium into the environment[1]. A typical treatment process for chromium-bearing wastes involves some form of a precipitation process. There are, however, several potential disadvantages in these traditional techniques. For example, prior treatment may be required to remove complexing agents that may inhibit precipitation [2].

Adsorption is capable of removing many metals over a wider pH range and to much lower levels than precipitation. Additionally, adsorption can often remove complexed metals which would not be removed by conventional treatment processes [3]. Removal of Cr(VI) from water and wastewater by adsorption has been reported by many investigators [4 - 9]. Cost effective sorbents are needed for its application. Natural materials that are available in large quantities, or certain waste products from industrial or agricultural operations, may have potential as inexpensive sorbents [10].

This study was conducted to evaluate the effectiveness of natural sepiolite in the removal of Cr(VI) from aqueous solution. Experiments were carried out at packed beds. Effects of pH, temperature, concentration and flow rate on adsorption were investigated.

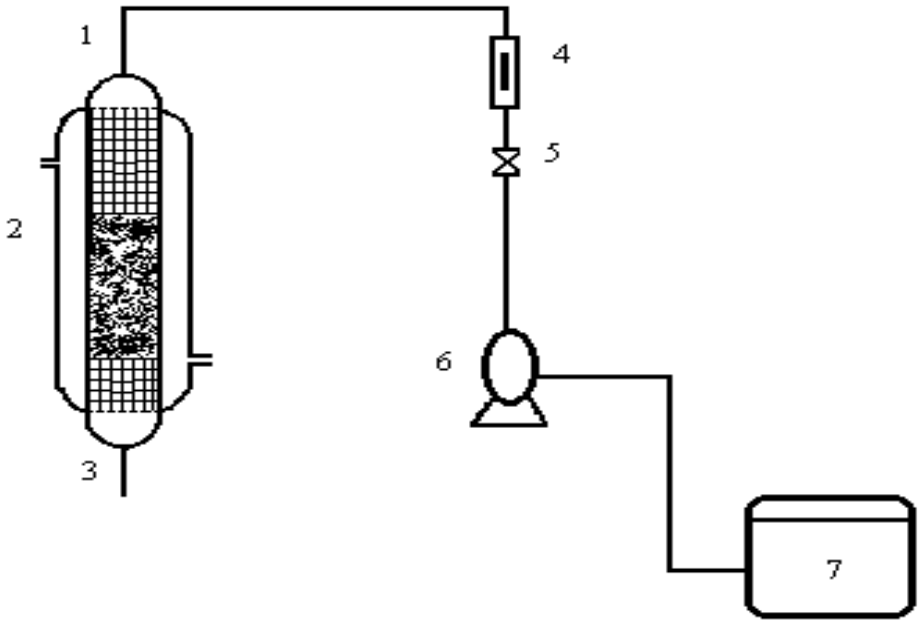
MATERIALS AND METHODS

A fixed-bed adsorber was employed in experiments (Figure 1). A pyrex-glass column was packed with spherical glass beads. Natural sepiolite was placed to the middle part of the bed. It is a clay mineral, which consists of mainly magnesium and silicon oxides. Turkish sepiolites can be divided into two classes as white and beige sepiolite. The beige sepiolite used in this study was obtained from Eskisehir-Sivrihisar area of Turkey. It was crushed in a ball mill and sieved. The portion whose size was higher than 3.15 mm was used as an adsorbent in experiments.

Before starting each experiment, distilled water was passed through the bed until Cr(VI) ions were not observed at the effluent. Cr(VI) solution in three different concentrations and the industrial wastewater from a plating plant were separately fed to the top of the column. Stock solutions of chromium were prepared (5, 10, and 15 ppm of sodium chromate in distilled water). All chemicals used were of analytical reagent grade.

The solutions were passed through the column at a constant flow rate over each experiment. Flow was measured with a rotameter precalibrated by timing the discharge of measured volumes. The temperature was maintained at a constant value by means of external heating, in which the hot water taken from a thermostatical water bath was circulated in a jacket surrounding the column.

