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Soils

METHODOLOGIES TO PREDICT THE MOBILITY AND AVAILABILITY OF HAZARDOUS METALS IN SLUDGE-AMENDED SOILS

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ABSTRACT

Research was undertaken to develop practicable laboratory methods for assessing the solubility and plant-availability of potentially hazardous trace metals accumulating in arid-zone soils receiving sewage effluents or sludges. Sequential extraction of field soil samples with potassium nitrate, water, sodium hydroxide, sodium EDTA, and nitric acid was shown to provide reliable estimates of trace metal solubility and uptake (leaf concentration by barley, irrespective of soil texture, rate of sludge application, or time during a three-year study period. The total concentrations of the trace metals Ni, Cu, Zn, Cd, and Pb accumulated in two sludge-amended field soils during the project period were comparable with the amounts of these metals sorbed by untreated soils at soil solution concentrations below 100 parts per billion. At these low metal concentrations, the sorption isotherms often exhibited a sigmoid shape that precludes interpretation of terms of conventional distribution function techniques. Organic forms of the trace metals, particularly copper, were found to increase in importance with the sludge application rate. These organic-metal compounds could be characterized conveniently by electron spin resonance spectrometry.

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I. INTRODUCTION

A. Description of the Problem

The irrigation of agricultural land with reclaimed sewage effluent or the disposal of sewage sludge on the land can cause an accumulation of potentially hazardous trace metals, such as Ni, Cu, Zn, Cd, and Pb, in the upper portions of the soil profile. This accumulation can affect water quality in two principal ways: (1) an increased soluble concentration and mobility of metals in the subsurface zone contacting groundwater and (2) an increased availability of the metals to crops that may be grown on the land receiving the effluent or sludge. These two water quality problems cannot be managed well without reliable methods for predicting both the soil solution concentrations and plant-availability of the metals in soils.

The present study was undertaken to establish practicable laboratory methodologies for predicting hazardous trace metal solubility and plant-availability in arid-zone field soils receiving sewage effluents or sludges. The approach selected was to do laboratory experiments on soil samples collected from lots in a long-term field investigation being conducted by Dr. A. C. Chang at the Moreno Field Station of the University of California (Chang et al., 1979, 1983). The laboratory experiments were developed on the basis of the research objectives listed in Sec. I.B. The data from these experiments will be presented in Sec. II to IV below with the benefit also of the extensive data collected by Dr. Chang and his coworkers in connection with their field study, which included crop production and the monitoring of soil characteristics.

B. Research Objectives

(1) To determine the chemical forms of the trace metals Ni, Cu, Zn, Cd, and Pb in field soils to which liquid and composted sewage sludge has been applied and to apply the results to predict the concentrations of plant-available metals in the soils.

(2) To evaluate the desorption of the trace metals Ni, Cu, Cd, and Pb by arid-zone soils as a means to predict the solubility of trace metals in soil solutions.

(3) To determine the principal components of the metal-complexing, soluble organic fraction of the field soils receiving sewage sludge amendments and to determine the changes these organic components undergo in the soil as time passes.

II. CHEMICAL FORMS OF TRACE METALS IN SLUDGE-AMENDED SOILS

A. Sequential Extraction Procedure and Statistical Methods

1. Soil samples

Soil samples were collected in September 1979, 1980, 1981, and 1982 from the surface horizons (0-15 cm) of Greenfield sandy loam (coarse-loamy, mixed, thermic Typic Haploxeralf) and Domino loam (fine-loamy, mixed, thermic Xerollic Calciorthid) in randomized experimental plots at the Moreno Field Station of the University of California, Riverside. The plots on Greenfield sandy loam and one half the plots sampled on Domino loam had received composted sewage sludge amendments at the rate of either 0, 22.5, 45.0, or 90.0 Mg ha^{-1} $year^{-1}$. The composted sludge was obtained from the Joint Water Pollution Control Plant operated by Los Angeles County Sanitation Districts. These plots also had been cropped annually with barley (Hordeum vulgare) from 1975 to 1982, but were cropped with sorghum (Sorghum bicolor (L.) Moench) only through 1978. The other sampled plots on Domino loam had received digested liquid sewage sludge (3.0% total solids), at the four application rates indicated above, from the spring of 1976 through the spring of 1978. The source of the liquid sludge was the same, but the material was not dewatered prior to its application on the field plots. These plots had been cropped with sorghum throughout the same period as the sludge was applied, but had carried barley crops for the longer period, 1975 to 1982. Full details of the field investigation, which utilized the soils in a study of anaerobically digested sewage sludge as a soil amendment, have been reported by Chang et al. (1983).

Some properties of the surface samples of the two soils investigated, determined by standard methods (Page, 1982), are listed in Table II-1. Detailed field observations and textural analyses indicated that soil properties were rather uniform across each experimental plot and through the upper 15 cm of the soil profile. Chemical analyses showed that almost all (> 90%) of the trace metal accumulation from sludge applications on the plots was in the upper 15 cm of the soil profile (Chang et al, 1983). Accordingly, it was expected that studies of the surface soils in the plots would give significant information concerning trace metal chemistry.

TABLE II-1

PROPERTIES OF THE FIELD SOILS

Soil Series	Particle size fractions			CEC	Organic C	pH (sat. paste)
	Sand	Silt	Clay			
	----------%----------			cmol(+)/kg	%	
Domino	42	35	23	14	4.3	7.8
Greenfield	62	27	11	8.7	0.8	7.1

2. Trace metal fractionation

The total concentration of Ni, Cu, Zn, Cd, and Pb in the soil samples was determined in duplicate shortly after collection in each sampling year. Atomic absorption spectrophotometry (AAS) with a Perkin-Elmer model 5000 was used on filtered extracts obtained from 2-g samples that had been digested in 12.5 mL of 4M HNO_3 at 80°C overnight. The five trace metals also were fractionated in duplicate in 2-g soil samples with a sequential extraction procedure modified after that of Stover et al. (1976). The modified extraction procedure, developed on the basis of studies with model trace metal compounds, (Lund et al., 1980), is believed to give data concerning the fraction of trace metal in exchangeable, sorbed, organic, carbonate, and sulfide forms. These solid-phase chemical forms were determined by sequential extractions with 25 g of the following reagents: 0.5M KNO_3, 16 hours; deionized water, 2 hours (extract three times and combine data); 0.5M NaOH, 16 hours; 0.05M Na_2 EDTA, 6 hours; 4M HNO_3, 16 hours at 80°C. During each extraction, the soil suspension was shaken. After extraction the suspension was centrifuged and the resulting supernatant solution was decanted and filtered. Standard solutions for the metals were prepared for each extraction step in a background solution of the extracting reagents. The amount of metal extracted by a given extracting reagent was calculated according to this equation:

$$\mu g \text{ extracted} = C \times 25 \text{ g} - C' \times M, \qquad \text{(II-1)}$$

where C is the concentration (μg/g) of the metal in the extracted solution; C' is the concentration (μg/g) of the metal in the solution extracted in the preceding step of the sequence; and M is the mass of solution (g) carried over to the present extraction from the preceding one. Equation (II-1) contains a correction for the amount of metal in the solution entrained in the soil sample after the centrifugation which follows each extraction. This correction is made in lieu of washing the sample with deionized water after centrifugation, as suggested by Stover et al. (1976), because washing may lead to metal losses and, therefore, low recovery of metal from the soil in a given extraction step.

Although the amounts of exchangeable, sorbed, organic, carbonate, and sulfide forms of trace metals are believed to correlate well with sequential extraction KNO_3, H_2O, NaOH, EDTA, and HNO_3, it is not critical to the present study that this correlation be exact. Therefore, the fractionation of the five trace metals usually will be reported acccording to the extracting reagent employed (e.g., KNO_3) instead of the expected soil solid-phase fraction (e.g., exchangeable). The complete set of duplicate laboratory data on the five extracted fractions in the two field soils is presented in Sec.II.E.

3. Plant samples

Each year at the time of heading, the barley flag leaf was sampled. The leaf samples were rinsed with tap water to remove particulates, washed in a solution of nonionic detergent, rinsed again with tap water, and given a final rinse with deionized water. The cleansed leaf tissue samples were dried at 60°C and ground to pass a 1 mm sieve. The total concentration of Zn was determined in duplicate with the Perkin-Elmer

5000 on filtered extracts obtained from 200-mg samples that had been digested in 1.0 mL of a 2:1 mixture (v/v) of 70% $HClO_4$ and concentrated HNO_3 at 70°C overnight.

4. DTPA-extractable zinc

DTPA-extractable zine in the soils was determined following the method of Soltanpour and Schab (1977). Ten-gram soil samples were extracted for 15 min with 20 mL of a solution containing 0.005M DTPA and 1M NH_4HCO_3 adjusted to pH 7.6. Zinc was determined by AAS on the filtered extract.

5. Statistical analysis

a. extracted fractions

Mean values and mean deviations were calculated each year for the total concentration and for both the sum and individual extracted-fraction concentrations of Ni, Cu, Zn, Cd, and Pb in the soil samples collected from the control plots and the plots receiving 45 Mg ha^{-1} $year^{-1}$ sewage sludge. The mean percentage of each trace metal in each extracted fraction of the sludge-amended soil samples was calculated as the ratio of the mean mass of metal extracted in the fraction to the sum of metal mass extracted in all five fractions. The standard deviation of this mean percentage, σ_p, was estimated with the formula (see, e.g., Baird, 1962, Chap. 3):

$$\sigma_p = P[(\sigma_M/M)^2 + (\sigma_S/S)^2]^{1/2} \qquad \text{(II-2)}$$

where P is the mean percentage of metal in a given fraction, M is the mean mass of metal in the fraction, and σ_M is the mean deviation of M. In Eq. (II-2), S is the sum of mean extracted metal mass in all five fractions and σ_S is the estimated standard deviiaton of S, calculated with the formula (Baird, 1962, Chap. 3)

$$S = \left[\sum_{i=1}^{5} \sigma_{Mi}^2\right]^{1/2} \qquad \text{(II-3)}$$

where σ_{Mi} is the mean deviation for the mean mass of metal in fraction i (i = 1,...,5). The values of σ_p^2 obtained with Eq. (II-2) were used as estimates of sample variance for a pairwise comparison among mean percentages of a given metal in a given extracted fraction for the years 1979 to 1982. Two mean percentage values corresponding to 2 different years were considered significantly different if the ratio of their positive difference to the standard deviation of that difference exceeded Student's t for two degrees of freedom at the 95% level of confidence (see, e.g., Steel and Torrie, 1960, Chap.5).

b. bioavailability correlations

Linear correlation analysis was performed comparing the mean barley leaf concentration of Zn (Zn_{leaf}) with the percentage of Zn in sequentially-extracted fraction F (Zn_F) obtained in a single replicate extraction. This latter quantity is calculated as 100 times the mass of Zn found in fraction F divided by the mass of Zn found by summing all five extracted

fractions Zn_T). The significance of the resulting r^2-values in the correlations was tested at the P = 0.01 level with standard tables (Steel and Torrie, 1980).

Following the approach of Bingham et al. (1982), values of Zn_{leaf} were compared with values of $(Zn_S Zn_T)/100$ according to the equation:

$$Zn_{leaf} = A\{1-\exp[-k(Zn_S Zn_T)/100]\} \qquad \text{(II-4)}$$

where $Zn_S = Zn_{KNO_3} + Zn_{H_2O}$ and both A and k are adjustable parameters. These latter parameters and their 95% confidence intervals were determined using a modified Gauss-Newton iteration technique (Helwig and Council, 1979).

B. Effect of Sewage Sludge on Metal Extractability

1. Total metal concentrations

The total content of Ni, Cu, Zn, Cd, and Pb in the soil samples collected in 1979 is listed in Table II-2. The general conclusion to be drawn from these data is that the trace metal contents of the two soils, at any level of sludge application, reflect the trace metal composition of the sludges. In order of metal content in the composted sludge is Zn > Pb > Cu > Ni > Cd, and this is the order of metal content in both the Domino and Greenfield soils regardless of the sludge application rate. In the liquid sludge, the order of metal content is Zn > Cu > Pb > Ni > Cd, which is also the order found in the Domino soil receiving this sludge.

In the soils amended with composted sludge, the total content of Ni, Cu, Zn, Cd, and Pb increased approximately proportionally to the sludge application rate. However, in the Domino soil amended with liquid sludge, the increase in metal content is less than proportional to the sludge application rate. This result does not appear to be caused by migration of the metals to lower parts of the soil profile after sludge application, nor by the slightly greater metal uptake by crop plants grown on the liquid sludge-amended soil (Chang et al., 1979, 1983). Instead, it is probably caused by differences in soil bulk density. At the same metal input, the composted sludge application introduced far greater amounts of organic solids and resulted in a greater reduction of the soil bulk density. Therefore, in a given volume of sampled soil, there was a greater proportion of sludge in the soil treated with composted sludge. On a mass basis, the metal concentration in this soil thus would increase at a greater rate.

2. Trace metal distribution

The experimental precision at any step of the sequential extraction procedure was always better than 5% and the sums of extracted trace metal contents were always near, though usually systematically higher than the total metal contents listed in Table II-2. The sequential extraction procedure used by Stover et al. (1976) on sludges tended to produce sums of extracted contents of Ni, Cu, Zn, and Cd, which were 15 to 25% below the total metal contents, whereas the extracted sum of extracted Pb was slightly above its total content. In the present study, the sums of extracted Zn, Cd, and Pb in the Domino soil samples and of Zn and Pb in

TABLE II-2

TOTAL CONTENT OF METALS (1979)

Rate	Ni	Cu	Zn	Cd	Pb
$Mg \cdot ha^{-1} \cdot year^{-1}$	----------	----------	$mg\ kg^{-1}$	----------	----------
			Domino soil/composted sludge		
0.0	8.3 ± 1.0†	17 ± 2	76 ± 8	<0.05	8.9 ± 0.9
22.5	19.7 ± 0.1	46.8 ± 0.0	168 ± 1	1.56 ± 0.06	53 ± 1
45.0	32.3 ± 0.0	74.3 ± 0.0	293 ± 1	3.13 ± 0.00	89.9 ± 0.6
90.0	58.5 ± 0.5	141 ± 2	469 ± 6	6.8 ± 0.3	181.8 ± 0.2
			Domino soil/liquid sludge		
0.0	10.2 ± 0.1	21.0 ± 0.1	91.1 ± 0.4	0.1 ± 0.0	8.65 ± 0.05
22.5	21.6 ± 0.2	46.7 ± 0.9	169 ± 1	1.75 ± 0.0	28.8 ± 0.5
45.0	27.9 ± 0.8	64 ± 3	232 ± 10	2.8 ± 0.2	43 ± 3
90.0.	42 ± 1	98 ± 7	360 ± 17	4.8 ± 0.2	72 ± 8
			Greenfield soil/composted sludge		
0.0	6.3 ± 0.1	13.5 ± 0.1	66.2 ± 0.6	0.1 ± 0.0	6.15 ± 0.05
22.5	15.8 ± 0.5	36.3 ± 0.7	138 ± 1	1.25 ± 0.0	40 ± 2
45.0	23.5 ± 0.5	57.9 ± 0.4	209 ± 2	2.50 ± 0.0	70 ± 2
90.0	45 ± 2	114 ± 3	376 ± 12	5.3 ± 0.2	149 ± 7

†Mean deviation of measurements on 2 different soil samples

the Greenfield soil samples were, on the average, equal to the total metal contents within experimental error. The sums of extracted Ni and Cu in both soils and of Cd in the Greenfield soil tended to be systematically larger, by about 15%, than the total metal contents. These small, systematic differences may have been the result of the 4M HNO_3 extraction in the sequential procedure being more effective than the 4M HNO_3 extraction to obtain the total metal content because of preconditioning of the soil sample by the previous extractions with NaOH and EDTA in the sequential procedure.

The amounts of trace metals extracted with either KNO_3 or H_2O from the soil samples always were very small. In general, the percent of the total metal content (in Table II-2) extracted by KNO_3 and H_2O combined was < 6% for any metal and any soil sample. Moreover, this percent showed no trend with the type of sludge or its rate of application.

The trends in the NaOH-, EDTA-, and KNO_3-extractable metal contents with sludge application rate are illustrated in Fig. II-1 for the case of the Domino soil amended with composted sludge. The extracted metal contents are reported as percentages of the total metal contents given in the first four rows of Table II-2. Precisely, the same trends as shown in Fig. II-1 were found for the Domino soil receiving liquid sludge and the Greenfield soil receiving composted sludge.

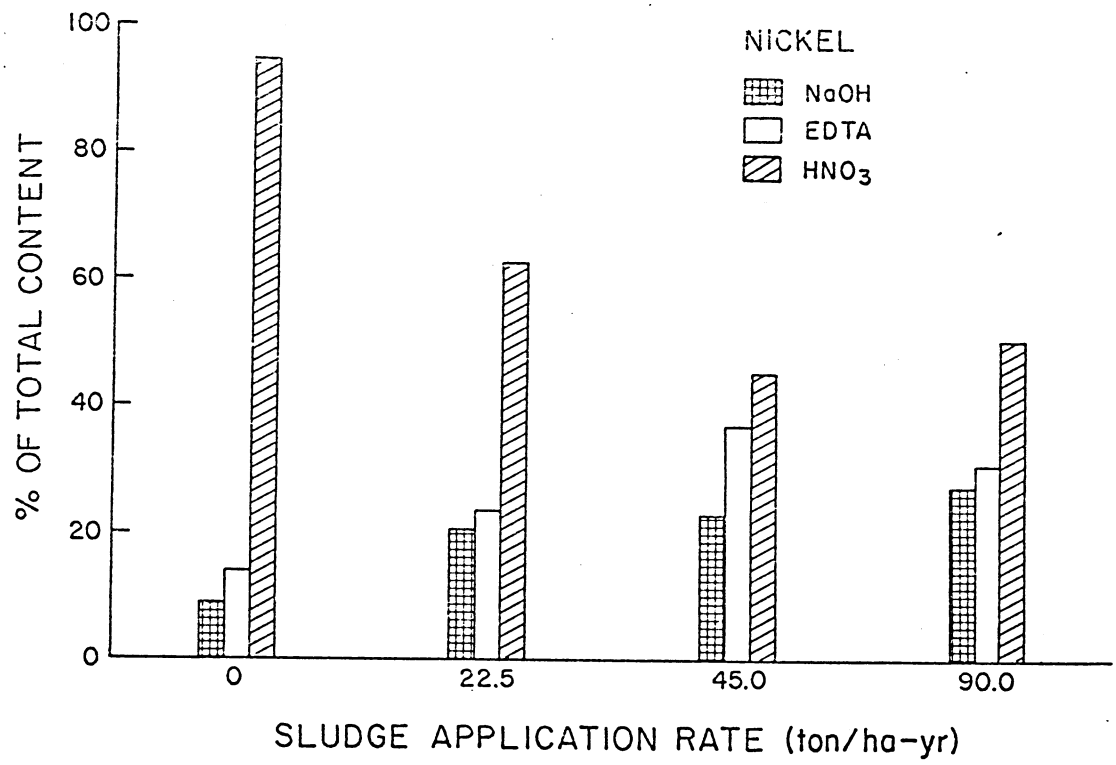
Figure II-1 shows that, as the sludge application rate increases, the percentage of Ni in the NaOH fraction increases, while that in the HNO_3 fraction drops. The percentage of Ni in the EDTA fraction increases also, but not systematically. It appears, therefore, that organic Ni and, to some extent, carbonate Ni are the principal chemical forms of Ni induced through sludge applications. However, even at the highest rate of sludge application, the HNO_3 fraction amounts to about one-half the total Ni content, and the order of fraction content is HNO_3 > EDTA > NaOH.

The trend observed for Ni is even more pronounced for Cu (Fig. II-1). At the highest sludge application rate, the NaOH fraction amounts to 60% of the total Cu content. The order of Cu contents in the fractions is NaOH > EDTA > HNO_3. This ordering is the reverse of that found for Cu in sludges by Stover et al. (1976), but it is similar to that reported by Schalscha et al. (1981) and is consistent with the known affinity of Cu for organic matter ligands.

The trends shown for Zn in Fig. II-1 are similar to those for Ni in that the NaOH fraction increases while the HNO_3 fraction decreases with increasing sludge application rate. In this case, there is also an increase in the EDTA fraction, with the result that EDTA > HNO_3 > NaOH at the highest application rate. The percentage of Zn accounted for in the NaOH fraction at this rate is almost identical with that of Ni.

It was not possible to fractionate Cd successfully in the Domino and Greenfield soils at the zero sludge application rate because the total metal contents were so small (compare Table II-2). Therefore, only fractions determined in the sludge-amended soil are described in Fig. II-1. The trends shown are similar to those for Zn, except for variability in the EDTA and HNO_3 fractions. This same variability appeared in the Greenfield soil amended with composted sludge and in the Domino soil amended with liquid sludge. Possibly this variability is an experimental artifact connected with the low absolute amount of Cd in the soil-sludge mixtures.

In the case of Pb, the dominant fraction is EDTA regardless of the sludge application rate. The NaOH fraction undergoes no significant change with sludge application rate, whereas the HNO_3 fraction may decrease slightly.



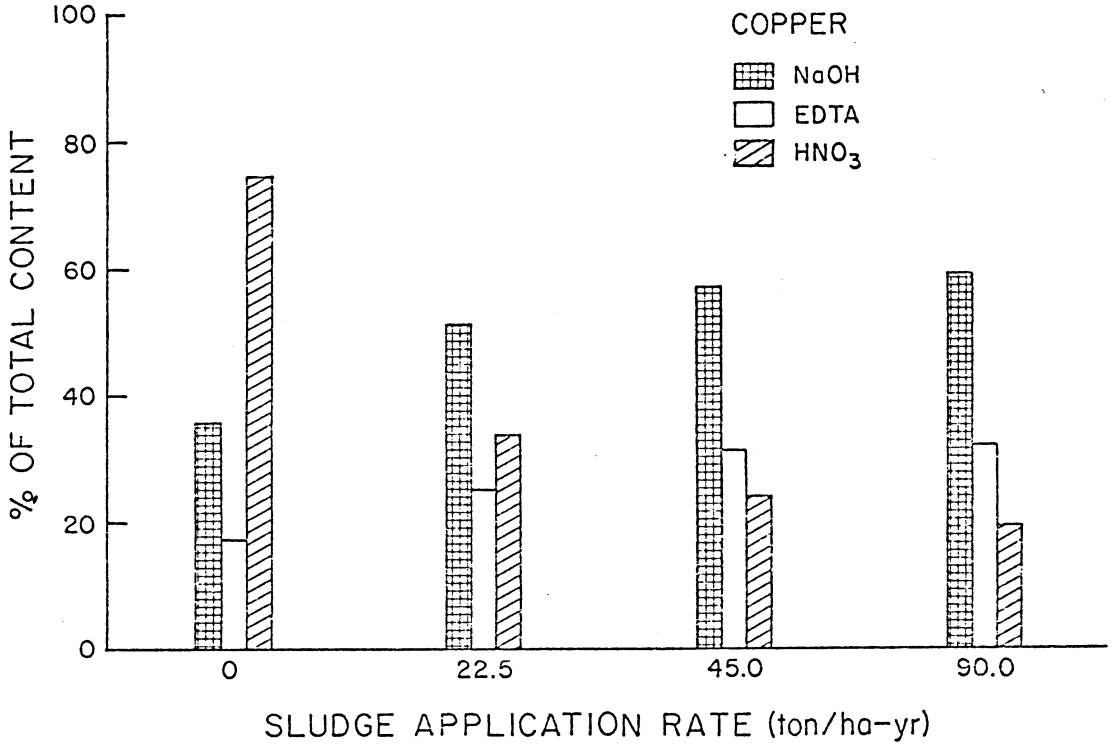
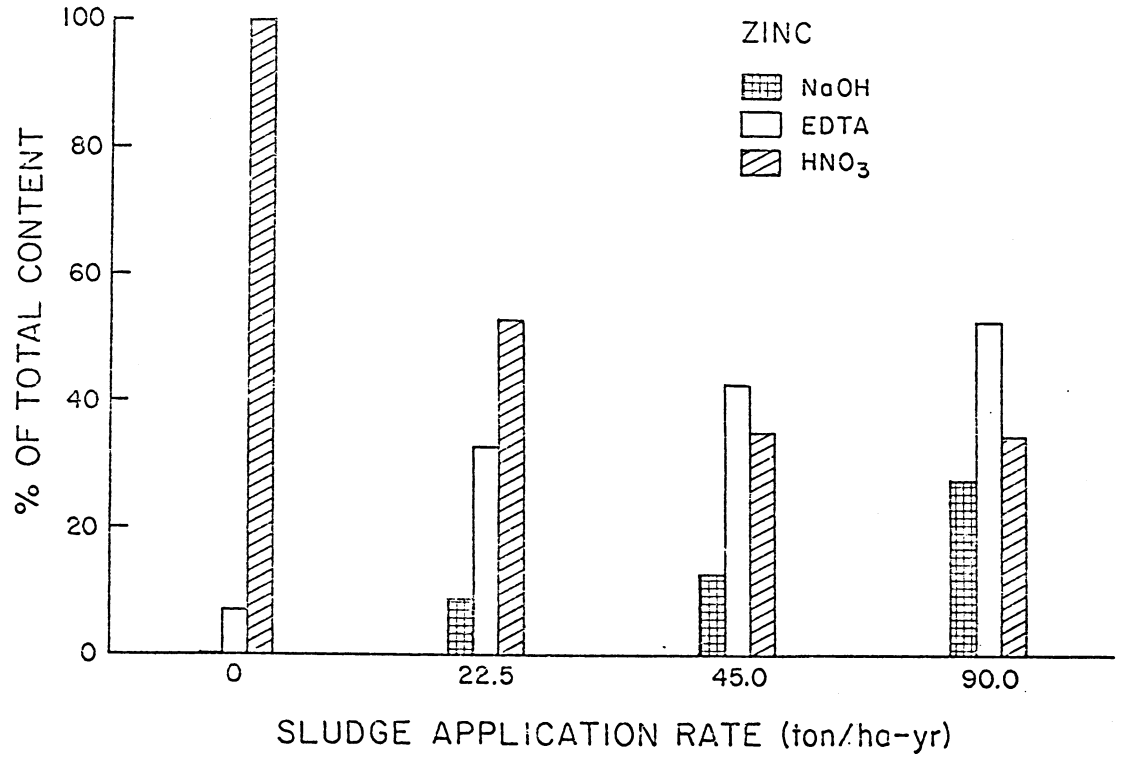


FIGURE II-1. Distribution of Ni and Cu among the NaOH, EDTA and HNO_3 fractions of Domino soil amended with composted sludge.



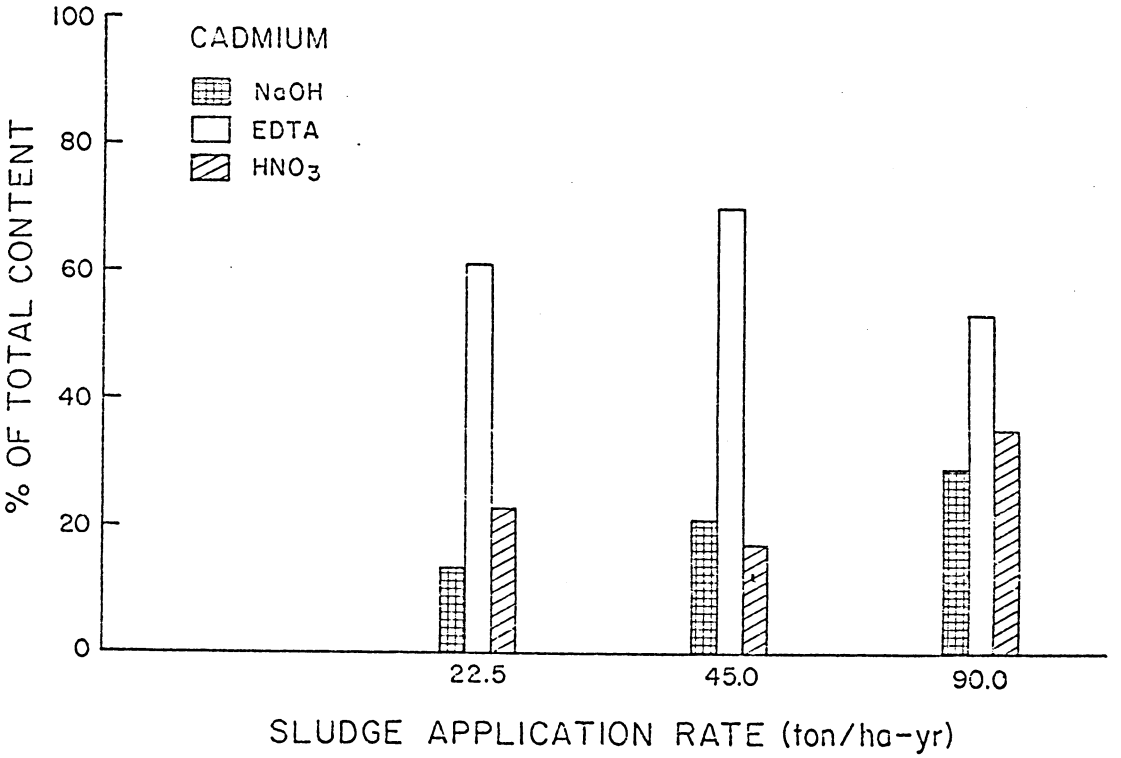


FIGURE II-1. Distribution of Zn and Cd among the NaOH, EDTA and HNO_3 fractions of Domino soil amended with composted sludge.

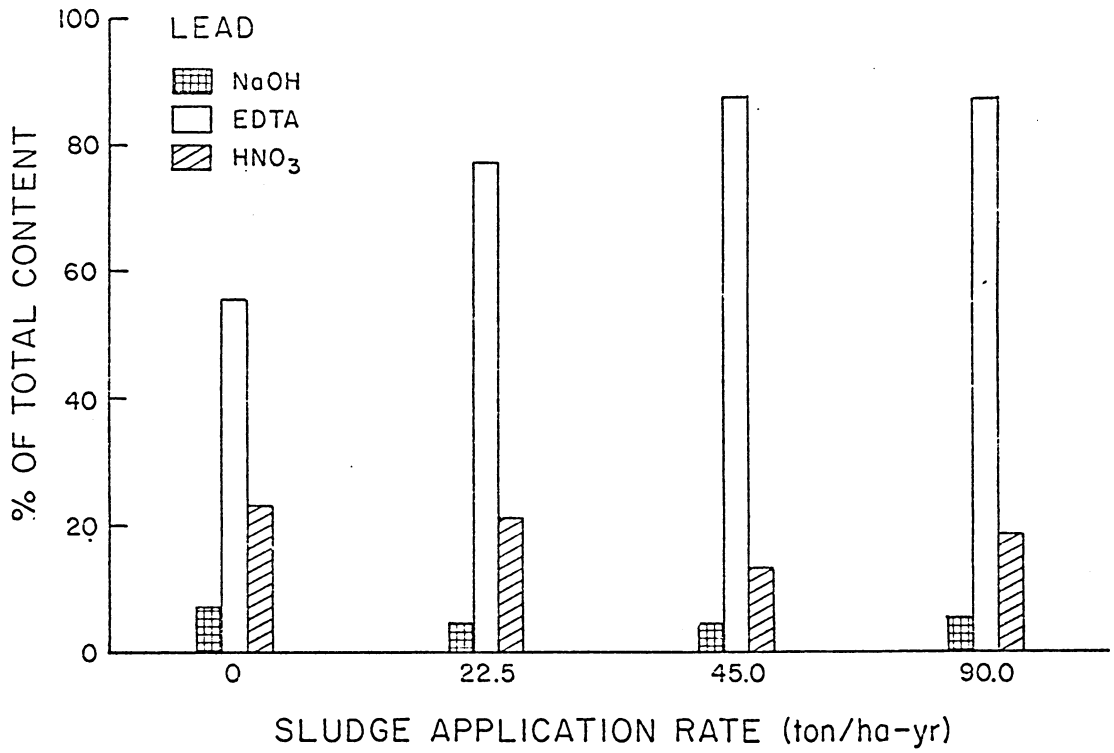


FIGURE II-1. Distribution of Pb among the NaOH, EDTA and HNO_3 fractions of Domino soil amended with composted sludge.

3. Conclusions

The accumulation of Ni, Cu, Zn, Cd, and Pb in arid-zone field soils amended with composted or liquid sludge was found to be governed by the content of the metals in the sludges and the rate of sludge application. Soil properties and sludge properties other than metal content did not appear to play the dominant role in determining metal accumulation, although the rate of accumulation in field plots amended with liquid sludge was less than in plots amended with composted sludge and accumulation was greater in the more clay Domino soil than in the Greenfield soil.

The percentage of total Ni, Cu, Zn, Cd, and Pb in the field soils which was extractable with KNO_3 and H_2O was very low, averaging between 1.1 and 3.7%, regardless of the type of sludge. These results are comparable with those for water extraction reported by Bradford et al. (1975) for soil at the bottom of a sludge-drying pond; in the case of Zn, Cd, and Pb, with those reported as exchangeable plus sorbed by Stover et al. (1976); and in the case of Ni, Cu, and Zn, with those reported by Schalscha et al. (1980, 1981). The low percentages of the metals in the (KNO_3 + H_2O) fraction could signify a low availability of the metals to crop plants, since the readily soluble form of a trace metal is often

regarded as the most biovailable. However, recent work (Silviera and Sommers, 1977; Latterell et al., 1978) suggests that, e.g., KNO_3 fractions of trace metals represent potential mobility more than potential bioavailability in sludge-amended soils.

