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CHANGES IN OPERATIONAL FRACTIONS OF TRACE METALS IN TWO SOILS DURING TWO-YEARS OF REACTION TIME FOLLOWING SEWAGE SLUDGE TREATMENT

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Two soils amended with a sewage sludge were incubated at two ambient temperatures (15°C and 25°C) under greenhouse conditions for two years. Representative samples of the sewage sludge and periodically collected soil samples were subjected to an operationally-defined sequential extraction procedure. This procedure fractionated metals in three broad fractions through the use of CH₃COOH, NH₂OH·HCl and H₂O₂ in sequence. In the sludge, the greatest percentages (63–98%) of the five metals studied (Cd, Cu, Ni, Pb and Zn) were extracted by H₂O₂/NH₄OAc. The application of sludge to soils substantially increased the contents of metals in all the three fractions. Cadmium, Ni and Zn in the sludge-amended soils were predominantly in the CH₃COOH extractable fraction while Cu and Pb were extracted primarily by H₂O₂/NH₄OAc. The contents of Cd, Cu, Pb and Zn extractable by CH₃COOH increased in samples collected over a period of two years while that of Ni did not show any significant change. Metal concentrations extractable by NH₂OH·HCl remained more or less constant over the residual period. Metal contents extracted in H₂O₂/NH₄OAc decreased considerably during the first year before stabilising for the rest of the study period. The different incubation temperatures had no obvious effect on CH₃COOH and NH₂OH·HCl extractable metal fractions. However, the observed decline in metal contents, over time, extracted by H₂O₂/NH₄OAc was faster in samples collected from the warm environment (25°C) than those from cooler conditions (15°C).

The observed trend of increasing metal concentrations in CH₃COOH- extractable fraction and decrease in the H₂O₂/NH₄OAc fraction coincided with decreasing soil organic matter status due to microbial oxidation of sludge-added organic matter. The importance of trace metal fractionation and transformation between fractions are discussed.

KEY WORDS: Metal fractions, sludge treatment, reaction time, temperature.

INTRODUCTION

The total concentration of a metal in a soil may not provide the best indication of its

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bioavailability. Several workers have found that the total content does not correlate with its availability to crop plants and does not show how the metal is bound in the soil. On the other hand, speciation techniques fractionate trace metals in forms of different solubilities and mobilities. Therefore, information about the forms of trace elements/pollutants is considered potentially valuable in predicting their availability to plants, their movement in the soil profile and transformation between different forms in soils^{1,2,3}. Trace metals in soils may be present in several different physico-chemical forms: (i) as simple or complexed ions in solution; (ii) as exchangeable ions; (iii) associated with soil organic fraction; (iv) occluded by or coprecipitated with metal oxides, carbonates, or phosphates and other secondary minerals; or (v) as ions in the crystal lattices of primary minerals^{2,3,4}.

There are a number of different sequential extraction procedures in use^{2,4,5,6,7,8,9,10,11}. However, those of Stover *et al.*¹² and Tessier *et al.*¹³ have been the most widely used by environmental scientists. Tessier *et al.*¹³ extracted trace metals in sediment samples into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and residual. The corresponding reagents used to extract these forms were: 1M MgCl₂, 1M NaOAc, 0.4M NH₂OH·HCl in 25% (v/v) CH₃COOH (pH~2), 30% H₂O₂ + HNO₃/NH₄OAc, HF/HClO₄. On the other hand, the procedure of Stover *et al.*¹² involving KNO₃, 0.5M KF, 0.1M Na₄P₂O₇, 0.1M EDTA and HNO₃ has been used to fractionate metals into exchangeable, adsorbed, organically bound, carbonate and sulfide forms, respectively.

The main problem in designing an extraction scheme for trace metals is in selecting reagents that are effective and specific in solubilising a given form of the element from the soil. Ordering these reagents into a sequential procedure, however, makes it possible to improve selectivity and avoid, or at least minimise, solubilisation of multiple phases in one extract¹⁴. Salts of strong acids and bases, for example CaCl₂^{4,8}, KNO₃^{7,9,10}, MgCl₂¹³ have been used to extract metals that are exchangeable or weakly adsorbed. It is believed that these salts will not attack silicate or hydrous oxide phases; however, there is a possibility of re-adsorption of released metal cations. On the other hand, salts of weak bases, such as CH₃COONH₄, can affect the pH of the soil with the possible precipitation of metal hydroxides⁴. This precipitation, however, is prevented by the formation of metal acetate complexes. Solutions of weak neutral salts such as CaCl₂¹⁵ and NaNO₃¹⁶ have also been used to extract metals which are weakly adsorbed. For the extraction of metals bound to Fe-Mn oxides (specifically adsorbed), use of NH₂OH·HCl in CH₃COOH or HNO₃ has been shown to be very specific and effective^{6,13,17}. For metals associated with the carbonate fraction of soils, acidified (pH 5) CH₃COONa or a mixture of CH₃COONa and CH₃COOH has been found to dissolve all the carbonates without attacking silicates¹³. Although EDTA can effectively extract carbonate bound metals, it also removes organically bound metals because of the high stability constants of its complexes. Therefore, unless metals associated with the organic fraction of the soil are first extracted, as in the procedures of Stover *et al.*¹² and Miller & McFee¹⁸, an EDTA extraction could overestimate the carbonate-bound metals. The reagents Na₄P₂O₇ and K₄P₂O₇, H₂O₂ or NaOCl were primarily intended for the extraction of organically bound metals but have also been shown to remove metals associated with carbonates and sulfides^{4,12,19}. Therefore, unless sulfide bound metals are first removed, use of these reagents does not provide a well-defined organic phase. This implies that it is not only the selection of an effective reagent for dissolving a specific form of trace metals, but

the sequence of various steps in an extraction procedure which needs to be carefully considered. In a nine-step sequential extraction procedure, 0.5M $\text{Pb}(\text{NO}_3)_2$ was found to be more specific than CH_3COOH in extracting "specifically adsorbed" metals, therefore it was placed before CH_3COOH in the sequence¹⁴. Similarly Chao's $\text{NH}_2\text{OH}\cdot\text{HCl}$ reagent and $\text{K}_4\text{P}_2\text{O}_7$, used for Mn oxide and organically bound metals, respectively, were found to solubilise significantly different amounts of Cu and Mn depending on the sequence, with $\text{K}_2\text{P}_2\text{O}_7$ dissolving more metals when used first.

Elaborate sequential extraction schemes appear to furnish detailed information about the different "states of existence" of an element in soils and sediments. However, as discussed above, given the practical difficulty in isolating a specific form of metals, overlap between the adjacent forms can occur. Thus such a procedure only represents an arbitrary division between different metal forms. For this reason, the scheme proposed by Ure *et al.*²⁰, on behalf of the European Community Bureau of Reference, for the standardisation of speciation procedures was adopted for the comprehensive study described in this paper. This sequential extraction scheme fractionates the solid phase metals into three broad operationally defined fractions as listed below:

1. Acetic acid-soluble
2. Hydroxylammonium hydrochloride-soluble
3. Hydrogen peroxide-soluble

These successive forms represent a progressively decreasing degree of mobility in soils. Metals not solubilised in this three-step sequential extraction procedure are not considered to be of environmental significance. The specific aims of these experiments were to study the influence of sewage sludge application on the defined metal fractions of Cd, Cu, Ni, Pb and Zn and to monitor possible changes in the fractions of metals over a period of two years.