FIGURE 1 - Experimental Set-up



1-Liquid inlet 2-Packed bed 3-Liquid outlet 4-Rotameter 5-Valve 6-Pump 7-Reservoir

TABLE 1 – Experimental Conditions

Column Diameter, cm	4.5
Column Height, cm	36
Inert Packing	Spherical Glass
Height of Inert Packing (Top-Bottom), cm	12-12
Adsorbent	Beige Sepiolite
Height of Adsorbent, cm	12
Amount of Adsorbent, g	200
Porosity of Adsorbent, %	75
Size of Adsorbent (min), cm	3.15
Solution Exit Temperature, ^{o}C	15, 30, 45
Flow Rate, mL/s	0.56, 1.11, 2.00
Chromium Concentration in Synthetic Solution, ppm	5, 10, 15
pH of Synthetic Solution	4, 9
Chromium Concentration in Industrial Wastewater, ppm	17
pH of Industrial Wastewater	2

FIGURE 2 - An Example Curve for Concentration Distribution

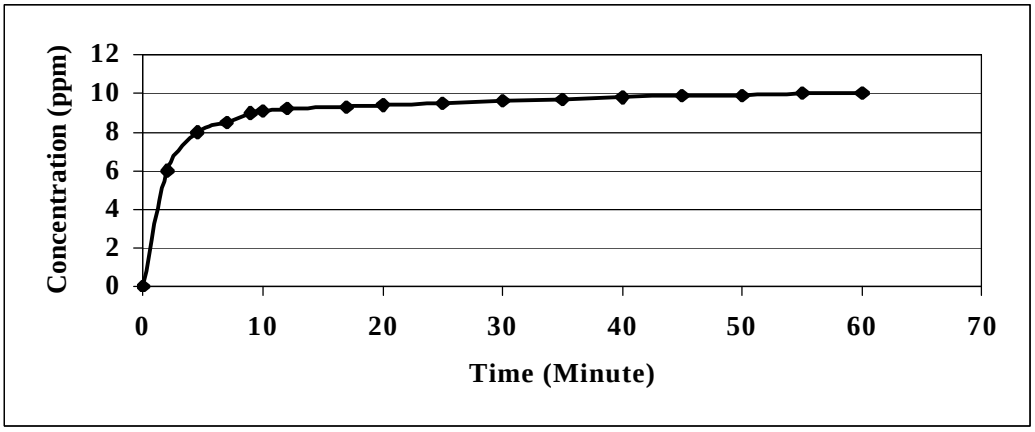


FIGURE 3 - Effects of Liquid Flow Rate and Temperature on the Adsorption Yield (C_o=5 ppm, pH=4)

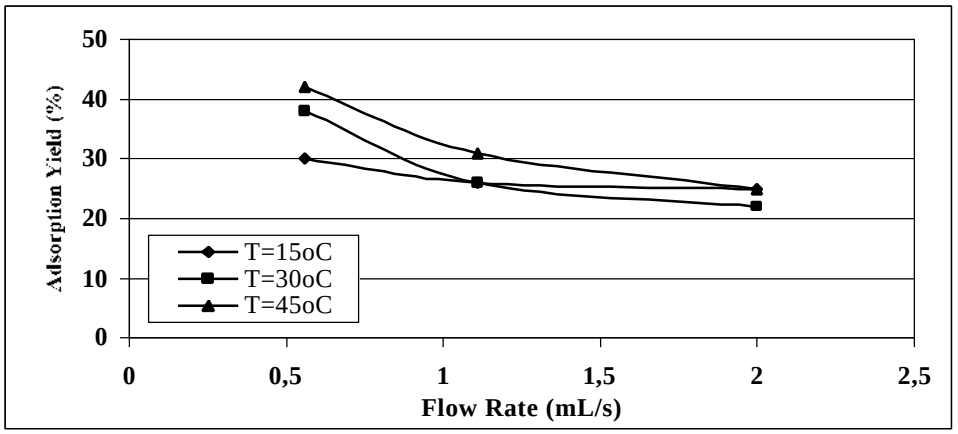
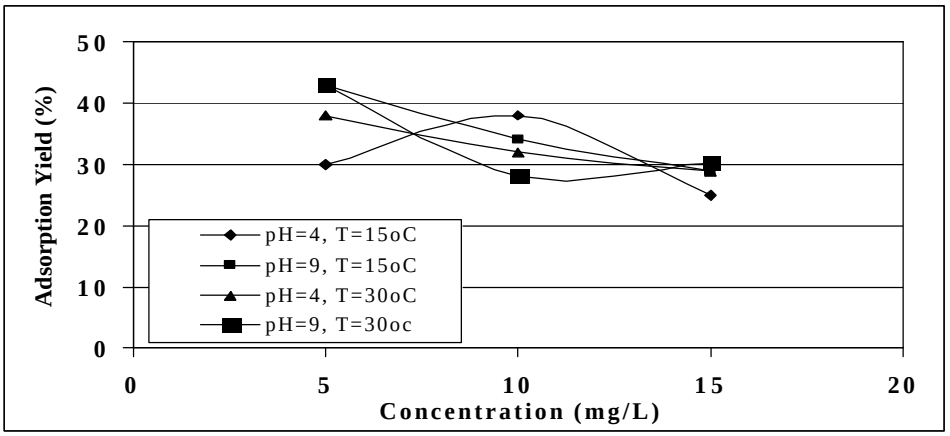