The application of sewage sludge to the field soils tended to reduce HNO_3 fraction percentages for all of the trace metals, regardless of the type of sludge or soil. The application of sludge tended to increase the NaOH fraction percentages for all of the trace metals except Pb, for which there was no change. Sludge applications also increased the EDTA fraction percentages for all of the metals. For Ni, the order of fraction percentages at the highest sludge application rate was HNO_3 > EDTA ≈ NaOH, and for Pb, it was EDTA >> HNO_3 > NaOH. These results indicate that the chemical behavior of Zn, Cd, and Pb in the soils may be similar whereas that of Ni and Cu differ both from this group and from one another.

It is evident that the application of sludge shifted the solid phases containing Ni, Cu, Zn, Cd, and Pb in the soils away from those extractable with more severe reagents, such as 4M HNO_3, to those extractable with milder reagents, such as dilute NaOH and EDTA. This shift suggests that sludge applications would provide the metals in labile chemical forms that could be more readily available to crop plants than in the nonamended soils.

C. Effect of Time on Metal Extractability

1. Introduction

A conclusion drawn in Sec.II B was that the application of sewage sludge tended to shift the solid-phase forms of the metals away from those extractable with 4M HNO_3 to those extractable with dilute NaOH and Na_2EDTA. The recently published investigations by Banin et al. (1981) and Schalscha et al. (1980, 1982) regarding the extractable forms of trace metals in arid-zone field soils which have been irrigated with sewage effluent for many years support this conclusion. Banin et al. (1981) found that the percentage of Ni, Cu, Zn, Cd, and Pb extractable with hydroxylamine hydrochloride/acetic acid in three Israeli coastal plain soils was increased through irrigation with sewage effluent for periods up to 28 years. [The extracting reagent used by Banin et al. (1981) is approximately equivalent to the combination of NaOH followed by EDTA in the procedure used by Sposito et al. (1982)]. Schalscha et al. (1982) employed a sequential extraction procedure very similar to that used in the present study to examine the solid-phase forms of Ni, Cu, Zn, and Pb in Chilean Inceptisols that had been irrigated with untreated sewage effluent for up to 50 years. The percentage of the total content of each metal that could be extracted by the milder reagents in the procedure ($Na_4P_2O_7$ and EDTA) was much larger in these soils than in soils that had never received sewage effluent. Schalscha et al. (1982) were led by their experimental results to suggest that "...the accumulation of wastewater-originated metals in organic complexes and inorganic precipitates enables them to become reactive whenever the chemical equilibrium in the soil shifts. The somewhat labile nature of the deposited heavy metal would enrich the soil with plant-available metals for a long time to come."

The pattern that emerges from these recent studies suggests that the repeated application of sewage sludge or effluent over a long period of time in an arid-zone agricultural soil will tend to promote trace metal accumulation into solid organic or inorganic phases which are readily extractable with comparatively mild reagents. In this section, the effect of time on the distribution of Ni, Cu, Zn, Cd, and Pb among extractable fractions of the two field soils will be examined with the objective of ascertaining any trend toward metal accumulation into labile or quasilabile solid phases.

2. Total metal concentrations

The sum of extracted concentrations of Ni, Cu, Zn, Cd, and Pb in the soil samples from the control plots and the field plots receiving 45.0 Mg ha^{-1} $year^{-1}$ are listed in Table II-3 for the four sampling years. According to Chang et al. (1979, p. 3-7), an application rate of 45.0 Mg ha^{-1} $year^{-1}$ of the sewage sludge should meet the N and P requirements of most field crops, whereas the rates of 22.5 and 90.0 Mg ha^{-1} $year^{-1}$ used in their investigation either would satisfy only the P requirement or would exceed both the N and P requirements of most crops. Therefore, the data obtained from the field plots receiving 45.0 Mg ha^{-1} $year^{-1}$ will be presented as representative of the effects of a typical application of the Los Angeles County sewage sludge as an agricultural amendment.

Within the precision of the data, three principal trends can be discerned in Table II-3: (i) The summed metal concentrations in the soils receiving composted sludge tend to increase with time, whereas those in the soil that received liquid sludge do not. (ii) The summed metal concentrations tend to be larger in the Domino soil receiving composted sludge than in the Greenfield soil for any year. (iii) The order of decreasing metal concentration in the soils receiving composted sludge is Zn > Pb ≈ Cu > Ni > Cd, whereas that in the Domino soil receiving liquid sludge is Zn > Cu > Pb > Ni > Cd, for any year. These same three trends were observed in the mean values of the total metal concentrations obtained through a single extraction with HNO_3 (see Sec.II.E), which differed little from the summed metal concentrations, as discussed in Sec.II.B.

The observed increase in metal concentration with time in the soils receiving composted sludge reflects the continued accumulation of trace metals in the soils with continued sludge application, a trend also noted by Banin et al. (1981) and Schalscha et al. (1982). Of course, the constancy in metal concentration in the Domino soil receiving liquid sludge confirms the expectation based on the cessation of sludge application 1 year before the first soil samples were collected for the present study (1979) and the finding of no significant downward movement of trace metals from the surface horizons of the two field soils as of 1981 (Sec.II.B.). The ordering of metal concentration observed in the soils for any year is essentially that in the applied sludge materials, as indicated in Table II-4.

The positive differences in metal concentrations between the Domino and the Greenfield soils receiving the composted sewage sludge reflect the same trend as reported by Banin et al. (1981), who found the total concentrations of Cr, Ni, Cu, Zn, Cd and Pb in an Israeli clay loam soil

TABLE II-3

SUM OF EXTRACTED TRACE METAL CONCENTRATIONS IN SURFACE SOILS FROM FIELD PLOTS RECEIVING 45 Mg ha^{-1} $year^{-1}$ SEWAGE SLUDGE

Soil/Sludge	Control † 1979-1982	1979	1980	1981	1982
	mg/kg soil				
Nickel					
Greenfield/ composted	7 ± 1	27.3 ± 0.7	40.4 ± 0.5	36 ± 2	48 ± 3
Domino/ composted	9.9 ± 0.6	34.6 ± 0.6	53.5 ± 0.3	59 ± 3	82.6 ± 0.8
Domino/liquid	12 ± 2	30.6 ± 0.6	33.0 ± 0.8	27.7 ± 0.05	30.1 ± 0.4
Copper					
Greenfield/ composted	16 ± 2	64 ± 1	92 ± 1	80 ± 2	112 ± 3
Domino/ composted	24 ± 1	85 ± 1	128 ± 1	139 ± 2	196 ± 3
Domino/liquid	30 ± 3	65.0 ± 0.8	73 ± 4	60 ± 1	63.6 ± 0.7
Zinc					
Greenfield/ composted	78 ± 12	210 ± 2	288 ± 7	388 ± 21	343 ± 12
Domino/ composted	106 ± 23	259 ± 2	334 ± 12	594 ± 9	584 ± 10
Domino/liquid	114 ± 19	222 ± 4	249 ± 6	273 ± 14	217 ± 1
Cadmium					
Greenfield/ composted	0.09 ± 0.03	2.96 ± 0.02	4.4 ± 0.1	4.2 ± 0.4	6.6 ± 0.7
Domino/ composted	0.2 ± 0.1	3.5 ± 0.1	3.0 ± 0.1	2.4 ± 0.1	3.1 ± 0.1
Domino/liqui	0.3 ± 0.1	2.8 ± 0.2	3.0 ± 0.1	2.4 ± 0.1	3.1 ± 0.1
Lead					
Greenfield/ composted	10 ± 2	73.3 ± 0.4	94 ± 1	87 ± 6	108 ± 4
Domino/ composted	12 ± 3	95.9 ± 0.7	137 ± 3	153 ± 5	182 ± 4
Domino/liquid	16 ± 4	41 ± 1	50 ± 4	43 ± 3	48 ± 1

† Mean and standard deviation for 4 years' data on field plots receiving no sludge application

TABLE II-4

AVERAGE TOTAL CONCENTRATIONS † AND EXTRACTED PERCENTAGES ‡ OF TRACE METALS IN THE APPLIED SLUDGE MATERIALS

Sludge or Fraction	Ni	Cu	Zn	Cd	Pb
	mg/kg^{-1}				
Composted	332 ± 84	833 ± 122	2665 ± 663	41 ± 14	1539 ± 297
Liquid	530 ± 44	1324 ± 275	4074 ± 407	79 ± 17	1057 ± 439
NaOH fraction, %	24	60	28	22	-
EDTA fraction, %	32	23	58	52	-
HNO_3 fraction, %	26	16	10	36	-

† Mean plus standard deviation for samples obtained during 1975-1979 and extracted overnight at 80°C with 4M HNO_3

‡ Emmerich et al., 1982a.

TABLE II-5

EXTRACTED PERCENTAGES† OF Ni IN SURFACE SOILS

FROM FIELD PLOTS RECEIVING 45 Mg ha^{-1} $year^{-1}$ SEWAGE SLUDGE

Year	NaOH fraction	EDTA fraction	HNO_3 fraction
		Greenfield/Composted	
1979	28b	20a	51b
1980	23ab	28b	46ab
1981	19a	40b	36a
1982	19a	34b	41ab
		Domino/Composted	
1979	21a	34c	42a
1980	19a	22a	58c
1981	19a	30bc	48abc
1982	20a	26b	49b
		Domino/Liquid	
1979	25c	23bc	48ab
1980	20b	17a	56b
1981	24bc	27c	46a
1982	15a	21ab	52b

† Entries in each subcolumn that have the same superscript letter are not significantly different at the 95% level of confidence.

to be greater than those in sandy loam soils, and by Schalscha et al. (1982), who made this kind of comparison for Mn, Cu, and Zn in Chilean soils.

3. Trace metal distributions

Tables II-5 to II-9 show the percentage distribution of Ni, Cu, Zn, Cd, and Pb among the sequentially extracted fractions of the Greenfield and the Domino soils, respectively, that received sewage sludge at the rate of 45.0 Mg ha^{-1} $year^{-1}$. In the Greenfield soil, Ni and Cu show an increase in the EDTA fraction with time; Zn and Cd tend to show a decrease in the HNO_3 fraction and some evidence of an increase in the EDTA fraction, and Pb shows no change in distribution with time. For samples collected in the year 1979, the predominant extractable fractions of the metals in the Greenfield soil were: Ni (HNO_3), Cu (NaOH), Zn (HNO_3), Cd (EDTA), and Pb (EDTA). This result agrees with the data reported for Ni, Cu, and Cd (but not Zn) in Holland sandy loam (fine-loamy, mixed, mesic Ultic Haploxeralf) by Emmerich et al. (1982) and with the data reported for Ni, Cu, and Zn in Chilean soils by Schalscha et al. (1980, 1982). However, by the year 1982, the predominant fraction of Zn in the Greenfield soil had shifted to the EDTA-extractable fraction and Ni and Cu had begun to achieve parity between the EDTA fraction and the

TABLE II-6

EXTRACTED PERCENTAGES[†] OF Cu IN SURFACE SOILS

FROM FIELD PLOTS RECEIVING 45 Mg ha^{-1} $year^{-1}$ SEWAGE SLUDGE

Year	NaOH fraction	EDTA fraction	HNO_3 fraction
		Greenfield/Composted	
1979	52b	23a	23ab
1980	45a	28b	25b
1981	42a	32b	19a
1982	46a	30ab	20a
		Domino/Composted	
1979	49b	27a	21a
1980	44a	29a	24b
1981	41a	36b	18a
1982	44a	33b	20a
		Domino/Liquid	
1979	50b	22a	27b
1980	47ab	23ab	28ab
1981	43a	28ab	24a
1982	45ab	25b	27b

[†]Entries in each subcolumn that have the same superscript letter are not significantly different at the 95% level of confidence

TABLE II-7

EXTRACTED PERCENTAGES[†] OF Zn IN SURFACE SOILS

FROM FIELD PLOTS RECEIVING 45 Mg ha^{-1} $year^{-1}$ SEWAGE SLUDGE

Year	NaOH fraction	EDTA fraction	HNO_3 fraction
		Greenfield/Composted	
1979	24b	32a	43c
1980	19a	41b	38b
1981	22ab	49ab	26a
1982	39a	36ab	32a
		Domino/Composted	
1979	14b	46a	38c
1980	12a	49a	37abc
1981	13ab	59b	26a
1982	21a	44a	34b
		Domino/Liquid	
1979	12b	38a	48a
1980	7a	37a	54b
1981	8a	47a	42a
1982	10a	32a	56b

[†]Entries in each subcolumn that have the same superscript letter are not significantly different at the 95% level of confidence

TABLE II-8

EXTRACTED PERCENTAGES[†] OF Cd IN SURFACE SOILS

FROM FIELD PLOTS RECEIVING 45 Mg ha^{-1} $year^{-1}$ SEWAGE SLUDGE

Year	NaOH fraction	EDTA fraction	HNO_3 fraction
		Greenfield/Composted	
1979	23a	38a	32b
1980	21a	53b	26a
1981	28a	57ab	15a
1982	26a	46ab	24ab
		Domino/Composted	
1979	20a	63c	15a
1980	27b	45b	27b
1981	26ab	46ab	26ab
1982	25b	39a	32c
		Domino/Liquid	
1979	25ab	47b	23c
1980	27a	49b	21bc
1981	39b	53b	7a
1982	29a	34a	17b

[†]Entries in each subcolumn that have the same superscript letter are not significantly different at the 95% level of confidence

TABLE II-9

EXTRACTED PERCENTAGES[†] OF Pb IN SURFACE SOILS

FROM FIELD PLOTS RECEIVING 45 Mg ha^{-1} $year^{-1}$ SEWAGE SLUDGE

Year	NaOH fraction	EDTA fraction	HNO_3 fraction
		Greenfield/Composted	
1979	5c	69a	24a
1980	1a	70a	26a
1981	3abc	76a	20a
1982	3b	70a	27a
		Domino/Composted	
1979	4b	82b	13a
1980	1a	70a	27b
1981	2a	74ab	24b
1982	2ab	69a	28b
		Domino/Liquid	
1979	3b	73a	23a
1980	0a	66a	33b
1981	0a	72a	28ab
1982	1a	63a	35b

[†]Entries in each subcolumn that have the same superscript letter are not significantly different at the 95% level of confidence

fraction predominant in 1979. Also, as noted previously in Sec. II-B, and by Emmerich et al. (1982a), Schalscha et al. (1982), and Banin et al. (1981), the percentage of total metal extracted by KNO_3 and water (the difference between 100 and the sum of the percentages in any row of Tables II-5 to II-9) was always very small, being less than 5% in general.

Tables II-5 to II-9 show that a tendency to shift to the EDTA-extractable fraction in the Domino soil receiving composted sludge was apparent for Cu, but not for any of the other trace metals. The predominant fraction of Cd and Pb in the Domino soil was the EDTA-extractable fraction in 1979 and subsequent measurements indicate a decline in percentage in favor of the HNO_3 fraction. Nickel does not show any particular trends with time over the four years of the study. The same lack of a distinct trend is evident in the distribution of all five trace metals among the sequentially extracted fractions for the Domino soil that received liquid sludge until 1978. In this case, as already indicated in Table II-3, the total metal concentrations also are not increasing with time.

Emmerich et al. (1982a), as summarized in Table II-3, reported that Ni is distributed approximately evenly among the NaOH-, EDTA-, and HNO_3-extractable fractions in the composted Los Angeles County sewage sludge; that Cu is mostly in the NaOH-extractable fraction, and that Zn and Cd are mostly in the EDTA-extractable fraction. No data were reported for Pb. After 7 years of application of this sludge to the Greenfield soil, the major portion of Ni (82%) remains in either the EDTA or the HNO_3 fraction; Zn has become more equally distributed among the NaOH, EDTA, and HNO_3 fractions; and the distributions of Cu and Cd remain similar to those in the sludge. In the Domino soil, Ni predominates in the HNO_3 fraction after 7 years of sludge application, but the major fractions of Cu, Zn, and Cd remain as they were in the sludge. These results suggest that, with the exception of Ni, the extractable fractions of the trace metals were determined principally by the nature of the composted sludge applied continually to the Greenfield and Domino soils. For Ni, the persistent importance of the HNO_3 fraction indicates the role of the soil in determining the chemical form of the metal (see Fig. II-1). This role appears to be enhanced in the case of liquid sludge application, since both Ni and Zn were primarily in the HNO_3 fraction (Tables II-6 and II-8), which is the strongly predominating one for both metals in the control plots on Domino soil (Fig. II-1). However, Cu shifts from the HNO_3 fraction in the nonamended Domino soil to the NaOH fraction after liquid sludge amendment, and Cd may be decreasing its significance in the HNO_3 fraction.

An important result in this study is the general increase of the percentage of EDTA-extractable trace metals, except Pb, in the Greenfield soil with the passage of time. This shift may be the result of the gradual decomposition of the composted sludge in the soil environment, since the degradation of organic materials might be expected to be relatively rapid in the Greenfield soil given its coarse texture. The finer-textured Domino soil did not exhibit any pronounced tendency for the accumulation of metals in the EDTA fraction; rather, there was a tendency for Cd and Pb to shift into the HNO_3 fraction.

The EDTA-extractable fraction usually has been identified, in soils and sludges, with inorganic precipitates, quite possibly carbonates (Stover et al., 1976; Emmerich et al., 1982; Schalscha et al., 1982). The HNO_3 fraction is associated with resistant secondary minerals (Soon and Bates, 1982). If these identifications are valid, then it may be concluded that the five trace metals studied tended often to shift toward inorganic precipitated chemical forms with time in the two sludge-amended soils.

Schalscha et al. (1982) noted that this kind of trend was prominent, except possibly for Cu, in Chilean soils that had received sewage effluent for up to 50 years. Banin et al. (1981) observed that the precipitated forms of Ni, Cu, Cd, and Pb, as estimated with a hydroxylamine hydrochloride/acetic acid extraction, tended to increase in Israeli soils irrigated with sewage effluent for up to 28 years. All of these studies on arid-zone soils circumstantially indicate the importance of inorganic, precipitated chemical forms of potentially hazardous trace metals during the long-term application of sewage sludges or effluents to agricultural land. In the present study, these precipitates can be associated to a reasonable degree with carbonate compounds, since the latter are extracted efficiently by EDTA (Stover et al., 1976) and bicarbonate ion is expected to be an abundant product of the gradual decomposition of organic matter in sewage sludge applied to well-aerated, arid-zone soils.

D. Bioavailability Correlations

The perspective that fractions of trace metals extracted from sludge-amended soils can be useful indicators of trace metal bioavailability in a correlative sense has recently found wide acceptance in published investigations of trace metal uptake by crop plants (White and Chaney, 1980; Haq et al., 1980; Iyengar et al., 1981; Soon and Bates, 1982). Its success as a working principle for both plant nutrition and phytotoxicity research seems apparent. In their comprehensive survey of trace metal extraction methods, van Valin and Morse (1982) have summarized a consensus view as applied to sediments: "...the concept of an operationally defined metal reactivity is generally used, rather than attempting to individually characterize each sedimentary phase. The relative reactivity has been defined by the type of chemical leaching necessary to liberate a fraction of a particular metal. It is assumed to be largely dependent on the original phase from which the metal was liberated. Distinct chemical phases that respond similarly are, therefore, treated as equivalent phases. Using this approach, sediments and their trace metals can be characterized by comparative degree of susceptibility to chemical conditions rather than by the relative amounts of specific chemical phases within each. It should be noted, however, that this behavior can frequently be closely correlated with individual phases." This statement is equally applicable to soil, with "reactivity" associated with bioavailability, according to the view adopted in this project.

In the present section, a study is made of the relationship between zinc (Zn) bioavailability, as expressed by Zn concentration in barley flag leaves, and the fractions of Zn extracted sequentially from surface horizons of the two sludge-amended, arid-zone field soils discussed in Sec. II.A through II.C. In addition to determining correlations between Zn uptake and extracted Zn fractions, comparisons are made between Zn extracted sequentially by the series of reagents described in Sec. II.A and Zn extracted by DTPA (1,4,7-triazaheptane-1,1,7-pentaacetic acid). DTPA-extractable Zn is considered widely to represent the labile, plant-available pool of Zn in both natural soils and those to which sewage sludge has been applied (Rule and Graham, 1976; White and Chaney, 1980; Haq et al., 1980; Gough et al., 1980; Havlin and Soltanpour, 1981). Thus the principal objective was to associate sequentially-extracted fractions of Zn in sludge-amended soils with categories of lability and bioavailability.

The percentages of Zn recovered in the sequentially-extracted fractions (Zn_F), the total mass of Zn extracted in the five fractions per unit mass of soil (Zn_T), and the DTPA-extractable Zn (Zn_{DTPA}) are listed in Tables II-10 to II-12 for each soil/sludge combination. The percentage of Zn extracted with KNO_3 followed by distilled water has been combined into a single figure ($Zn_S = Zn_{KNO_3} + Zn_{H_2O}$) because the water-extractable Zn was always extremely small. Shown also, in the ninth column of each table, are the average barley leaf concentrations (Zn_{leaf}) for the crops grown on the experimental plots.

Bingham et al. (1982, 1983), Pavan and Bingham (1982), and Pavan et al. (1982) have shown that trace metal uptake by crop plants is correlated strongly with the activity of the free cationic species of the metal in a saturation extract of the cropped soil. The quantity of Zn extracted with KNO_3 and H_2O in the sequential extraction procedure [i.e., the product ($Zn_S Zn_T/100$)] may be associated with the activity of Zn^{2+} in a saturation extract because Zn_S represents essentially the water-soluble fraction in the soil and the dominant soluble form of Zn in sludge-amended soils appears to be Zn^{2+} (Emmerich et al., 1982b; Behel et al., 1983). Activity coefficient corrections that relate the concentration of a bivalent metal cation to its activity should not affect the general relation between metal uptake and the dominant soluble form of the metal (Bingham et al., 1982). Figure II-2 shows a graph of Zn_{leaf} plotted against the amount of Zn extracted by (KNO_3 + H_2O) in sequence for the Domino soil receiving composted sludge amendments. These data, based on columns 4, 9 and 10 in Table II-11, include all four rates of sludge application over all four years of the present study. The trend of the data, showing a sharp rise in Zn_{leaf} followed by a more gradual rise, also occurs for Zn uptake by barley grown on the Greenfield soil receiving composted sludge and on the Domino soil receiving liquid sludge (data not shown), but there is more scatter in these data than in Fig. II-2 for KNO_3-extractable Zn values greater than 6 mg kg^{-1}. The general curvilinear trend in Fig. II-2 is similar to that observed by Bingham et al. (1982) for Cd concentrations in Swiss chard and by Pavan et al. (1982) for Al concentrations in coffee. The data points can be fit to Eq. (II-4):

$$Zn_{leaf} = A \{1 - \exp[-k(Zn_S Zn_T)/100]\} \qquad \text{(II-4)}$$

TABLE II-10

VALUES OF Zn_F, Zn_{DTPA}, Zn_{leaf}, and Zn_T FOR GREENFIELD SANDY LOAM RECEIVING AMENDMENTS OF COMPOSTED SLUDGE

Year	Rate	Replicate	Zn_S†	Zn_{NaOH}	Zn_{EDTA}	Zn_{HNO_3}	Zn_{DTPA}	Zn_{leaf}	Zn_T‡
	Mg ha^{-1} yr^{-1}		----------%----------				------mg kg^{-1}-------		
1979	0	A	1.74	0.48	6.57	91.21	5.14	21.8	62.60
		B	1.50	0.00	7.02	91.48	5.36	21.8	63.28
	22.5	A	2.22	14.42	34.25	49.11	20.70	32.3	137.80
		B	1.81	16.14	26.15	55.90	23.39	32.3	148.69
	45	A	1.47	24.14	31.36	43.03	43.56	28.8	210.82
		B	1.37	24.40	32.00	42.22	49.39	28.8	208.84
	90	A	0.92	30.24	44.89	23.96	108.60	31.5	461.70
		B	0.89	29.15	40.55	29.40	103.50	31.5	455.24
1980	0	A	0.83	0.25	40.07	58.85	4.81	16.0	93.09
		B	0.54	0.09	40.78	58.59	4.92	16.0	93.09
	22.5	A	1.29	14.02	35.43	49.26	22.93	30.2	174.48
		B	1.01	13.43	40.25	45.31	28.72	30.2	176.98
	45	A	0.98	19.54	42.03	37.45	59.46	34.8	294.71
		B	0.89	19.28	40.93	38.90	70.20	34.8	281.75
	90	A	0.65	22.65	43.62	33.08	135.11	38.8	470.51
		B	0.63	21.24	51.76	26.37	141.58	38.8	553.03
1981	0	A	2.89	0.61	6.93	89.57	4.70	22.6	74.51
		B	2.15	0.64	8.18	89.03	5.25	22.6	77.61
	22.5	A	2.42	13.91	43.76	39.91	32.02	46.8	206.38
		B	2.06	14.87	45.21	37.86	19.69	46.8	214.49
	45	A	2.18	21.54	51.69	24.60	65.26	54.5	350.33
		B	1.96	22.54	46.89	28.60	74.95	54.5	325.05
	90	A	1.19	22.62	56.59	19.61	128.88	56.5	645.84
		B	1.23	23.03	53.87	21.87	122.23	56.5	640.84
1982	0	A	1.01	1.32	5.37	92.31	5.14	22.8	71.48
		B	1.18	1.34	6.39	91.08	4.70	22.8	74.00
	22.5	A	1.45	21.85	35.48	41.23	35.09	38.5	216.45
		B	1.53	22.81	33.42	42.24	25.54	38.5	208.46
	45	A	1.52	31.13	34.29	33.06	43.37	80.5	355.90
		B	1.50	29.61	37.85	31.04	52.29	80.5	350.34
	90	A	1.07	29.11	41.11	28.71	149.75	77.0	626.01
		B	1.00	29.04	41.76	28.20	147.12	77.0	602.23

†$Zn_S = Zn_{KNO_3} + Zn_{H_2O}$

‡Zn_T = sum of Zn extracted in fractions S, NaOH, EDTA, and HNO_3

TABLE II-11

VALUES OF Zn_F, Zn_{DTPA}, Zn_{leaf}, and Zn_T FOR DOMINO LOAM RECEIVING AMENDMENTS OF COMPOSTED SLUDGE

Year	Rate	Replicate	Zn_S†	Zn_{NaOH}	Zn_{EDTA}	Zn_{HNO_3}	Zn_{DTPA}	Zn_{leaf}	Zn_T‡
	Mg ha^{-1} yr^{-1}		----------%----------				-------mg kg^{-1}------		
1979	0	A	1.60	0.00	6.81	91.59	4.26	23.9	81.88
		B	1.29	0.00	6.52	92.19	4.26	23.9	83.58
	22.5	A	2.09	8.74	32.79	56.39	25.70	34.0	166.82
		B	1.76	8.86	35.91	53.47	20.12	34.0	172.60
	45	A	1.77	14.19	46.12	37.92	57.37	28.5	267.03
		B	1.58	13.74	46.53	38.16	52.78	28.5	270.33
	90	A	0.84	24.87	44.70	29.59	127.71	34.0	545.83
		B	0.76	23.12	46.27	29.85	130.05	34.0	531.99
1980	0	A	0.66	0.25	39.30	59.79	5.03	20.8	140.46
		B	0.63	0.04	40.27	59.06	4.70	20.8	135.70
	22.5	A	0.92	7.48	40.01	51.59	29.70	26.4	246.87
		B	1.00	7.17	37.93	53.90	32.86	26.4	238.02
	45	A	1.03	12.18	47.46	39.33	85.40	31.8	338.87
		B	1.00	12.30	50.97	35.72	87.37	31.8	328.90
	90	A	0.59	15.59	53.16	30.66	191.50	33.4	694.67
		B	0.65	16.02	51.71	31.62	201.42	33.4	656.17
1981	0	A	2.33	0.68	11.27	85.72	5.36	26.3	107.26
		B	1.98	0.48	9.46	88.08	5.10	26.3	99.36
	22.5	A	1.25	10.35	53.18	35.22	39.69	36.0	324.63
		B	1.60	9.77	51.59	37.04	14.94	36.0	303.80
	45	A	1.24	13.23	58.60	26.94	46.39	45.0	602.77
		B	1.12	13.00	60.24	25.65	72.83	45.0	585.62
	90	A	0.79	15.66	61.96	21.59	155.43	55.5	1051.71
		B	0.68	15.97	63.79	19.56	144.83	55.5	1034.68
1982	0	A	0.69	0.92	7.02	91.37	5.47	17.5	97.07
		B	0.67	0.80	7.15	91.38	5.70	17.5	98.78
	22.5	A	1.25	14.21	40.53	44.00	49.49	29.8	285.32
		B	1.29	14.72	38.80	45.19	62.98	29.8	282.73
	45	A	1.01	21.19	43.82	33.99	139.59	33.3	574.42
		B	1.00	20.90	44.63	33.46	129.15	33.3	594.01
	90	A	1.05	22.81	46.11	30.03	226.46	52.5	821.28
		B	0.96	22.53	46.36	30.14	253.83	52.5	858.75

†$Zn_S = Zn_{KNO_3} + Zn_{H_2O}$

‡Zn_T = sum of Zn extracted in fractions S, NaOH, EDTA, and HNO_3

TABLE II-12

VALUES OF Zn_F, Zn_{DTPA}, Zn_{leaf}, and Zn_T FOR DOMINO LOAM RECEIVING AMENDMENTS OF LIQUID SLUDGE

Year	Rate	Replicate	Zn_S †	Zn_{NaOH}	Zn_{EDTA}	Zn_{HNO_3}	Zn_{DTPA}	Zn_{leaf}	Zn_T ‡
	Mg ha^{-1} yr^{-1}		----------%----------				------mg kg^{-1}------		
1979	0	A	1.51	0.66	11.10	86.74	6.15	20.0	93.30
		B	1.22	0.51	9.55	88.71	5.25	20.0	89.49
	22.5	A	2.43	7.73	33.72	56.13	16.44	29.8	162.49
		B	2.46	4.03	29.07	64.44	29.37	29.8	131.47
	45	A	3.35	12.12	37.10	47.43	42.81	27.8	218.28
		B	3.03	12.25	38.12	46.59	52.78	27.8	225.72
	90	A	2.05	18.95	49.12	29.88	66.36	51.1	392.70
		B	2.16	16.75	48.45	32.65	62.87	51.1	357.28
1980	0	A	0.90	0.62	11.30	87.19	7.30	22.0	102.14
		B	0.85	1.11	13.97	84.06	6.49	22.0	104.81
	22.5	A	1.21	4.27	31.05	63.47	27.28	30.0	178.03
		B	1.18	4.32	30.29	64.21	24.61	30.0	186.04
	45	A	1.56	6.97	35.93	55.54	44.68	31.3	242.16
		B	1.63	7.70	37.24	53.43	49.87	31.3	255.81
	90	A	1.84	9.98	40.75	47.43	59.46	34.8	324.24
		B	1.66	9.55	38.09	50.69	56.46	34.8	338.72
1981	0	A	1.29	1.51	22.23	74.96	8.24	26.0	129.41
		B	1.86	1.40	23.11	73.63	7.41	26.0	126.84
	22.5	A	2.10	5.50	42.43	49.97	28.40	33.3	214.85
		B	1.83	5.54	41.78	50.86	28.08	33.3	219.64
	45	A	1.95	8.35	49.16	40.54	30.85	36.5	281.73
		B	2.22	8.59	44.91	44.28	37.36	36.5	264.01
	90	A	1.88	11.50	55.29	31.32	66.36	42.5	475.29
		B	2.30	11.57	53.30	32.84	59.25	42.5	439.94
1982	0	A	1.09	1.70	13.24	83.97	10.05	26.3	157.00
		B	1.26	2.19	14.39	82.16	8.59	26.3	118.67
	22.5	A	1.67	6.69	29.47	62.16	25.54	31.5	179.43
		B	1.64	6.76	30.04	61.56	21.29	31.5	182.76
	45	A	2.21	9.69	32.12	55.19	31.69	31.5	217.20
		B	2.09	9.98	32.53	55.40	33.54	31.5	216.39
	90	A	2.24	12.81	36.63	48.32	52.47	52.5	291.89
		B	2.18	13.59	35.91	48.32	69.16	52.5	285.76

†$Zn_S = Zn_{KNO_3} + Zn_{H_2O}$

‡Zn_T = sum of Zn extracted in fractions S, NaOH, EDTA, and HNO_3

TABLE II-13

LINEAR CORRELATIONS BETWEEN Zn_{leaf} (Y) AND % Zn IN EXTRACTED SOIL FRACTIONS (X) FOR SLUDGE-AMENDED SOILS †

Soil/Sludge	Years	Fraction	Regression Equation	N ‡	r^2
Domino/compost	1979-80,1982	NaOH	Y=22.22+0.582X	20	0.702**
Domino/compost	1981	NaOH	Y=23.15+1.744X	8	0.892**
Greenfield/compost	1979-80,1982	NaOH	Y=21.33+0.554X	20	0.652**
Greenfield/compost	1981	NaOH	Y=22.63+1.501X	8	0.986**
Domino/liquid	1979-82	NaOH	Y=21.56+1.549X	32	0.711**
Domino/compost	1979-80,1982	EDTA	Y=19.05+0.257X	22	0.496**
Domino/compost	1981	EDTA	Y=20.11+0.445X	8	0.755**
Greenfield/compost	1979-80,1982	EDTA	Y=21.36+0.256X	18	0.721**
Geenfield/compost	1981	EDTA	Y=17.11+0.715X	8	0.982**
Domino/liquid	1979-82	EDTA	Y=15.60+0.527X	28	0.865**
Domino/compost	1979-80,1982	HNO_3	Y=39.21-0.209X	22	0.659**
Domino/compost	1981	HNO_3	Y=56.15-0.364X	8	0.782**
Greenfield/compost	1979-80,1982	HNO_3	Y=41.37-0.227X	18	0.700**
Greenfield/compost	1981	HNO_3	Y=66.89-0.497X	8	0.996**
Domino/liquid	1979-82	HNO_3	Y=57.06-0.417X	26	0.936**

†Zn_{leaf} expressed as mg Zn/kg leaf (Y-variable)

‡number of data-pairs used in the regression analysis

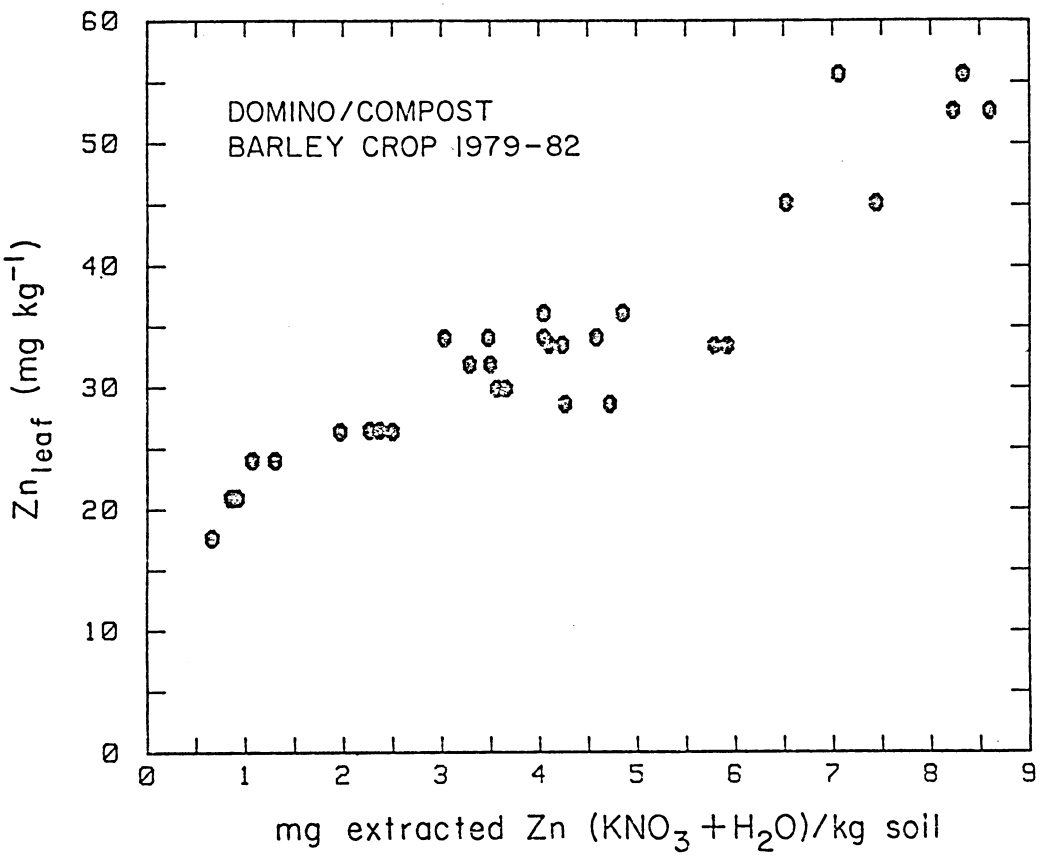


Figure II-2. Relationship between Zn_{leaf} in barley and Zn extracted in sequence by KNO_3 and H_2O from the Domino soil receiving composted sewage sludge.