MATERIALS AND METHODS

Soils and treatments

The soil treatments referred to here are described elsewhere²¹. Briefly, bulk samples of two soils were collected from agricultural land in Southern England. One was a sandy soil of the Worlington/Methwold series. The other soil was a sandy loam of the Bursledon series (gleyed brown earth). The prepared soils (air dried <5 mm) were mixed with the appropriate amounts of sludge and allowed to air dry before further grinding and passing through <2 mm nylon sieve and placing in PVC pots. Anaerobically digested sewage sludge, containing ~10% solids, was obtained from a sewage works in England. The soils and sludge were analysed for various physico-chemical properties, using standard procedures²², and are shown in Table 1. The sludge was applied at the rates of 0, 190, and 570 g DW per pot, which was equivalent to 0, 50, and 150 t ha⁻¹ in the field (on an area basis). Each pot contained 4.6 kg air-dried soil-sludge mixture. These will hereafter be referred to as control, low sludge and high sludge treatments. All the treatments were replicated six times. In order to

Table 1 Physico-chemical characteristics of the soils

<i>Parameter</i>	<i>Sandy loam</i>	<i>Breckland sand</i>	<i>Sludge</i>
pH (H ₂ O)	5.40	6.58	5.92 [‡]
Bulk Density [§] , kg m ⁻³	1.18	1.45	0.63
Organic Matter, %	3.61	1.41	—
CaCO ₃ equivalent, %	1.00	0.75	—
CEC, cmol _c kg ⁻¹	18.94	4.75	—
Sand, g kg ⁻¹	586	922	—
Silt, g kg ⁻¹	207	47	—
Clay, g kg ⁻¹	177	5	—
Free Mn, µg g ⁻¹	118	77	—
Reducible Fe, µg g ⁻¹	1200	200	—
Total oxides (%), based on ICP-AES analysis of HF-HClO ₄ extracts			
MnO	0.04	0.03	0.06
Fe ₂ O ₃	2.88	2.63	1.63
Al ₂ O ₃	5.90	2.28	4.62
MgO	0.35	0.08	1.06
CaO	0.35	0.21	9.52
Na ₂ O	0.36	0.16	0.34
K ₂ O	1.01	0.73	0.33
Total Metal (µg g ⁻¹ air dry soil)*			
Zinc	58.16	27.40	1408
Copper	18.25	7.70	1031
Lead	52.10	14.38	706
Nickel	16.11	7.63	259
Cadmium	0.45	0.12	40

§ Bulk density in "loose state"

‡ pH of the dry sewage sludge

* sewage sludge was oven dried for this analysis

investigate the effects of temperature, the soils were kept under two incubation temperatures of $15 \pm 2^\circ\text{C}$ and $25 \pm 3^\circ\text{C}$ at field capacity moisture status for a period of 24 months. The two soils were sampled on days 1, 15, 30, 60, 120, 240, 360, 540 and 720 following the commencement of these studies; a constant sampling depth of soil 0–10 cm was maintained throughout the sampling plan. The samples were air dried at room temperature and ground to pass through a <0.25 mm nylon sieve and then stored in sealed polyethylene bags at $<5^\circ\text{C}$ ready for analysis. In addition to sequential extraction, the periodically taken soil samples were also analysed for pH and organic matter.

Sequential extraction scheme

The sequential extraction scheme used in these experiments fractionates trace metals in three operationally defined fractions²³, and involved the following steps:

Step 1: The weak acid soluble trace metals were extracted by shaking duplicate 2g soil sample of soil (<0.25 mm air dried) with 80 cm³ of 0.11M CH₃COOH (AnalaR grade) for 16 hours in 200-cm³ shaking bottles. The suspensions were separated by centrifuging at 4000

rpm and subsequently filtering through Whatman 542 filter paper into 30-cm³ Sterilin tubes. The filtrates were stored at <5°C until required for analysis.

Step 2: The residue from Step 1, was resuspended in 80 cm³ of 0.10M acidified NH₂OH·HCl (pH 2) (AnalaR grade) and extracted over night (16 hours). The extract was then separated by centrifugation and filtration through the filter paper into the sample bottles.

Step 3: The residue from Step 2, was rinsed with deionised-distilled water and transferred by rinsing with 10 cm³ of 30% H₂O₂ into a 100-cm³ borosilicate glass beaker. The contents were digested at 85 ± 5°C and then taken to low volume on a sand bath; the beakers during the H₂O₂ digestion were kept covered with watch glasses. The digestion was repeated with 10 cm³ of H₂O₂ in order to complete the oxidation of organic matter and sulfides. The moist oxidised residue was then rinsed back into a shaking bottle with 100 cm³ of acidified CH₃COONH₄ (pH 5), extracted by shaking for 16 hours and then separated by centrifugation and filtration into the sample bottles. The addition of CH₃COONH₄ is designed to prevent the re-adsorption of extracted metals on to the oxidised soil residue⁶.

All the equilibrations were carried out at room temperature (22 ± 2°C) unless otherwise stated. The overnight shaking period (16 hours) was chosen for convenience and to give maximum time for the dissolution and desorption of ions in soils. Preliminary experiments were carried out to optimise the volumes of the extracting solutions to be used. The experiments were performed by extracting the metals from a fixed amount (2g) of the in-house reference soil with varying volume of the solutions. The results showed that the optimised volumes of the extractants were the same as the Ure and Griepink protocol used above. The bottles/tubes were weighed before and after each solution was added to determine true extraction volumes. The soil residues were not washed between extractions. The amount of a metal extracted by a given extractant was calculated according to this equation:

$$M_{\text{ex}} = C \cdot M - C^1 \cdot M^1 \quad (1)$$

where M_{ex} is metal extracted ($\mu\text{g g}^{-1}$); C is the metal concentration ($\mu\text{g g}^{-1}$) in the extracting solution; C^1 is the concentration ($\mu\text{g g}^{-1}$) in the preceding step of the sequence; M is the mass of extracting solution (g) and M^1 is the mass of the solution (g) carried over to the present extraction from the preceding one. Equation (1) contains a correction for the amount of metal in the solution entrained in the sample after centrifugation which follows each extraction step. In addition to reagent blank checks, an in-house reference soil was used throughout the sequential extraction procedure. The in-house reference soil, like other soil samples was air-dried before being finely ground to pass through a <0.25 mm sieve and thoroughly homogenised and stored in moisture-free cold conditions (<5°C).

Trace metal analysis

The total contents of trace metals in soils and sludge samples were determined following their digestion in HF and HClO₄¹³. Total metal concentrations were then analysed by flame atomic absorption spectrometry. Total metal contents were determined in samples from only the first sampling. All the filtrates from sequential extraction were first analysed by flame

atomic absorption spectrophotometer (FAAS). For those samples with concentrations below the limit of detection by FAAS, electrothermal atomic absorption spectrophotometry, ETAAS, (Philips PU 9200/PU 9390) was used.

RESULTS

The analysis of variance (ANOVA) for the contents of five trace metals (Cd, Cu, Ni, Pb and Zn) extractable by three reagents (CH_3COOH , $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$) together with the total extractability (i.e. sum of the three forms) were computed using the GENSTAT-5 computer programme²⁴. The effects of various treatments and their interactions were evaluated by the Least Significant Difference Test at $p \leq 0.05$. Table 2 gives the mean concentrations of metals extracted in the samples of the in-house reference soil. The extractability of Cd, Cu and Pb by acetic acid over the nine samplings varied within $\pm 12.37\%$, $\pm 13.87\%$ and $\pm 17.37\%$, respectively. Although a relative standard deviation of $> \pm 10\%$ between the batches seems somewhat high on the precision scale, it was considered acceptable since precision is known to decrease at low concentrations (Table 2). Metal extraction by $\text{NH}_2\text{OH}\cdot\text{HCl}$ in the reference samples was shown to be consistent and within experimental errors (Table 2). However, the contents of Cd extracted by $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ varied by $\pm 14.05\%$ around its mean of $0.26 \mu\text{g g}^{-1}$, which was outside the usual precision limit of $\pm 10\%$. Given the low concentrations of Cd in all the three metal fractions and Pb in the acetic acid-soluble fraction, the observed variability in metal extractions using this procedure appeared to be within experimental errors. This showed that the results obtained in this study are reproducible and the procedure is relatively error free.