FIGURE 4 - Effects of pH and Concentration on the Adsorption Yield (u_L=0.56 mL/s)



When the solution reached the column outlet, the samples from the effluent were collected in short intervals and tested for chromium. For this purpose, they were reacted with an excess amount of $(NH_4)_2Fe(SO_4)_2$ in an acidic medium. The remaining Fe^{2+} (unreacted with CrO_4^{2-}) was back titrated with $KMnO_4$ [11]. The reaction equations are following :

$$CrO_4^{2-} + 3\ Fe^{2+} + 8\ H_3O^+ \rightarrow Cr^{3+} + 3\ Fe^{3+} + 12\ H_2O$$

$$MnO_4^- + 5\ Fe^{2+} + 8\ H_3O^+ \rightarrow Mn^{2+} + 5\ Fe^{3+} + 12\ H_2O$$

Analysis of the industrial wastewater was performed by an atomic absorption spectrometric method [12]. In this way, distribution curves were obtained as concentration versus time, and adsorption capacities were calculated by using these curves [13]. The experimental conditions are presented in Table 1.

RESULTS AND DISCUSSION

In order to calculate the adsorption yield, concentration distribution curves were used (Figure 2). The area above the distribution curve, which represents the amount of chromium adsorbed in the column, was calculated by subtracting the area under the distribution curve that is evaluated either by integration or using numerical techniques, from the total area of the rectangle that consists of the areas above and below the distribution curve. The adsorption yield was then evaluated from the ratio of the amount of chromium adsorbed in the column to the total amount of chromium in the feed stream [14].

The influence of flow rate on the yield is represented in Figure 3. The yield was found to decrease with an increase in the liquid flow rate. The highest yield (about 43 %) observed in this study was achieved at low values (0.56 mL/s) of liquid flow rate. This result is attributed to the shortness of contact time between solid and liquid phases with increase in liquid flow rate.

As seen from Figure 3, the influence of temperature on the yield is not apparent. A decrease in yield may be expected because physical adsorption decreases with increasing temperature [15]. The adsorption efficiency, however, increases because ionic mobility and diffusion coefficient increase depending on increasing temperature. As a result of these adverse effects, a general perspective for temperature effect can not be drawn at the end of this study performed in a wide range of operating conditions.

The relation between the initial pH of the solution and adsorption yield is shown in Figure 4. The results show that an increase in pH resulted in increase on the yield at low and high values of concentrations. This effect is clearly seen at the region of low concentration. An adverse effect was, however, observed at the region of middle concentration and, the yield decreased with an increase in pH. Similar results were obtained in all of this study with the exception of a few experiments. This changing effect of pH may be due to the competing of chromate ions with the hydroxide ions for the adsorption on active sites of the sorbent [16].

As seen from Figure 4, the influence of concentration on the yield is not also linear. In some cases, the yield continuously decreased with increasing concentration. However, it sometimes tended to reach a maximum or minimum value. This result may be attributed to competition between chromate and hydroxide ions as mentioned above. However, this case may also be due to the change of contact efficiency between solid and liquid phases depending on flow properties in the column [17].

All of experimental results mentioned above were obtained with a laboratory made solution of sodium chromate under different operating conditions for the adsorption. Finally, the experiment was repeated by using an industrial wastewater at the liquid flow rate of 0.56 mL/s, where the highest yield was obtained, and a yield of 31 % was obtained. This value is lower than that obtained with the lab-made solution. This decrease is probably resulted from the differences in solution properties.

In this study, the applicability of natural sepiolite for adsorption of chromium (VI) was investigated and the experiments were carried out at a continuously operating system. Despite these apparent disadvantages compared to batch studies on activated packings, a yield of 43 % has been reached at low liquid flow rates. Further tests, including physical and chemical optimization of the column and testing the activated adsorbent with actual wastewaters in a wide range, are needed to evaluate and develop the adsorption capacity of sepiolite.