This is an expression derived from the differential equation for metal uptake suggested by Bingham et al. (1982, Eq. (1)), with the following parameter values: A = 50.4 $\pm$ 9.3 mg kg^{-1} and k = 0.308 $\pm$ 0.124. (The $\pm$ figures represent the 95% confidence intervals for the estimates of A and k.) These results suggest that Zn_S can be associated with a highly labile Zn pool in the sludge-amended soils which is readily bioavailable.

Table II-13 shows the results of linear correlation analysis relating Zn_{leaf} to the percentage of Zn in the sequentially-extracted fractions, Zn_{NaOH}, Zn_{EDTA}, and Zn_{HNO_3}. Linear regression equations, the number of data-pairs used, and r^2-values developed in the analysis are listed in the fourth, fifth, and sixth columns of the table. Differences in the number of data-pairs used for the same soil/sludge combination and grouping of years (e.g., Domino/liquid, 1979-82) reflect the elimination of outliers in some cases using the method of Snedecor and Cochran (1980). All of the correlations, were statistically significant at P = 0.01. Correlations for the year 1981 were significantly different from those for the years 1979-80, 1982 in the case of the soils receiving composted sludge. Thus separate linear regression equations were established, as shown in Table II-13. The reason for this difference evidently is connected with either the form of sludge applied or the fact that application was continual, since the plots receiving liquid sludge during the limited period 1976-78 did not produce an anomaly in 1981. It is possible that the sharp increase in Zn concentration in the

TABLE II-14

AVERAGE CONTENT[+] OF ZINC IN THE APPLIED SLUDGES

Composted Sludge Year	Zn	Liquid Sludge Year	Zn
	mg kg^{-1}		mg kg^{-1}
1975	2,005	1976	4,423
1976	2,070	1977	3,954
1977	2,966	1978	3,562
1978	3,035		
1979	5,885		
1980	4,732		
1981	4,095		
1982	2,911		

[+]as determined by atomic absorption spectrophotometry after digestion with perchloric acid

TABLE II-15

PROPOSED RELATIONSHIPS BETWEEN SEQUENTIALLY-EXTRACTED ZINC FRACTIONS AND ZINC BIOAVAILABILITY

Extracted Fraction	Chemical form in soil		Lability/bioavailability category	
$Zn_{KNO_3+H_2O}$	Water - soluble Zn	Zn_{DTPA}	Labile	bioavailable
Zn_{NaOH}	Organic Zn		Labile	
Zn_{EDTA}	Inorganic Zn precipitates		Quasilabile	
Zn_{HNO_3}	Resistant-mineral Zn		Nonlabile	

composted sludge during 1979-80 (Table II-14) played some role, but the lack of unusual behavior in Zn_{leaf} in 1982 suggests no consistent effect. The pH values of the soils in the plots--another possible source of lack of unusual behavior in Zn_{leaf} in 1982 suggests no consistent effect. The pH values of the soils in the plots--another possible source of influence on Zn_{leaf}--showed no significant departure in 1981 from previous trends (Chang et al., 1983).

The correlation between Zn_{leaf} and Zn_{NaOH} was positive and, for a given grouping of years, independent of the soil series. The slope of the regression line for the Domino soil was dependent on the form of sludge applied, being larger for the liquid sludge (except in 1981). The y-intercepts were independent of soil, sludge form, and grouping of years. The correlation between Zn_{leaf} and Zn_{EDTA} also was positive and showed trends similar to those for Zn_{NaOH}, except that the r^2-values usually were smaller. The correlation between Zn_{leaf} and Zn_{HNO_3} was negative and showed trends with soil, sludge form, and grouping of years like those for the other two extracted fractions.

The interpretation of the results in Table II-13 can be facilitated by examining the relation between the sequentially-extracted Zn fractions and DTPA-extractable Zn. Figure II-3 shows a plot of the total amounts of Zn extracted sequentially by KNO_3, H_2O, and NaOH against Zn_{DTPA}, based on the data in columns 4, 5, 8, and 10 in Tables II-10 to II-12. These data include all soil/sludge combinations for all four years, except for three points classified as outliers by the test of Snedecor and Cochran (1980). The least-squares regression line through the data points obeyed the equation:

$$Zn_{Ext} = -3.15 + 1.01\ Zn_{DTPA}\ (r^2 = 0.912^{**}) \qquad \text{(II-5)}$$

where $Zn_{Ext} = (Zn_S + Zn_{NaOH})Zn_T/100$. The y-intercept of this line is not significantly different from 0 at P=0.05. Equation (II-5) provides strong statistical evidence that the labile pool of Zn in the sludge-amended soils, as measured by Zn_{DTPA}, is congruent with the Zn extracted sequentially with KNO_3, H_2O, and NaOH.

The negative correlation between Zn_{leaf} and Zn_{HNO_3} indicates, as expected, that the Zn extracted at the end of the fractionation sequence by HNO_3 does not belong to the labile, plant-available pool of Zn in the sludge-amended soils. On the other end of the spectrum, the strong, positive correlations in Fig. II-3 and in the first five rows of Table II-13 indicate that the first three steps of the fractionation sequence extract the labile, plant-available Zn in the soils. These conclusions are illustrated in summary form in Table II-15. The position of the EDTA-extractable fraction appears to be borderline in respect to labile Zn. A statistically-significant positive linear correlation between Zn_{leaf} and Zn_{EDTA} exists, but the slope of the correlation is not as large as for Zn_{NaOH}, and EDTA-extractable Zn is not necessary to achieve a 1:1 relation between the partial sum of sequentially-extracted fractions, $(ZN_S + Zn_{NaOH})$, and Zn_{DTPA}. In Sec.II.C. it was suggested that EDTA-extractable trace metals result in part from a gradual process of inorganic precipitate formation (principally carbonates) that accompanies the decomposition of sewage sludge in arid-zone soils. On this

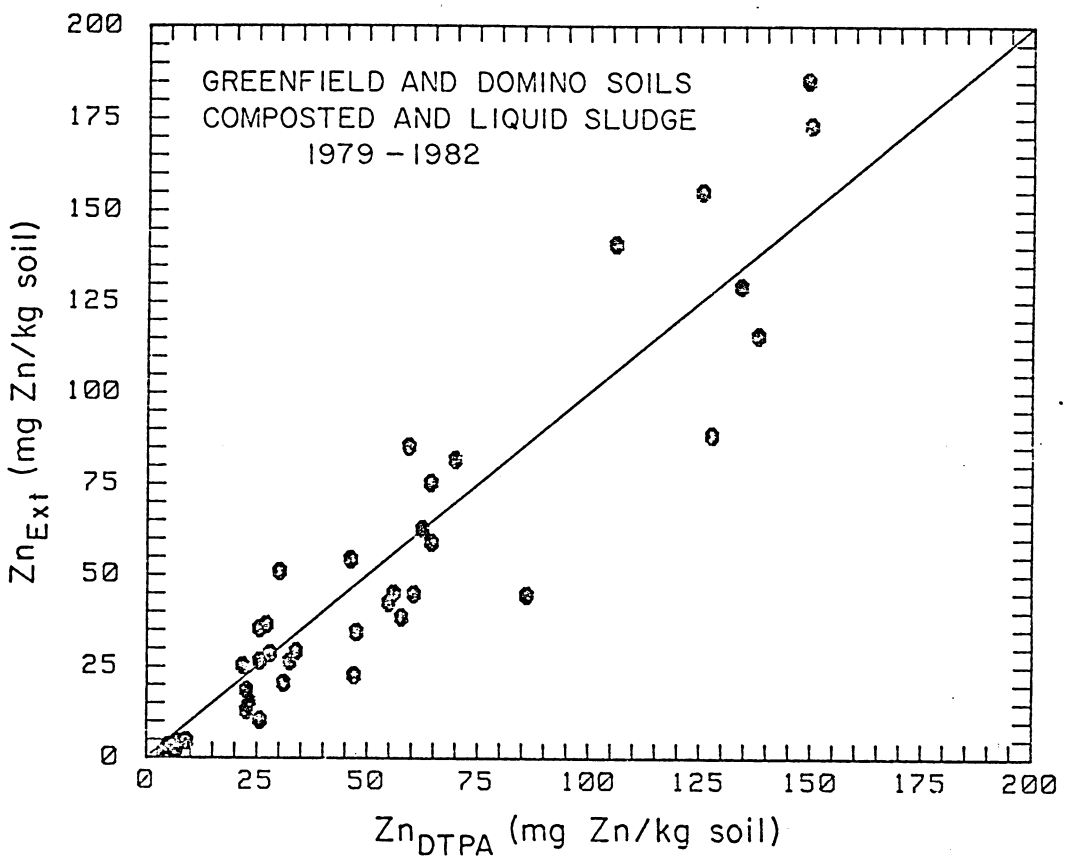


Figure II-3. Relationship between Zn extracted in sequence by KNO_3, H_2O, and NaOH and DTPA-extractable Zn for the Greenfield and Domino soils, including both types of sewage sludge at all four application rates during 1979-1982.

view, Zn_{EDTA} would represent a reservoir of potentially bioavailable, inorganic Zn that accumulates in soil with time.

The following conclusions can be drawn: (1) Zinc extracted by KNO_3 and H_2O in sequence is associated with the highly labile, soluble pool dominated by Zn^{2+}, which determines immediate bioavailability. (2) Zinc extracted by KNO_3, H_2O, and NaOH in sequence has a strong, positive, 1:1 correlation with DTPA-extractable Zn and, therefore, is associated with the labile, plant-available Zn pool. (3) EDTA-extractable Zn represents a quasilabile reservoir of potentially bioavailable Zn and HNO_3-extractable Zn is associated with a nonlabile pool that is not bioavailable. These general relationships between extracted Zn fractions and bioavailability were applicable to both soils investigated and did not depend on the type of sludge applied or the length of time of sludge application.

E. Appendix: Laboratory Data on Extracted Metal Fractions

The following 15 tables contain the complete set of duplicate laboratory data pertaining to the sequentially extracted fractions of

Ni, Cu, Zn, Cd, and Pb in the domino soil receiving either composted or liquid sludge and in the Greenfield soil receiving composted sludge amendments. Each table is labeled according to the format: METAL/SOIL/FORM OF SLUDGE. The columns in each table are headed uniformly in the format:

Column 1: Soil Sample Number ("Obs #")
Column 2: Year of Soil Sampling
Column 3: Sludge Application Rate, in megagrams per hectare per year
Column 4: KNO_3-Extractable Metal
Column 5: Water-Extractable Metal
Column 6: Sum of Columns 4 and 5
Column 7: NaOH-Extractable Metal
Column 8: EDTA-Extractable Metal
Column 9: HNO_3-Extractable Metal
Column 10: Sum of Columns 6, 7, 8, and 9
Column 11: Metal Extracted in a Single Extraction with 4M HNO_3

The units of the data in Columns 4 through 11 are milligrams of metal extracted per kilogram of dry soil.

These tabulated data appear to be the only set of sequential extraction data that includes both time and soil series as variables. They should provide a base for many future statistical modeling studies of trace metal chemistry in sludge-amended soils.

TABLE II-A-1

NICKEL/DOMINO/COMPOSTED

OBS#										
1	1979.00	0.00	0.00	.26	.26	.69	1.17	7.76	9.87	8.25
2	1979.00	0.00	.25	.25	.50	.70	1.17	7.86	10.22	8.25
3	1979.00	22.50	.25	.43	.67	3.95	4.04	12.65	21.30	19.69
4	1979.00	22.50	.12	.45	.57	4.13	5.38	11.97	22.05	19.69
5	1979.00	45.00	.36	.46	.82	7.36	11.87	14.29	34.34	32.25
6	1979.00	45.00	.25	.46	.71	7.09	11.94	15.05	34.79	32.25
7	1979.00	90.00	.85	.73	1.58	16.87	18.00	29.97	66.41	58.50
8	1979.00	90.00	.85	.68	1.53	15.45	18.62	28.88	64.47	58.50
9	1980.00	0.00	0.00	.11	.11	.83	.36	8.61	9.90	11.72
10	1980.00	0.00	.12	.15	.27	.70	.47	8.38	9.81	11.72
11	1980.00	22.50	.17	.27	.44	4.62	6.41	18.54	29.99	31.89
12	1980.00	22.50	.17	.32	.49	4.32	6.06	18.16	29.03	31.89
13	1980.00	45.00	.50	.46	.96	10.14	11.44	30.65	53.18	46.04
14	1980.00	45.00	.43	.37	.80	10.34	11.79	30.89	53.81	46.04
15	1980.00	90.00	1.21	.73	1.94	21.78	25.66	35.93	85.31	84.92
16	1980.00	90.00	.96	.64	1.59	21.05	24.80	45.83	93.27	84.92
17	1981.00	0.00	.23	0.00	.23	.71	.98	7.74	9.66	7.44
18	1981.00	0.00	.36	.07	.43	.66	.48	7.06	8.63	7.44
19	1981.00	22.50	.38	.47	.85	5.05	9.18	16.00	31.07	27.25
20	1981.00	22.50	.29	.29	.58	4.36	10.44	14.14	29.51	27.25
21	1981.00	45.00	.94	.70	1.64	11.72	16.91	29.97	60.24	59.06
22	1981.00	45.00	.94	.29	1.23	10.99	18.91	27.17	58.30	59.06
23	1981.00	90.00	1.65	1.94	3.59	20.25	30.15	47.01	101.00	86.62
24	1981.00	90.00	1.46	1.40	2.86	20.86	28.48	48.56	100.77	86.62
25	1982.00	0.00	0.00	2.42	2.42	0.00	1.03	8.87	12.33	10.43
26	1982.00	0.00	0.00	2.74	2.74	0.00	1.25	8.75	12.74	10.43
27	1982.00	22.50	.23	2.28	2.51	4.70	11.65	18.57	37.42	36.74
28	1982.00	22.50	.12	2.45	2.57	4.76	9.76	20.19	37.27	36.74
29	1982.00	45.00	.85	3.17	4.02	16.34	21.33	39.95	81.63	85.13
30	1982.00	45.00	.94	3.11	4.04	17.12	21.93	40.57	83.65	85.13
31	1982.00	90.00	2.25	3.41	5.66	27.31	33.66	56.31	122.93	65.25
32	1982.00	90.00	2.06	3.29	5.34	26.41	34.17	57.58	123.50	65.25

TABLE II-A-2

NICKEL/DOMINO/LIQUID

OBS#										
1	1979.00	0.00	0.00	.27	.27	1.19	1.40	8.38	11.23	10.20
2	1979.00	0.00	0.00	.15	.15	1.40	.70	8.77	11.03	10.20
3	1979.00	22.50	.25	.26	.50	4.99	6.80	10.53	22.81	21.57
4	1979.00	22.50	.73	1.04	1.77	4.34	7.30	9.86	23.27	21.57
5	1979.00	45.00	.73	.37	1.10	7.79	6.97	14.35	30.21	27.87
6	1979.00	45.00	.73	.25	.98	7.67	7.16	15.23	31.04	27.87
7	1979.00	90.00	.85	.26	1.11	14.58	15.19	16.96	47.83	41.75
8	1979.00	90.00	.73	3.06	3.79	13.76	13.28	17.46	48.30	41.75
9	1980.00	0.00	.08	.17	.24	1.37	.88	9.29	11.77	13.75
10	1980.00	0.00	0.00	.06	.06	1.30	1.01	9.29	11.65	13.75
11	1980.00	22.50	.14	.23	.36	4.10	3.62	14.38	22.47	24.44
12	1980.00	22.50	.06	.21	.27	4.17	3.78	15.52	23.73	24.44
13	1980.00	45.00	.43	.25	.68	6.52	5.28	18.18	30.66	30.60
14	1980.00	45.00	.43	.34	.77	6.80	5.95	19.22	32.73	30.60
15	1980.00	90.00	.90	.55	1.45	9.89	8.87	22.41	42.61	42.40
16	1980.00	90.00	.86	.36	1.23	9.68	7.30	24.00	42.20	42.40
17	1981.00	0.00	.05	.34	.39	1.20	2.74	8.24	12.56	10.44
18	1981.00	0.00	.15	.40	.54	1.27	2.57	7.80	12.17	10.44
19	1981.00	22.50	.36	1.01	1.37	4.21	4.84	11.62	22.04	23.44
20	1981.00	22.50	.47	.09	.56	4.69	5.62	11.53	22.39	23.44
21	1981.00	45.00	.83	.13	.96	6.37	7.87	12.61	27.80	24.87
22	1981.00	45.00	.84	.07	.91	6.65	7.20	12.80	27.55	24.87
23	1981.00	90.00	.93	.68	1.60	8.18	12.73	21.30	43.80	30.44
24	1981.00	90.00	.86	.48	1.35	8.97	10.74	20.22	41.28	30.44
25	1982.00	0.00	.04	1.47	1.51	0.00	1.19	5.29	7.98	14.82
26	1982.00	0.00	.14	1.44	1.58	0.00	1.31	5.37	8.26	14.82
27	1982.00	22.50	.09	2.85	2.93	2.66	5.38	14.21	25.19	23.43
28	1982.00	22.50	.06	2.84	2.90	2.63	6.51	13.34	25.37	23.43
29	1982.00	45.00	.28	3.29	3.57	4.42	6.42	15.45	29.86	27.23
30	1982.00	45.00	.34	3.27	3.61	4.67	6.18	15.95	30.40	27.23
31	1982.00	90.00	.57	1.01	1.58	5.35	10.13	19.17	36.23	40.02
32	1982.00	90.00	.61	.62	1.23	5.76	9.01	19.00	34.98	40.02

TABLE II-A-3

NICKEL/GREENFIELD/COMPOSTED

OBS#										
1	1979.00	0.00	0.00	.18	.18	.62	.50	5.37	6.66	6.30
2	1979.00	0.00	0.00	.45	.45	.51	.65	5.32	6.91	6.30
3	1979.00	22.50	0.00	.29	.29	4.23	5.83	7.68	18.02	15.75
4	1979.00	22.50	0.00	.56	.56	4.80	2.91	11.00	19.26	15.75
5	1979.00	45.00	0.00	.27	.27	7.43	5.48	14.24	27.42	23.50
6	1979.00	45.00	0.00	.27	.27	7.99	5.49	13.51	27.25	23.50
7	1979.00	90.00	.36	.55	.92	16.80	16.89	22.01	56.63	44.75
8	1979.00	90.00	.25	.42	.66	16.26	11.72	26.85	55.48	44.75
9	1980.00	0.00	.01	.12	.13	.82	.50	5.01	6.46	7.44
10	1980.00	0.00	0.00	.15	.15	.68	.50	4.78	6.11	7.44
11	1980.00	22.50	.54	.21	.75	4.82	4.72	11.87	22.15	24.05
12	1980.00	22.50	.53	.28	.80	4.83	6.71	10.38	22.72	24.05
13	1980.00	45.00	.84	.36	1.20	9.29	11.67	18.57	40.73	40.10
14	1980.00	45.00	.83	.38	1.21	9.52	11.03	18.28	40.03	40.10
15	1980.00	90.00	1.23	.48	1.71	15.32	18.32	30.49	65.84	92.17
16	1980.00	90.00	1.20	.59	1.79	20.14	20.14	41.00	83.06	92.17
17	1981.00	0.00	0.00	.17	.17	.38	1.08	4.43	6.07	5.56
18	1981.00	0.00	.06	.14	.20	.46	1.19	4.63	6.47	5.56
19	1981.00	22.50	.54	1.42	1.95	3.62	7.91	7.92	21.39	23.75
20	1981.00	22.50	.47	.71	1.17	3.25	8.40	7.89	20.70	23.75
21	1981.00	45.00	1.22	.34	1.55	7.22	15.51	12.10	36.38	34.38
22	1981.00	45.00	1.06	.16	1.22	6.63	13.07	13.81	34.73	34.38
23	1981.00	90.00	2.16	.46	2.61	13.46	24.45	25.65	66.16	67.75
24	1981.00	90.00	2.24	.43	2.67	13.56	23.62	28.75	68.59	67.75
25	1982.00	0.00	.03	1.57	1.59	0.00	1.19	5.29	8.06	7.89
26	1982.00	0.00	.14	1.76	1.90	0.00	1.31	5.37	8.58	7.89
27	1982.00	22.50	.44	1.13	1.57	3.85	11.88	11.18	28.47	34.11
28	1982.00	22.50	.47	1.51	1.98	4.02	11.11	11.06	28.18	34.11
29	1982.00	45.00	1.24	1.10	2.34	9.52	14.74	20.73	47.32	48.52
30	1982.00	45.00	1.27	1.46	2.73	8.87	18.35	18.94	48.89	48.52
31	1982.00	90.00	1.89	2.03	3.92	17.69	27.86	38.20	87.67	95.50
32	1982.00	90.00	1.88	1.43	3.31	16.81	28.45	35.35	83.91	95.50

TABLE II-A-4

COPPER/DOMINO/COMPOSTED

OBS#										
1	1979.00	0.00	.12	1.04	1.16	6.40	2.86	12.73	23.13	17.40
2	1979.00	0.00	.12	.95	1.07	6.00	13.00	2.90	22.97	13.21
3	1979.00	22.50	.25	1.76	2.00	23.20	11.35	15.50	52.05	46.82
4	1979.00	22.50	.25	1.79	2.04	24.84	12.37	16.14	55.37	46.82
5	1979.00	45.00	.36	2.28	2.65	42.44	22.47	17.47	85.02	74.32
6	1979.00	45.00	.36	2.12	2.49	41.63	24.10	18.04	86.25	74.32
7	1979.00	90.00	.73	3.98	4.71	85.35	44.28	25.50	159.84	141.00
8	1979.00	90.00	.73	3.25	3.97	81.73	45.82	26.50	158.01	141.00
9	1980.00	0.00	.15	1.00	1.14	5.77	2.77	15.32	24.98	24.62
10	1980.00	0.00	.16	.75	.91	5.10	2.91	13.87	22.79	24.62
11	1980.00	22.50	.25	2.42	2.67	30.44	21.84	23.26	78.21	75.83
12	1980.00	22.50	.26	2.13	2.38	29.29	19.84	21.42	72.93	75.83
13	1980.00	45.00	.83	4.97	5.81	55.86	36.66	30.85	129.18	119.42
14	1980.00	45.00	.61	2.75	3.36	55.92	37.12	31.02	127.41	119.42
15	1980.00	90.00	.95	5.25	6.21	112.00	68.11	32.23	218.54	227.58
16	1980.00	90.00	1.02	4.32	5.34	110.43	65.03	44.40	225.20	227.58
17	1981.00	0.00	.84	2.37	3.21	10.96	4.04	11.91	30.11	15.12
18	1981.00	0.00	.97	1.50	2.47	4.71	3.84	10.84	21.86	15.12
19	1981.00	22.50	.76	2.35	3.10	27.72	25.68	17.89	74.39	52.88
20	1981.00	22.50	.73	3.23	3.96	30.78	24.91	16.35	75.99	52.88
21	1981.00	45.00	1.03	5.73	6.76	57.62	51.05	27.09	142.52	117.69
22	1981.00	45.00	1.11	4.70	5.81	56.88	49.97	24.42	137.07	117.69
23	1981.00	90.00	1.61	8.49	10.09	97.73	85.66	38.56	232.04	173.50
24	1981.00	90.00	1.62	6.42	8.04	97.54	87.05	37.83	230.45	173.50
25	1982.00	0.00	0.00	3.05	3.05	6.89	4.20	13.54	27.68	23.51
26	1982.00	0.00	0.00	3.09	3.09	6.62	4.29	13.69	27.69	23.51
27	1982.00	22.50	0.00	4.43	4.43	37.90	30.02	21.57	93.92	36.00
28	1982.00	22.50	0.00	4.68	4.68	36.66	29.99	22.13	93.46	36.00
29	1982.00	45.00	0.00	6.31	6.31	84.96	63.68	39.70	194.65	104.69
30	1982.00	45.00	0.00	6.42	6.42	87.64	65.81	40.63	200.49	104.69
31	1982.00	90.00	.09	9.42	9.51	87.64	128.78	84.15	310.07	53.19
32	1982.00	90.00	0.00	9.31	9.31	123.52	96.75	55.40	284.98	76.81

TABLE II-A-5

COPPER/DOMINO/LIQUID

OBS#										
1	1979.00	0.00	.12	.83	.95	8.88	3.21	13.71	26.73	21.00
2	1979.00	0.00	.12	.76	.88	8.10	3.02	13.31	25.30	21.00
3	1979.00	22.50	.12	1.21	1.33	22.73	10.34	15.56	49.95	46.69
4	1979.00	22.50	.73	4.48	5.21	20.53	10.33	14.71	50.77	46.69
5	1979.00	45.00	.25	1.43	1.68	32.00	13.81	17.23	64.72	63.75
6	1979.00	45.00	.25	1.29	1.54	33.01	14.39	17.27	66.21	63.75
7	1979.00	90.00	.49	1.70	2.19	62.99	22.70	19.01	106.88	97.75
8	1979.00	90.00	.49	1.32	1.81	55.04	19.63	18.36	94.83	97.75
9	1980.00	0.00	.36	2.70	3.06	7.81	3.58	14.67	29.11	26.46
10	1980.00	0.00	.15	1.55	1.69	7.75	4.61	14.24	28.28	26.46
11	1980.00	22.50	.18	.93	1.12	20.72	11.62	17.93	51.37	55.75
12	1980.00	22.50	.25	.93	1.18	21.72	12.14	18.81	53.84	55.75
13	1980.00	45.00	.25	.99	1.24	31.75	15.39	19.71	68.09	66.92
14	1980.00	45.00	.20	1.38	1.57	36.98	17.94	21.00	77.49	66.92
15	1980.00	90.00	.29	5.47	5.76	48.89	22.16	20.59	97.40	90.88
16	1980.00	90.00	.15	3.14	3.29	47.56	21.43	22.56	94.84	90.88
17	1981.00	0.00	.57	1.52	2.09	7.29	6.93	12.34	28.65	22.63
18	1981.00	0.00	1.18	1.19	2.37	11.00	6.40	11.48	31.25	22.63
19	1981.00	22.50	1.40	2.05	3.45	18.46	13.82	13.66	49.39	48.13
20	1981.00	22.50	1.07	5.72	6.79	19.30	14.17	15.37	55.63	48.13
21	1981.00	45.00	1.13	1.75	2.88	25.41	17.44	14.34	60.06	44.81
22	1981.00	45.00	1.34	1.84	3.18	26.01	15.83	14.39	59.40	44.81
23	1981.00	90.00	.96	4.18	5.14	43.80	32.59	22.02	103.54	58.88
24	1981.00	90.00	.83	2.78	3.61	45.01	28.27	18.02	94.90	58.88
25	1982.00	0.00	0.00	3.02	3.02	11.52	8.67	19.88	43.08	34.25
26	1982.00	0.00	0.00	2.95	2.95	9.12	6.38	15.55	34.00	34.25
27	1982.00	22.50	0.00	3.29	3.29	24.78	14.73	17.09	59.88	22.50
28	1982.00	22.50	0.00	3.65	3.65	23.50	37.50	14.45	79.10	16.95
29	1982.00	45.00	0.00	1.73	1.73	28.23	15.94	17.11	63.00	24.81
30	1982.00	45.00	0.00	1.79	1.79	29.10	15.80	17.58	64.27	24.81
31	1982.00	90.00	0.00	3.00	3.00	38.71	22.59	21.17	85.46	91.25
32	1982.00	90.00	0.00	2.90	2.90	38.49	21.05	20.20	82.63	91.25

TABLE II-A-6

COPPER/GREENFIELD/COMPOSTED

OBS#										
1	1979.00	0.00	.12	.64	.76	4.12	1.21	9.88	15.95	13.50
2	1979.00	0.00	.12	.59	.71	3.95	.86	10.67	16.18	13.50
3	1979.00	22.50	.25	.81	1.05	19.66	8.71	11.35	40.77	36.25
4	1979.00	22.50	.25	.85	1.10	21.78	8.71	13.48	45.06	36.25
5	1979.00	45.00	.49	1.08	1.57	33.14	14.64	15.17	64.51	57.88
6	1979.00	45.00	.49	1.16	1.65	33.95	14.35	13.89	63.84	57.88
7	1979.00	90.00	.85	1.98	2.82	71.98	36.37	19.82	130.99	114.00
8	1979.00	90.00	.85	1.90	2.75	71.01	34.15	23.05	130.96	114.00
9	1980.00	0.00	.08	.90	.97	2.96	1.17	9.92	15.02	15.12
10	1980.00	0.00	.03	.78	.80	3.22	1.12	9.88	15.02	15.12
11	1980.00	22.50	.09	.86	.95	23.03	12.61	15.80	52.39	55.75
12	1980.00	22.50	.03	.78	.81	23.12	13.96	15.42	53.30	55.75
13	1980.00	45.00	.08	1.54	1.62	41.87	26.80	23.41	93.69	88.21
14	1980.00	45.00	.15	1.25	1.39	41.99	25.14	22.60	91.12	88.21
15	1980.00	90.00	.54	2.97	3.51	72.15	46.26	31.99	153.90	253.17
16	1980.00	90.00	.51	2.90	3.41	95.71	50.44	43.60	193.15	253.17
17	1981.00	0.00	.76	.96	1.71	2.15	1.71	8.48	14.04	12.69
18	1981.00	0.00	.58	.79	1.37	2.52	2.37	8.38	14.63	12.69
19	1981.00	22.50	.65	3.71	4.35	19.39	13.43	11.35	48.52	45.50
20	1981.00	22.50	.92	2.13	3.04	21.85	15.82	11.25	51.95	45.50
21	1981.00	45.00	1.31	2.29	3.59	34.51	28.78	15.19	82.07	65.12
22	1981.00	45.00	1.21	2.26	3.47	32.24	27.87	14.97	78.53	65.12
23	1981.00	90.00	1.54	3.67	5.22	71.48	16.42	23.80	116.92	131.87
24	1981.00	90.00	2.04	3.75	5.79	74.86	16.63	26.86	124.13	131.87
25	1982.00	0.00	0.00	2.59	2.59	3.35	2.13	10.74	18.80	19.09
26	1982.00	0.00	0.00	4.68	4.68	3.49	2.45	11.09	21.70	19.09
27	1982.00	22.50	0.00	3.42	3.42	29.22	20.23	17.24	70.11	30.81
28	1982.00	22.50	0.00	3.41	3.41	27.31	19.54	16.72	66.97	30.81
29	1982.00	45.00	0.00	3.98	3.98	52.16	32.31	22.29	110.73	79.00
30	1982.00	45.00	0.00	4.24	4.24	50.84	36.17	22.89	114.13	79.00
31	1982.00	90.00	0.00	5.05	5.05	91.80	70.48	35.11	202.44	120.38
32	1982.00	90.00	0.00	5.24	5.24	91.35	68.39	34.18	199.15	120.38