Total metal concentrations and recoveries

When the sums of metals extracted from each soil by the three extractants are compared with the total concentrations ($\text{HF}\text{-HClO}_4$ digestion) of the sludge-treated soils, percentage recoveries of metals were Zn 107, Cu 79, Pb 88, Ni 106 and Cd 116. In a Canadian study involving a large number of polluted soils, 100, 80 and 67% recoveries for Cd, Zn and Ni, respectively, were observed in a three-step sequential extraction procedure². On the other hand, in a sequential extraction of Ni, Cu and Cd, the sums obtained were greater than their

Table 2 Metal extraction in the samples of in-house reference soil

<i>Element</i>	<i>CH₃COOH Soluble ($\mu\text{g g}^{-1}$)</i>	<i>NH₂OH·HCl Soluble ($\mu\text{g g}^{-1}$)</i>	<i>H₂O₂/NH₄OAc Soluble ($\mu\text{g g}^{-1}$)</i>
Cadmium	0.22 ± 0.027	0.41 ± 0.044	0.26 ± 0.036
Copper	0.46 ± 0.06	2.04 ± 0.15	7.92 ± 0.45
Nickel	6.69 ± 0.52	6.81 ± 0.50	3.92 ± 0.38
Lead	2.15 ± 0.37	20.77 ± 1.41	11.73 ± 1.04
Zinc	6.78 ± 0.40	10.44 ± 0.56	12.60 ± 1.16

NB: Values are mean \pm SD based on the data from nine samplings

total contents¹⁰, by about 15%. In terms of metal recovery, this three-step sequential extraction scheme appears to be comparable with other such procedures.

Metal distribution in the sewage sludge

Figure 1 shows the distribution of the metals in the extracted fractions. The Cd in the sludge predominated in the $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ soluble fraction (63%), followed by the $\text{NH}_2\text{OH}\cdot\text{HCl}$ (28%) with the least in CH_3COOH extractable fraction (9%). Almost all of extractable Cu (98%) and Pb (96%) in the sludge is associated with $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ fraction, with CH_3COOH and $\text{NH}_2\text{OH}\cdot\text{HCl}$ fractions combined comprising only 2 – 4%. Nickel in the sludge predominated in the $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ fraction (73%), with 17% in CH_3COOH and $\text{NH}_2\text{OH}\cdot\text{HCl}$ extracting only 10%. The distributions of Cd, Ni and Zn fractions in the sludge were somewhat similar. On the other hand, Cu and Pb showed a similar pattern of behaviour which differed from that of the other three elements.

Metal fractions in soils during a two year reaction time

The application of sewage sludge to both soils substantially increased the amounts of metals that were extractable by the three reagents used in the sequential extraction procedure. The rates of sludge application were also clearly reflected in the extraction data (Figures 2–6). Overall, CH_3COOH extracted significantly higher amounts of Cd, Cu, Ni, Pb and Zn from the Breckland sand than from the sandy loam soil. On the other hand, $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ solubilised greater concentrations of all the metals from the sandy loam soil than from the Breckland sand. The extraction data for the individual metals shown in Figures 2–6 represent the combined data for both soils and temperatures.

CADMIUM

The Cd concentrations extracted from the periodically collected samples by CH_3COOH , $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ are presented in Figure 2. As shown in Figure 2a, Cd extractable by CH_3COOH increased significantly with time over successive samplings in both the low sludge and the high sludge treatments. Overall, Cd extracted by $\text{NH}_2\text{OH}\cdot\text{HCl}$ did not tend to change over the residual period of two years (Figure 2b). Cadmium extracted by $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ in both the sludge treatments showed a marked decline over the first 12 months (Figure 2c). For example, Cd in the first sampling was 0.52 and 1.37 $\mu\text{g g}^{-1}$ in the sandy loam soil for low and high sludge treatments, respectively. After a period of two years, the corresponding levels declined to 0.26 and 0.48 $\mu\text{g g}^{-1}$. The total Cd extractability (sum of the three extractable forms) tended to remain constant over the two year study period (Figure 7a). This implies that the observed decreasing trend in $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable Cd over time was compensated by an increasing fraction of CH_3COOH extractable Cd. The order of Cd concentrations in the fractions in the soils before their treatment with the sludge

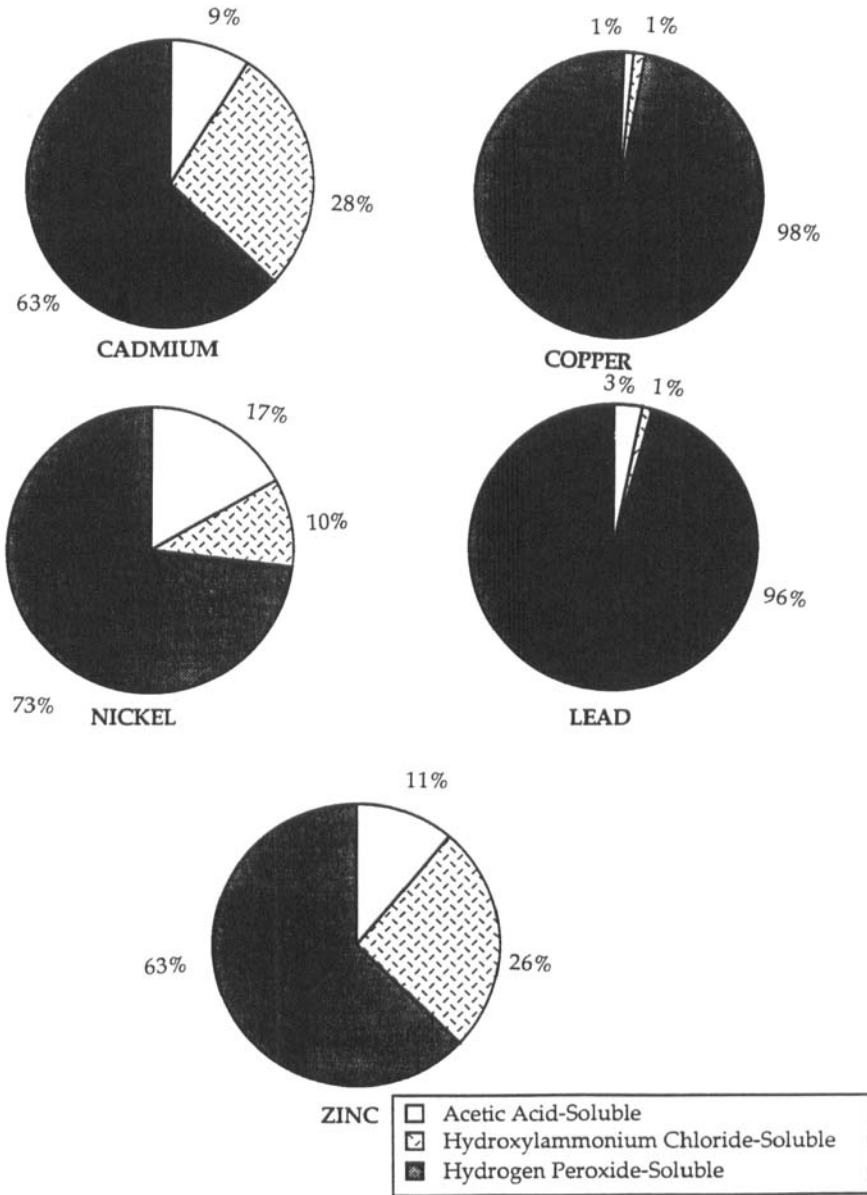


Figure 1 Cadmium distribution among CH_3COOH , $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable fractions in the sludge