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DETERMINING THE MAXIMUM AIR POLLUTANT CONCENTRATIONS FOR PLUME TRAPPING CONDITIONS

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SUMMARY

This note discusses the importance of the existence of a more stable layer above the plume in estimating the maximum air pollutant concentrations from point sources. The Gaussian plume model generally used to determine the critical concentrations is frequently not applicable during plume trapping conditions. In this paper a simple method based on the atmospheric diffusion equation is presented for describing the effect of mixing height on the dispersion of air pollutants and for determining the characteristics of maximum air concentrations during plume trapping conditions.

KEYWORDS: Dispersion model, maximum concentration, plume trapping, air pollution control.

INTRODUCTION

As discussed by Turner,[1] the fact that atmospheric pollutant concentration depends on both downwind distance and wind speed suggests that one may find simultaneously the critical distance and wind speed, at which the highest possible ground-level concentration may occur, the so-called critical concentration. [2-5]

The most important output of a diffusion model is the maximum concentration, which is related to the air pollution episode concentrations. [3] However, it is often desirable to determine the hypothetical worst-case meteorology that causes a maximum calculated concentration in dispersion prediction and to locate the receptor where the maximum occurs. [6-10]

When the atmospheric diffusion process is described by the usual Gaussian plume equation, incorporating reflection of the material at the ground to insure conservationtion of mass, the downwind air concentration C(x,y,z) from a continuous, elevated point source located at the point (0,0,h), is given by, [3]

$$C(x,y,z) = Q(x)[G_1(x,z) + G_2(x,z) + G_3(x,z)]Y(x,y) \quad \textbf{(1)}$$

with $Q(x) = q/(2\pi u s_y s_z)$, $Y(x,y) = \exp[-0.5(y/s_y)^2]$

$$G_1(x,z) = \exp\{-0.5[(z - H)/s_z]^2\}$$

$$G_2(x,z) = \exp\{-0.5[(z + H)/s_z]^2\}$$

and $G_3(x,z) = 0$, where, q is the source release rate (gs^{-1}), H is the effective stack height (m) taken to be the sum of the actual stack height (h) and the plume rise (? h),[11] u is the mean wind speed representative of the diffusing layer (ms^{-1}), and the diffusion parameters, s_y and s_z, are, respectively, the horizontal and vertical standard deviations (m) of the assumed Gaussian plume.

This equation estimates the concentration at the receptor located at x downwind, y crosswind, and at a height z above the ground, that results from an emission that has an effective stack height H. Physically, the wind speed in the Gaussian plume equation should represent an average between the ground surface and the level of the plume centerline. [3] However, for practical purposes, the wind speed profile is usually represented as a function of height by a power law. [12-15]

The characteristics of maximum ground-level air concentrations from a point source are determined by maximizing the Gaussian plume equation evaluated along the plume centerline. In the special case, in which the plume has reached its final height (i.e., for the level plume), by differentiating Equation (1) with respect to the downwind distance (x) and setting the resulting relation equal to zero, finally yields,

$$J_o S_z - W_o(S_y + S_z) = 0 \quad \textbf{(2)}$$

where $ds_y/dx = s_y S_y$, $ds_z/dx = s_z S_z$, $J_o = (H/s_z)^2$ and $W_o = 1$. Then Equation (2) can be solved by numerical iteration for x to obtain the location of the maximum ground-level concentration for any given wind speed.

Many investigators[3-10] have obtained the worst-case results for a Gaussian type of plume, when the dispersion parameters, s_y and s_z, can be expressed as functions of downwind distance from a stack and atmospheric stability category. [1] However, such an approach produces unreliable results when the plume is trapped between the ground surface and a stable layer aloft. Such a stable layer frequently caps the mixing height. The purpose of this study is to estimate the maximum air pollutant concentration for plume trapping conditions.[1]

MATERIALS AND METHODS

A difficulty is encountered in determining a reasonable value for the location of the maximum ground-level concentration, for plume trapping conditions. [1] Bierly and Hewson[16] and Turner[1] have suggested the use of Equation (1) that accounts for the multiple eddy reflections from both the ground and the stable layer, where z_i is the height of the stable layer and the parameter, $G_3(x,z)$, can be expressed as:

$$G_3(x,z) = \sum_{N=1}^{N=J} [T_1(x,z,N) + T_2(x,z,N) + T_3(x,z,N) + T_4(x,z,N) \quad \textbf{(3)}$$

where

$T_1(x,z,N) = \exp\{-0.5[(z - H - 2Nz_i)/s_z]^2\}$

$T_2(x,z,N) = \exp\{-0.5[(z + H - 2Nz_i)/s_z]^2\}$

$T_3(x,z,N) = \exp\{-0.5[(z - H + 2Nz_i)/s_z]^2\}$

$T_4(x,z,N) = \exp\{-0.5[(z + H + 2Nz_i)/s_z]^2\}$

and J =3 or 4 is sufficient to include the reflections of any significance. This equation is evaluated for receptors that are close to the source.[1]