TABLE II-A-7

ZINC/DOMINO/COMPOSTED

OBS#										
1	1979.00	0.00	0.00	1.31	1.31	0.00	5.57	75.00	81.88	76.00
2	1979.00	0.00	0.00	1.08	1.08	0.00	5.45	77.05	83.57	76.00
3	1979.00	22.50	.36	3.12	3.48	14.58	54.70	94.07	166.82	167.94
4	1979.00	22.50	.25	2.79	3.03	15.29	61.98	92.30	172.60	167.94
5	1979.00	45.00	.73	4.00	4.72	37.89	123.17	101.25	267.02	293.44
6	1979.00	45.00	.49	3.79	4.27	37.14	125.77	103.15	270.33	293.44
7	1979.00	90.00	.85	3.74	4.59	135.75	243.99	161.51	545.83	468.50
8	1979.00	90.00	.85	3.20	4.05	123.00	246.18	158.77	531.99	468.50
9	1980.00	0.00	.28	.65	.93	.35	55.20	84.99	141.46	91.25
10	1980.00	0.00	.21	.65	.85	.06	54.64	80.15	135.70	91.25
11	1980.00	22.50	.83	1.44	2.27	18.46	98.78	127.36	246.87	223.75
12	1980.00	22.50	.55	1.82	2.37	17.07	90.29	128.30	238.02	223.75
13	1980.00	45.00	1.20	2.31	3.50	41.27	160.82	133.29	338.87	338.75
14	1980.00	45.00	1.11	2.17	3.29	40.47	167.65	117.49	328.90	338.75
15	1980.00	90.00	2.16	1.96	4.11	108.30	369.26	213.01	694.67	600.00
16	1980.00	90.00	2.21	2.03	4.24	105.11	339.32	207.51	656.17	600.00
17	1981.00	0.00	.61	1.89	2.50	.73	12.09	91.94	107.26	83.13
18	1981.00	0.00	.36	1.62	1.97	.48	9.40	87.51	99.36	83.13
19	1981.00	22.50	.92	3.14	4.05	33.60	172.64	114.35	324.63	251.25
20	1981.00	22.50	.95	3.91	4.86	29.68	156.73	112.54	303.80	251.25
21	1981.00	45.00	2.06	5.38	7.45	79.76	353.20	162.37	602.77	500.00
22	1981.00	45.00	2.28	4.25	6.53	76.12	352.75	150.22	585.62	500.00
23	1981.00	90.00	3.69	4.64	8.34	164.70	651.65	227.03	1051.71	710.00
24	1981.00	90.00	3.42	3.65	7.07	165.28	659.98	202.36	1034.68	710.00
25	1982.00	0.00	0.00	.67	.67	.90	6.82	88.69	97.07	101.44
26	1982.00	0.00	0.00	.67	.67	.79	7.06	90.27	98.78	101.44
27	1982.00	22.50	1.04	2.53	3.57	40.55	115.65	125.55	285.32	128.12
28	1982.00	22.50	1.03	2.62	3.65	41.61	109.71	127.76	282.73	128.12
29	1982.00	45.00	2.62	3.18	5.80	121.70	251.70	195.22	574.42	331.25
30	1982.00	45.00	3.06	2.88	5.93	124.18	265.12	198.79	594.01	331.25
31	1982.00	90.00	5.09	3.51	8.61	187.30	378.72	246.66	821.28	247.50
32	1982.00	90.00	4.69	3.55	8.24	193.51	398.15	258.86	858.75	247.50

TABLE II-A-8

ZINC/DOMINO/LIQUID

OBS#										
1	1979.00	0.00	.12	1.79	1.91	.61	10.36	80.93	93.81	91.10
2	1979.00	0.00	0.00	1.46	1.46	.46	8.55	79.39	89.85	91.10
3	1979.00	22.50	.85	4.27	5.12	12.55	54.79	91.20	163.66	169.12
4	1979.00	22.50	1.95	2.45	4.40	5.29	38.22	84.73	132.64	169.12
5	1979.00	45.00	2.92	6.21	9.13	26.45	80.98	103.54	220.10	232.50
6	1979.00	45.00	2.92	5.74	8.66	27.66	86.05	105.17	227.54	232.50
7	1979.00	90.00	4.75	4.50	9.25	74.41	192.89	117.35	393.90	360.25
8	1979.00	90.00	4.51	4.73	9.23	59.84	173.09	116.66	358.82	360.25
9	1980.00	0.00	.22	1.23	1.45	.63	11.54	89.05	102.67	96.25
10	1980.00	0.00	.15	1.34	1.48	1.17	14.64	88.10	105.39	96.25
11	1980.00	22.50	.70	2.72	3.42	7.61	55.27	112.99	179.29	174.17
12	1980.00	22.50	.77	2.66	3.42	8.04	56.36	119.46	187.27	174.17
13	1980.00	45.00	1.97	3.52	5.49	16.89	87.01	134.49	243.87	216.67
14	1980.00	45.00	2.11	3.99	6.10	19.70	95.25	136.68	257.72	216.67
15	1980.00	90.00	3.48	4.77	8.25	32.37	132.12	153.77	326.51	290.42
16	1980.00	90.00	3.25	4.56	7.81	32.36	129.04	171.70	340.90	290.42
17	1981.00	0.00	.26	1.98	2.23	1.96	28.77	97.01	129.96	128.75
18	1981.00	0.00	.49	2.49	2.98	1.78	29.31	93.39	127.46	128.75
19	1981.00	22.50	.86	4.94	5.81	11.83	91.15	107.36	216.14	227.50
20	1981.00	22.50	1.44	3.39	4.83	12.16	91.76	111.71	220.45	227.50
21	1981.00	45.00	2.86	4.05	6.92	23.54	138.50	114.21	283.16	228.75
22	1981.00	45.00	2.75	4.41	7.16	22.68	118.56	116.91	265.30	228.75
23	1981.00	90.00	5.33	5.72	11.05	54.68	262.79	148.88	477.39	304.38
24	1981.00	90.00	6.41	5.63	12.03	50.88	234.47	144.46	441.85	304.38
25	1982.00	0.00	.27	2.17	2.44	2.67	20.79	131.84	157.74	131.69
26	1982.00	0.00	.22	2.00	2.22	2.60	17.07	97.50	119.39	131.69
27	1982.00	22.50	.76	3.06	3.82	12.01	52.89	111.53	180.24	85.00
28	1982.00	22.50	.36	3.56	3.92	12.36	54.91	112.51	183.69	85.00
29	1982.00	45.00	1.73	4.15	5.88	21.04	69.76	121.61	218.27	186.25
30	1982.00	45.00	1.82	3.98	5.81	21.59	70.40	119.87	217.66	186.25
31	1982.00	90.00	3.46	4.54	8.00	37.40	106.91	141.04	293.35	340.63
32	1982.00	90.00	3.33	4.39	7.71	38.83	102.63	138.07	287.24	340.63

TABLE II-A-9

ZINC/GREENFIELD/COMPOSTED

OBS#										
1	1979.00	0.00	0.00	1.09	1.09	.30	4.12	57.46	62.97	66.15
2	1979.00	0.00	0.00	.95	.95	0.00	4.44	57.89	63.28	66.15
3	1979.00	22.50	.73	2.33	3.06	19.88	47.20	67.67	137.80	138.37
4	1979.00	22.50	.61	2.08	2.69	24.00	38.88	83.12	148.69	138.37
5	1979.00	45.00	.85	2.26	3.11	50.89	66.11	90.71	210.82	209.00
6	1979.00	45.00	.73	2.14	2.87	50.96	66.84	88.18	208.84	209.00
7	1979.00	90.00	1.46	2.77	4.23	139.61	207.26	110.61	461.70	375.75
8	1979.00	90.00	1.34	2.73	4.06	132.72	184.62	133.84	455.24	375.75
9	1980.00	0.00	.12	.65	.77	.23	37.30	54.79	93.09	61.25
10	1980.00	0.00	0.00	.50	.50	.09	37.96	54.54	93.09	61.25
11	1980.00	22.50	.85	1.40	2.25	24.47	61.82	85.95	174.48	171.25
12	1980.00	22.50	.54	1.25	1.79	23.78	71.24	80.19	176.98	171.25
13	1980.00	45.00	1.44	1.45	2.88	57.59	123.87	110.37	294.71	255.00
14	1980.00	45.00	1.31	1.20	2.51	54.32	115.32	109.61	281.75	255.00
15	1980.00	90.00	1.51	1.54	3.04	106.59	205.24	155.64	470.51	673.75
16	1980.00	90.00	1.62	1.88	3.50	117.48	286.23	145.83	553.03	673.75
17	1981.00	0.00	.43	1.73	2.16	.46	5.17	66.73	74.51	88.13
18	1981.00	0.00	.32	1.36	1.67	.50	6.35	69.09	77.61	88.13
19	1981.00	22.50	1.94	3.05	4.99	28.72	90.31	82.37	206.38	224.38
20	1981.00	22.50	1.95	2.47	4.42	31.89	96.97	81.22	214.49	224.38
21	1981.00	45.00	4.12	3.50	7.62	75.47	181.07	86.18	350.33	294.37
22	1981.00	45.00	4.17	2.22	6.38	73.28	152.43	92.96	325.05	294.37
23	1981.00	90.00	4.62	3.05	7.66	146.09	365.47	126.63	645.84	536.87
24	1981.00	90.00	4.75	3.14	7.89	147.58	345.20	140.18	640.84	536.87
25	1982.00	0.00	.11	.61	.72	.95	3.84	65.98	71.48	81.31
26	1982.00	0.00	.17	.71	.88	.99	4.73	67.40	74.00	81.34
27	1982.00	22.50	1.60	1.54	3.13	47.29	76.80	89.24	216.45	108.13
28	1982.00	22.50	1.62	1.58	3.20	47.54	69.67	88.06	208.46	108.13
29	1982.00	45.00	3.62	1.50	5.12	104.56	115.17	111.06	335.90	316.25
30	1982.00	45.00	3.55	1.70	5.25	103.74	132.62	108.74	350.34	316.25
31	1982.00	90.00	4.57	2.12	6.69	182.21	257.36	179.75	626.01	825.00
32	1982.00	90.00	4.28	1.75	6.03	174.90	251.49	169.82	602.23	825.00

TABLE II-A-10

CADMIUM/DOMINO/COMPOSTED

OBS#										
1	1979.00	0.00	0.00	0.00	0.00	.28	.12	0.00	.39	.05
2	1979.00	0.00	0.00	0.00	0.00	.15	.13	0.00	.27	.05
3	1979.00	22.50	0.00	0.00	0.00	.28	.82	.42	1.52	1.56
4	1979.00	22.50	0.00	0.00	0.00	.14	1.09	.27	1.50	1.56
5	1979.00	45.00	0.00	0.00	0.00	.69	2.11	.52	3.31	3.13
6	1979.00	45.00	.12	0.00	.12	.71	2.26	.52	3.61	3.13
7	1979.00	90.00	.12	0.00	.12	1.94	3.57	2.48	8.11	6.75
8	1979.00	90.00	.12	0.00	.12	1.94	3.62	2.24	7.91	6.75
9	1980.00	0.00	.03	.03	.05	.01	.05	0.00	.11	5.000000E-04
10	1980.00	0.00	.03	.01	.04	.02	.05	0.00	.10	5.000000E-04
11	1980.00	22.50	0.00	.06	.06	.61	1.59	.57	2.83	2.70
12	1980.00	22.50	0.00	.03	.03	.60	1.51	.49	2.63	2.70
13	1980.00	45.00	.05	.08	.13	1.63	2.62	1.59	5.96	5.41
14	1980.00	45.00	.06	.04	.09	1.56	2.64	1.55	5.85	5.41
15	1980.00	90.00	.09	.03	.12	3.02	6.37	4.08	13.58	10.00
16	1980.00	90.00	.06	.04	.09	3.06	6.06	3.93	13.14	10.00
17	1981.00	0.00	0.00	.19	.19	.08	.06	0.00	.32	5.000000E-04
18	1981.00	0.00	0.00	.06	.06	.08	.08	0.00	.21	5.000000E-04
19	1981.00	22.50	.09	.06	.14	.90	1.73	.56	3.32	2.73
20	1981.00	22.50	.05	.04	.09	.77	1.78	.21	2.86	2.73
21	1981.00	45.00	.14	.10	.24	2.02	3.34	2.19	7.79	7.69
22	1981.00	45.00	.11	5.000000E-03	.12	1.91	3.58	1.65	7.24	7.69
23	1981.00	90.00	.21	.15	.36	3.82	5.98	4.19	14.34	12.19
24	1981.00	90.00	.12	.11	.23	3.24	6.41	4.42	14.29	12.19
25	1982.00	0.00	.09	.30	.40	.06	.14	0.00	.59	.03
26	1982.00	0.00	.09	.35	.45	.06	.15	0.00	.66	.03
27	1982.00	22.50	.09	.37	.46	1.08	2.37	.81	4.71	4.24
28	1982.00	22.50	.08	.39	.46	1.03	2.04	1.06	4.59	4.24
29	1982.00	45.00	.15	.40	.54	2.86	4.55	3.88	11.82	11.64
30	1982.00	45.00	.17	.39	.56	3.02	4.78	3.84	12.20	11.64
31	1982.00	90.00	.26	.30	.55	4.75	6.80	5.73	17.83	4.25
32	1982.00	90.00	.21	.37	.57	4.57	7.11	5.87	18.12	4.25

TABLE II-A-11

CADMIUM/DOMINO/LIQUID

OBS#										
1	1979.00	0.00	0.00	0.00	0.00	.15	.26	0.00	.41	.10
2	1979.00	0.00	0.00	0.00	0.00	0.00	.15	0.00	.15	.10
3	1979.00	22.50	.12	0.00	.12	.56	1.04	.29	2.01	1.75
4	1979.00	22.50	.12	1.10	1.22	.14	.66	0.00	2.01	1.75
5	1979.00	45.00	.12	0.00	.12	.83	1.30	1.33	3.59	2.75
6	1979.00	45.00	.12	0.00	.12	.58	1.34	1.28	3.32	2.75
7	1979.00	90.00	0.00	0.00	0.00	1.61	2.88	1.74	6.22	4.75
8	1979.00	90.00	0.00	0.00	0.00	1.45	2.37	1.58	5.40	4.75
9	1980.00	0.00	0.00	.04	.04	.14	.23	0.00	.41	.09
10	1980.00	0.00	0.00	.03	.03	.06	.25	0.00	.33	.09
11	1980.00	22.50	.01	.03	.04	.47	.93	.39	1.82	1.63
12	1980.00	22.50	.03	.02	.04	.47	.96	.46	1.93	1.63
13	1980.00	45.00	.06	.03	.09	.77	1.36	1.18	3.39	2.40
14	1980.00	45.00	.06	.02	.08	.85	1.53	1.31	3.77	2.40
15	1980.00	90.00	.03	.05	.08	1.30	1.99	1.86	5.21	3.76
16	1980.00	90.00	.04	.01	.05	1.21	1.82	2.21	5.29	3.76
17	1981.00	0.00	0.00	.06	.06	.11	.28	0.00	.44	5.000000E-04
18	1981.00	0.00	0.00	.02	.02	.06	.33	0.00	.40	5.000000E-04
19	1981.00	22.50	0.00	.09	.09	.63	.93	.24	1.89	1.81
20	1981.00	22.50	0.00	0.00	0.00	.59	1.13	0.00	1.71	1.81
21	1981.00	45.00	0.00	.02	.02	.92	1.34	.30	2.58	2.06
22	1981.00	45.00	0.00	0.00	0.00	.97	1.22	.41	2.61	2.06
23	1981.00	90.00	.09	.08	.16	1.95	2.37	1.91	6.39	3.31
24	1981.00	90.00	.06	.03	.09	1.61	2.04	1.90	5.63	3.31
25	1982.00	0.00	.04	.16	.20	.31	.51	0.00	1.01	.40
26	1982.00	0.00	.05	.20	.25	.25	.46	0.00	.95	.40
27	1982.00	22.50	.12	.35	.47	.71	.73	.66	2.57	2.23
28	1982.00	22.50	.12	.34	.46	.70	.94	.50	2.60	2.23
29	1982.00	45.00	.36	.35	.70	.89	1.04	1.03	3.67	2.58
30	1982.00	45.00	.17	.38	.55	.89	1.02	1.03	3.49	2.58
31	1982.00	90.00	.05	.23	.28	.12	.21	0.00	.60	4.20
32	1982.00	90.00	.09	.25	.34	.13	.23	0.00	.70	4.20

TABLE II-A-12

CADMIUM/GREENFIELD/COMPOSTED

OBS#										
1	1979.00	0.00	0.00	0.00	0.00	0.00	.15	0.00	.15	.10
2	1979.00	0.00	0.00	0.00	0.00	0.00	.14	0.00	.14	.10
3	1979.00	22.50	.12	0.00	.12	.32	.94	.17	1.54	1.25
4	1979.00	22.50	0.00	0.00	0.00	.30	.56	.60	1.46	1.25
5	1979.00	45.00	.12	0.00	.12	.68	1.13	.95	2.88	2.50
6	1979.00	45.00	.12	0.00	.12	.66	1.12	.95	2.84	2.50
7	1979.00	90.00	0.00	0.00	0.00	1.85	3.09	1.63	6.57	5.75
8	1979.00	90.00	.12	0.00	.12	1.65	2.55	2.13	6.45	5.75
9	1980.00	0.00	0.00	.03	.03	0.00	.07	0.00	.09	5.000000E-04
10	1980.00	0.00	0.00	.03	.03	0.00	.07	0.00	.09	5.000000E-04
11	1980.00	22.50	0.00	.04	.04	.23	1.05	.50	1.82	1.97
12	1980.00	22.50	0.00	.03	.03	.18	1.35	.20	1.75	1.97
13	1980.00	45.00	0.00	.03	.03	.92	2.37	1.14	4.46	3.94
14	1980.00	45.00	0.00	.04	.04	.92	2.22	1.10	4.27	3.94
15	1980.00	90.00	0.00	.03	.03	1.89	3.85	2.30	8.05	11.51
16	1980.00	90.00	0.00	.03	.03	2.47	4.62	3.21	10.33	11.51
17	1981.00	0.00	0.00	0.00	0.00	0.00	.09	0.00	.09	5.000000E-04
18	1981.00	0.00	0.00	.02	.02	0.00	.08	0.00	.09	5.000000E-04
19	1981.00	22.50	0.00	.18	.18	.50	1.14	.01	1.82	2.63
20	1981.00	22.50	0.00	.06	.06	.56	1.25	0.00	1.87	2.63
21	1981.00	45.00	.04	0.00	.04	1.16	2.63	.48	4.30	4.38
22	1981.00	45.00	0.00	0.00	0.00	1.13	2.15	.73	4.02	4.38
23	1981.00	90.00	.04	.02	.05	2.25	4.16	2.11	8.56	9.06
24	1981.00	90.00	.03	0.00	.03	2.31	3.96	2.35	8.64	9.06
25	1982.00	0.00	.05	.25	.30	.04	.12	.21	.66	0.00
26	1982.00	0.00	.09	.25	.34	.13	.23	0.00	.70	.04
27	1982.00	22.50	.09	.18	.26	1.17	2.12	.34	3.89	4.33
28	1982.00	22.50	.11	.17	.28	1.03	2.01	.40	3.72	4.33
29	1982.00	45.00	.16	.10	.26	1.56	2.72	1.83	6.37	6.06
30	1982.00	45.00	.17	.12	.29	1.84	3.41	1.28	6.82	6.06
31	1982.00	90.00	.17	.16	.33	3.03	5.09	3.76	12.20	7.06
32	1982.00	90.00	.17	.18	.35	2.97	5.33	3.33	11.98	7.06

TABLE II-A-13

LEAD/DOMINO/COMPOSTED

OBS#										
1	1979.00	0.00	0.00	.79	.79	.40	4.86	2.11	8.16	8.88
2	1979.00	0.00	0.00	.52	.52	.96	4.96	1.97	8.42	8.88
3	1979.00	22.50	0.00	.99	.99	2.26	37.54	12.66	53.44	52.69
4	1979.00	22.50	0.00	.92	.92	2.29	43.96	9.86	57.02	52.69
5	1979.00	45.00	0.00	1.08	1.08	3.90	78.50	11.78	95.26	89.88
6	1979.00	45.00	0.00	1.15	1.15	3.89	79.38	12.18	96.59	89.88
7	1979.00	90.00	0.00	.82	.82	10.06	159.57	33.87	204.33	181.75
8	1979.00	90.00	0.00	.84	.84	9.20	156.74	33.78	200.56	181.75
9	1980.00	0.00	.81	.51	1.31	0.00	3.56	7.12	11.99	13.92
10	1980.00	0.00	.27	1.04	1.32	0.00	3.54	6.99	11.85	13.92
11	1980.00	22.50	0.00	1.34	1.34	.19	51.51	20.02	73.06	67.92
12	1980.00	22.50	0.00	1.17	1.17	.57	50.53	19.10	71.36	67.92
13	1980.00	45.00	.98	2.57	3.56	2.12	93.95	36.36	135.98	104.17
14	1980.00	45.00	.43	1.43	1.85	1.54	98.25	36.85	138.48	104.42
15	1980.00	90.00	.06	2.93	2.99	4.11	192.93	49.12	249.14	215.83
16	1980.00	90.00	0.00	1.10	1.10	3.83	192.85	51.28	249.05	215.83
17	1981.00	0.00	.36	.37	.73	0.00	6.19	7.03	13.95	7.50
18	1981.00	0.00	.61	.52	1.13	0.00	6.42	6.54	14.08	7.50
19	1981.00	22.50	.61	1.61	2.22	.76	54.80	19.32	77.09	76.88
20	1981.00	22.50	.73	.86	1.59	.80	57.21	17.32	76.92	76.88
21	1981.00	45.00	.49	.90	1.39	2.64	114.33	39.46	157.80	167.50
22	1981.00	45.00	.12	.77	.89	1.94	112.47	32.40	147.69	167.50
23	1981.00	90.00	.73	2.11	2.84	5.37	201.27	70.31	279.78	243.75
24	1981.00	90.00	.73	1.10	1.83	4.22	205.85	58.00	269.90	243.75
25	1982.00	0.00	0.00	1.90	1.90	0.00	5.04	8.28	15.22	14.13
26	1982.00	0.00	0.00	.13	.13	1.67	6.14	7.88	15.80	14.13
27	1982.00	22.50	0.00	1.93	1.93	0.00	62.72	23.64	88.28	41.25
28	1982.00	22.50	0.00	.57	.57	.21	60.54	26.31	87.62	41.25
29	1982.00	45.00	.36	2.76	3.13	.09	125.01	50.94	179.17	110.00
30	1982.00	45.00	0.00	1.14	1.14	5.55	127.36	51.17	185.22	110.00
31	1982.00	90.00	0.00	1.70	1.70	7.67	206.70	76.34	292.40	91.88
32	1982.00	90.00	0.00	1.74	1.74	7.38	217.79	82.45	309.35	91.88

TABLE II-A-14

LEAD/DOMINO/LIQUID

OBS#										
1	1979.00	0.00	0.00	.29	.29	0.00	7.60	1.88	9.76	8.65
2	1979.00	0.00	0.00	.43	.43	0.00	6.41	3.41	10.25	8.65
3	1979.00	22.50	0.00	.65	.65	1.23	21.70	4.88	28.45	28.75
4	1979.00	22.50	0.00	2.32	2.32	1.57	20.86	4.04	28.78	28.75
5	1979.00	45.00	0.00	.53	.53	1.36	29.37	9.15	40.41	42.88
6	1979.00	45.00	0.00	.49	.49	1.26	30.94	10.02	42.70	42.88
7	1979.00	90.00	0.00	.78	.78	2.43	61.69	11.72	76.61	71.75
8	1979.00	90.00	0.00	.80	.80	1.85	50.67	11.64	64.96	71.75
9	1980.00	0.00	1.59	4.38	5.97	0.00	5.79	7.13	18.88	16.59
10	1980.00	0.00	.29	2.84	3.13	0.00	7.49	7.26	17.87	16.59
11	1980.00	22.50	.06	.70	.76	0.00	18.95	11.82	31.52	36.84
12	1980.00	22.50	.72	.82	1.54	0.00	20.68	12.88	35.09	36.84
13	1980.00	45.00	.22	.15	.37	0.00	30.88	16.05	47.30	48.75
14	1980.00	45.00	.34	.82	1.16	0.00	35.90	16.97	54.03	48.75
15	1980.00	90.00	1.74	5.47	7.22	.82	49.55	17.74	75.31	55.42
16	1980.00	90.00	0.00	3.45	3.45	.52	46.85	20.48	71.28	55.42
17	1981.00	0.00	.61	.72	1.33	0.00	10.31	6.57	18.21	13.75
18	1981.00	0.00	0.00	.76	.76	0.00	12.41	6.95	20.12	13.75
19	1981.00	22.50	.61	.98	1.59	0.00	19.86	12.03	33.47	40.63
20	1981.00	22.50	.85	.75	1.60	.36	54.00	11.23	67.19	40.63
21	1981.00	45.00	.73	.63	1.36	0.00	33.70	11.85	46.90	33.75
22	1981.00	45.00	.97	.48	1.45	0.00	30.12	13.39	44.96	33.75
23	1981.00	90.00	0.00	1.63	1.63	.25	61.07	22.87	85.81	57.50
24	1981.00	90.00	0.00	.53	.53	.27	55.99	21.11	77.88	57.50
25	1982.00	0.00	0.00	1.10	1.10	0.00	10.05	12.88	24.03	18.44
26	1982.00	0.00	0.00	.94	.94	0.00	8.70	8.88	18.51	18.44
27	1982.00	22.50	0.00	1.19	1.19	.98	23.36	13.28	38.81	39.06
28	1982.00	22.50	0.00	.92	.92	1.73	23.20	12.54	38.38	39.06
29	1982.00	45.00	0.00	.80	.80	0.00	29.09	17.12	47.01	50.13
30	1982.00	45.00	.12	.53	.65	.44	30.84	16.26	48.17	50.13
31	1982.00	90.00	0.00	4.04	4.04	.86	40.83	19.62	65.36	186.25
32	1982.00	90.00	.61	1.47	2.08	.85	38.12	19.57	60.62	186.25

TABLE II-A-15

LEAD/GREENFIELD/COMPOSTED

OBS#										
1	1979.00	0.00	0.00	.58	.58	0.00	4.46	2.60	7.64	6.15
2	1979.00	0.00	0.00	.64	.64	0.00	5.40	.27	6.30	6.15
3	1979.00	22.50	0.00	.41	.41	.95	33.92	4.95	40.23	39.62
4	1979.00	22.50	0.00	.30	.30	1.66	30.31	15.04	47.31	39.62
5	1979.00	45.00	0.00	1.14	1.14	3.90	51.03	17.60	73.66	69.50
6	1979.00	45.00	0.00	.88	.88	3.96	50.54	17.64	73.01	69.50
7	1979.00	90.00	0.00	1.31	1.31	10.62	137.55	26.45	175.92	148.50
8	1979.00	90.00	0.00	1.14	1.14	10.36	123.55	36.32	171.36	148.50
9	1980.00	0.00	.42	4.41	4.82	0.00	3.64	4.34	12.80	10.67
10	1980.00	0.00	.06	2.79	2.85	0.00	3.64	4.51	11.00	10.67
11	1980.00	22.50	0.00	2.44	2.44	.57	36.74	16.90	56.65	53.75
12	1980.00	22.50	0.00	2.35	2.35	.20	46.76	13.58	62.88	53.75
13	1980.00	45.00	0.00	2.96	2.96	1.28	66.70	24.63	95.57	82.09
14	1980.00	45.00	0.00	2.21	2.21	.73	65.32	23.51	91.76	82.09
15	1980.00	90.00	0.00	2.64	2.64	4.44	128.21	42.94	178.24	265.42
16	1980.00	90.00	0.00	2.04	2.04	4.81	163.74	45.57	216.15	265.42
17	1981.00	0.00	.36	.98	1.35	0.00	4.54	4.38	10.27	14.38
18	1981.00	0.00	0.00	1.02	1.02	0.00	5.73	4.20	10.95	14.38
19	1981.00	22.50	.36	1.11	1.48	0.00	41.61	10.46	53.54	68.75
20	1981.00	22.50	.61	.86	1.47	0.00	43.81	10.00	55.28	68.75
21	1981.00	45.00	.61	.23	.84	.94	69.44	15.49	86.71	91.25
22	1981.00	45.00	0.00	.78	.78	3.76	62.68	19.66	86.88	91.25
23	1981.00	90.00	.12	.73	.85	4.33	145.95	36.95	188.08	209.38
24	1981.00	90.00	.97	.71	1.69	3.80	149.69	42.21	197.38	209.38
25	1982.00	0.00	0.00	.58	.58	0.00	2.96	5.29	8.82	10.19
26	1982.00	0.00	0.00	.44	.44	.06	3.60	5.63	9.73	10.19
27	1982.00	22.50	0.00	.99	.99	1.02	48.37	14.65	65.04	41.88
28	1982.00	22.50	0.00	.76	.76	1.44	42.18	14.37	58.75	41.88
29	1982.00	45.00	0.00	.43	.43	3.64	73.43	31.00	108.50	102.50
30	1982.00	45.00	0.00	.16	.16	3.43	76.67	26.55	106.81	102.50
31	1982.00	90.00	0.00	1.40	1.40	7.51	153.19	58.95	221.04	166.87
32	1982.00	90.00	0.00	1.19	1.19	7.71	150.80	50.98	210.68	166.87

III. TRACE METAL SORPTION ON ARID-ZONE SOILS

A. Materials and Methods

1. Soil samples

Bulk samples of five arid-zone soils collected and described by Mahler et al. (1978) were sub-sampled for use in the present study. The general physical and chemical properties of the soils are given in Table III-1. It is apparent that a range of textures, cation exchange capacities, $CaCO_3$ and organic C contents is represented. The classification of the five soils according to the Soil Taxonomy is as follows:

Altamont: fine, montmorillonitic, thermic Typic Chromoxerert

Domino: fine-loamy, mixed, thermic Xerollic Calciorthid

Greenfield: coarse-loamy, mixed, thermic Typic Haploxeralf

Holtville: coarse-loamy, mixed, montmorillonitic (calcareous), hyperthermic Typic Torrifluvent

Simona: coarse-loamy, mixed, thermic, shallow Typic Paleorthid

2. Sorption experiments

One-gram samples of soil were weighed into clean, dry teflon centrifuge tubes. The appropriate aliquots of water, 0.05M $NaClO_4$, and trace metal perchlorate solution, each adjusted to pH 5.0 with perchlorate solution, were then added. This produced 0.025-liter solutions which were 0.01M in $NaClO_4$ and 0, 2, 5, 7, 10, 20, 40, 60, 80 and 100 $mg \cdot L^{-1}$ in metal perchlorate in the cases of cadmium, copper, and nickel. For the lead study, 0, 20, 50, 70, 100, 200, 400, 600, 800 or 1000 $mg \cdot L^{-1}$ lead perchlorate was added for the Altamont, Domino, and Greenfield soils, and 0, 200, 500, 700, 1000, 2000, 4000, 6000, 8000 or 10,000 $mg \cdot L^{-1}$ lead perchlorate were added to the Holtville and Simona soils. Blanks were prepared for each metal at each concentration to determine the initial concentrations. All samples were prepared in duplicate.

The tubes were purged with nitrogen, capped with teflon-lined stoppers and shaken at 25°C in a shaking water bath for 24 h. At the end of this period, the samples were removed from the bath, centrifuged on a Sorvall desktop SS-3 automatic centrifuge at 10,000 RPM for 10 minutes. The supernatants were filtered into screw cap polycarbonate sample bottles through 0.4 um Nuclepore polycarbonate filters held in polycarbonate supports. The samples then were analyzed for metal using either a Perkin-Elmer model 103 or model 5000 atomic absorption spectrophotometer, with standards prepared from 1000 $mg \cdot L^{-1}$ Fisher-analyzed reference standards. The pH values of the supernatant solutions were measured using Colorplast pH papers or a set of Beckman glass and calomel electrodes. The supernatant solution concentrations were determined by estimates derived from second-order regression analyses of standard mean deviations for duplicate samples were generally within 5%. The copper sorption study was repeated using the procedure outlined above, except with 0.02M $NaClO_4$ and three replicates.

TABLE III-1

PROPERTIES OF THE SOILS USED AS SORBENTS

Soil Series	Particle-size Fractions Sand	Silt	Clay	Textural Designation	Cation-Exchange Capacity cmol(+)/kg	Organic Carbon (%)	$CaCO_3$ Content (%)	pH_p (sat. paste)
	-------%--------							
Altamont	40.3	22.2	37.5	clay loam	37.9	3.0	0.0	5.7
Domino	15.7	65.6	18.7	silt loam	14.0	1.4	1.2	7.4
Greenfiel	62.0	27.0	11.0	sandy loam	8.7	0.8	0.0	7.1
Holtville	7.3	52.1	40.6	silty clay	27.3	0.9	12.8	7.7
Simona	63.9	20.9	15.2	f. sandy loam	7.8	0.7	28.3	7.8

Experiments were conducted to check for sorption of metals onto the polycarbonate filters and the effect of warming during centrifugation. The latter was performed by centrifugation of duplicate samples in refrigerated and unrefrigerated centrifuges. There was no effect due to sorption or warming, and an unrefrigerated centrifuge was used for convenience. The metal analysis was checked for interferences due to sodium content, pH adjustment, and soluble organic compounds. The effect of organic compounds was determined using an overnight ultraviolet irradiation in the presence of 0.01 mL of 30% H_2O_2 per 25 ml of supernatant. This procedure removed the organic materials and the metals in the supernatant were determined before and after irradiation. In all cases, there were no significant effects due to these factors.