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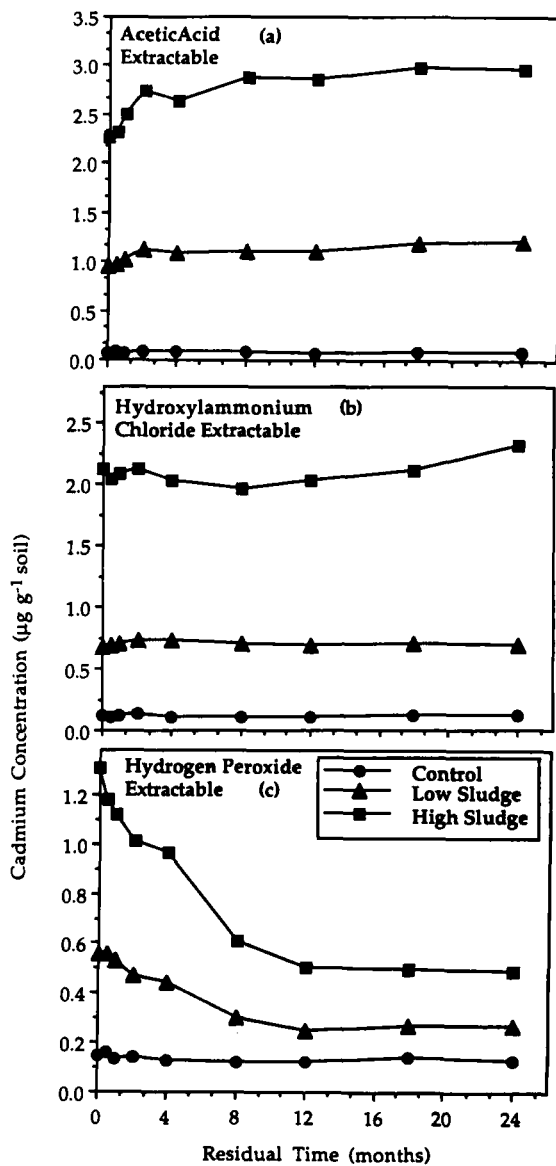


Figure 2 Cadmium distribution among CH_3COOH , $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable fractions, based on data averaged over both soils and temperatures

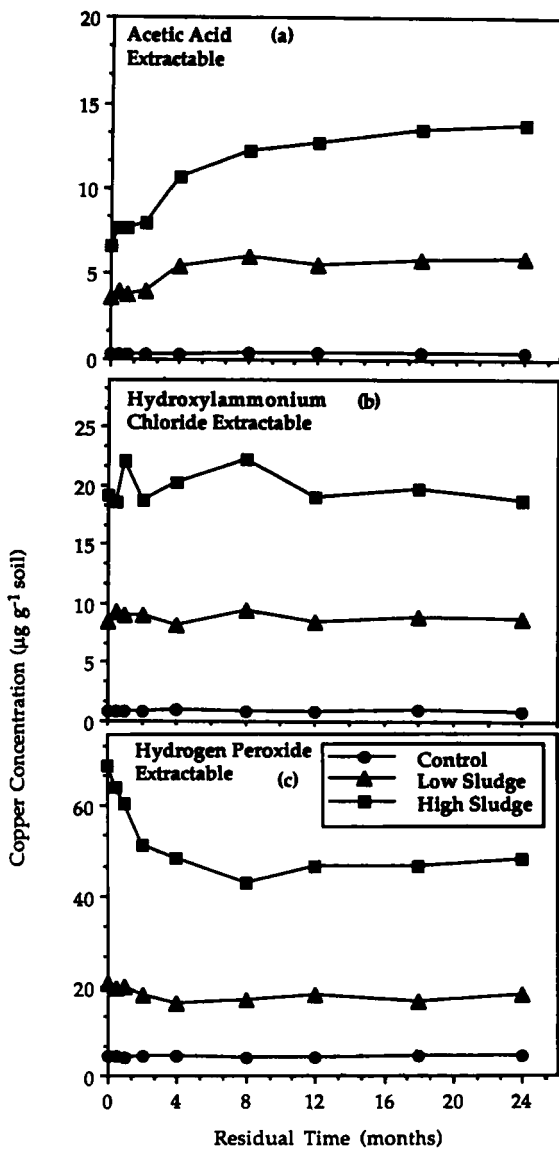


Figure 3 Copper distribution among CH₃COOH, NH₂OH·HCl and H₂O₂/NH₄OAc extractable fractions, based on data averaged over both soils and temperatures.

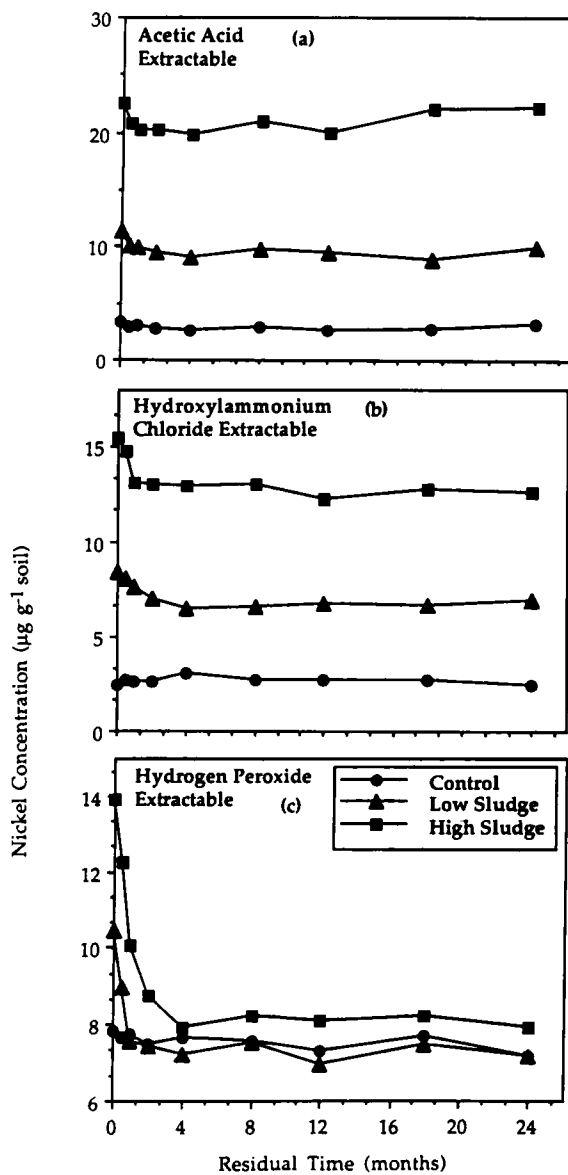


Figure 4 Nickel distribution among CH_3 , $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable fractions, based on data averaged over both soils and temperatures