The characteristics of maximum concentrations are determined by maximizing the function (1) subject to the constraint conditions (3). Then the location of the maximum ground-level air concentration for plume trapping conditions can be calculated by solving the nonlinear equation:

$$J_I S_z - W_I(S_y + S_z) = 0 \quad \textbf{(4)}$$

where

$J_I = (I + I_1 + I_2)\,(H/s_z)^2$

$W_I = I + I_A + I_B$

$s_y s_z I = \exp[-0.5(H/s_z)^2]$

$$s_y s_z I_A = \sum_{N=1}^{N=J} \exp\{-0.5[(H - 2Nz_i)/s_z]^2\}$$

$$s_y s_z I_B = \sum_{N=1}^{N=J} \exp\{-0.5[(H + 2Nz_i)/s_z]^2\}$$

$$s_y s_z I_1 = \sum_{N=1}^{N=J} \{[(H - 2Nz_i)/H]^2 \exp\{-0.5[(H - 2Nz_i)/s_z]^2\}\}$$

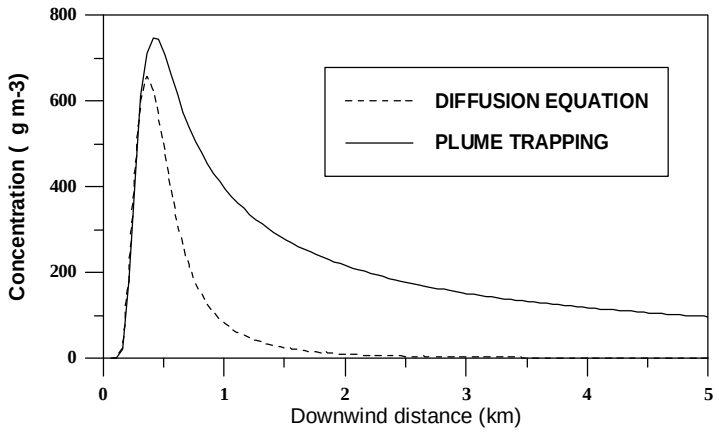
$$s_y s_z I_2 = \sum_{N=1}^{N=J} \{[(H + 2Nz_i)/H]^2 \exp\{-0.5[(H + 2Nz_i)/s_z]^2\}\}$$

RESULTS AND DISCUSSION

A simple numerical algorithm[10] suitable for execution on a small personal computer was used to calculate the maximum ground-level concentration for plume trapping conditions. To illustrate a simple calculation, the suggested methodology was used, where H = 80 m, u(h) = 4 m s^{-1}, q = 100 g s^{-1} and z_i = 120 m, for a Pasquill stability class. The widely used Briggs plume rise equations, [11] and the Pasquill-Gifford dispersion parameters s_y and s_z recommended by Turner[1] are selected for this stack-problem. [17,18]

Based on Equations (1) and (3), Figure 1 presents the ground-level concentrations as a function of the downwind distance.

FIGURE 1
The ground-level concentrations as a function of the downwind distance, for A Pasquill stability class.



The curve constructed from the above relationships with an inversion layer at a height of 120 m is shown as solid line, and the curve computed with the Gaussian diffusion equation is shown as dashed line. Solving the nonlinear Equation (2) for the Gaussian plume model, the maximum concentration C_{MAX} is calculated to be 657 μg m^{-3} at a distance x_{MAX} of 0.365 Km. Also, solving the nonlinear Equation (4) for plume trapping conditions, the maximum concentration C_{MAX} is calculated to be 750 μg m^{-3} at a distance x_{MAX} of 0.434 Km. Therefore, it appears possible that the classical atmospheric diffusion equation underestimates the maximum concentrations.

Figure 1 shows the importance of the existence of a more stable layer above the plume in estimating the maximum air concentrations. Thus, the mixing height can significantly affect the analysis of the characteristics of maximum concentrations, when modeling the dispersion of air pollutants from point sources.

It is obvious then, in view of Figure 1 and Equations (1)-(4), that the Gaussian plume model used to determine the worst-case maximum air concentrations (e.g., the critical concentrations) is generally not applicable during plume trapping conditions. It is for these reasons that the ability to determine realistic values for z_i to characterize the presence of a more stable layer above the plume is very important, in estimating the maximum air concentrations from point sources. Consequently, by the incorporation of the suggested relationship (4) into the set of equations used in the Gaussian plume models, it may be possible to represent properly the effect of mixing height on the dispersion of air pollutants and to determine the characteristics of maximum air concentrations during plume trapping conditions.