B. Nickel, Copper, Cadmium, and Lead Sorption Characteristics

Sorption isotherm data at 25°C for the metals Ni, Cu, Cd, and Pb on five arid-zone soils are given in Tables III-2 to III-6. These data are presented in the conventional form of the amount sorbed, in units of milligrams of metal per kilogram of soil, versus the concentration in the aqueous solution phase, in units of grams of metal per cubic meter of solution. (Both of the units used correspond to what is loosely termed "parts per million".).

Graphs of the sorption data for the Domino and Greenfield soils (Tables III-2 and III-6) are shown in Fig. III-1 to III-4. It is instructive to compare these graphs with the data in Table II-3, which lists the sum of extracted trace metal concentrations in the Domino and Greenfield soils receiving a representative 45 Mg $ha^{-1}yr^{-1}$ in sludge applications. For the Domino soil, the largest trace metal concentrations over the period 1979-1982 are, mg metal per kg soil: Ni (83), Cu (196), Cd (3), and Pb (182). These concentrations correspond to aqueous solution concentrations at or below 0.05 g m^{-3} except for Ni (0.1 g m^{-3}). Thus, only the very lowest portions of the sorption isotherms in Fig. III-1 to III-4 are directly relevant to metal enrichment by sludge

TABLE III-2

METAL SORPTION ON DOMINO SOIL

Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m^3 soln)	Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m^3 soln)
Nickel		**Copper**	
22.8	0.0350	161	0.0050
71.9	0.0740	216	0.0215
137	0.153	285	0.0340
176	0.259	320	0.0345
238	0.392	386	0.256
468	1.49	399	0.0600
540	1.66	638	0.327
913	4.90	684	0.432
1260	10.3	1160	0.941
1510	17.9	1600	2.21
1800	26.0	2050	4.57
		2370	10.3
Cadmium		**Lead**	
1.27	0.0042	28.9	0.0155
53.0	0.0255	294	0.0175
109	0.161	2200	0.0280
117	0.0419	2980	0.0787
150	0.181	5350	1.63
158	0.0585	8710	9.11
208	0.194	10500	30.2
511	0.354	10600	35.7
970	1.10	12000	88.5
1390	2.46	13100	126
1900	6.83	13600	133
2110	9.03	14300	203
		15200	265
		15400	343
		15300	435
		15600	435

TABLE III-3

METAL SORPTION ON GREENFIELD SOIL

Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m^3 soln)	Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m^3 soln)
Nickel		**Copper**	
20.5	0.0720	155	0.00900
69.3	0.120	225	0.00150
132	0.310	270	0.0110
168	0.527	337	0.0270
229	1.06	575	0.339
324	0.891	626	0.270
418	3.40	983	2.80
493	3.48	1050	2.64
778	10.2	1360	9.27
1006	20.6	1590	20.5
1125	33.2	1770	31.97
1263	47.5		
Cadmium		**Lead**	
0.586	0.00405	227	0.0140
52.2	0.0271	279	0.0060
107	0.210	358	0.0130
115	0.0767	647	0.127
148	0.229	1750	0.0160
156	0.107	2060	0.0620
202	0.392	3030	0.489
216	0.225	4940	20.6
475	0.657	5310	23.1
492	1.11	7100	76.2
908	3.53	7740	144
1240	8.20	7750	182
1680	15.3	8420	235
1730	23.8	8500	311
		9170	313
		9130	411
		9200	507
		9010	514
		8680	615
		8900	706
		8070	728

TABLE III-4

METAL SORPTION ON ALTAMONT SOIL

Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m^3 soln)	Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m^3 soln)
Nickel		**Copper**	
22.7	0.0670	83.5	0.0210
71.6	0.114	122	0.163
71.8	0.204	137	0.143
136	0.233	190	0.240
144	0.146	205	0.185
175	0.305	238	0.245
188	0.289	308	0.283
237	0.450	609	0.346
250	0.315	1090	0.409
480	1.04	1570	0.490
566	0.626	2070	0.728
999	1.50	2530	0.982
1430	3.63		
1790	6.46		
2180	10.9		
Cadmium		**Lead**	
0.844	0.0113	24.6	0.0140
52.7	0.0241	159	0.0060
109	0.134	211	0.0130
117	0.0474	290	0.127
151	0.165	581	0.0160
158	0.0684	1130	0.0620
208	0.184	1670	0.489
510	0.390	2190	20.6
971	1.047	2970	23.1
1393	2.29	5370	76.2
1960	4.25	8830	144
2140	7.42	10900	182
		11300	235
		13700	311
		15100	313
		16000	411
		17800	507
		19300	514
		19300	615
		20400	706
		21500	728
		21700	

TABLE III-5

METAL SORPTION ON HOLTVILLE SOIL

Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m^3 soln)	Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m^3 soln)
Nickel		**Copper**	
15.3	0.0700	199	0.129
65.4	0.0675	269	0.135
131	0.118	316	0.149
137	0.136	387	0.181
173	0.115	688	0.214
183	0.197	1170	0.267
237	0.150	1650	0.338
246	0.171	2150	0.537
492	0.241	2610	0.757
568	0.266		
893	0.804		
1010	0.709		
1460	2.40		
1810	5.57		
2170	10.9		
Cadmium		**Lead**	
1.20	0.00210	46000	276
53.1	0.0152	87600	732
117	0.0229	112000	1860
159	0.0339	124000	3530
209	0.146	138000	5050
221	0.0499		
489	0.113		
514	0.225		
986	0.448		
1420	1.15		
2003	2.57		
2210	4.87		

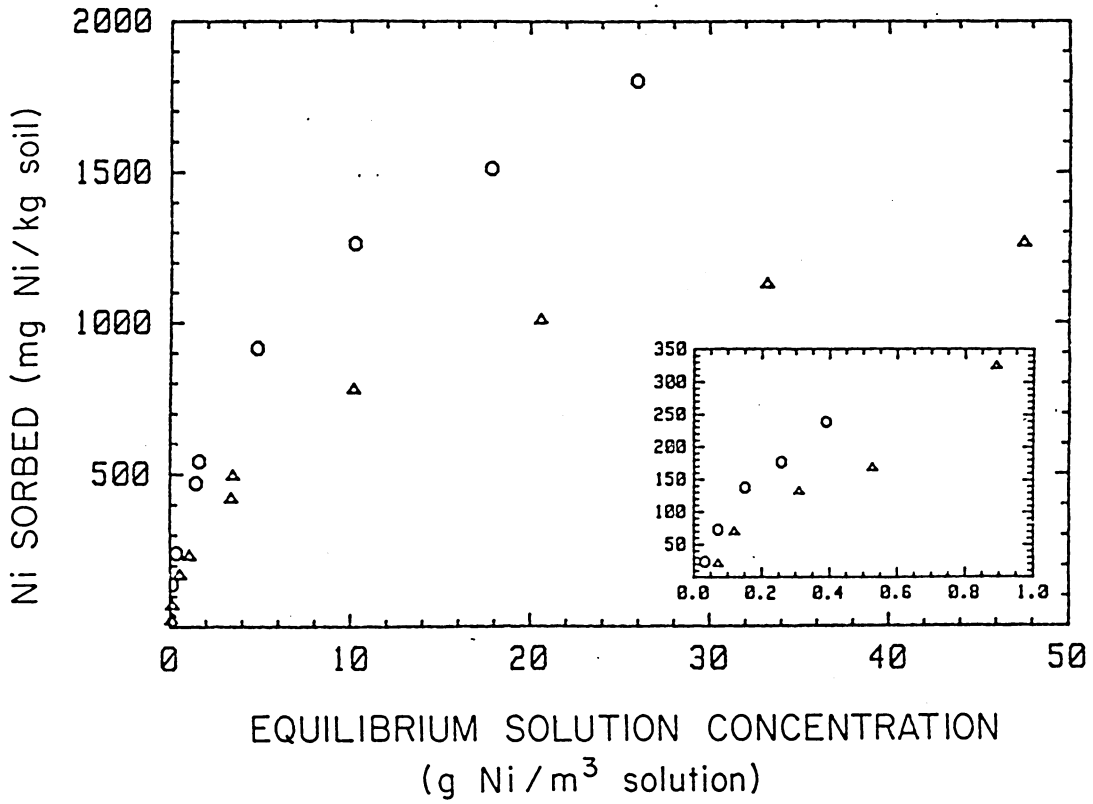


Figure III-1. Nickel sorption on Domino (O) and Greenfield (Δ) soils

TABLE III-6

METAL SORPTION ON SIMONA SOIL

Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m3 soln)	Total Quantity Adsorbed (mg/kg soil)	Equilibrium Solution Conc'n (g/m3 soln)
Nickel		**Copper**	
6.49	0.0230	77.7	0.0601
56.3	0.0320	148	0.0576
124	0.0305	196	0.0470
165	0.0140	267	0.0642
230	0.0225	569	0.0789
487	0.0610	1050	0.114
560	0.160	1530	0.189
894	0.346	2030	0.315
990	1.15	2490	0.586
1380	4.95		
1613	13.0		
1860	22.9		
Cadmium		**Lead**	
0.672	0.00393	143000	648
51.8	0.0532	186000	1040
117	0.0301	176000	3570
159	0.00960		
221	0.0198		
491	0.0486		
517	0.126		
929	0.115		
993	0.163		
1450	0.224		
2060	0.336		
2280	2.04		

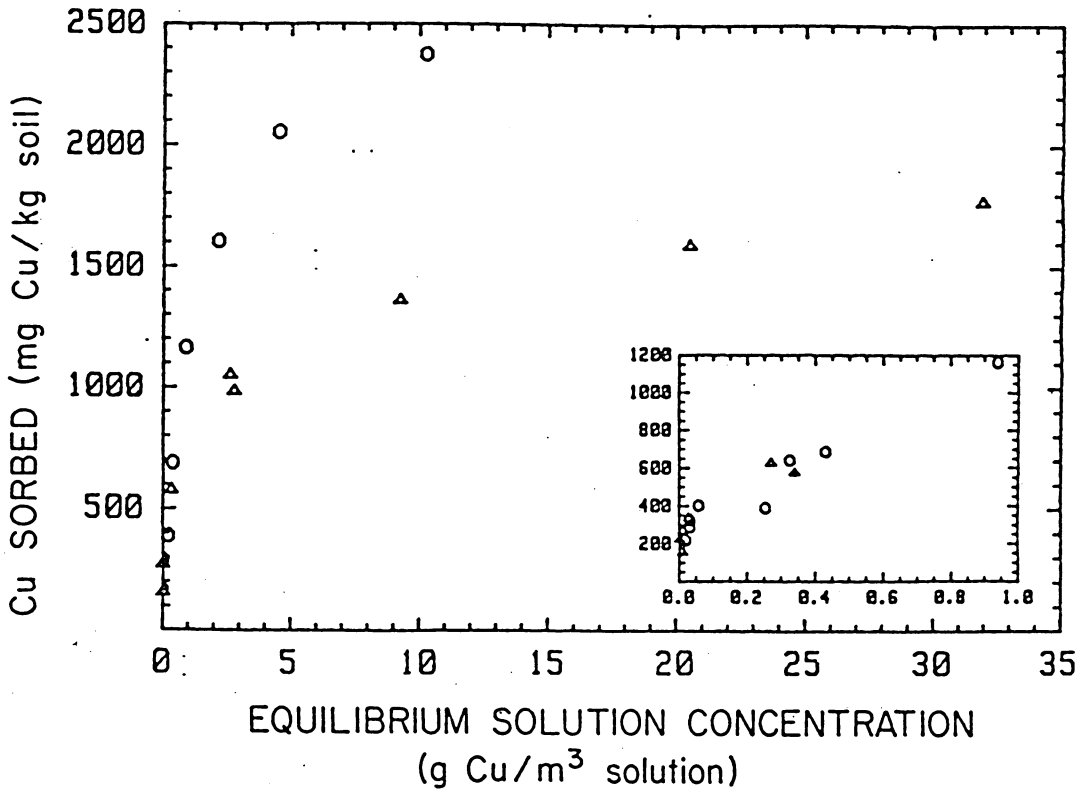


Figure III-2. Copper sorption on Domino (O) and Greenfield (Δ) soils

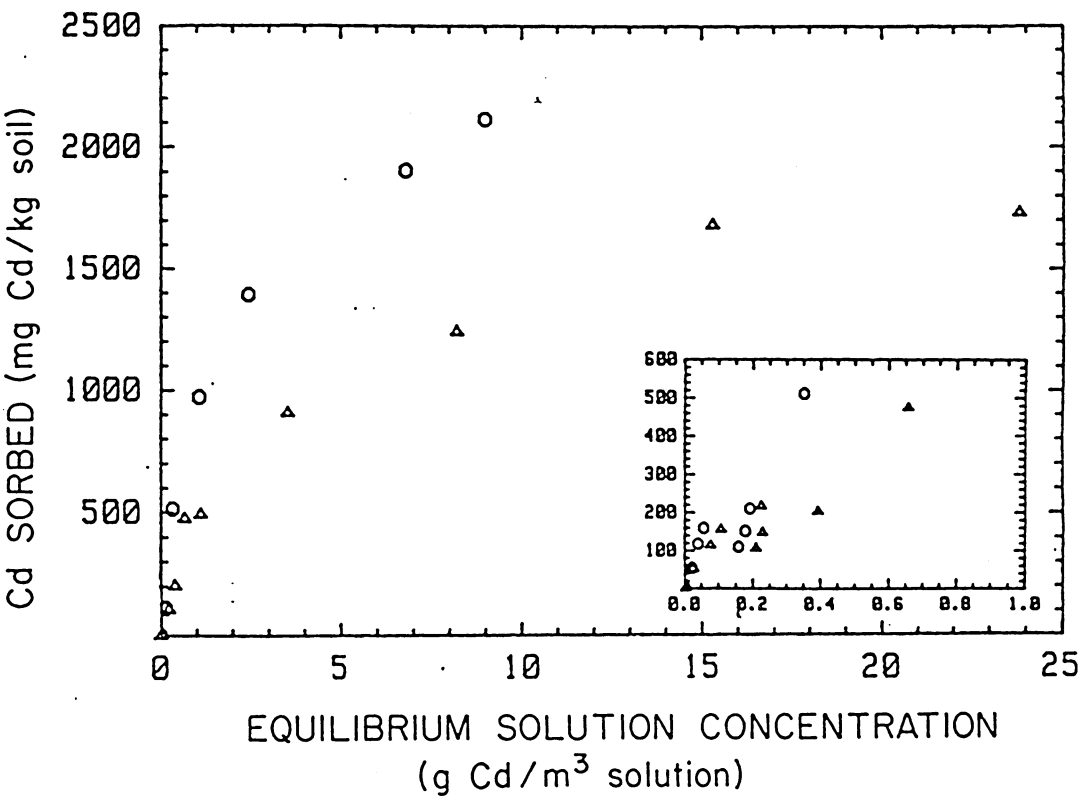


Figure III-3. Cadmium sorption on Domino (O) and Greenfield (Δ) soils

application in the present study. In the case of the Greenfield soil, the largest trace metal concentrations in Table II-3 are: Ni (48), Cu (112), Cd (7), and Pb (108). The corresponding aqueous solution concentrations in Fig. III-1 to II-4 are also at or below 0.05 g m^{-3} except for nickel. By way of reference, it can be noted that the proposed ambient human health water quality criteria for these four trace metals recommended by the Environmental Protection Agency are, in g m^{-3}: Ni (0.013), Cu ($\leq$ 0.006), Cd (0.91), and Pb (0.05). These aqueous solution concentrations also pertain to the very lowest portions of the sorption isotherms in Fig. III-1 to III-4.

Except for Ni, the trace metal sorption isotherms in Fig. III-1 to III-4 exhibit variability and irregular behavior that preclude a meaningful computation of the distribution coefficient for the very lowest range of aqueous solution concentrations (< 1 g m^{-3}) shown in the small inset graphs. There is some indication in Ni, Cd, and Pb sorption by the Greenfield soil that the isotherm is initially concave to the x-axis. This behavior appears even more clearly in Fig. III-5, which shows Cu sorption isotherms at low concentrations for the Altamont and Simona soils. The concave nature of the isotherm implies that the distribution coefficient

$$K_d = \text{metal sorbed/metal in solution} \qquad \text{(III-1)}$$

will not be a monotonically decreasing function of the amount of metal sorbed, as observed at higher metal concentrations in previous studies (Navrot et al., 1978), but instead will increase to a maximum value, then decrease thereafter as a function of the amount sorbed. It is not possible to correlate this kind of trend in K_d with soil characteristics, as proposed by Navrot et al. (1978). Reference to Table III-1 shows that the Altamont and Simona soils differ greatly in their chemical properties, yet both produce sigmoid sorption isotherms with respect to copper.

C. Detailed Characteristics of Copper Sorption

The unusual results illustrated in Fig. III-5 motivated a more detailed study of copper sorption by five soils listed in Table III-1. Tables III-7 to III-11 list the resulting data in a conventional format. The corresponding isotherm plots are in Fig. III-6 to III-10. A sigmoid character in the graphs is apparent at low concentrations for the Greenfield, Altamont, Holtville, and Simona soils. This observation alone indicates that the feature is independent of clay content, cation exchange capacity, organic carbon percentage, calcium carbonate content, and probably pH value in the soils (see Table III-1). However, the Cu concentration in the aqueous solution phase at which the sharp rise in the isotherm occurs appears to increase as the organic carbon concentration in the soil increases: Simona $\leq$ Greenfield < Holtville < Altamont. This trend suggests that soluble organic matter in the soil solution may complex Cu and preclude its sorption until the complexation capacity is exceeded. This "titration" of the soluble organic matter by Cu would correspond to the sharp sigmoid rise in the sorption curve and, therefore, would occur at a larger Cu concentration the greater is the organic matter content of the soil. Further research is required in

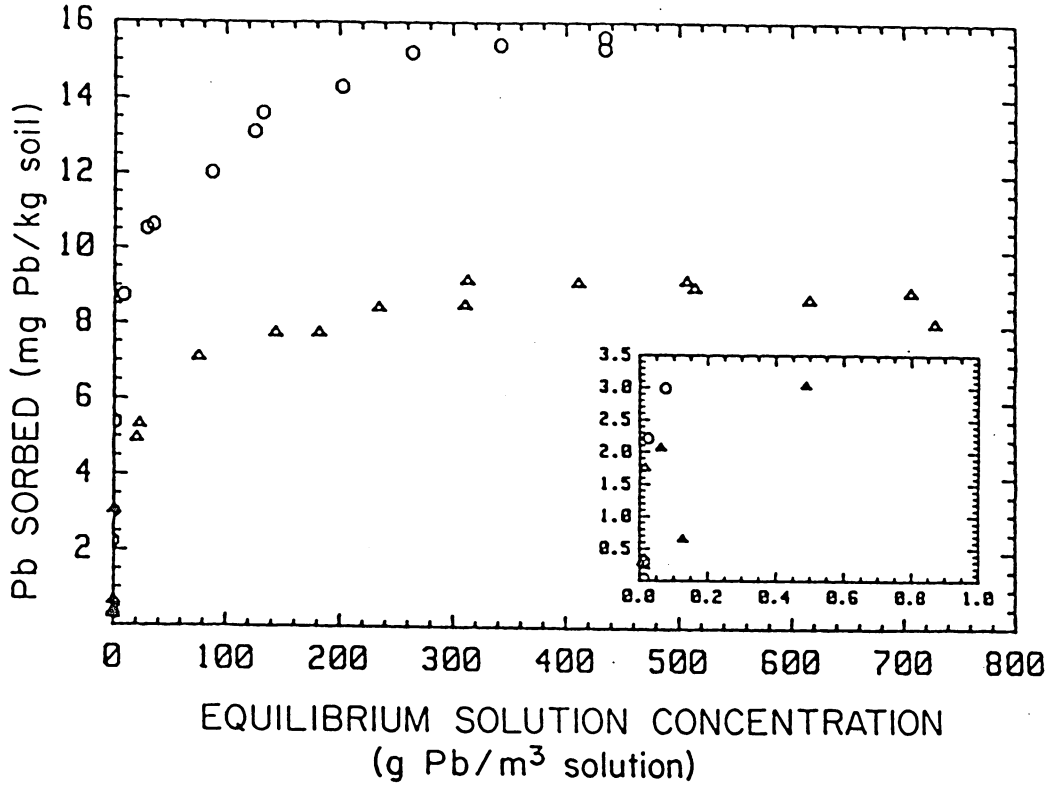


Figure III-4. Lead sorption on Domino (O) and Greenfield (Δ) soils

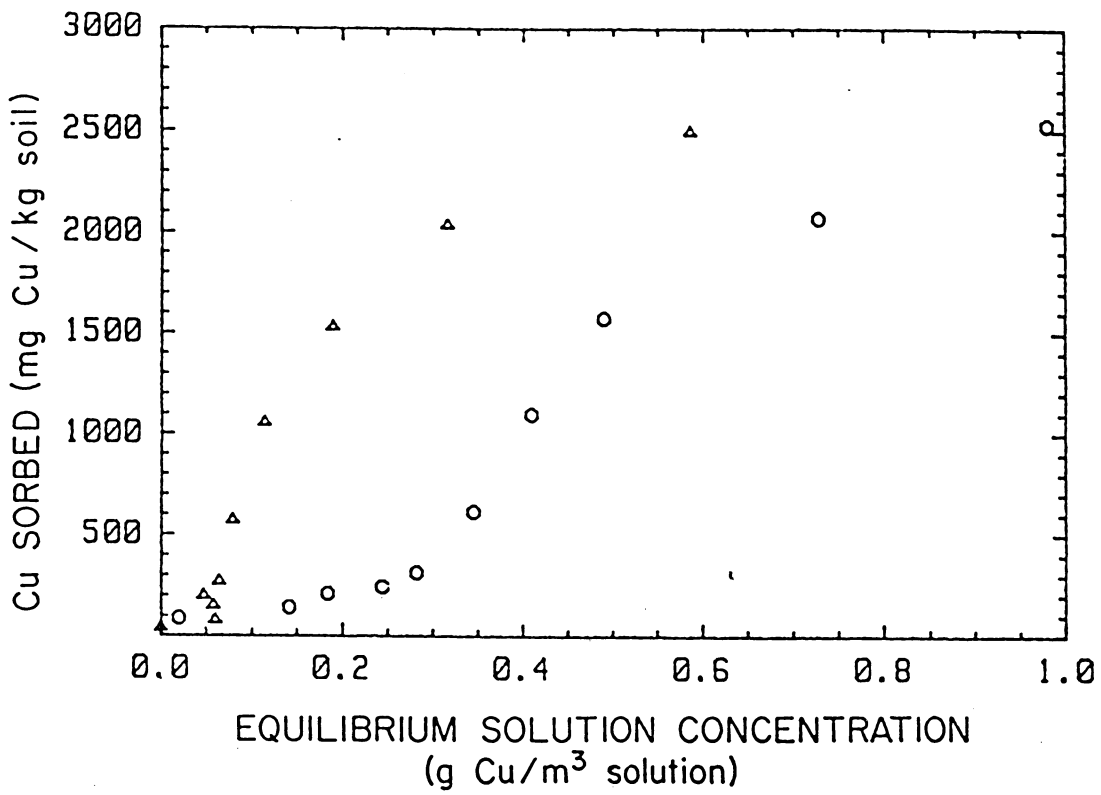


FIGURE III-5. Copper sorption on Altamont (O) and Simona (Δ) soils

TABLE III-7

COPPER SORPTION ON SIMONA SOIL

Equilibrium Solution Concentration ($g \cdot m^{-3}$)	Amount Sorbed $mg\ kg^{-1}$
.0419	36.3
.0252	85.1
.0839	137
.0922	213
.101	295
.109	511
.138	1044
.252	1481
.327	2109
.415	2452

TABLE III-8

COPPER SORPTION ON HOLTVILLE SOIL

Equilibrium Solution Concentration ($g \cdot m^{-3}$)	Amount Sorbed $mg\ kg^{-1}$
.0700	160
.105	209
.131	263
.131	338
.157	360
.184	646
.224	1161
.323	1632
.603	2259
.761	2606

TABLE III-9

COPPER SORPTION ON ALTAMONT SOIL

Equilibrium Solution Concentration ($g \cdot m^{-3}$)	Amount Sorbed $mg\ kg^{-1}$
.0149	83.7
.0827	120
.159	194
.234	230
.274	203
.331	496
.407	873
.589	1510
.732	2004
1.056	2345

TABLE III-10

COPPER SORPTION ON GREENFIELD SOIL

Equilibrium Solution Concentration ($g \cdot m^{-3}$)	Amount Sorbed $mg\ kg^{-1}$
.0875	98.8
.0984	145
.120	223
.164	276
.339	343
.492	564
2.908	1018
10.813	1279
20.685	1579
30.995	1761

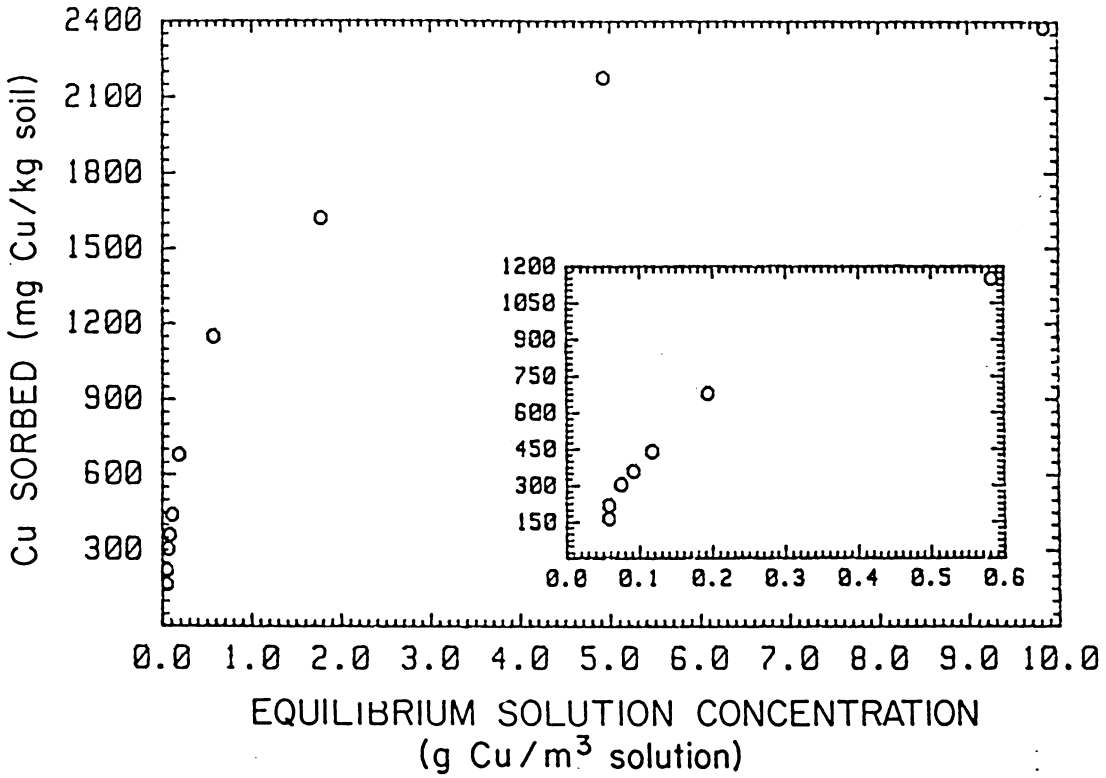


FIGURE III-6. Copper Sorption on Domino Soil

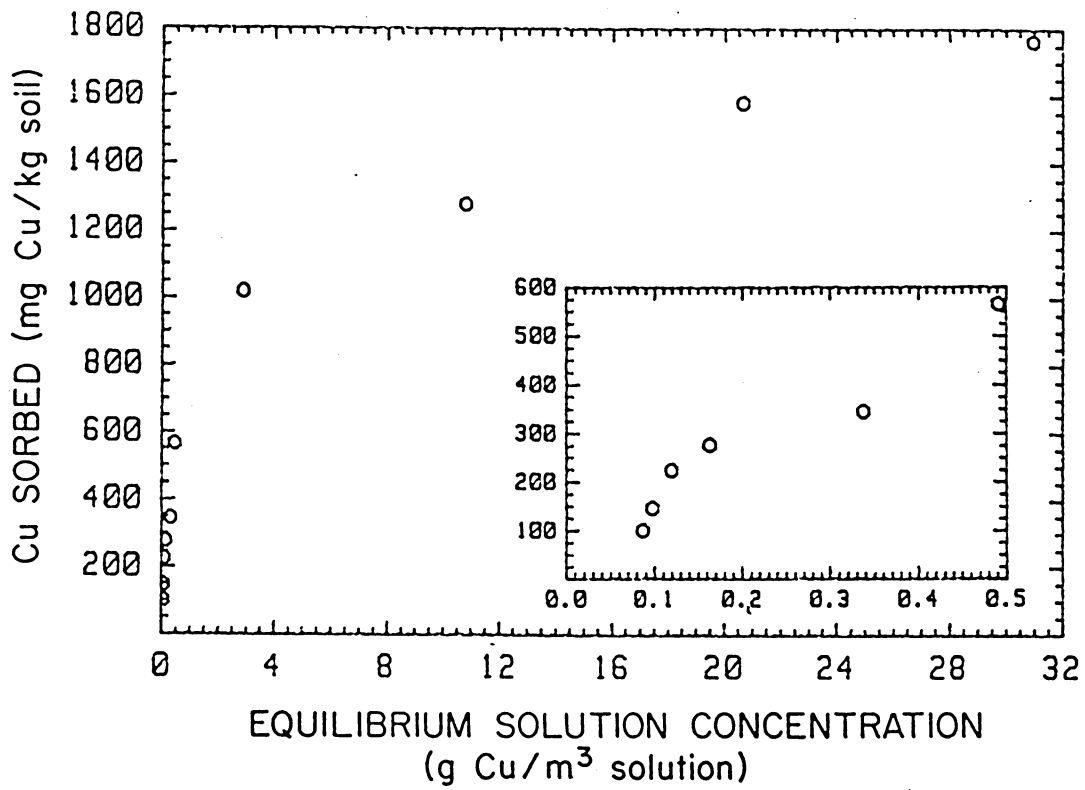


FIGURE III-7. Copper Sorption on Greenfield Soil

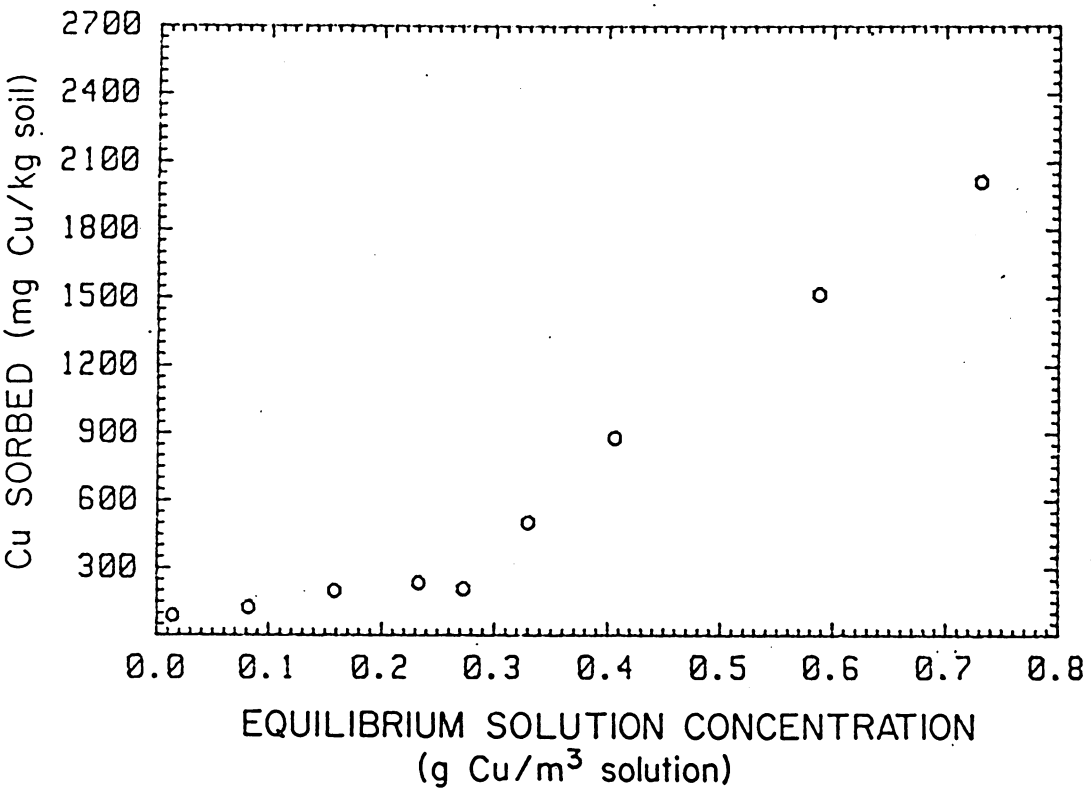


FIGURE III-8. Copper Sorption on Altamont Soil

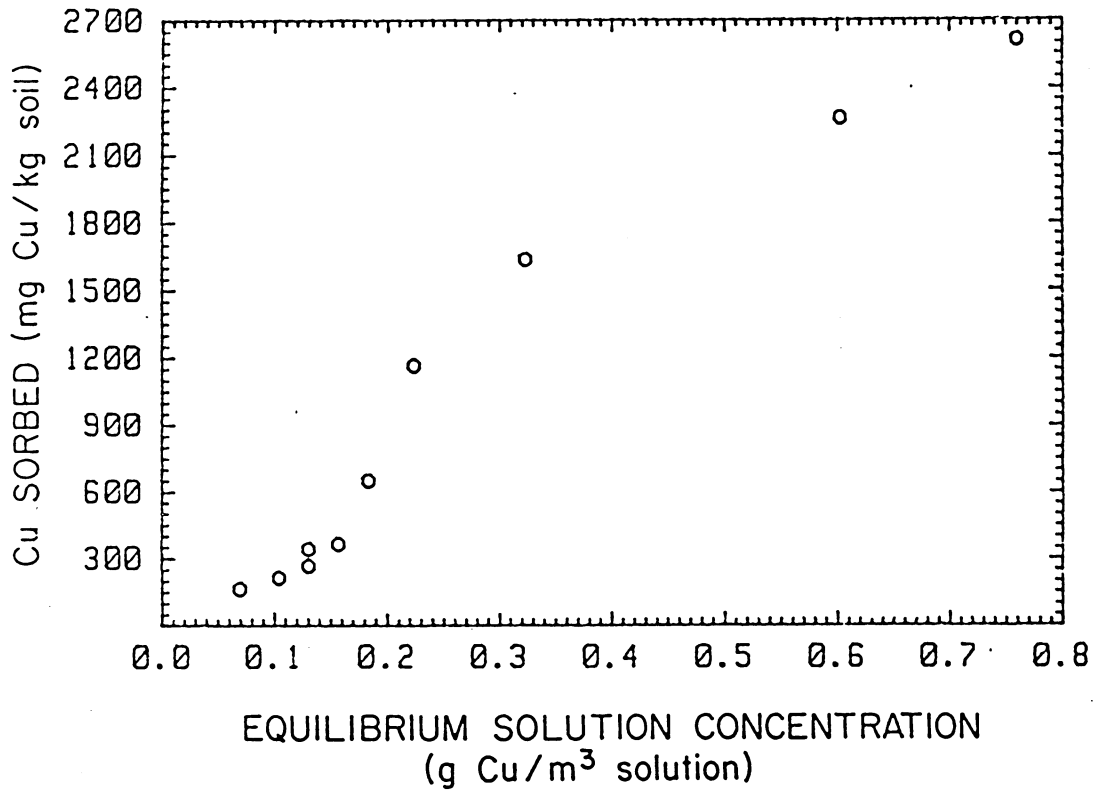


FIGURE III-9. Copper Sorption on Holtville Soil

TABLE IV-1

ANALYTICAL PROPERTIES† OF DOMINO FULVIC ACID

Fulvic acid	C	H	N	S	O	COOH	LAS	LS‡
	%					meq/g	%	
Domino	36.0±0.4§	7.9 ±0.1	4.7 ±0.2	6.0 ±0.0	45.5	7.9±0.2	0.97±0.01	3.45±0.01
Sludge (avg)¶	40.8	6.6	2.8	8.2	41.6	0.68	5.3	4.5
Soil (avg)#	45.7	5.4	2.1	1.9	44.8	8.2	--	--