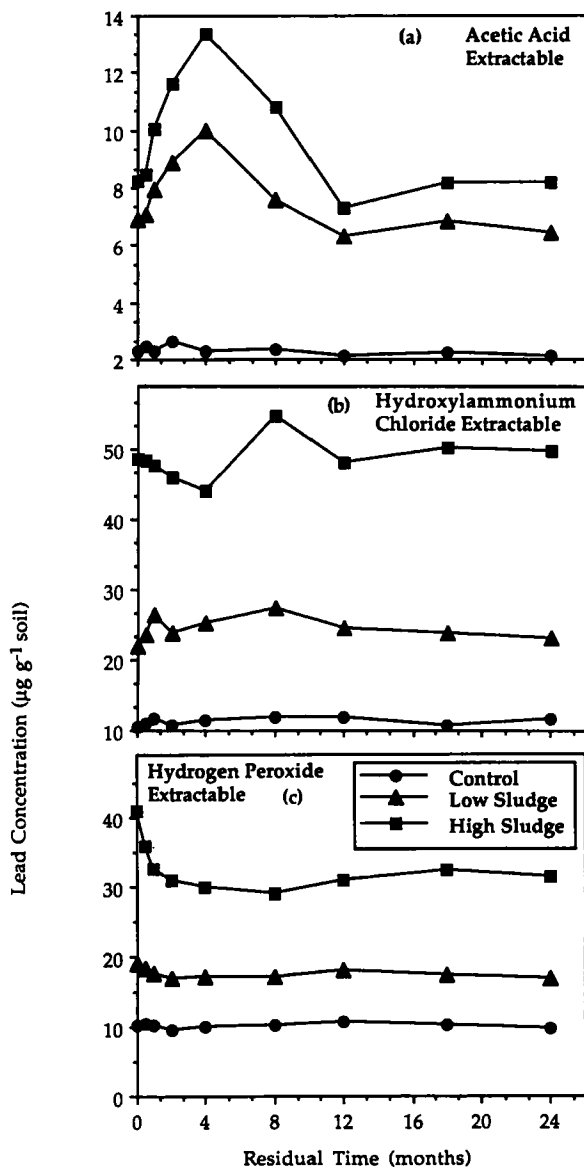


Figure 5 Lead distribution among CH_3COOH , $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable fractions, based on data averaged over both soils and temperatures

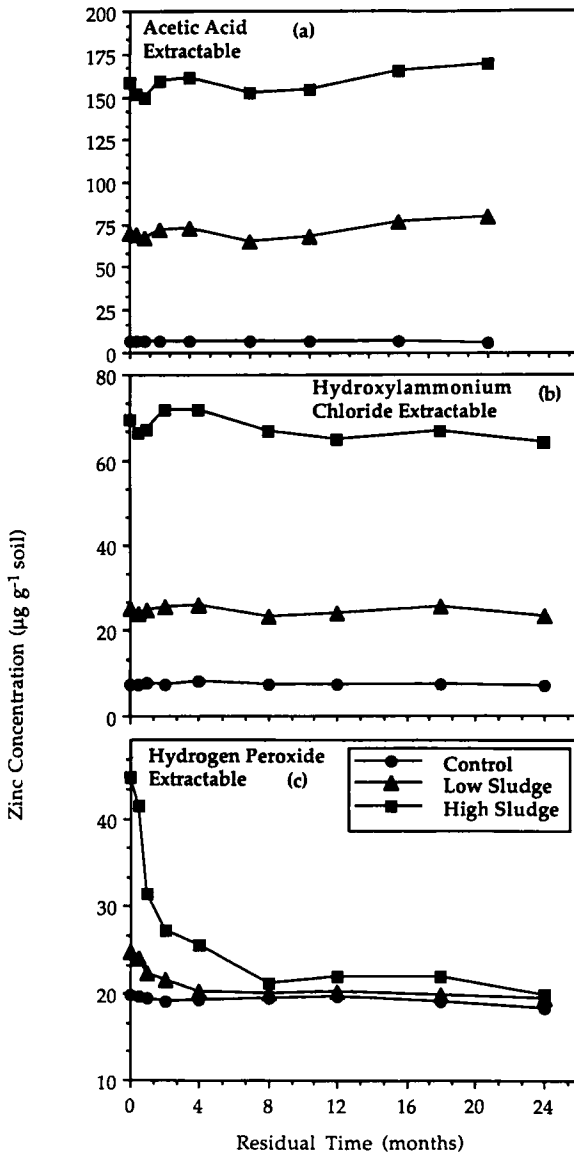


Figure 6 Zinc distribution among CH_3COOH , $\text{NH}_2\text{OH}\cdot\text{HCl}$ and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable fractions, based on data averaged over both soils and temperatures

was: $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc} \geq \text{NH}_2\text{OH}\cdot\text{HCl} > \text{CH}_3\text{COOH}$ for both soils. The order in both sludge-treated soils after two years had changed to $\text{CH}_3\text{COOH} > \text{NH}_2\text{OH}\cdot\text{HCl} > \text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$. This shift in Cd fractions in favour of that extractable with CH_3COOH following the sludge application, means that Cd in the soil is dominantly present in a relatively mobile form. Thus, it is more readily available to plants in sludge-amended soils than uncontaminated soils.

COPPER

The order of Cu concentrations in the extracted fractions in both control soils is $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc} > \text{NH}_2\text{OH}\cdot\text{HCl} > \text{CH}_3\text{COOH}$ which is similar to the distribution of these fractions in the experimental sludge. The contents of Cu associated with the CH_3COOH fraction varied significantly during the two year residual period. The pooled Cu extraction