In conclusion, because of the numerical simplicity of the methodology adopted here, this search algorithm should become useful for regulatory applications.[19-22] It may be used in applications in design of stacks, air quality management, and air pollution episode control planning.

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PERSONAL EXPOSURE TO ASBESTOS DURING REMOVAL OF ASBESTOS-CONTAINING WINDOW CAULKING AND FLOOR TILE/PIPE INSULATION

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SUMMARY

Results suggest that exposure during asbestos abatement of windows and floor tile/pipe insulation are generally below the occupational exposure standard or level (Permissible Exposure Limit – PEL). A graphic evaluation for probability of overexposure suggests it is unlikely that exposure will exceed the PEL when performing asbestos abatement of window caulking. Depending on the means employed, some risk of overexposure may exist for floor tile/pipe insulation as reported in this study. However, when methodology of sample analysis and distribution of airborne asbestos are considered, likelihood of overexposure for those performing abatement of floor tile/pipe insulation becomes low. These findings do not support current regulatory requirements in the United States for abatement of window caulking and floor tile. However, previous investigations have suggested a much higher exposure concentration for those removing insulation. Issues of legislating science in the asbestos abatement industry are discussed.

KEYWORDS: asbestos abatement, asbestos-containing materials, management of asbestos, occupational exposure, personal protection, legislating science

INTRODUCTION

Asbestos-containing material (ACM) is a common building component in the United States and is currently a frequently used building material in many countries of the world (1,2). Abatement/removal of windows that have ACM, mostly in the form of caulking, is a regulated activity in the United States (3). When ACM exists as a component or part of a building material, most specifications require precautions for protection of personnel, environment and public health. Previous publications suggest that exposure below the occupational exposure limit (OEL) occur when windows having asbestos-containing caulking are removed (3), and both low and high concentrations have been reported for asbestos-containing floor tile and asbestos-containing pipe insulation (2-6).

This study provides personnel (occupational) exposure data on airborne asbestos during removal of window caulking while windows remained in place and abatement of floor tile and pipe insulation. These data provide information on historical exposure during various abatement practices. Issues associated with exposure to airborne asbestos are presented and discussed.

MATERIALS AND METHODS

Personal air samples (occupational exposure) were collected from the breathing zone (2) of workers during asbestos abatement of asbestos-containing window caulking and floor tile/pipe insulation. Exposure was measured as a time-weighted average (TWA) (eight hour weighted average) or excursion limit (EL) (7). Window caulking was reported to be 2-5% asbestos, floor tile 2-5% asbestos and pipe insulation 25% asbestos, all chrysotile. These materials are defined as ACM according to United States Occupational Safety and Health Administration (OSHA) regulations (7). This work was performed in a public building located in the mid-Atlantic region of the US in 2000 over approximately a twenty-day period. Removal consisted of caulking from approximately fifty windows, 20,000 square feet of floor tiles and 2,000 linear feet of insulation.

Removal of caulking was performed by scraping material off window edges using a putty knife or similar device. All caulking was on the outside of the building. Wetting was minimal during this removal. After caulking was removed, windows and frames were physically removed (3). Pipe insulation and floor tiles were removed after establishing a containment barrier system. Procedures employed followed the general OSHA requirements for ACM removal (4,6,7). Removal of floor tiles and pipe insulation was conducted at the same time. Mastic associated with floor tile was not an ACM. Wetting was employed for pipe insulation, but was limited in application for removal of floor tiles.

Samples were collected on 25-mm diameter electrically conductive extension cowl cassettes with mixed cellulose ester filter membrane using a calibrated low flow personal pump (2,3). The air sample pump was operated at a nominal flow rate of 2 lpm (2). Analysis was performed by the NIOSH 7400 method, which employs polarized light microscopy (PCM) (7). Blank cassettes were analyzed as controls and no fibers were reported.

Exposure was reported by summary statistics (arithmetic mean – AM, geometric mean - GM, standard deviation – SD, geometric standard deviation – GSD, and range). Samples were calculated at the value reported by the laboratory (3). Some samples were reported as < 0.01 f/cc and these values were included in calculations at 0.01 f/cc. Outliers were determined using the Grubbs test.

Probability (confidence coefficient) of overexposure for at least 5% of employees' above the occupational exposure limit (OSHA Permissible Exposure Limit – PEL or OEL) was determined using a graph method (8). Standard of comparison for this graph method was the OSHA PEL (0.1 f/cc-TWA) or EL (1.0 f/cc-30 minutes/day) (7). Statistical calculations were performed at the 95% level (3).