†All data expressed on an ash- and water-free basis

‡LAS = anionic surfactants of the linear alkyl benzene sulfonate type; LS = anionic surfactants of the lauryl sulfate type

§Mean deviation of measurements on 3 samples of fulvic acid

¶Compiled from Sposito et al. (1976), Holtzclaw and Sposito (1978, 1979), and unpublished data

#From Schnitzer (1978), Table V

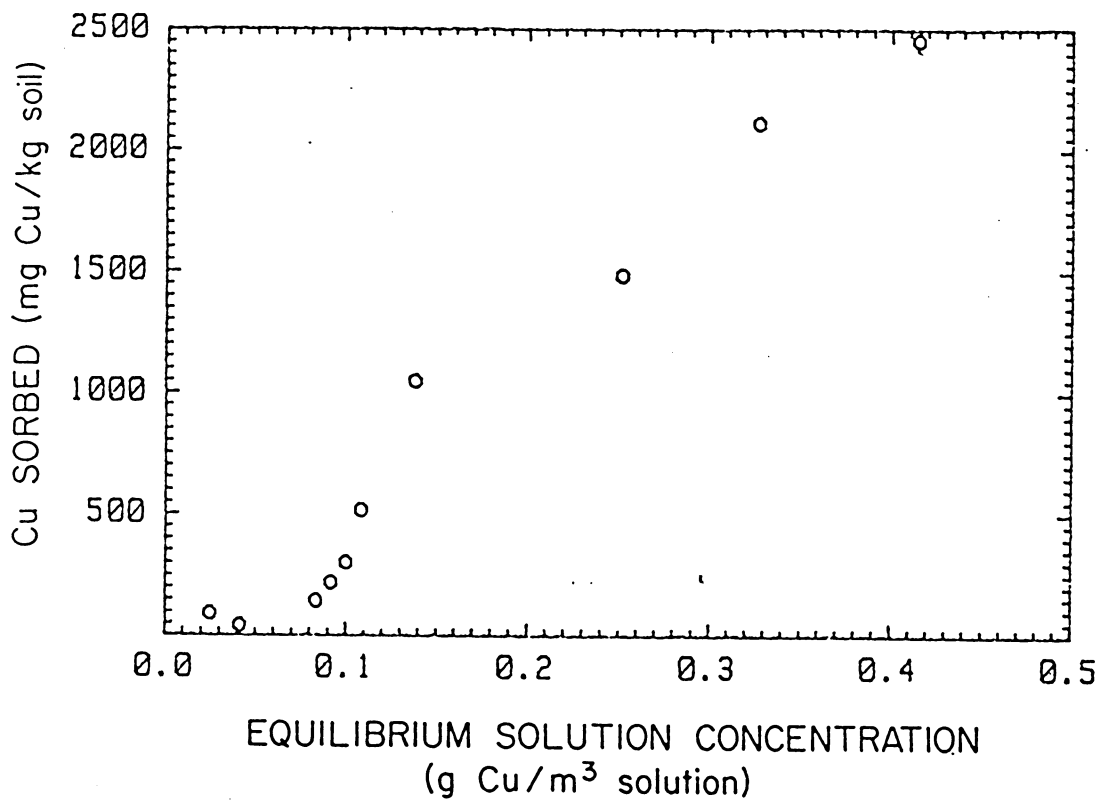


FIGURE III-10. Copper Sorption on Simona Soil

TABLE III-11

COPPER SORPTION ON DOMINO SOIL

Equilibrium Solution Concentration ($g \cdot m^{-3}$)	Amount Sorbed $mg\ kg^{-1}$
.0593	161
.0593	216
.0762	301
.0931	356
.112	437
.195	676
.584	1147
1.787	1613
4.936	2169
9.855	2373

order to evaluate this hypothesis, which has important implications for the effect of sludge organic matter on metal sorption characteristics in sludge-amended soils.

IV. ORGANIC COMPONENTS OF SLUDGE-AMENDED SOILS

A. The Fulvic Acid Fraction

Although the chemical nature of organic matter in soil has not been fully elucidated, there is a general consensus that the fulvic acid fraction of soil organic matter is an important set of compounds which can react strongly with trace metals (see, e.g., Schnitzer, 1978). Given the apparent increase in the organic fraction of trace metals in arid-zone soils amended with sewage sludge described in Sec. II.B, the question arises as to what is the nature of the fulvic acid fraction in these soils. In Sec. II.B it was demonstrated that the content of Ni, Cu, Zn, Cd, and Pb in the surface horizons of soils was governed by the total content of the metals in the applied sludges. If this result is extrapolated to the fulvic acid fraction, then its properties in soil should be governed by those of the fulvic acid fraction of the sludge. This hypothesis depends on the assumption that significant alteration of the sludge fulvic acid does not occur after it is incorporated into a field soil. If that assumption is not correct, the fulvic acid fraction of the soil could resemble that extracted from natural soil organic matter more than that extracted from sewage sludge. To help settle this question, a detailed study was made of a fulvic acid extracted from one of the soils investigated in Sec. II. This soil, Domino loam, had received 90 $Mg \cdot ha^{-1} \cdot year^{-1}$ of composted, anaerobically digested sewage sludge in an experimental field plot for three years prior to sampling.

It also had been cropped in consecutive seasons to barley and sorghum (Chang et al., 1983). Thus, both ample time and the necessary field conditions had existed for biologically-mediated transformations of the applied sludge in the soil environment.

1. Materials and methods

a. fulvic acid

A soil sample was collected from the upper 15 cm of a Domino loam (fine-loamy, mixed, thermic, Xerollic Calciorthid) in an experimental plot at the Moreno Field Station of the University of California, Riverside. The experimental plot, part of a long-term field study to assess the utility of sewage sludge as an agricultural amendment (Chang et al., 1983), had received a total of 270 Mg/ha of composted sludge and had been cropped with barley and sorghum prior to the sampling date in September 1978. (The sludge had been applied to the plot since 1975 at the rate of $Mg \cdot ha^{-1} \cdot year^{-1}$). Full details of the field experiment are given by Chang et al. (1983).

The soil sample was air-dried and made to pass through a 2-mm sieve, then mixed well and stored in a plastic bag before extraction of its fulvic acid fraction. This fraction was extracted from the soil and purified according to the method of Holtzclaw et al. (1976), except that the series of pH adjustments followed by centrifugation to remove "β-humus" was omitted to enhance the recovery of fulvic acid. The purified fulvic acid was lyophilized and stored in a vacuum dessicator over P_2O_5.

b. analytical procedures

Elemental analyses of the fulvic acid samples for carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O) were carried out generally as described by Sposito et al. (1976), with the samples digested for the N and S analyses as recommended by Nelson and Sommers (1972). Sulfur was analyzed by the barium chloranilate method (Beaton et al., 1968).

Carboxyl groups in the fulvic acid samples were determined by the calcium acetate method of Holtzclaw and Sposito (1979). This method does not suffer from the interferences of the older calcium acetate method of Schnitzer and Gupta (1965).

Amino acids and hexosamines were determined in the fulvic acid sample extracted from the sludge-amended Domino soil as described by Holtzclaw et al. (1980).

The content of unaltered anionic surfactant of the linear alkyl benzene sulfonate type was determined in the Domino fulvic acid sample by the method of Holtzclaw and Sposito (1978). This method cannot determine anionic surfactants of the laury sulfate type. These were determined by quantitative gas chromatographic analysis of the organic fraction of a hydrolyzed fulvic acid sample. This method has been described in detail by Schaumberg et al. (1982).

The infrared spectra of the fulvic acid samples were obtained as described by Sposito et al. (1976). Briefly, about 0.6 ml of fulvic acid solution were placed on a AgCl plate and evaporated to dryness over P_2O_5 in a vacuum dessicator kept in total darkness. The spectra were recorded on a Perkin-Elmer Model 621 spectrophotometer in the 4,000 to 400 cm^{-1} wave-number range. A blank AgCl plate was utilized in the reference beam.

Proton titration curves for the fulvic acid samples were determined potentiometrically as described by Sposito et al. (1977). The Domino fulvic acid sample was dissolved in deionized water, and this solution was filtered prior to a titration experiment. The titrated solution of Domino fulvic acid was found to contain 6×10^{-5} mol Al, 3×10^{-4} mol P, and 9×10^{-4} mol S per gram of fulvic acid. These small amounts of inorganic impurities would make at most a very small range between 3.0 and 11.0 and, therefore, they could be ignored when the curve was analyzed as discussed by Sposito et al. (1977). Titration curves were determined in duplicate for both fulvic acids at 25°C in the background solution of 0.1M KCl.

2. Results and discussion

The elemental composition of the fulvic acid extracted from the sludge-amended Domino soil as determined in the present study is given in Table IV-1. This table also lists the average composition of sludge-derived fulvic acid, compiled from data obtained previously in the authors' laboratory, and of fulvic acid extracted from natural soil organic matter, compiled recently by Schnitzer (1978). The Domino fulvic acid differs from the average sludge-derived fulvic acid primarily in its nitrogen content, which is about 60% greater than in an average sludge-derived fulvic acid. The content of C, H, S, and O in the Domino fulvic acid does not differ from that in an average sludge-derived fulvic acid by more than the normal variation of elemental composition in the latter [see Sposito et al. (1976), Table 1]. On the other hand, the Domino fulvic acid differs from the average soil fulvic acid outside the range of normal variation in respect to the content of C, H, N, and S [see Schnitzer (1978), Table II]. These results indicate that the Domino fulvic acid more resembles a sludge-derived fulvic acid than a fulvic acid extracted from natural soil organic matter. This is true particularly in the case of the sulfur content, which is about three times that of the average soil fulvic acid.

The content of carboxyl groups in the Domino fulvic acid, given also in Table IV-1, is much larger than what is typical in sludge-derived fulvic acid, but is quite well within the normal range for soil fulvic acids (Schnitzer, 1978, Table IV).

The Domino fulvic acid was found to have a small content of unaltered LAS-type anionic surfactants and a significant content of unaltered LS-type surfactants. The content of the latter is within the normal range of LS content for sludge-derived fulvic acid (Schaumberg et al., 1982), which suggests that this type of surfactant has not degraded much in the Domino soil. Schaumberg et al. (1980) have presented laboratory data which indicate that anionic surfactants can degrade rapidly in soils under aerobic, alkaline conditions. The data

in Table IV-1 suggest that these conditions, although they existed in the Domino soil, were not sufficient for the degradation of anionic surfactants.

The distribution of amino acids and hexosamines in the Domino fulvic acid is given in Table IV-2. These data can be compared with those obtained by Holtzclaw et al. (1980) for sludge-derived fulvic acids and with those compiled by Kowalenko (1978) for soils. Generally, the Domino fulvic acid is like a sludge-derived fulvic acid in that it is relatively higher in acidic amino acids and in S-containing amino acids than is the average soil organic matter. It is also similar to sludge-derived fulvic acid in having a relatively low content of basic amino acids and in not having detectable amounts of ornithine or hydroxyproline. The contents of glucosamine and galactosamine are quite similar to what is found in sludge fulvic acid (Holtzclaw et al., 1980, Table 1). The comparison can be brought into sharper relief through an examination of Table IV-3, which gives the nitrogen distribution in Domino fulvic acid. It is apparent that the relative proportions of NH_3-N and amino acid-N are reversed in relation to what is found in sludge fulvic acid (third column). However, the glucosamine/galactosamine ratio is about the same in Domino and the average sludge fulvic acid (approximately 0.13), but is about an order of magnitude smaller than what is typical in soil fulvic acid (Holtzclaw et al., 1980).

The infrared spectrum of the Domino fulvic acid showed bands assignable to H-bonded OH (3350 cm^{-1}), aliphatic CH(2920 and 28.50 cm^{-1}), carboxyl (1723, 1215, and 950 cm^{-1}), amide and aromatic C = C(1612 and 1560 cm^{-1}), phenolic OH(1400 cm^{-1}), polysaccharide (1045 cm^{-1}), and sulfonyl (1370, 1090, and 608 cm^{-1}) units (Stevenson and Goh, 1971; Sposito et al., 1976). The sharp sulfonyl absorption near 600 cm^{-1} is characteristic of sludge-derived fulvic acid (Sposito et al., 1976; Schaumberg et al., 1980, 1981) and suggests that the Domino fulvic acid has retained much of the nature of the sewage sludge material. The significant percentages of anionic surfactants in the Domino fulvic acid (Table IV-1) support this conclusion.

The proton titration curve for Domino fulvic acid is shown in Fig. IV-1. This curve was determined for a solution of 0.5 g $liter^{-1}$ fulvic acid in exactly the same fashion as in Exp. 13 and 14 reported by Sposito et al. (1977, Table 2 and Fig. 2, upper curve). The titration curve clearly shows three classes of acidic functional groups, just as found for sludge-derived fulvic acid in the pH range 3.0 to 11.0 by Sposito et al. (1977). In the present study, the titration curve could be reproduced closely by Eq. [1] in Sposito et al. (1976):

$$n_H = \sum_{i=II}^{IV} \frac{n_H^i \, {}^cK_{HA_i}[H]}{1 + {}^cK_{HA_i}[H]} \qquad \text{(IV-1)}$$

The values of the parameters n_H^i and ${}^cK_{HA_i}$ (i = II, III, IV), determined according to the method of Sposito et al. (1977), are listed in Table IV-4. The solid line in Fig. IV-1 is Eq. IV-1 plotted with these parameters. It is evident from Table IV-4 that the ${}^cK_{HA_i}$ (i = II, III, IV) are essentially the same as the condition proton stability constants determined for sludge-derived fulvic acid by Sposito et al. (1976).

TABLE IV-2

DISTRIBUTION OF AMINO ACIDS AND HEXOSAMINES

IN DOMINO FULVIC ACID (ASH- AND WATER-FREE BASIS)

Fraction	Relative molar distribution†	Percent of total fulvic acid mass	Percent N contributed‡
		Amino Acids	
Acidic			
Aspartic	15.33 ± 0.26§	0.5514 ± 0.0601	0.1646 ± 0.0064
Glutamic	14.00 ± 0.12	0.5686 ± 0.0736	0.1505 ± 0.0071
Basic			
Arginine	0.39¶	0.0495¶	0.0159¶
Histidine	0.55 ± 0.10	0.0659 ± 0.0085	0.0177 ± 0.0022
Lysine	1.53 ± 0.06	0.1717 ± 0.0037	0.0327 ± 0.0007
Neutral			
Phenylalanine	1.24 ± 0.01	0.15634± 0.0099	0.0133 ± 0.0009
Glycine	16.72 ± 0.17	0.8079 ± 0.0369	0.1796 ± 0.0082
Alanine	10.44 ± 0.02	0.7133 ± 0.0409	0.1122 ± 0.0064
Valine	6.26 ± 0.09	0.5620 ± 0.0384	0.0641 ± 0.0014
Isoleucine	2.41 ± 0.07	0.2419 ± 0.0201	0.0259 ± 0.0021
Serine	6.86 ± 0.43	0.5513 ± 0.0040	0.0736 ± 0.0006
Threonine	4.73 ± 0.09	0.4314 ± 0.0616	0.0508 ± 0.0019
Proline	5.87 ± 0.36	0.5151 ± 0.0589	0.0633 ± 0.0072
Leucine	3.45 ± 0.08	0.3476 ± 0.0273	0.0372 ± 0.0030
Tyrosine	1.23¶	0.1603¶	0.0126¶
Sulfur-containing			
Methionine	1.09 ± 0.7	0.1245 ± 0.0147	0.0118 ± 0.0014
Cysteic acid	4.09 ± 1.25	0.5174 ± 0.1840	0.0448 ± 0.0159
Half-Cystine	0.67¶	0.0794¶	0.0069¶
		Hexosamines	
Glucosamine		0.6163 ± 0.0483	0.0482 ± 0.0038
Galactosamine		5.0642 ± 0.4714	0.3961 ± 0.0369
		Unknown	
	4.52 ± 0.23#	0.4760 ± 0.0500	0.0487 ± 0.0051

†Mole per cent of total amino acid present
‡Grams of acid or grams hexosamine per gram of fulvic acid x % N in amino acid or hexosamine
§Mean deviation (two replicates)
¶Based on 1 determination only
#Based on the average mole weight of the amino acids detected

TABLE IV-3

NITROGEN DISTRIBUTION IN DOMINO AND IN AN AVERAGE

SLUDGE-DERIVED FULVIC ACID

Fraction	Domino	Sludge
Total N †	4.67	2.8
Unhydrolyzed-N‡	9.57	8.2
Hydrolyzed-N‡	90.43	91.8
Unidentified-N‡	1.04	1.2
NH_3-N ‡	56.81	29.6
Amino Acid-N‡	23.07	33.6
Glucosamine-N‡	1.03	3.3
Galactosamine-N‡	8.48	24.1

†Percent of ash- and water-free mass of fulvic acid
‡Percentage of total N
§Compiled from Holtzclaw et al. (1980)

TABLE IV-4

PARAMETERS IN EQ. (IV-1) OBTAINED FROM PROTON

TITRATION CURVES OF FULVIC ACIDS

Exp. No.	Fulvic acid	n_H^{II}	$\log^c K_{II}$	n_H^{III}	$\log^c K_{III}$	n_H^{IV}	$\log^c K_{IV}$
		mmol g^{-1}		mmol g^{-1}		mmol g^{-1}	
1	Domino	4.10	3.90	4.85	6.475	3.00	9.30
2	Domino	4.25	3.90	5.12	6.475	2.63	9.30
13	Sludge†	0.66	3.78	2.07	6.59	1.64	9.61
14	Sludge†	0.60	3.63	1.83	6.36	1.42	9.56

† Data from Sposito et al. (1977)

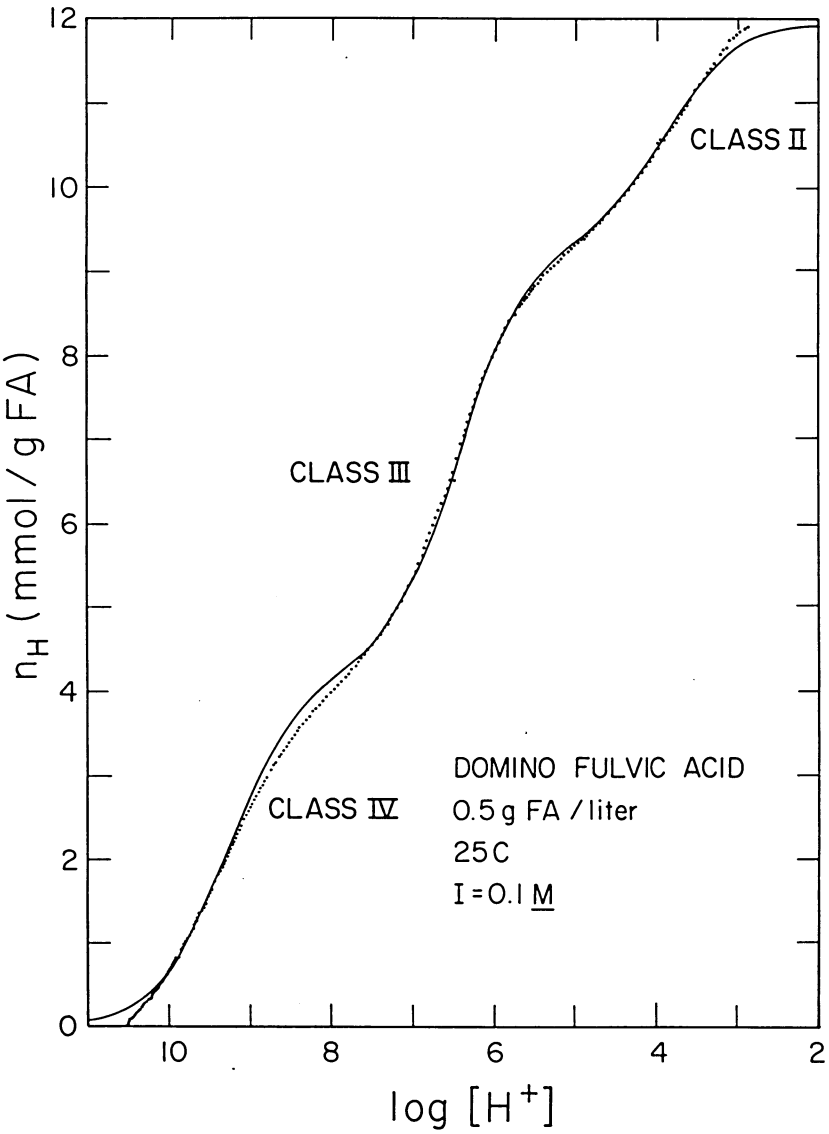


FIGURE IV-1. Proton titration curve for Domino fulvic acid. The parameter n_H is the number of mmol of protons bound per gram of fulvic acid (ash- and water-free basis) at a given molar concentration of protons, [H+]. The line through the data points is a plot of Eq. (IV-1) using the data in the second row of Table IV-4.

However, the values of the n_H^i are considerably larger for the Domino fulvic acid. The value of n_H^{II}, 4.2 mmol g^{-1}, may be compared with the COOH content of 7.9 mmol g^{-1} given in Table IV-1. The class II acidic functional groups are believed to be primarily COOH groups; apparently some, but not all, of the class III acidic functional groups also are carboxyls. This partitioning of the carboxyl groups into both classes II and III also occurred in a sample of sludge-derived fulvic acid studied by Holtzclaw and Sposito (1979). They found 0.63 mmol

g^{-1} of class II groups, but found 0.74 mmol g^{-1} COOH groups in this fulvic acid.

3. Conclusions

The fulvic acid fraction of a Domino soil which had received sewage sludge applications for three years in a field experiment bears a greater resemblance to a typical sludge-derived fulvic acid than to a fulvic acid extracted from natural soil organic matter. In particular, the properties of the Domino fulvic acid were quite different from those of a podzol fulvic acid employed as a reference material in this study. The principal data concerning the Domino fulvic acid which led to this conclusion are:

(i) the large sulfur content and the presence of sulfonyl groups;

(ii) the significant content of anionic surfactants and S-containing amino acids;

(iii) the very low glucosamine/galactosamine ratio, and

(iv) the close similarity of the shape of the proton titration curve to that for sludge-derived fulvic acid.

On the other hand, the Domino fulvic acid differed from a typical sludge-derived fulvic acid in having a much greater content of carboxyl and other titratable functional groups. This greater acidity was likely caused by the partial oxidation of the sludge organic matter in the Domino soil.

B. Electron Spin Resonance Studies on Copper-Fulvic Acid Complexes

On the basis of the sequential extraction data presented in Sec. II.B and II.C, it is expected that the organic complexes formed between copper(II) and fulvic acid extracted from sewage sludge will be a determinative factor in the partitioning of copper(II) among soluble chemical species in agricultural soils receiving sewage sludge applications. The macroscopic properties of these complexes have been measured with electrochemical techniques (Sposito and Holtzclaw, 1979; Sposito et al., 1979, 1981), but the results obtained yield no unambiguous information concerning structure and bonding. On the basis of an application of the Scatchard plot method (Stevenson, 1982, Chap. 15) to copper (Cu) titration experiments with sludge-derived fulvic acid (FA), Sposito et al. (1979) concluded that two classes of Cu-FA complexes existed, with the more stable class involving the sulfonic acid functional groups that are unique to sludge FA. This tentative conclusion, though in general accord with a significant body of literature on Cu(II) complexation by humic substances (Stevenson, 1982, Chap. 15), awaits substantiation by experiments aimed directly at elucidating structural chemistry.

Electron spin resonance (ESR) spectrometry is a useful technique for obtaining structural information about Cu(II)-FA complexes with few inherent limitations which could make interpretation problematic (Saar and Weber, 1982). There appear to be no published ESR studies of

sludge-derived FA, but Boyd et al. (1983) have obtained ESR spectra recently for Cu(II) complexes with sludge humic acid (HA). Their data suggested that complexed Cu^{2+} ions formed only two equatorial bonds exclusively with HA oxygen donor ligands and that various synthetic N-ligands were able to replace one or two equatorial water molecules to give Cu-HA-synthetic ligand adducts. Boyd et al. (1983) also suggested that Cu^{2+} may form axial bonds with sludge HA donor ligands originating from proteinaceous materials. ESR spectral data obtained by Lim (1981) for solutions prepared by adding Cu as $Cu(ClO_4)_2$ to a water-soluble organic fraction extracted from a sewage sludge suggested that four, in-plane ligands--principally sulfonate, aliphatic carboxyl, and N-containing groups--complexed Cu by acting independently as monodentate ligands. However, the ESR parameters on which the interpretation by Lim (1981) was based were limited to only $g_{\perp}$ values because of resolution problems.

Electron spin resonance data obtained for soil- and peat-derived HA and FA complexes with Cu(II) are in conflict with the published data on sludge-derived HA. McBride (1978), for example, concluded that only a single bond involving exclusively oxygen donors (COO^-) formed between Cu^{2+} and soil HA. Other ESR studies of Cu complexes with soil or peat FA and HA and their subfractions (Goodman and Cheshire, 1973, 1976; Cheshire et al., 1977; Lakatos et al., 1977; Bresnahan et al., 1978; Abdul-Halim et al., 1981) have suggested the existence of tridentate, and often quadridentate, equatorial chelation for the complexed Cu^{2+} ions.

Senesi and Sposito (1984) very recently have obtained ESR data for sludge-derived FA containing residual Cu (Cu/FA molar ratio $\simeq$ 0.0002) which also indicate that Cu^{2+} is bonded in polydentate, inner-sphere complexes, with both oxygen and nitrogen donor ligands arranged in an equatorial plane. The present study was undertaken to determine whether this conclusion can be extended to higher Cu/FA molar ratios as would be expected to occur in sludge-amended field soils.

1. Materials and methods

a. fulvic acids

Two FA samples extracted from anaerobically-digested sewage sludges were chosen for investigation on the basis of their significantly different chemical compositions. The sludge materials were gathered from surface matter in drying beds at the Ontario and Rialto wastewater treatment facilities in Southern California. The methods of extraction and purification of these FA samples and the techniques for preparing K/H fulvates in nitrate background have been described previously (Holtzclaw et al. 1976; Sposito et al., 1976; Sposito et al., 1977). The major elemental composition, copper (Cu) content, carboxyl group content, and ash content of the FA samples are reported in Table IV-5 (Sposito et al., 1976; Holtzclaw and Sposito, 1979). The copper content was determined by atomic absorption spectrometry (Perkin-Elmer model 5000) on acid-digested FA samples. Sposito et al. (1978) have reported a number-average relative molecular mass ($\overline{M}_n$) of 243 for the Rialto FA; a similar value may apply to Ontario FA.

TABLE IV-5

ELEMENTAL COMPOSITION (WATER- AND ASH-FREE BASIS), Cu CONTENT, CARBOXYL GROUP CONTENT, AND ASH CONTENT OF THE FULVIC ACIDS

Fulvic acid	C	H	N	S	O†	Cu	COOH	Ash
	---------- % ----------					mg/kg	mol/kg	%
Ontario	30.3±1.7‡	5.20±0.16	1.77±0.18	11.56±0.95	51.1	214	0.614±0.022	2.49±0.04
Rialto	45.2±3.0	7.31±0.28	3.06±0.06	4.46±0.65	40.0	301	0.740±0.034	3.82±0.08

†Calculated by difference: % O = 100 - % (C + H + N + S)

‡Mean deviation computed on data for three different samples of fulvic acid

TABLE IV-6

SPECTRAL PARAMETERS DERIVED FROM ESR SPECTRA AT 77 K OF UNTREATED AQUEOUS SOLUTIONS AND OF PRECIPITATES

Cu/FA ratio	$g_{\parallel}$ (± 0.001)	$\lvert A_{\parallel} \rvert$ (± 2 $cm^{-1} \times 10^4$)	$g_{\perp}$ (± 0.001)	g_{app} (± 0.001)	ligand atoms and molecules
Rialto FA					
0.08:1†	2.295	166	2.059	--	4 O/3 O,1 N (4 FA)
	2.273	192			2 O,2 N (4 FA)
0.8:1†	2.344§	154	2.073	--	4 O ($2H_2O$, 2 FA)
	2.310	181			4 O (4 FA)
8:1†	2.378	156	2.111	--	4 O ($2H_2O$, 2 FA)
				2.143	4 O ($4H_2O$)
8:1‡	2.358	155	2.087	--	4 O ($2H_2O$, 2 FA)
80:1‡	--	—	--	2.165	4 O ($4H_2O$)
80:1‡	2.361	153	2.083	--	4 O ($2H_2O$, 2 FA)
Ontario FA					
0.08:1†	2.269	181	2,057	—	2 O, 2 N (4 FA)
0.8:1†	2.348	158	2.076	--	4 O ($2H_2O$, 2 FA)
8:1†	—	—	—	2.167	4 O ($4H_2O$)
80:1†	--	—	--	2.169	4 O ($4H_2O$)
80:1†	2.361	153	2.080	--	4 O ($2H_2O$, 2 FA)

† aqueous solutions

‡ precipitates

§ main absorption

b. copper-fulvic acid complexes

Stock solutions of reagent-grade $Cu(ClO_4)_2$ were mixed with FA solutions as described by Senesi et al. (1984) to produce nominal Cu/FA molar ratios of 80, 8, 0.8, and 0.08. The pH value of each mixture was adjusted to 5.0 $\pm$ 0.1 by the addition of KOH. The mixtures then were shaken at laboratory temperature for 24 hours, following which the pH value was adjusted to 5.0 $\pm$ 0.1 again and the mixtures were centrifuged at 27,000 x g for 20 min. Precipitates were formed in the Rialto FA mixtures with Cu/FA molar ratios of 80 and 8, and in the Ontario FA mixture with molar ratio 80. These three precipitates accounted for approximately 20, 10, and 5 percent of the FA added initially. The subsequent treatment of the precipitates followed the procedure described by Senesi et al. (1984). Solutions of copper(II)-fulvic acid complexes also were prepared in water-glycerol mixtures (1:1 v/v).

The Cu^{2+} ions not complexed by FA were eliminated from supernatant solutions, obtained by centrifugation, with the resin method described by Senesi et al. (1984). Briefly, the solutions were passed twice at a rate of about 0.5 mL min^{-1} through a column of macroporous, strongly-acidic cation exchange resin (Bio-Rad AG-MP-50 cation exchange resin, H-form). The mass of dry resin employed was arranged to provide an exchange capacity ten times greater than the estimated total number of moles of charge of Cu(II) in the supernatant solutions. The pH values of the solutions dropped to 1.0 after passage through the resin, but were adjusted immediately to 5.0 $\pm$ 0.1 again with KOH.