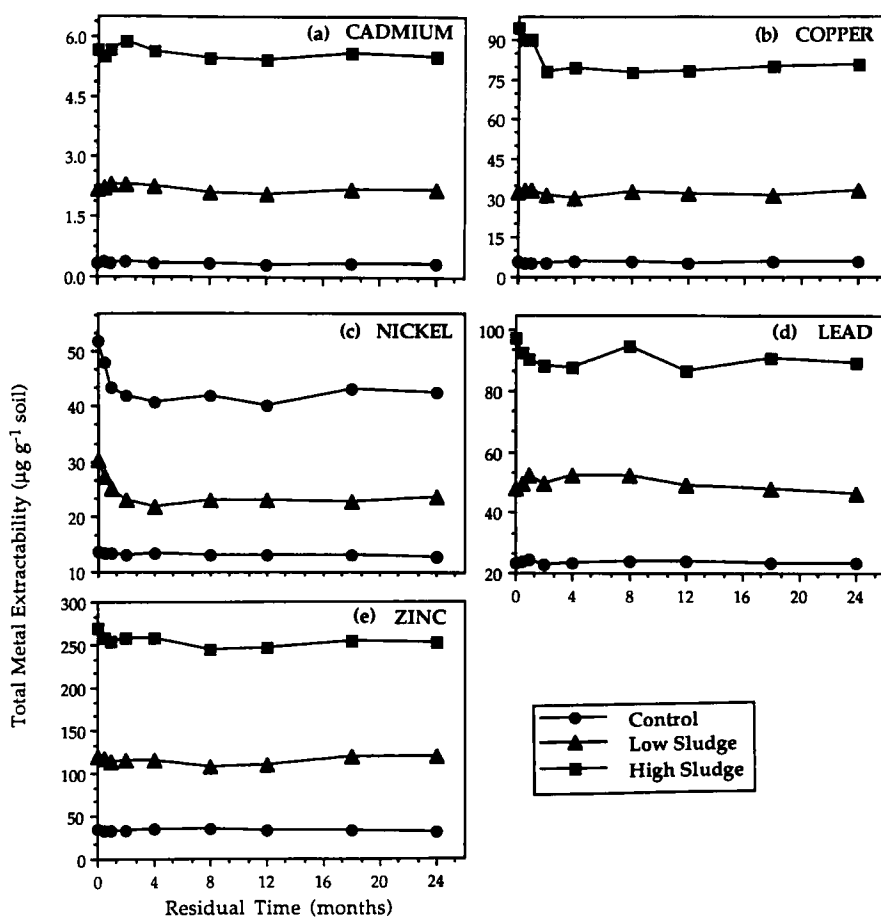


Figure 7 Total metal extractability during the two years reaction period

data for this fraction are depicted in Figure 3a. This figure shows that CH_3COOH soluble Cu increased in both the sludge treatments and its extraction continued to increase during the first twelve months (Figure 3a). As shown in Figure 3b, $\text{NH}_2\text{OH}\cdot\text{HCl}$ extractable Cu showed some significant ($p \leq 0.05$) variations over the residual period, but after a two-year period, this fraction of Cu was more or less similar to its initial levels. The variability in mean Cu concentrations extracted by $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ from the different samplings is shown in Figure 3c. It is evident from this figure that the Cu extracted by $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ in both sludge treatments decreased substantially over the first 8 months before stabilising for the rest of the study period (Figure 3c). The total Cu extraction data showed that Cu in the low sludge treatment in the last sampling was similar to that in the first sampling (Figure 7b). However, the total Cu extracted from the high sludge treatment showed a significant declining trend particularly during the first four months, indicating a possible transformation of the applied Cu to a form which is not affected by this extraction procedure. It is interesting to note that, despite a decline in Cu extracted by $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ (mainly associated with the organic fraction of soils) in the sludge-amended soils, this is still the most dominant Cu fraction. The order remains the same as it was before the application of sludge i.e. $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc} > \text{NH}_2\text{OH}\cdot\text{HCl} > \text{CH}_3\text{COOH}$. Despite the observed increase in CH_3COOH soluble Cu during the reaction time, no shift in the Cu fractions was observed. This indicates that Cu equilibrium in soils may not change over time. In other words, Cu mobility in soils or plant availability may not increase over time following sludge application.

NICKEL

The CH_3COOH extractable Ni in the sludge-treated soils varied significantly over the period of two years. However, Ni extractability after this two year period is similar to its values in the first sampling (Figure 4a). As shown in Figure 4b, Ni extractable by $\text{NH}_2\text{OH}\cdot\text{HCl}$ declined in the initial samplings but subsequently remained more or less constant over rest of the study period. The Ni concentrations in the final sampling, for both the sludge treatments, were somewhat less than their levels in the first samples (Figure 4b). Application of sewage sludge influenced the Ni fraction associated with $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extraction. As shown in Figure 4c, this form of Ni declined rapidly in the first four months from both sludge treatments before stabilising for rest of the study period. When the total Ni extraction data were examined, the extractability by this sequential extraction scheme showed a clear declining trend during the first four months before stabilising for the rest of the study period (Figure 7c). This suggests that the $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ pool was not contributing towards the other two fractions (CH_3COOH and $\text{NH}_2\text{OH}\cdot\text{HCl}$), but rather went into some other form which was not extractable by this sequential extraction procedure or leached below the sampling depth. In the unsludged sandy loam soil, the order of the Ni contents in the fractions is $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc} > \text{CH}_3\text{COOH} > \text{NH}_2\text{OH}\cdot\text{HCl}$, and that in the unsludged Breckland sand is $\text{CH}_3\text{COOH} > \text{H}_2\text{O}_2/\text{NH}_4\text{OAc} > \text{NH}_2\text{OH}\cdot\text{HCl}$. The order of extractable Ni in the fractions of both sludged soils shifted to $\text{CH}_3\text{COOH} > \text{NH}_2\text{OH}\cdot\text{HCl} > \text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$. This shift in Ni fractions in favour of that extractable with a milder reagent (CH_3COOH) suggests that sludge application would provide Ni in soils in relatively mobile forms.

LEAD

As shown in Figure 5a, CH_3COOH extractable Pb showed an initial increase over the first 4 months followed by a decrease to levels similar to those at the beginning (Figure 5a). The sludge application resulted in increased concentrations of Pb extractable by $\text{NH}_2\text{OH}\cdot\text{HCl}$ (Figure 5b). Lead contents in this fraction in the last sampling are similar to their values in the first sampling, with some significant changes in between. Like Cd, Cu and Ni, the $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable Pb concentration declined in both the low and high sludge treatments in the first 6–8 months but it remained more or less constant in the later samplings (Figure 5c). The total extraction data showed that Pb removed by this sequential extraction procedure tended to remain more or less constant over time in the low sludge treatment. However, in the high sludge treatment it showed a significant decreasing trend during the first four months of the residual period (Figure 7d). The order of fractions in both control soils was $\text{NH}_2\text{OH}\cdot\text{HCl} > \text{H}_2\text{O}_2/\text{NH}_4\text{OAc} > \text{CH}_3\text{COOH}$. Despite most of the Pb (96%) being in $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ fraction in the sludge, the order of the extractable fractions in both sludge treated soils after a two year period was the same as in the control soils. This suggests that sludge application may not affect the Pb equilibrium in soils. In other words, Pb mobility or plant availability may not increase in soils following sludge application.

ZINC

Overall, the Zn extracted by CH_3COOH showed an increasing trend over time from both sludge treatments (Figure 6a). The Zn contents extracted by $\text{NH}_2\text{OH}\cdot\text{HCl}$ over the two years study period are shown in Figure 6b. The extractability in both sludge treatments after a period of two years, is similar to its initial levels (Figure 6b). The addition of sewage sludge resulted in considerably higher Zn concentrations in the $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ -fraction. As shown in Figure 6c, Zn extracted by the $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ decreased markedly in both sludge treatments over the first four months. Examination of the total extraction data showed that the sum of the three Zn fractions removed by this sequential extraction procedure decreased over time in the high sludge treatment during initial few samples (Figure 7e), suggesting a possible conversion of Zn into some other form. On the other hand, the total extractability in the low sludge treatment did not show any significant change over time (Figure 7e). The order of Zn fractions in both control soils was $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc} > \text{CH}_3\text{COOH} > \text{NH}_2\text{OH}\cdot\text{HCl}$ and that for the sludge-treated soils was $\text{CH}_3\text{COOH} > \text{NH}_2\text{OH}\cdot\text{HCl} > \text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ after a residual period of two years. This means that Zn in the sludge-amended soils is dominantly present in a relatively more mobile fraction compared to the unamended soils.

The effect of temperature on metal fractions

Overall, no significant differences in the amounts of Cd, Cu, Ni, Pb and Zn extracted by CH_3COOH were observed in the samples collected from both the cool and warm incubation conditions. However, when the extraction data were compared within a treatment for

individual metals, the extractability was found to show significant variations for Ni and Pb but with no clear trends. As with CH_3COOH , the difference in temperature had no effect on the concentration of any of the five metals extracted by $\text{NH}_2\text{OH}\cdot\text{HCl}$ in any of the sludge treatments. On the other hand, the difference in temperature had significant effects on $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable metals. Metals extracted from samples in both cool ($15 \pm 2^\circ\text{C}$) and warm ($25 \pm 3^\circ\text{C}$) ambient conditions are shown in Figure 8. Overall, the observed decline in metal contents extracted by $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ over time (Figures 2–6) in the sludge-treated soils occurred at a faster rate in the samples taken from the warm conditions compared to the samples from the cool conditions (Figure 8). However, after a two year reaction time, metal contents extracted from samples collected from both warm and cool conditions are