RESULTS AND DISCUSSION

Exposure to airborne asbestos fibers during abatement of window caulking and floor tile/pipe insulation is shown in the table. Only one value in the category floor tile/ pipe insulation exceeded the OSHA PEL. No EL sample exceeded the exposure standard. Probability of overexposure, using AM, for OSHA PEL samples for window caulking was less than 20% and greater than 80% for pipe insulation/floor tile. The elevated probability for pipe insulation/floor tile is a result of the GSD. If the GSD is reduced to 2.5 a probability of about 40% results. Examination of these data in regard to means (AM and GM) and range, neither exposure scenario from window caulking or floor tile/pipe insulation appear to have a high likelihood of exceeding the OSHA PEL.

Due to the small sample sizes and concentrations near or below 0.01 f/cc analysis of distribution was not performed (2). Previous investigations of distribution for airborne asbestos have suggested non-normality and a fit that is best represented by a logarithmic form (2). If the GM is used in determining probability of overexposure for window caulking and floor tile/pipe insulation rather than AM, a confidence coefficient is reduced to less than 5% and around 50%, respectively. Since PCM counts all fibers, not only asbestos fibers, a likelihood of even a lower probability of overexposure exists (2). With the "designated time period" of exposure (40 hours per week, 50 weeks a year, for 45 years) in determining risk associated with the OSHA PEL as compared to these exposure results, it is unlikely that acceptable risk as identified by OSHA will be exceeded (9). These data in total suggest that likelihood of workers on average exceeding the occupational exposure standards (OEL/PEL) is low and potential for "risk" of occupational diseases associated with ACM above that defined by OSHA is unlikely (2).

TABLE 1 - Summary statistics for personal sample concentrations, in f/cc, TWA or EL, during abatement of floor tile and mastic from different schools.

Location of Samples	Number	AM	GM	SD	GSD	Range
Window Caulking	9	0.008	0.006	0.004	2.3	<0.002-0.013
Window Caulking	4 (EL)	0.05	0.03	0.04	5.6	<0.004-0.08
FT/Pipe Insul*	10	0.024	0.01	0.04	3.8	<0.002-0.13
FT/Pipe Insul*	1 (EL)	0.005	ND	ND	ND	ND

*exposure to floor tile (FT) and pipe insulation was collected at the same time because removal was performed at or about the same time and same work location, ND – not determined, EL – excursion limit. One value for pipe insul/FT (0.13 f/cc-TWA) was an outlier at 95% and 99%.

The method recognized by OSHA for analyzing personal air samples is PCM (7). However, at least one study (10) has suggested that analysis for personal samples be performed by transmission electron microscopy (TEM). This study (10) reported exposure levels (structures) associated with removal of floor tile above the PEL. Criticism of this study is that the number of samples collected was limited and employed area sampling rather than personal measurements (2). TEM includes structures or fibers that are smaller than 5 um in length, where as PCM counts only fibers 5 um or larger. The central question at issue is the importance of structures that are 5 um or smaller. One report suggests (11) that these smaller fibers/structures are of less importance for health outcome and it is implied that the most appropriate measure of exposure to airborne asbestos be by PCM analysis. However, caution must be exercised in comparison of the two methods, PCM and TEM, since they primarily measure two different populations of airborne materials. Personal sampling has also been suggested to exhibit higher concentrations than area samples (2) and TEM higher concentrations of airborne fibers (structures) as compared to PCM (10). However, these relationships are not universally observed (12).

Many specifications require use of respiratory protection without regard to historical exposure levels and potential deficits as a result of these devices (2). Exposure concentrations reported in this investigation do not support employment of respiratory protection (2). This is an example of respiratory protection potentially having more harm than good if implemented (2).

These data, along with previous studies (2,3,5), suggest health risks from abatement of floor tile and window caulking is low. Other investigations have reported elevated exposure during removal of insulation (4,6) and results reported in this investigation appear to be different. Even though one sample (0.13 f/cc-TWA) did exceed the OSHA PEL, it was an outlier and it is likely that this result is actually below the exposure standard based sample analysis methodology (9). As suggested (2), the most appropriate indicator of exposure is summary data and not individual measurements. Some have proposed that if one value exceeds the OEL, all protective practices should be based on this criterion (13). This has become commonly known as the "one strike and your out" rule. Since asbestos results in chronic disease, a one-strike rule would not be related to prediction, based on epidemiological information, of future occupational disease (2). Since the primary purpose of occupational sampling is for prevention of occupational disease, AM or GM is suggested to be a better predictor value. Applying a one-strike criterion, there is no identification of protection requirements, exposure groups or limitations of conditions with the sample. Such criterion also does not provide any variation for interpreting data and identifies only a single fixed value.