Copper in the Cu-FA complexes was analyzed with a Perkin-Elmer model 5000 atomic absorption spectrophotometer (AAS). Samples of air-dried complexes obtained by evaporation of aliquots of the aqueous Cu/FA solutions at laboratory temperature were dissolved in acidified distilled water prior to AAS analysis.

c. ESR measurements

Electron spin resonance spectra were recorded at 77 K and at room temperature (RT) on a Bruker ER-200 D spectrometer operating at X-band frequency with a 100 kHz magnetic field modulation. Data were recorded as the first derivative of the absorption spectra. A microwave attenuation value of 13 dB was found to provide the least saturated signal and best resolved spectra; the corresponding microwave power varied between 9.1 and 9.2 mW. The modulation amplitude was fixed at 6.3 G (1 G = 10^{-4}T). The microwave frequency was set between 9.31 and 9.38 GHz for the spectra recorded at 77 K and 9.72 and 9.75 GHz for those recorded at RT. The magnetic field scanned was 2000 G usually, with center-field at 3000 G. In some cases, the field scan was limited to 1000 G with a center-field at 3100 G. Additional information pertaining to the scan range, gain, time constant, and scan time for each spectrum is given in the figures presented under Results and Discussion.

Spectra were recorded at 77 K for aqueous and water-glycerol solutions of the Cu(II)-FA complexes, including the solutions obtained after treatment with the H-form cation exchange resin. Spectra at 77 K and at RT were recorded for the precipitates obtained after centrifugation of the Cu-FA solutions and on air-dried solid samples obtained after evaporation of the Cu-FA solutions (both before and after passage

through the cation exchange resin). Quartz tubes (4 mm O.D./3 mm I.D.) supplied by Wilmad, Rt. 40, Oak Rd., Buena, NJ, were employed as sample containers.

The physical parameters of principal interest that can be derived from the analysis of the ESR spectra are the g-values ($g_{\parallel}$ and $g_{\perp}$) and the hyperfine coupling constants ($A_{\parallel}$ and $A_{\perp}$.) The g-values provide a measure of the interaction between the applied magnetic field and unpaired electrons. For a free electron, g = 2.0023. The g-values for organic radicals do not vary greatly from this figure, whereas in transition metal ion complexes, spin-orbit coupling can cause appreciable departures from this value depending on the contributions from atomic orbital motions. The hyperfine constants reflect coupling between an unpaired electron and the parent nucleus, if the latter has non-zero spin. Additional interactions of an unpaired electron with nuclei having non-zero spin in atoms bound to a central metal ion also are possible. These give rise to super-hyperfine splitting of the ESR absorption peaks.

The following three equations (Wertz and Bolton, 1972) were used for the calculation of g- and A-values from the experimental spectra:

$$g = \frac{h\nu_{el}}{\beta H} = 0.714484 \frac{\nu_{el}}{H} \qquad \text{(IV-2)}$$

where υ_{el} is the microwave frequency, expressed in MHz, H is the value of the field where the peak is centered, conventionally expressed in gauss, h is Planck's constant, and β is the Bohr magneton.

$$A\ (\text{MHz}) = 2.80247 \left(\frac{g}{g_e}\right) \times a \qquad \text{(IV-3)}$$

where g_e = 2.0023 (electron free-spin g-factor), g is calculated with Eq. (IV-2) and a is the hyperfine constant calculated from the ESR spectrum and expressed in gauss.

$$A\ (\text{cm}^{-1}) = \frac{A}{c} = 0.333564 \times 10^{-4} \times A\ (\text{MHz}) \qquad \text{(IV-4)}$$

where c is the speed of light in vacuum.

2. Interpretive theory of ESR parameters

Information regarding the nature of the binding sites in Cu-FA complexes can be derived from the magnitude of $g_{\parallel}$ and $A_{\parallel}$ (absorption at lower H-field), which are dependent, among other things, on the kind of ligand atoms coordinating to the Cu(II) center. The observed values of $g_{\parallel}$ for Cu(II) in a non-distorted, square planar coordination (tetragonal symmetry) range from about 2.4 in complexes with oxygen ligands to less than 2.1 in complexes with sulfur atoms. A similar variation exists in $g_{\perp}$ (absorption at higher H-field), although less information can be derived from the $g_{\perp}$ factors because they vary within a more limited range. The g-values are given by the following

equations for Cu(II) in tetragonal coordination with the unpaired electron in $d_{x^2-y^2}$:

$$g_{\parallel} = 2.0023 + \frac{8\lambda}{\Delta E(d_{x^2-y^2} - d_{xy})} \qquad \text{(IV-5)}$$

$$g_{\perp} = 2.0023 + \frac{2\lambda}{\Delta E(d_{x^2-y^2} - d_{xz,yz})} \qquad \text{(IV-6)}$$

where λ is the spin-orbit coupling constant (-83 cm^{-1}) and ΔE, which ranges from 1.4 to 1.8 cm^{-1}, is the separation in energy between the two sets of orbitals. According to Eq. (IV-5) and (IV-6) the variability of g-factors with the different type and number of ligand atoms coordinating to Cu^{2+} is dependent on the variation in the crystal field splitting, ΔE, from one complex to another. But, since the observed values of g are always smaller than those predicted on the basis of Eq. (IV-5) and (IV-6), it is possible that there exists, at least partly, a delocalization of the unpaired electron toward the ligands, i.e., covalency effects (Vänngård, 1972). This delocalizing effect of the ligands is related directly to their electronegativity. The combined effects of the crystal field splittings and the covalency effects give the following empirical order of decreasing g-factors for different donor atoms: O > N > S.

The behavior of the hyperfine coupling constants, $A_{\parallel}$ and $A_{\perp}$, is illustrated by the following simplified theoretical expressions, assuming the symmetry to be tetragonal with the unpaired electron in the $d_{x^2-y^2}$ orbital:

$$A_{\parallel} = P\left(-k - \frac{4\alpha^2}{7} + (g_{\parallel} - 2.0023) + \frac{3(g_{\perp} - 2.0023)}{7}\right) \qquad \text{(IV-7)}$$

$$A_{\perp} = P\left(-k + \frac{2\alpha^2}{7} + \frac{11\,(g_{\perp} - 2.0023)}{14}\right) \qquad \text{(IV-8)}$$

The constant P is equal to about 3.5 cm^{-1}. The terms containing the factor α^2 arise from dipole-dipole interactions between the magnetic moments associated with the spin motion of the electron and the nucleus. Delocalization of the unpaired electron toward the ligand atoms reduces this contribution as α^2 decreases from unity. The terms containing the g-factors derive from the coupling between the orbital motion and the nucleus. The values assumed by the hyperfine coupling constants, $A_{\parallel}$ and $A_{\perp}$, are, therefore, directly related, through the terms containing the factor α^2 in the Eq. (IV-7) and (IV-8), to the delocalization of the unpaired electron toward the neighboring atoms (electronegativity) and, through terms of the type (g - 2.0023), to the g-factors. The g-factors also depend partly on the electronegativity of the atoms coordinated (covalency effects). It follows that the values of $A_{\parallel}$ and $A_{\perp}$ increase

and those of $|A_{\parallel}|$ and $|A_{\perp}|$ decrease with increasing electronegativity of the ligand atoms (O < N < S).

In conclusion, an opposite empirical relationship exists between the electronegativity of the ligand atoms and the values assumed both by the g-factors and the hyperfine splitting constants, $|A|$. Care should be exercised in any interpretation of the kind and the number of atoms coordinating, however, since small deviations from square planar coordination will shift $A_{\parallel}$ to smaller values, while larger deviations wil also shift $g_{\parallel}$ to smaller values (closer to g = 2) as well (Peisach and Blumberg, 1974).

3. Results and discussion

a. copper-fulvic acid solutions with excess copper

Aqueous solutions. Figure IV-2 shows ESR spectra recorded at 77 K for aqueous solutions of $Cu(ClO_4)_2$ and Rialto FA after centrifugation but before resin treatment to remove excess Cu^{2+} ions. The lineshapes of the spectra clearly are dependent on the Cu/FA molar ratio, as observed previously by Senesi et al. (1984) for copper(II)-soil FA solutions. Only one broad peak with a g_{app} = 2.165 (Fig. IV-2a) is shown by the solution with the highest Cu/FA ratio (80), whereas the spectrum of the solution with Cu/FA = 8 (Fig. IV-2b) shows a weak resolution at the lowest fields. The spectrum in Fig. IV=2c) (Cu/FA = 0.8) shows a much better resolved pattern. The latter two spectra gave spin Hamiltonian parameters ($g_{\parallel}$, $|A_{\parallel}|$ and $g_{\perp}$) typical of non-rotating Cu^{2+} ions in inner-sphere complexes, consistent with a $d_{x^2-y^2}$ groundstate of Cu(II) bonded with a number of ligands other than water and arranged in distorted octahedral coordination (tetragonal symmetry) (Senesi and Sposito, 1984). A more accurate analysis of the spectrum for a Cu/FA ratio of 0.8 indicates the presence of two, four-featured components at $g_{\parallel}$, the minor component showing a lower $g_{\parallel}$-value and a higher coupling constant $|A_{\parallel}|$ than the principal one (Table IV-6). The solution prepared with the lowest Cu/FA ratio (0.08) gave a spectrum that was highly structured at $g_{\parallel}$ and at $g_{\perp}$, but difficult to interpret because of the low signal-to-noise ratio at the high gain employed to detect the very low Cu(II) content. Two quadruplets may exist at $g_{\parallel}$.

An examination of the ESR parameters in Table IV-6 suggests that different types and numbers of FA binding sites are involved in Cu(II) complexation at different Cu/FA ratios. When the amount of added Cu is very low (Cu/FA = 0.08) the energetically strongest coordinating sites with least sterical hindrance should be engaged in binding of Cu. The $g_{\parallel}$-value and $|A_{\parallel}|$ coupling constant of the two ESR components suggest 2 O, 2 N and 4 O (or 3 O, 1 N) binding sites. At a Cu/FA ratio of 0.8, other available FA functional groups become involved in the binding of Cu. The ESR parameters in Table IV-6 indicate an almost exclusive participation of oxygen ligand atoms in FA in the coordination. The g - values and $|A|$ constants of the main component approach those of the Cu^{2+} aquo-ion, suggesting that Cu/FA interactions are largely ionic and involve surface oxygen atoms. The absence in this spectrum of low intensity ESR resonances attributable to the more nitrogenated binding sites in FA may be a result of the much lower relative intensity of

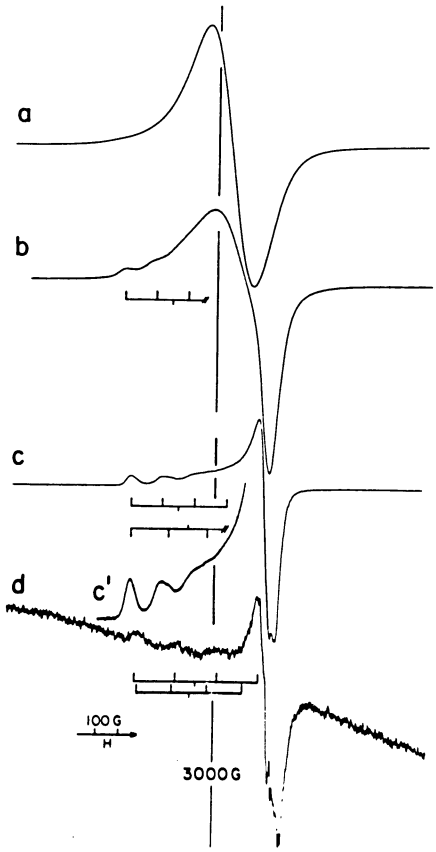


FIGURE IV-2. ESR spectra (scan range, 2 kG) of untreated Cu-Rialto FA aqueous solutions at 77 K and at Cu/FA ratios of:

(a) 80 (gain, 8×10^2; time constant, 10 ms; scan time, 500 s)
(b) 8 (gain, 1.25×10^4; time constant, 50 ms; scan time, 500 s)
(c) 0.8 (gain, 1×10^4; time constant, 50 ms; scan time, 200s)
(c') 0.8 (gain, 4×10^4; time constant, 100ms; scan time, 500s)
(d) 0.08 (gain, 5×10^5; time constant, 1s; scan time, 1 ks)

this latter signal with respect to the much more intense new ones. The percentage of available oxygen functional groups (mainly sulfonic, carboxylic, and phenolic OH) in sewage sludge FA is indeed much higher than that of nitrogen groups (Table IV-5) (Holtzclaw and Sposito, 1978; Schaumberg et al., 1982; Holtzclaw et al., 1980).

When the Cu/FA ratio is increased to 8, $g_{\parallel}'$, $g_{\perp}$, and $|A_{\parallel}|$ approach values appropriate for Cu^{2+} aquo-ions, indicating the presence of more hydrated (probably only monodentate or bidentate) FA complexes. The broad, partially superimposed line at g_{app} reflects the significant presence of free Cu^{2+} ions. At a Cu/FA ratio of 80, the dipolar broadened absorption produced by the excess of Cu^{2+} aquo-ions prevails over any other ESR signal. The high Cu/FA ratio used in these two systems caused the formation of precipitates whose ESR spectra at 77 K

in the solid state are shown in Fig. IV-3. A well resolved quadruplet is evident at $g_{\parallel}$. The calculated $g_{\parallel}$, $g_{\perp}$ and $|A_{\parallel}|$ values (Table IV-6, rows 6 and 8) appear to be very similar for the two precipitates, indicating that the same type of binding sites for Cu(II) are present in the complexes with FA, despite the different Cu/FA ratios of the parent solutions. Perhaps the excess of Cu^{2+} ions induces the aggregation of a certain number of small FA molecules and complexes through the formation of highly cross-linked structures. The increased relative molecular mass of the aggregated particles may exceed FA solubility limits and produce precipitation. The $g_{\parallel}$ and $|A_{\parallel}|$ values calculated for the precipitates are consistent with 4 O ligand atoms in the binding environment. The particularly high $g_{\parallel}$ values and low $|A_{\parallel}|$ constants may reflect a significant involvement of water molecules in the coordination.

Turning now to the ESR spectra obtained for Cu(II) complexes with Ontario FA, one notes that the spectra at the two lowest Cu/FA ratios (not shown) are characterized by only one well-resolved quadruplet at $g_{\parallel}$. The $g_{\parallel}$, $|A_{\parallel}|$ and $g_{\perp}$ parameters (Table IV-7) are very similar to those calculated for one of the quadruplets in the corresponding ESR spectra of the Cu(II)-Rialto FA solutions. The ESR data are consistent, respectively, with a 2 O, 2 N FA binding site for the sample at Cu/FA = 0.08 and with a 4 O mixed FA and water binding configuration for the sample prepared at Cu/FA = 0.8. The absence, in the ESR spectra of Ontario FA, of the other component present in the spectra of the Rialto FA complexes and attributed to a 4 O and/or 3 O, 1 N binding site can be ascribed to the low carboxyl group content of the Ontario FA with respect to the Rialto FA (Table IV-6).

When the Cu/FA ratio is 8.0, the dipolar-broadened ESR signal of Cu^{2+} aquo-ions does not allow resolution of ESR features specific to Cu(II)-FA complexes. This fact, together with the absence of a precipitate at this molar ratio, suggests a lower overall ability of Ontario FA oxygen-containing functional groups to form complexes with Cu^{2+} ions. At the highest Cu/FA ratio (80:1), the behavior of Ontario FA resembles qualitatively that of Rialto FA in the ESR spectra of both the aqueous solution and precipitate, the latter spectrum showing ESR data (Table IV-7) almost identical to those of the corresponding Rialto FA precipitate. The lower amount of precipitate obtained in the case of Ontario FA seems to illustrate the lower capacity of oxygen functional groups in Ontario FA to form aggregates through Cu^{2+} bridges among FA molecules and/or primary Cu(II)-FA complexes.

In general, the ESR results for the two different sludge FA tend to confirm the hypothesis that at least two distinct sets of complexes may be formed between sludge FA and Cu^{2+} (Sposito et al., 1979). The most acidic functional groups in FA (i.e., sulfonic acid groups), together with nitrogen groups appear to be the first involved in the formation of Cu(II) complexes. The more weakly acidic functional groups (e.g., carboxyl groups) then may act to form secondary, more aggregated (i.e., higher relative molecular mass) complexes.

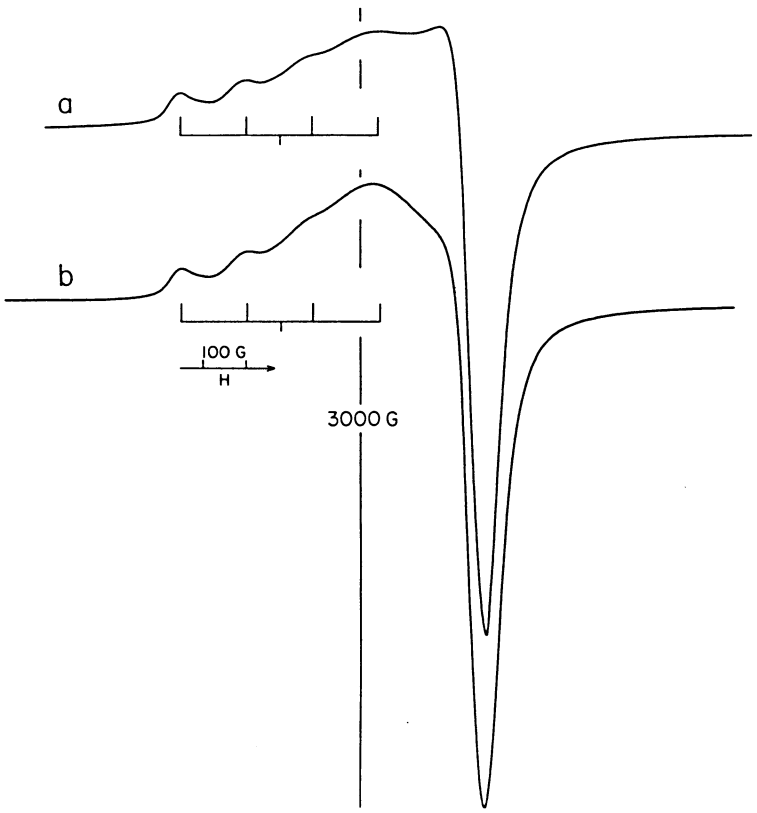


Figure IV-3. ESR spectra (scan range, 2 kG) at 77 K for precipitates from Cu-Rialto FA solutions at Cu/FA ratios of:
(a) 80 (gain, 2.5×10^3; time constant, 20 ms; scan time, 500 s)
(b) 8 (gain, 4×10^3; time constant, 20 ms; scan time, 500 s)

Water-glycerol solutions. Figures IV-4 shows ESR spectra recorded at 77 K for water-glycerol solutions of Cu(II)-Rialto FA. When glycerol is present at the lowest Cu/FA ratio (0.08), the spectrum is characterized by two, almost congruent four-featured components at $g_{\parallel}$. The $g_{\parallel}$ and $|A_{\parallel}|$ values for the minor component (Table IV-7) appear to be not very different from those calculated for the component that corresponds to the 2 O, 2 N FA binding site for Cu(II) in aqueous solution alone. Therefore, the complexation reactivity of this site is large, despite the massive presence of glycerol ligands. On the other hand, the major component in the spectrum shows a $g_{\parallel}$-value and a $|A_{\parallel}|$ constant higher and lower, respectively, than those corresponding to the second component detected in the aqueous solution spectrum, indicating a partial replacement, at this site, of one FA oxygen or nitrogen ligand group by one glycerol molecule.

TABLE IV-7

SPECTRAL PARAMETERS DERIVED FROM ESR SPECTRA AT 77 K FOR WATER-GLYCEROL SOLUTIONS

Cu/FA Ratio	$g_{\parallel}$ (±0.001)	$\lvert A_{\parallel}\rvert$ (± 2 $cm^{-1} \times 10^4$)	$g_{\perp}$ (±0.001)	Ligand atoms and molecules
Rialto FA				
0.08:1	2.316†	164	2.066	4 O (4 FA)
	2.268	187	2.052	2 O, 2 N (4 FA)
0.8:1	2.357†	150	2.073	4 O (2 glyc, 2 FA)
	2.309	172		4 O (4 FA)
8:1	2.404†	138	2.079	4 O (4 glyc)
	2.364	159		4 O (2 glyc, 2 FA)
80:1	2.407	138	2.081	4 O (4 glyc)
Ontario FA				
0.08:1	2.292†	171	2.064	4 O/3 O, 1 N (4 FA)
	2.265	185	2.051	2 O/2 N (4 FA)
0.8:1	2.407	138	2.078	4 O (4 glyc)
	2.358†	150	2.064	4 O (2 glyc, 2 FA)
8:1	2.407†	138	2.081	4 O (4 glyc)
	2.370	150		4 O (2 glyc, 2 FA)
80:1	2.405	138	2.085	4 O (4 glyc)

TABLE IV-8

SPECTRAL PARAMETERS DERIVED FROM ESR SPECTRA (77 K) OF SAMPLES DRIED BEFORE RESIN TREATMENT

Cu/FA Ratio	$g_{\parallel}$ (±0.001)	$\lvert A_{\parallel}\rvert$† (±2$cm^{-1} \times 10^4$)	$g_{\perp}$ (±0.001)	Number of lines at $g_{\perp}$‡	g_{app} (±0.001	Ligand atoms and molecules
Rialto FA						
0.08:1	2.277§	182/193	2.058	5 lines	--	2 O, 2 N (4 FA)
	2.261	186	2.049	5 lines		2 O, 2 N (4 FA)
0.8:1	2.302	177	2.058	5 lines	--	4 O (4 FA)
	2.277	182/193	2.051	5 lines		2 O, 2 N (4 FA)
8:1	2.325	156	2.111	—	—	4 O ($1H_2O$,3 FA)
80:1	—	—	--	—	2.176	4 O ($4H_2O$)
Ontario FA						
0.08:1	2.264	181	2.057	—	—	2 O,2 N (4 FA)
0.8:1	2.303	177	2.057	—	—	4 O (4 FA)
	2.278	182/196	2.048	5 lines		2 O,2 N (4 FA)
8:1	—	—	--	—	2.138	4 O ($4H_2O$)
80:1	—	—	--	—	2.141	4 O ($4H_2O$)

†two values for $\lvert A_{\parallel}\rvert$ refer to couples of peaks at the same $g_{\parallel}$

‡$\lvert A_{\perp}^{14N}\rvert = (20 \pm 2\ cm^{-1}) \times 10^{-4}$

§main absorption

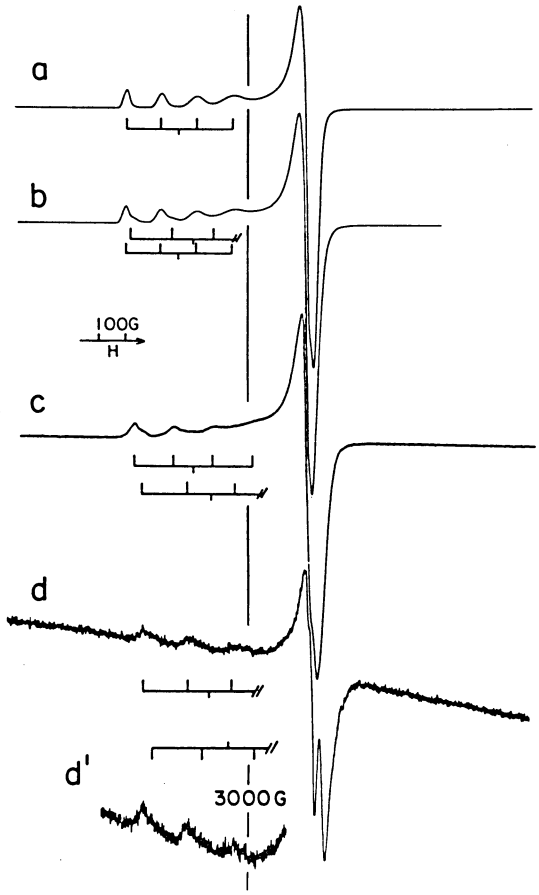


Figure IV-4. ESR spectra (scan range, 2 kG) of Cu-Rialto FA water-glycerol solutions at 77 K and at Cu/FA ratios of:
(a) 80 (gain,8×10^{2};time constant,20 ms;scan time,500 s)
(b) 8 (gain,4×10^{3};time constant,20 ms;scan time,500 s)
(c) 0.8 (gain,2×10^{4};time constant,50 ms;scan time,500 s)
(d) 0.08 (gain,2.5×10^{5};time constant,500 ms;scan time,1 ks)
(d') 0.08 (gain,5×10^{5};time constant,500 ms;scan time,1 ks)

At a Cu/FA ratio of 0.8, there is again an ESR spectrum characterized by a major and a minor absorption at $g_{\parallel}$. The ESR parameters may be associated with two different coordination environments, both involving 4 O ligand atoms, suggesting the replacement of water molecules by glycerol ligands at one of the sites. The ESR spectrum obtained for the Cu/FA = 8 system appears quite different from that obtained in the absence of glycerol. The spectrum is well resolved into: (a) a principal, four-feature component that may be assigned to non-rotating, mixed glycerol-water complexes formed with excess Cu^{2+} (Table IV-7) and (b) a very minor component showing a $g_{\parallel}$-value and $|A_{\parallel}|$ constant somewhat lower and higher, respectively, than those calculated for the anisotropic component detected in the corresponding aqueous solution spectrum (Fig. IV-2c and Table IV-6). The latter result may be explained by the substitution of water with glycerol as the ligand in monodentate (or bidentate) complexes of Cu in the less strong binding sites of FA. The low gain used in the recording of this spectrum, together with the superposition of the spectrum of the glycerol-water-excess-Cu^{2+} complexes, does not make it possible to detect, under these conditions, the small ESR signal from stronger Cu-FA complexes that can be observed in the spectra of water-glycerol solutions at the lowest Cu/FA ratios.

The ESR spectrum obtained in the presence of glycerol at the highest Cu/FA ratio gives parameters identical to those previously calculated from the spectra obtained from Cu^{2+}-water-glycerol complexes in the absence of FA (Senesi et al., 1984), thus indicating the prevalence of Cu^{2+}-aquo-glycerol complexes in this system.

The analysis of the ESR spectra obtained at 77 K for the water-glycerol solutions of Cu(II) -Ontario FA solutions (not shown) gave the Hamiltonian spin parameters listed in the second part of Table IV-7. At the lowest Cu/FA ratio (0.08), two components are resolved in the spectrum. The minor one shows a $g_{\parallel}$-value and $|A_{\parallel}|$ constant very similar to those calculated for the unique feature recorded in the absence of glycerol, whereas the major one gives a higher $g_{\parallel}$-value and a lower $|A_{\parallel}|$ constant. One explanation of these results is that glycerol molecules may have displaced one or two FA nitrogen ligand atoms in the original 2 O, 2 N FA binding sites, giving rise to a predominant 3 O, 1 N or 4 O coordination for Cu(II), while a smaller number of stronger 2 O, 2 N FA binding sites are left unchanged.

When Cu/FA = 0.8, again two components are well resolved in the ESR spectrum. The $g_{\parallel}$-value and $|A_{\parallel}|$ constant of the main component appear very similar to those calculated for the major component present in the ESR spectrum obtained at the same Cu/FA ratio for the water-glycerol solution of the Cu(II)-Rialto FA solution and, therefore, may be interpreted in the same way. On the other hand, the minor component shows ESR parameters almost identical to those corresponding to Cu^{2+} ions complexed completely by O-ligand atoms of the solvents (Senesi et al., 1984). This result appears to indicate: (a) a complete displacement of the Cu^{2+} ions from the weaker, fully oxygenated FA binding sites and/or (b) the participation of glycerol in the coordination of a slight excess of free Cu^{2+} aquo-ions not complexed by the Ontario FA in aqueous solution alone.

The ESR spectrum obtained for Cu/FA = 8 is resolved also in two components whose parameters are almost identical to those of the components of the corresponding Cu(II)-Rialto FA solution. The ESR parameters for the main component are typical of Cu^{2+} ions fully coordinated by solvent molecules. The parameters for the minor component clearly indicate the existence of monodentate (or bidentate) Cu(II)-FA complexes partly involving solvent molecules. These results are of interest because the presence of glycerol seems to make possible the detection of the weak ESR signal of Cu(II)-Ontario FA complexes, even in the presence of a large excess of free Cu^{2+} ions in the system. The resolution of the signal was not possible in the original aqueous solution where the broad ESR absorption masked almost completely the weak ESR signal of the Cu-FA complexes. The ESR of Cu-Ontario FA solution at the highest Cu/FA ratio, similar to the Cu-Rialto FA system, is characterized by a unique feature consistent with Cu^{2+} ions fully coordinated by glycerol and/or water ligands.

Air-dried samples. The spectra recorded at 77K for air-dried solutions of both Rialto and Ontario FA at Cu/FA = 80 showed no significant differences in lineshape and ESR parameters (Table IV-8) with respect to spectra obtained for the untreated solutions (i.e., one broad almost symmetric line). On the other hand, the ESR spectra of the air-dried samples obtained from solutions at lower Cu/FA ratios (Fig. IV-5) show remarkable differences, both in the lineshape and spin Hamiltonian parameters (Table IV-8), from those of the corresponding untreated solutions. Couples of very closely spaced, but well-separated, peaks forming two quadruplets centered on the same g -value appear clearly in these spectra. This feature can be attributed to hyperfine coupling from ^{63}Cu and ^{65}Cu nuclei. No evidence has been seen previously for such a well-resolved feature in the ESR spectra of either Cu(II)-FA or Cu(II)-HA systems. Another typical characteristic of these spectra is the intense, well-resolved structure at $g_\perp$. Here a larger number of lines are better resolved than in the $g_\parallel$-structures observed previously for either Cu- soil FA complexes after the elimination of excess Cu^{2+} ions (Senesi et al., 1984) or residual Cu-sludge FA complexes (Senesi and Sposito, 1984). The lines in the $g_\perp$ region may be the result of: (a) heterogeneity, (b) copper nucleus hyperfine structure, (c) ligand nuclei super-hyperfine structure, (d) the "overshoot" line, and/or (e) lower than axial symmetry (Malklin and Malmstrom, 1970). According to the discussion given by Senesi and Sposito (1984), and to the results of studies on copper(II) proteins (Vänngård, 1972), these lines should be assigned preferentially to heterogeneous superhyperfine coupling of the unpaired electron to a number of nitrogen ligand nuclei. In order to do a better analysis of the intensely-structured pattern at $g_\perp$, ESR spectra were recorded in a narrower scan range (1 kG), thereby permitting a more accurate calculation of the $|A_\perp^{14}N|$ constants (Fig. IV-6 and Table IV-8). Two groups of 5 lines each can be singled out, each group corresponding to two equivalent nitrogen atoms coordinating the copper(II). The shoulders in Fig. IV-6 are excluded from this interpretation, but they may indicate an even higher heterogeneity.

A general decrease in $g_\parallel$-values and increase in $|A_\parallel|$ constants (Table IV-8) were observed for these spectra with respect to the ESR

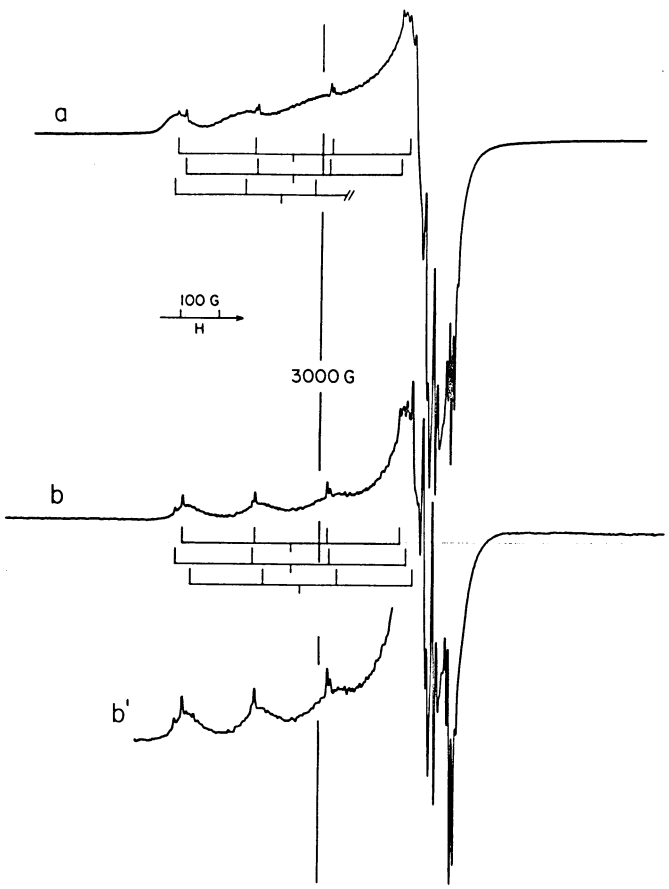


Figure IV-5. ESR spectra (scan range 2 kG) at 77 K for Cu-Rialto FA air-dried samples from untreated aqueous solutions at Cu/FA ratios of:
(a) 0.8 (gain,$8x10^3$;time constant,100 ms,scan time,500 s)
(b) 0.08 (gain,$3.2x10^4$;time constant,500 ms;scan time,1 ks)
(b') 0.08 (gain,$6.3x10^4$;time constant,1 s;scan time,1ks)

parameters of the corresponding untreated solutions, suggesting a more intense participation of FA nitrogen ligands, evidently as a result of the elimination of the solvent upon drying. Two components are found in the ESR spectrum of the Cu-Rialto FA solid sample at Cu/FA = 0.08. The ESR parameters of one are very similar but those of the other quite different from the corresponding parameters of the untreated solution. The latter component appears consistent with a more nitrogenated FA binding site than that present in the aqueous solution. In addition, the $g_{\perp}$ region appears very structured. However, the corresponding Cu-Ontario FA sample shows an ESR spectrum with only one component at $g_{\parallel}$ characterized by parameters very similar to those shown by the untreated solution and with no structure in the $g_{\perp}$ region. These

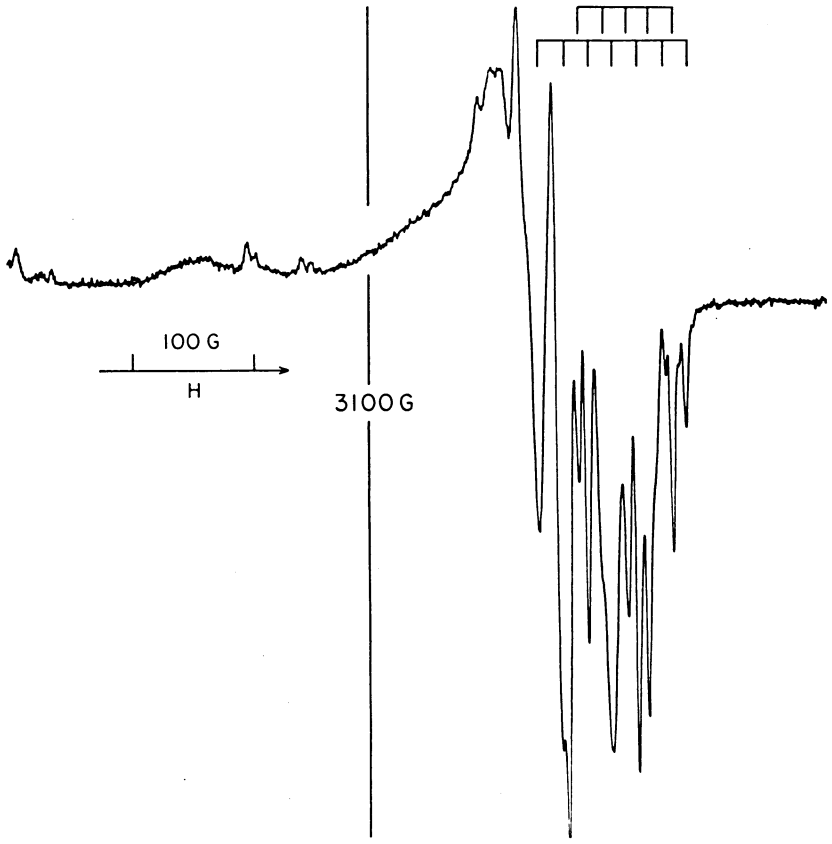


Figure IV-6. ESR spectrum (scan range 1 kG) at 77 K for Cu-Rialto FA air-dried sample from untreated aqueous solution at Cu/FA = 0.08 (gain, 3.2×10^4; time constant, 500 ms, scan time, 1 ks)

features may be the result of the much higher content of nitrogen in the Rialto FA with respect to the Ontario FA (Table IV-5).