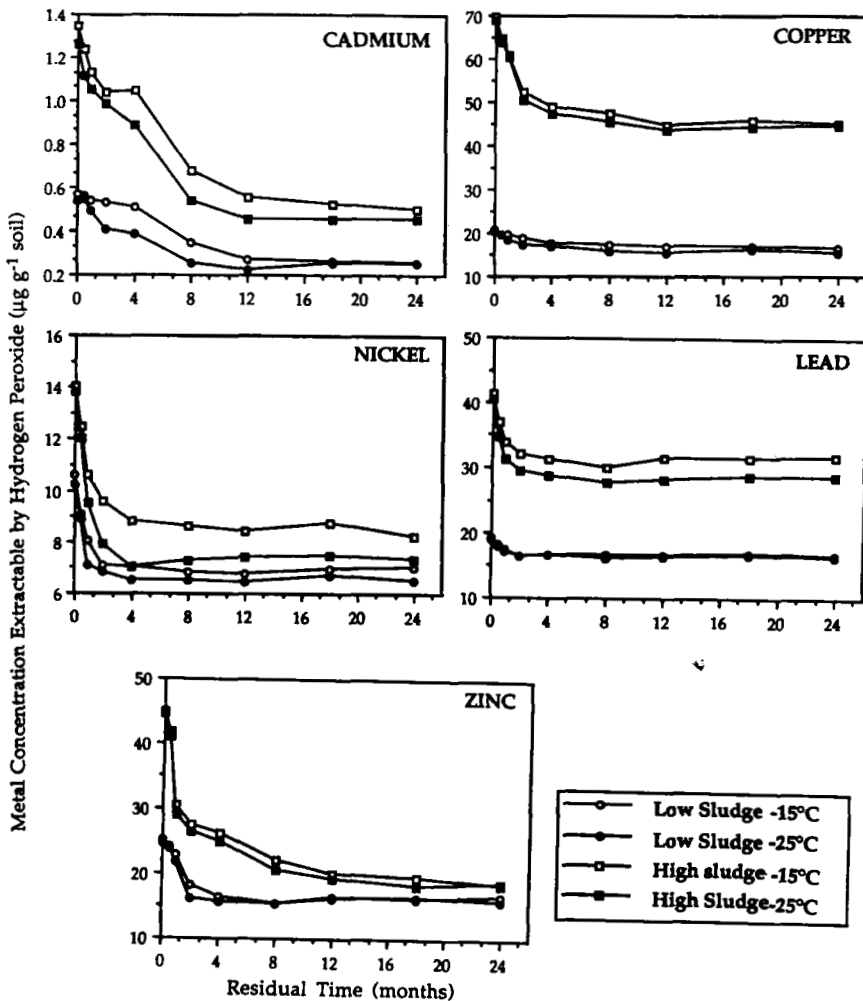


Figure 8 Effects of difference in temperature on $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable metals during the two-years study period, based on data averaged over both soils

not significantly different; this is particularly true for the low sludge treatment. Individually, this trend due to the temperature difference was more marked in the high sludge treatment of both soils. This implies that the trend of a decreasing $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ soluble fraction of metals during the residual period was similar in samples from both the cool and warm incubation conditions. However, this trend was only delayed in samples from the cool environment compared to those from the warm conditions.

DISCUSSION AND CONCLUSIONS

Reliable measurement of metal fractions is essential for understanding the behaviour of trace metals in soils or sediments, but is difficult, partly because the distribution of fractions may change during sampling and extraction. Given the practical difficulty in isolating a specific fraction of metals, overlap between the adjacent fractions can occur; therefore metal fractions extracted by employing a sequential extraction procedure are operational in nature and only approximate to the real situation. Nevertheless, the results of the sequential extraction procedure used in these experiments indicated that the predominant fractions of Cd, Cu, Ni, Pb and Zn in the sludge were extractable in $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$. This reagent, along with $\text{K}_4\text{P}_2\text{O}_7$, $\text{Na}_4\text{P}_2\text{O}_7$ and NaOCl have all been used to extract metals associated with the organic fraction of sludges and soils but, as discussed previously, these reagents also remove metals present in as sulfides. Having used $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$, it can be concluded that the metals in the experimental sludge were predominantly (63–98%) in the organo-sulfide phase. Other workers have shown that Pb, Zn and Cu were predominantly in the organic fraction of sewage sludges, using 0.1M $\text{Na}_4\text{P}_2\text{O}_7$ as the extractant²⁵. While almost all of the Cu (98%) and Pb (96%) in the experimental sludge were in $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ -soluble fraction, the percentages of Cd, Ni and Zn in this fraction were somewhat lower but similar (63–75%), indicating a possible similarity in behaviour when the latter group of metals are added to soils. The sequential extraction recovery data showed that <21% of the total Cu, Pb and Cd in the sludge was not dissolved by any of the three extractants used in this scheme. Similarly, metal extraction in the sludge-treated soils showed that a substantial amount of total (HClO_4 -HF soluble) Cu (21%) and Pb (12%) is probably present in silicate minerals which are not dissolved by any of the extractants. Nevertheless, the total extractabilities (sum of the fractions of an element) of applied metals were similar to those found by others^{2,10}. Because of the drastic treatment required to dissolve silicates, it was recognised that the metals that are not dissolved in the three steps of the scheme used here, would probably represent an environmentally inactive phase of metals.

The application of sewage sludge increased the contents of the metals that were soluble in 0.11M CH_3COOH . This reagent being the weakest in the extraction procedure is considered to extract metals which are loosely held; therefore metals extracted with 0.11M CH_3COOH represent the most mobile phase of metals in this extraction scheme. The concentrations of metals solubilised by CH_3COOH were considerably higher in the Breckland sand than in the sandy loam soil regardless of the sludge application rate. The coarse texture, lower CEC, lower contents of free oxides of Fe and Mn and of organic matter in the Breckland sand probably accounted for the higher recoveries of applied metals from this

soil (Table 1). The diversity of reagents, used by researchers to dissolve a specific metal fraction, makes comparison with other published data difficult. Nevertheless, numerous reports in the literature indicate that amounts of weak acid soluble and DTPA/EDTA extractable metals increase in soils following their additions to soils and these are likely to reflect bioavailability,^{26,27,28,29,30}. While the contents of Cd, Cu and Zn extracted by CH₃COOH showed an increasing trend over the two years, the contents of Ni and Pb first increased and then declined to their initial levels in the same period of time. The apparent increase in CH₃COOH soluble metals with time may have been due to (i) a decrease in soil pH (Figure 9) over time and consequent dissolution of relatively insoluble phases of metals such as carbonates, phosphates and hydroxides; (ii) a decrease in soil organic matter (Figure 10) and consequent release of metals due to a decline in number of adsorption sites in soil-sludge mixture; (iii) the formation of soluble organo-metal complexes which do not participate in adsorption reactions; (iv) microbial release of metals complexed with sludge-added organic matter; or (v) microbial oxidation of metal sulfides to sulfates.

Treating soils with the sewage sludge increased the concentration of trace metals which were extractable with acidified NH₂OH·HCl. This reagent has been used to extract metals

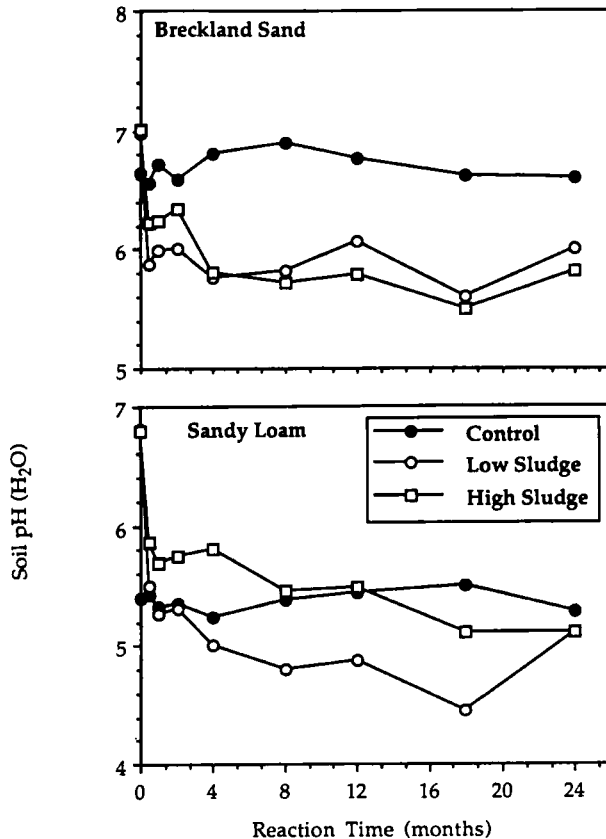


Figure 9 Effects of sludge application on soil pH monitored during the two-years study period