These data demonstrate that regulatory requirements for asbestos abatement should be based on anticipated exposure and not on a blanket rule for all ACM. Previous studies (2) have supported this concept that some of the asbestos regulations are nothing more than a legislation of science. Further, these regulations would not survive the scrutiny of court action if they were proposed solely as "expert" statements or information. Criteria instituted by courts in the United States (Daubert v. Merrell Dow Pharmaceuticals, Inc., 527 U.S. 579, 1993; Kumho Tire Co. v. Carmichael, 526 U.S. 137, 1999) require evidence to stand the rigor of reliability and acceptable methodological practice (14). This would not be the situation for stringent regulatory controls associated with abatement of ACM for floor tile and window caulking.

Regulatory criteria should be based on the type of materials, practice and anticipated exposure associated with these activities. Results presented here and in other studies (2,3,5) suggest that low exposure is the more likely outcome from abatement of floor tile and caulking. Employment of "strict" control practices for removal of floor tile and caulking ACM is suggested to be more based on legislating of science than scientific evidence. Importance of "scientifically" evaluating respiratory protection in comparison to competing risks is also suggested (2). Additional studies are warranted for abatement of different types of building materials and practices employed.

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The Preliminary Announcement

MEETING OBJECTIVES

The Brno Meeting will have an unofficial motto: „The environmental chemistry and ecotoxicology of environmental chemicals is a fascinating, multidisciplinary, active and energetic area of present science". Our common interest will help to the development of environmental chemistry and ecotoxicology in the countries of Central and Eastern Europe and New Independent States. For these reasons all experts round the Globe are invited and welcomed.

MAIN TOPICS

I. Advances and Trends in Environmental Chemistry

Session Chairs: Prof. Dr. Kevin C. Jones,
Dr. Michael Mc Lachlan, Prof. Dr. Ivan Holoubek

More detailed, sophisticated and refined studies on the environmental inter-phase exchange; measurements of the physicochemical properties of environmental chemicals at different environmental conditions as a base for validation of environmental models; studies on the form, bioavailability/sequestration and remediation of pollutants in soils, sediments and ground waters; study of environmental fate traditional and new type of environmental pollutants; global scale inventories, budgets and models; measurements vs, modelling – advantages, disadvantages, limitation.

II. Advances and Trends in Environmental Analytical Chemistry

Session Chairs: Prof. Dr. Harun Parlar,
Dr. Anton Kocan, Assoc. Prof. Dr. Aleš Hrdlicka

Further improvement of analytical methods to identify and quantify new or hitherto unknown compounds, notably metabolites, stereoisomers and more polar types; development of new methods or new combination of methods for determination of environmental pollutants and study of environmental processes;

III. Advances and Trends in Ecotoxicology and Ecological Risk Assessment

Session Chairs: Prof. Dr. Paul Vasseur,
Prof. Dr. Colin Janssen, Dr. Miroslav Machala

Studies to further elucidate and quantify the subtle effects of environmental pollutants on human and wildlife, mechanisms of harmful effects including molecular modelling of biodegradation, transformation and toxicity mechanisms; study of phytotoxic effects of chemicals and

their effects on soil microbial populations and soil fauna; bioavailability of chemicals in terrestrial and aquatic ecosystems; the advances in the test of toxicity; effects of real environmental mixtures;

IV. Environmental Safety; Environmental Policy, Regulations, Enforcement

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MEETING PROGRAMME

The 7th Regional SECOTOX Meeting will take place in the Hotel Santon, Brno, Czech Republic, September 16-18, 2002. Platform and poster sessions will provide information on key topics from environmental chemistry and ecotoxicology. Meeting exhibition will be suitable platform for contacts with industry, publishers, consultants and will inform about new publications, software, products, services in the field of environmental chemistry, ecotoxicology and risk analysis. The abstracts of all platform and poster presentations will be published in Symposium Proceedings and selected contributions will be published in the special issue of the Fresenius Environmental Bulletin.

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The Symposium is held in Brno, metropolis of the South Moravia. Brno is the second largest city in the Czech Republic with population of more than 400 thousand and it is the administrative, cultural and economic centre of the region. The official language of the Symposium and the Proceedings is English.

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