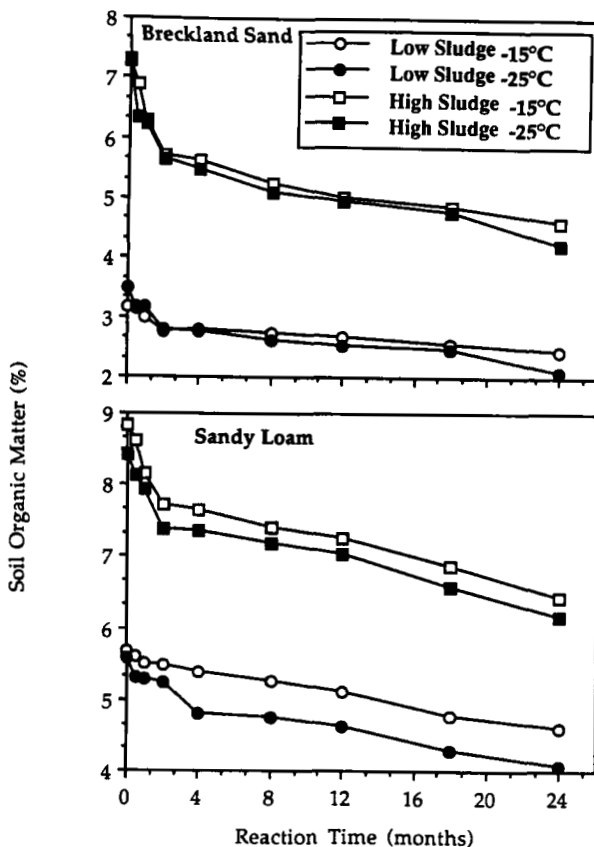


Figure 10 Effects of sludge application on soil organic matter contents monitored during the two-years study period

in soils which are specifically adsorbed on Fe-Mn hydrous oxides^{13,17,29}. Unlike the CH_3COOH , this reagent extracted far greater amounts of metals from the sandy loam soil compared to the Breckland sand regardless of the sludge application rate. The sandy loam soil contains far higher contents of Fe and Mn oxides (Table 1) compared to the Breckland sand and consequently, appeared to have adsorbed more of the sludge-applied metals which were extracted by $\text{NH}_2\text{OH}\cdot\text{HCl}$. The $\text{NH}_2\text{OH}\cdot\text{HCl}$ extractable metals data showed that this reagent extracted more or less similar amounts of the metals in periodically taken samples, indicating no significant change over time in this fraction of metals.

As with the $\text{NH}_2\text{OH}\cdot\text{HCl}$ soluble fraction, $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ dissolved greater contents of metals from the sandy loam soil than from the Breckland sand. These differences in control soils seem to have resulted in higher metal extractability in the sandy loam soil. Since metals in the sludge were predominantly present in the $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ -soluble fraction, application of the sludge to soils would be expected to have increased contents of $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable metals compared to the controls. Metal contents in this fraction started to

decrease over the residual time period; the decline was faster in the beginning but by the end of the first year this phase of metals had more or less stabilised. After two years, Cu and Pb extractable by $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ had declined by nearly 20% from their values at the beginning of the experiment. On the other hand, reductions in Cd, Ni and Zn were 45, 62 and 55%, respectively, over the same period of time. Since Cu and Pb were mainly present in this form in the sludge, these results suggest that the extractable fractions of Cu and Pb were determined principally by the nature of their fractions in sludge. The general decrease in $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ soluble metals may have been due to the microbial oxidation of sludge-added organic matter and a consequent release of metals bound to the organic matter. These metals released from the organic fraction appeared to have transferred into other fractions. The extractability in sludged soil samples collected from the two incubation temperatures was similar in the first sampling, but $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extracted considerably lower amounts of metals in samples collected subsequently from the warm environment. The biodegradation of sludge-added organic matter in soils is expected to be faster at the higher ambient temperature owing to greater microbial activity in warm conditions (Figure 10). The increased biochemical activity implies that metals associated with the sludge-added organic matter are released as the biodegradation process proceeds. This trend is reflected in lower metal concentrations extracted by $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ from the samples collected from the warm environment. However, such differences had disappeared by the last sampling, probably due to the stabilization of sludge-added organic matter in the soils.

The data for the total amounts of metal extracted (sum of three forms) showed that Cd, Cu, Pb and Zn remained more or less constant over the two years in the sludged soils, while Ni total extractability decreased over the same period of time. In the case of Cd, Cu, Pb and Zn, the decreasing trend in $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ seems to have contributed towards the increasing trend in CH_3COOH soluble metals, making the total extractability more or less similar in all the periodic samplings. The decrease in $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ extractable Ni did not result in a consequent increase in its CH_3COOH extractable fraction. This decrease in total Ni extractability over time may have been due to a possible fixation in minerals which cannot be dissolved by any of the reagents used in this sequential extraction procedure or to its leaching below the depth of sampling.

The sequential extraction data have shown that transformation between different fractions, particularly the CH_3COOH and $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ fractions, occurs in soils following treatment with sewage sludge. The results are consistent with the studies of long-term sludge treatment at Lee valley and Luddington field experiments³¹. Results from a long-term study on the behaviour of metals in soils showed that proportions of Pb, Cu, Zn, Ni and Cd in at least one of the three fractions (CaCl_2 , NaOH and EDTA) increased during the first 10 years of sewage sludge additions³². The general increase in CH_3COOH soluble metals over time suggests an increased metal mobility and plant availability over time in soils following their treatment with sewage sludge. This increasing extractability with time of the CH_3COOH soluble metals from the sludge treatments is in agreement with the plant metal uptake data from the same soils reported elsewhere³³. Metals present in these three fractions represent environmentally active metals in soils; successive fractions representing a decreasing degree of bioavailability to plant or mobility in soils. It should be stressed here that almost all of the Cu (98%) and Pb (96%) in the sludge were soluble in $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$, and it is possible

that these two metals may have different behaviour in sludged soils than the other three elements. It is also important to recognise here that before treating the soils with sewage sludge, the general order of metal contents in the fractions was $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc} > \text{NH}_2\text{OH}\cdot\text{HCl} > \text{CH}_3\text{COOH}$. After a period of two years following the sludge application, the order of metals changed to $\text{CH}_3\text{COOH} > \text{NH}_2\text{OH}\cdot\text{HCl} > \text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ for Cd, Ni and Zn, whereas no change in the ordering was seen for Cu and Pb. It is clear from the order of metal fractions that the forms of Cd, Ni and Zn changed, following the application of sludge. There was a decrease in the proportions extracted by strong reagent such as H_2O_2 and an increase in amounts extracted by 0.11M CH_3COOH , the weakest of the three reagents. This shift in fractionation of metals suggests that the application of sludge provides increased concentrations of the more mobile forms of Cd, Ni and Zn. In contrast, the absence of a shift between fractions of Cu and Pb suggests that the mobility of these elements may not change (i.e. remain relatively low).

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