The ESR spectra and parameters of the air-dried samples from solutions at a Cu/FA ratio of 0.8 appear almost identical for the two FA, but they are very different from the corresponding aqueous solution data. In both cases, two well-resolved components may be detected. Both show $g_{\parallel}$-values lower and $|A_{\parallel}|$ constants higher than in the spectra obtained for aqueous solutions. Moreover, a very structured pattern of lines appears at $g_{\perp}$, suggesting that drying the solutions with an increased Cu/FA ratio produced a differentiation of two binding site classes in both FA: (a) a stronger one, more nitrogenated and detectable also at the lowest Cu/FA ratio, and (b) a weaker one, more oxygenated, originating from the displacement of water molecules by FA oxygen-containing functional groups.

The $g_{\parallel}$ -value of the single resolved feature in the spectrum of the air-dried sample prepared from the Rialto FA solution at Cu/FA = 8 also was lower than that for the aqueous solutions, indicating again a partial replacement of water ligand molecules by FA oxygenated groups upon drying.

The ESR spectra recorded at RT on the dried Cu-FA systems showed lineshapes very similar, but less resolved, than the spectra recorded at 77 K. In particular, the resolution of the peaks arising from the hfs coupling from the two copper isotopes often was very poor and a reduced number of lines was observed in the $g_{\perp}$ region. Spin Hamiltonian parameters thus were calculated with more difficulty, but their values (Table IV-9) are not substantially different from those calculated from the spectra at 77 K.

b. resin-treated copper-fulvic acid solutions

Aqueous solutions. The ESR spectra recorded at 77 K and related spin Hamiltonian parameters for aqueous solutions of the Cu-sludge FA complexes after resin treatment were always very well resolved and fundamentally similar for both the Rialto and Ontario FA at all Cu/FA ratios. Similar observations were made after resin treatment of Cu-soil FA aqueous solutions (Senesi et al., 1984). The present findings confirm the efficacy of the resin treatment for the elimination of interferences caused by excess Cu^{2+} aquo-ions in the ESR analysis of Cu(II)-FA complexes, thereby ameliorating the ESR analysis at lower Cu/FA ratios and allowing it at higher Cu/FA ratios.

Figures IV-7 to and IV-8 show the ESR spectra obtained, respectively, for the Rialto FA and the Ontario FA complexes with copper(II). Table IV-10 lists the ESR parameters calculated from these spectra. Two well-resolved components at $g_{\parallel}$ may be easily seen in all the ESR spectra, showing $g_{\parallel}$ -values and $|A_{\parallel}|$ couplings consistent with a 4 O and a 2 O, 2 N FA-binding site for Cu^{2+} in FA. In two cases, the $g_{\perp}$-values appear to be somewhat different from those observed typically. The ESR component at lower field for the Cu-Rialto FA complex originating from a solution at Cu/FA = 0.08 yields a lower than usual $g_{\parallel}$-value (2.290) that may be attributed to a partial involvement of N-ligand atoms in the coordination (3 O, 1 N) and/or to the low resolution of this spectrum. The higher than usual $g_{\parallel}$ -value (2.286) of the ESR component at higher field for the Cu-Rialto FA complex prepared from a solution at a Cu/FA = 8 may be the result of a greater participation of oxygen in the coordination (3 O, 1 N) and/or the partial superposition of different spectral components.

A third, minor ESR component at $g_{\parallel}$ is detectable in many of the spectra. The particularly low $g_{\parallel}$ -value and high $|A_{\parallel}|$ coupling constant of this component suggest a 1 O, 3 N coordinating site for Cu^{2+}. A similar value for $g_{\parallel}$, but lower for $|A_{\parallel}|$ was attributed to the 1 O, 3 N site in $[Cu(bipy)(py)]^{++}$ by Walker et al. (1972), whereas Boyd (1983) attributed the $\bar{g}$ = 2.25 and $|A_{\parallel}|$ = 179 and 181 x 10^{-4} cm^{-1} values to a CuO_2N_2 chromophore in the adducts, Cu-sludge HA:phen = 1:1 and Cu-sludge HA:en = 1:1.

Further information about Cu^{2+}-sludge FA complexation mechanisms may be obtained by a comparative ESR study of the samples, before and

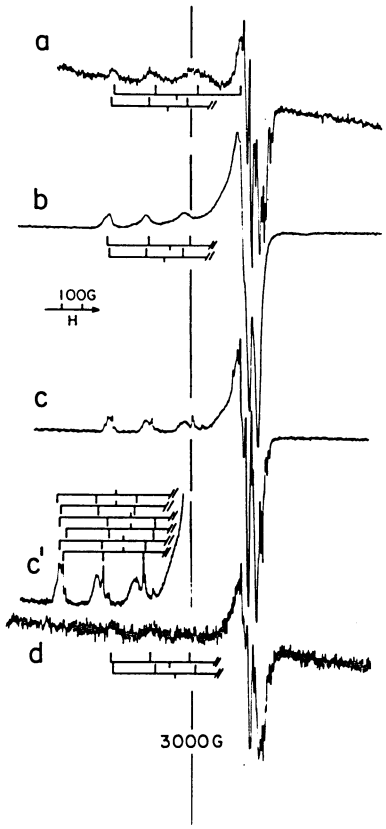


Figure IV-7. ESR spectra (scan range, 2 kG) of Cu-Rialto FA aqueous solutions at 77 K after the resin treatment and at Cu/FA ratios of:

(a) 80 (gain,5×10^{5};time constant,1 s;scan time, 1 ks)
(b) 8 (gain,6.3×10^{4};time constant,200 ms;scan time, 500 s)
(c) 0.8 (gain,8×10^{4};time constant;200 ms;scn time,500 s)
(c') 0.8 (gain,2×10^{5},time constant,500 ms;scan time, 1 ks)
(d) 0.08 (gain,8×10^{5};time constant, 1 s; scan time, 2 ks)

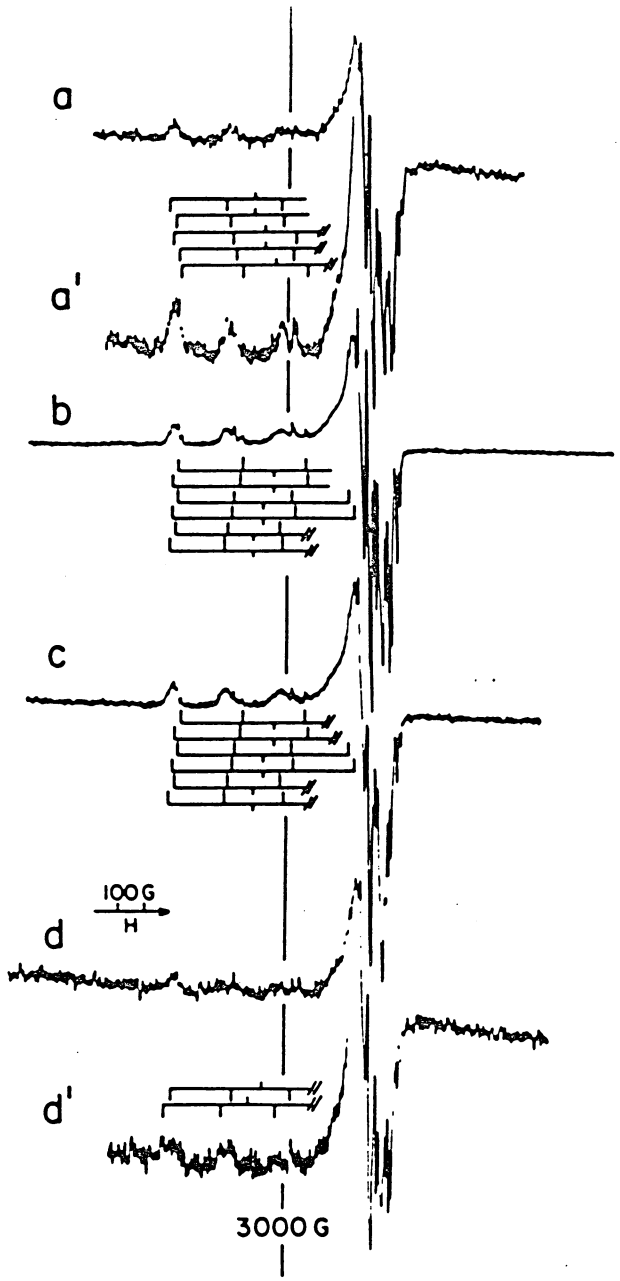


Figure IV-8. ESR spectra (scan range, 2 kG) of Cu-Ontario FA aqueous solutions at 77 K after resin treatment and at Cu/FA ratios of:

(a) 80 (gain, 3.2×10^5; time constant, 500 ms; scan time, 500s)
(a') 80 (gain, 8×10^5; time constant, 1 s; scan time, 1 ks)
(b) 8 (gain, 8×10^4; time constant, 200 ms; scan time, 500s)
(c) 0.8 (gain, 1.25×10^5; time constant, 200 ms; scan time, 500s)
(d) 0.08 (gain, 6.3×10^5; time constant, 1 s; scan time, 1 ks)
(d') 0.08 (gain, 1×10^6; time constant, 1 s; scan time, 1 ks)

TABLE IV-9

SPECTRAL PARAMETERS DERIVED FROM ESR SPECTRA (RT) OF SAMPLES DRIED BEFORE RESIN TREATMENT AND OF PRECIPITATES

Cu/FA Ratio	$g_{\parallel}$ ($\pm$0.001)	$\lvert A_{\parallel}\rvert$ ($\pm 2 cm^{-1} \times 10^{4}$)	$g_{\perp}$ ($\pm$0.001)	Number of lines at $g_{\perp}$ †	g_{app} ($\pm$0.001)	Ligand atoms and molecules
Rialto FA						
0.08:1‡	2.273¶	175	2.048	3 lines	--	2 O,2 N (4FA)
	2.253	182	2.043	3 lines		1 O,3 N (4FA)
0.8:1‡	2.294	177	2.058	3 lines	--	4 O/3 O,1 N (4 FA)
	2.277	175	2.048	3 lines		2 O,2 N (4 FA)
8:1‡	2.323	156	2.111	--	--	4 O (1 H_2O,3 FA)
80:1‡	--	--	--	--	2.184	4 O ($4H_2O$)
8:1§	2.345	152	2.085	--	--	4 O ($2H_2O$,2 FA)
80:1§	2.363	141	2.085	--	--	4 O ($2H_2O$,2 FA)
Ontario FA						
0.08:1‡	2.248	185	2.058	--	--	1 O,3 N (4 FA)
0.8:1‡	2.301	177	2.058		--	4 O (4 FA)
	2.279	177	2.048	2x3 lines		2 O,2 N (4 FA)
8:1‡	--	--	--	--	2.115	4 O ($4H_2O$)
80:1‡	--	--	--	--	2.118	4 O ($4H_2O$)
80:1§	2.351	147	2.077	--	--	4 O ($2H_2O$,2 FA)

† $\lvert A_{\perp}^{14}N\rvert = (20 \pm 2\ cm^{-1}) \times 10^{-4}$
‡dried samples
§precipitates
¶main absorption

TABLE IV-10

SPECTRAL PARAMETERS DERIVED FROM ESR SPECTRA (77 K) OF AQUEOUS SOLUTIONS AFTER RESIN TREATMENT

Cu/FA Ratio	$g_{\parallel}$ (±0.001)	$\lvert A_{\parallel}\rvert^{\dagger}$ (±2 $cm^{-1} \times 10^{4}$)	$g_{\perp}$ (±0.001)	Number of lines at $g_{\perp}$ ‡	FA Ligand Atoms	Total Cu mg/kg
Rialto FA						
0.08:1	2.290	179	2.058	5 lines	4 O/3 O,1 N	521
	2.273	187	2.038	7 lines	2 O,2 N	
0.8:1	2.300§	169/184	2.055	—	4 O	894
	2.275§	184/196	2.038	5 lines	2 O,2 N	
	2.249	197/212		7 lines	1 O,3 N	
8:1	2.303	169	2.065	—	4 O	745
	2.286	188	2.045	—	3 O,1 N	
80:1	2.302	175	2.056	—	4 O	596
	2.273	190	2.043	2x5 lines	2 O,2 N	
Ontario FA						
0.08:1	2.307	178	2.055		4 O	470
	2.278	190	2.040	2x5 lines	2 O,2 N	
0.8:1	2.300§	169/183	2.057		4 O	706
	2.277§	182/193	2.038	5 lines	2 O,2 N	
	2.250	197/210		7 lines	1 O,3 N	
8:1	2.300§	172/184	2.055		4 O	1058
	2.279§	182/193	2.037	5 lines	2 O,2 N	
	2.250	194/213		7 lines	1 O,3 N	
80:1	2.300§	172/184	2.056		4 O	764
	2.277§	182/193	2.041	5 lines	2 O,2 N	
	2.251	202		7 lines	1 O,3 N	

† two values for $\lvert A_{\parallel}\rvert$ refer to couples of peaks at the same $g_{\parallel}$

‡ $\lvert A_{\perp}^{14N}\rvert = (20 \pm 2\ cm^{-1}) \times 10^{-4}$

§ main absorption

TABLE IV-11

SPECTRAL PARAMETERS DERIVED FROM ESR SPECTRA (77 K) OF SAMPLES DRIED AFTER RESIN TREATMENT

Cu/FA Ratio	$g_{\parallel}$ (±0.001)	$\lvert A_{\parallel}\rvert^{\dagger}$ (±2 $cm^{-1} \times 10^{4}$)	$g_{\perp}$ (±0.001)	Number of lines at $g_{\perp}$ ‡	FA Ligand atoms
Rialto FA					
0.08:1	2.291§	174	2.052	—	4 O/3 O,1 N
	2.277	182/193	2.039	2x5 lines	2 O,2 N
0.8:1	2.295¶	172/183	2.054	—	4 O/3 O,1 N
	2.277¶	182/193	2.040	2x5 lines	2 O,2 N
8:1	2.303	175	2.052	—	4 O
	2.295	172/189	2.042	5 lines	3 O,1 N
	2.274	182/196		5 lines	2 O,2 N
80:1	2.299§	172	2.054	—	4 O
	2.278	182/193	2.043	2x5 lines	2 O,2 N
Ontario FA					
0.08:1	2.293¶	175	2.054	—	4 O,3 O,1 N
	2.277¶	182/193	2.042	2x5 lines	2 O,2 N
0.8:1	2.297§	172	2.058	—	4 O/3 O,1 N
	2.278	182/193	2.042	2x5 lines	2 O,2 N
8:1	2.301¶	169	2.054	—	4 O
	2.278¶	182/193	2.040	2x5 lines	2 O,2 N
80:1	2.299	169	2.051	—	4 O
	2.291¶	—		—	3 O,1 N
	2.279¶	182/193	2.041	2x5 lines	2 O,2 N

† two values for $\lvert A_{\parallel}\rvert$ refer to couples of peaks at the same $g_{\parallel}$

‡ $\lvert A_{\perp}^{14N}\rvert = (20 \pm 2\ cm^{-1}) \times 10^{-4}$

§ very low absorption

¶ main absorption

after the resin teatment. Almost no difference in the spectra is opbserved for the Cu-Rialto FA samples at the lowest Cu/FA ratio, whereas more informative ESR results are obtained by comparing the Cu-Ontario FA samples at this ratio. Two components are resolved in the spectrum obtained after the resin treatment, one having ESR parameters similar to those obtained before the treatment, the other having parameters consistent with a 4 O binding site not detectable in the spectrum recorded before the treatment with the resin.

For both the Rialto and the Ontario FA prepared at a Cu/FA ratio of 0.8, the ESR data obtained before the resin treatment suggested the participation of both FA and water molecules in the ligation of Cu^{2+}, whereas the absence of the broad line indicated the probable lack of Cu^{2+} aquo-ions. A comparison of these results with those obtained after the resin treatment indicates that the acidic exchanger has partially removed the less strongly bonded (external) Cu^{2+} ions from the mixed Cu-FA-water complexes. This should result in a complete replacement of water in the final complexes and in a rearrangement into more differentiated types of coordination sites involving only FA ligand atoms.

For the solutions prepared at the highest Cu/FA ratios, the effect of the resin acts both by fixing the excess of free Cu^{2+} ions and by producing the same type of rearrangements in the preexisting Cu-FA-water complexes, thus creating well-differentiated binding sites almost identical to those observed in the solutions obtained from initially lower Cu/FA ratios.

The total copper contents determined in the resin-treated Cu-FA complexes are much higher than the residual copper contents in the two sludge FA (Senesi and Sposito, 1984) and increases with increasing the Cu/FA ratio (Table IV-10). These results indicate that no saturation of the complexation capacity of FA occurs up to a Cu/FA ratio of 80 pointing to the existence of a series of different complexation equilibria deriving from the formation of Cu-FA complexes of decreasing strength. Each equilibrium obviously should be more and more shifted towards complex formation as the Cu concentration increases in the solution. Copper contents lower than those expected according to this trend are found when a precipitate is obtained from the original Cu-FA mixture, the deviations appearing to be roughly proportional to the amount of precipitate. These results suggest that an increasing portion of the more reactive FA molecules were precipitated, thus diminishing the amount of Cu^{2+} coordinated as soluble Cu-FA complexes.

The $g_{\perp}$ region of the ESR spectra of all the resin-treated Cu-FA complexes appears very richly structured. Because of this, enlarged ESR spectra were recorded in a narrower scan range (1 kG) centered closer to the $g_{\perp}$ field (3100 G) (Fig. IV-9). Similar structure-patterns were noted previously only in the ESR spectra of untreated, dried samples prepared at the lowest Cu/FA ratios. Accordingly, the patterns may be assigned tentatively to superhyperfine coupling of the unpaired electron to two (5 lines) or three (7 lines) nitrogen atoms in heterogeneous binding sites.

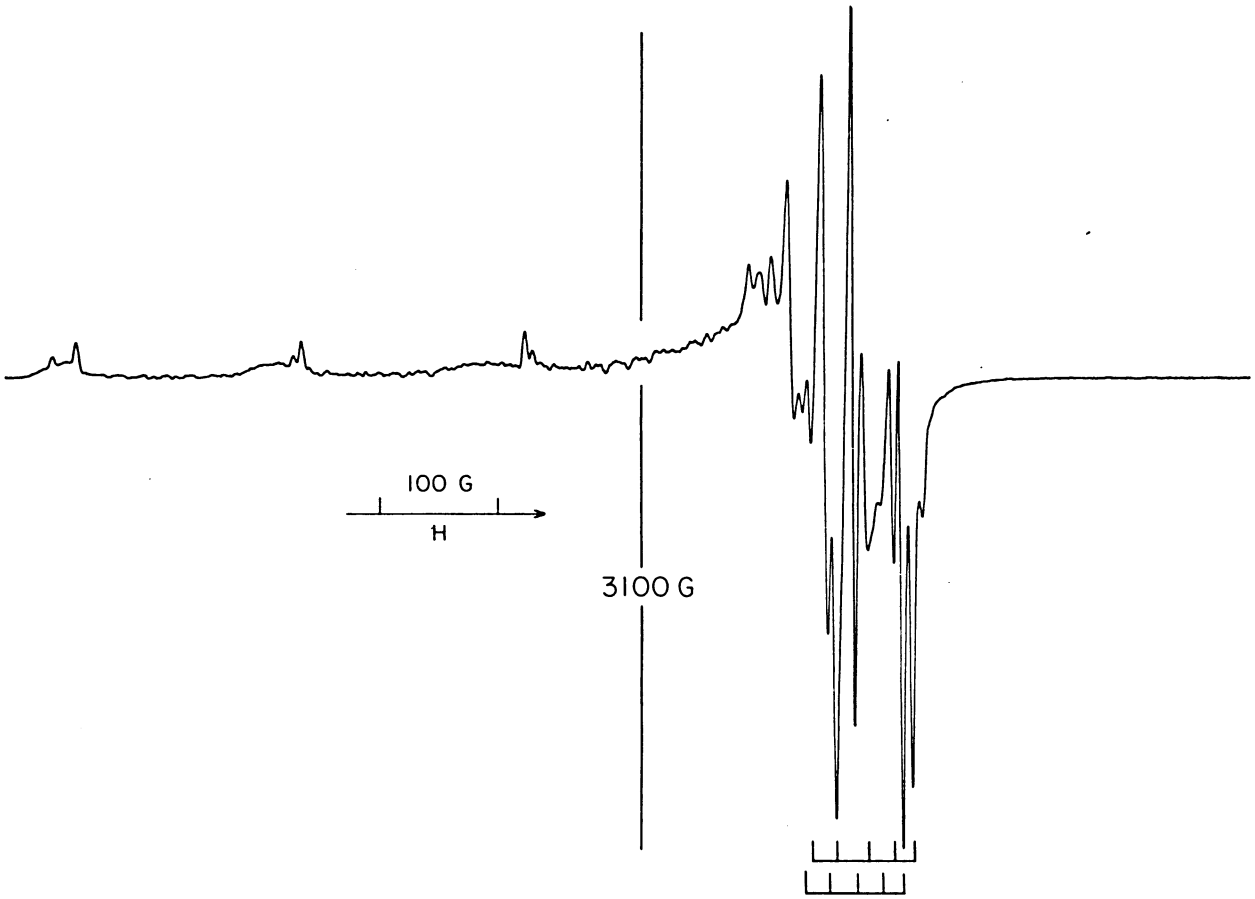


Figure IV-9. ESR spectrum (scan range, 1 kG) of a Cu-Ontario FA aqueous solution at 77 K after resin treatment and at Cu/FA = 8 (gain, 8×10^4; time constant, 200 ms; scan time, 500 s)

Air-dried samples. The air-drying of the resin-treated Cu/FA solutions produces both qualitative and quantitative variations in the ESR spectra with respect to the corresponding aqueous solution samples (Fig. IV-10 and IV-11, Table IV-11). The well-resolved ESR absorption centered at the lowest field (highest $g_{\parallel}$-value) and assigned to a 4 O binding site in the aqueous solution is often strongly reduced in intensity in the spectra of the dried samples. A small lowering in the $g_{\parallel}$-value and the disappearance of the couples from the two copper isotopes is also observable for this component. The ESR component centered at the highest field (lowest $g_{\parallel}$-value) and attributed to a 1 O, 3 N site in the aqueous solution disappeared in these spectra. These results, together with the characteristic presence in the spectra of a component with intermediate $g_{\parallel}$-values, suggest a significant rearrangement of the 4 O and 1 O, 3 N sites into 2 O, 2 N sites upon drying of the Cu-FA complexes. A similar effect was observed upon drying the Cu-soil FA systems (Senesi et al., 1984). The $g_{\perp}$ region of

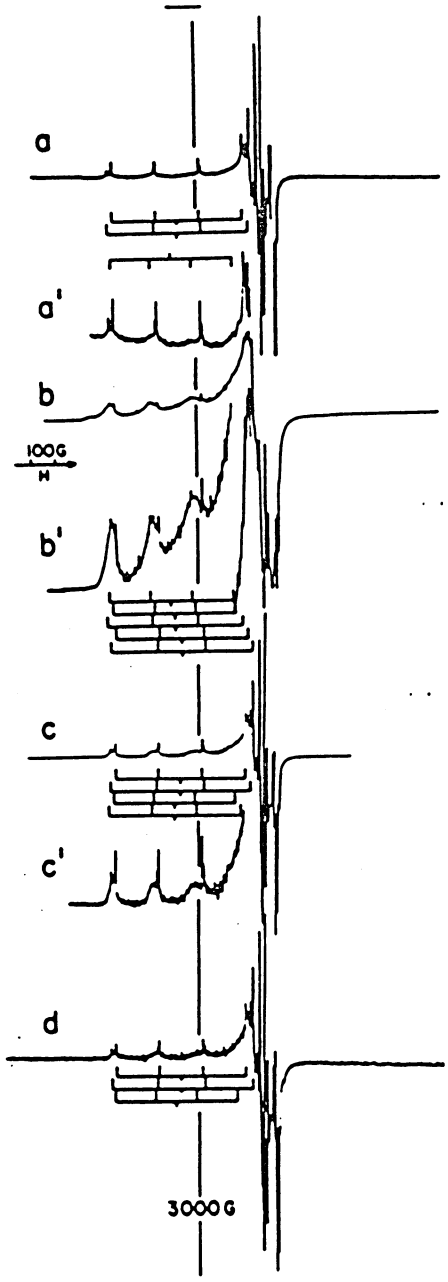


Figure IV-10. ESR spectra (scan range, 2 kG) at 77 K of Cu-Rialto FA air-dried samples from resin-treated solutions at Cu/FA ratios of:
(a) 80 (gain, 2×10^{4}; time constant, 50 ms; scan time, 500 ms)
(a') 80 (gain, 5×10^{4}; time constant, 100 ms; scan time, 500 s)
(b) 8 (gain, 8×10^{3}; time constant, 100 ms; scan time, 500 s)
(b') 8 (gain, 4×10^{4}; time constant, 100 ms; scan time, 500 s)
(c) 0.8 (gain, 1.25×10^{4}; time constant, 100 ms; scan time, 500 s)
(c') 0.8 (gain, 5×10^{4}; time constant, 100ms; scan time, 500 s)
(d) 0.08 (gain, 8×10^{4}; time constant, 200 ms; scan time, 500 s)

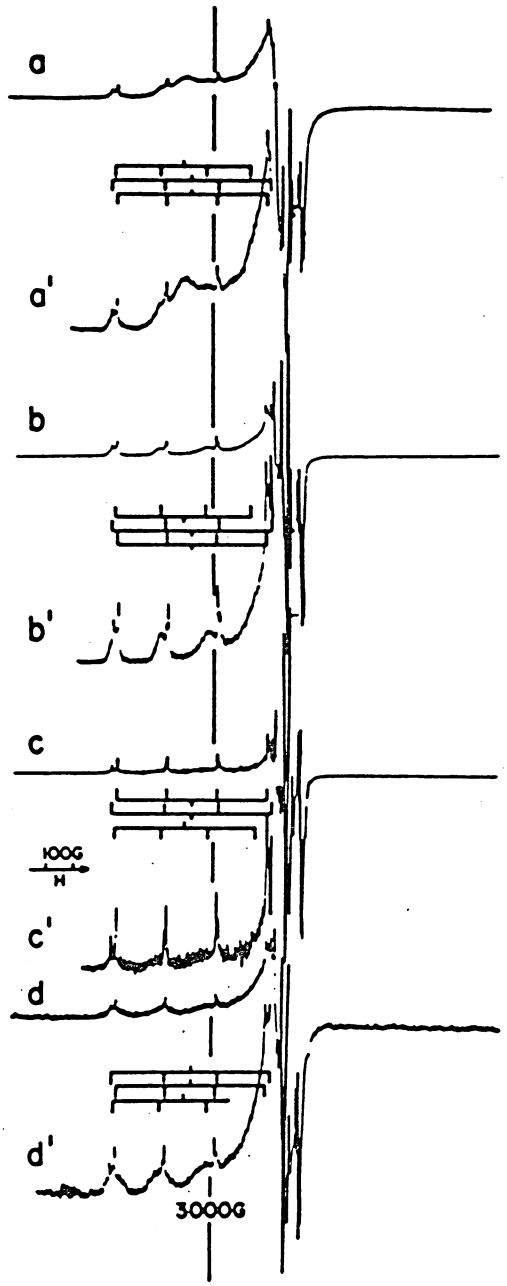


Figure IV-11. ESR spectra (scan range, 2 kG) at 77 K of Cu-Ontario FA air-dried samples from resin-treated solutions at Cu/FA ratios of:

(a) 80 (gain, 2×10^4; time constant, 50 ms; scan time, 500 s)
(a') 80 (gain, 5×10^4; time constant, 100 ms; scan time, 500 s)
(b) 8 (gain, 1.25×10^4; time constant, 100 ms; scan time, 500 s)
(b') 8 (gain, 5×10^4; time constant, 100 ms; scan time, 500 s)
(c) 0.8 (gain, 2×10^4; time constant, 100 ms; scan time, 500 s)
(c') 0.8 (gain, 8×10^4; time constant, 100 ms; scan time, 500 s)
(d) 0.08 (gain, 8×10^4; time constant, 100 ms; scan time, 500 s)
(d') 0.08 (gain, 2×10^5; time constant, 500 ms; scan time, 1 ks)

the ESR spectra of the dried samples is very structured, the total number of lines and their position appearing almost identical to those observed in the corresponding spectra of aqueous solutions (Fig. IV-12 and Table IV-11).

A particularly well-resolved and finely-structured ESR spectrum is observed for the Cu-Rialto FA dried sample prepared from a solution at a Cu/FA ratio of 8. The component centered at $g_{||}$= 2.286 appears to be differentiated into two components, at $g_{||}$ = 2.295 and $g_{||}$ = 2.274. A fine structure probably caused by super-hyperfine coupling to nitrogen nuclei at $g_{||}$ is also particularly evident in this spectrum, despite the fact that a quantitative analysis cannot be carried out. An additional, typical feature centered at $g_{||}$ = 2.291 and showing no nuclear hfs splitting is present uniquely in the ESR spectrum of the dried sample prepared at the highest Cu/FA ratio from Ontario FA. An identical feature was observed for the corresponding spectrum of a dried sample of Cu-soil FA solution (Senesi et al., 1984). The broadening of the anisotropy of this component may be attributed to intense dipole interactions in the solid state in the presence of very high Cu content. The absence of this feature in the corresponding Cu-Rialto FA spectrum seems to confirm the higher overall ability of Rialto FA to complex Cu.

The ESR spectra obtained at RT and related spin Hamiltonian parameters (Table IV-12) for the dried samples are very similar to the corresponding data obtained at 77 K (Table IV-11). As observed previously, the spectra at RT look, in general, less resolved. In particular, the peaks at $g_{||}$ arising from the two copper isotopes often are not resolved and the number of lines observable in the $g_{||}$ region is somewhat reduced.

4. Conclusions

The principal conclusions to be drawn from the results of this study are:

(1) The spin Hamiltonian parameters of the Cu-sludge FA complexes are consistent with equatorial coordination of the oxygen and nitrogen donor ligands to Cu^{2+}. This arrangement contrasts with the axial coordination suggested for Cu-sludge humic acid complexes by Boyd et al. (1983) on the basis of ESR spectra.

(2) In the untreated Cu-FA solutions, a quadridentate, nearly square-planar, chelate is formed exclusively with FA donor ligands only at low Cu/FA molar ratios, whereas at the higher ratios solvent molecules become more and more involved, giving rise to a partial FA ligand dissociation and a final bidentate (or monodentate) chelate with FA.

(3) Resin treatment can remove Cu^{2+} bonded weakly to FA in mono- or bidentate sites, but the same kinds of binding sites generally are involved in the Cu^{2+} ligation by the two sludge FA both before and after the resin treatment.

(4) Different classes of binding sites, involving different oxygen and nitrogen functional groups of FA, participate successively in the coordination of Cu^{2+} under different experimental conditions: aqueous solution --> water-glycerol solutions --> dried sample.

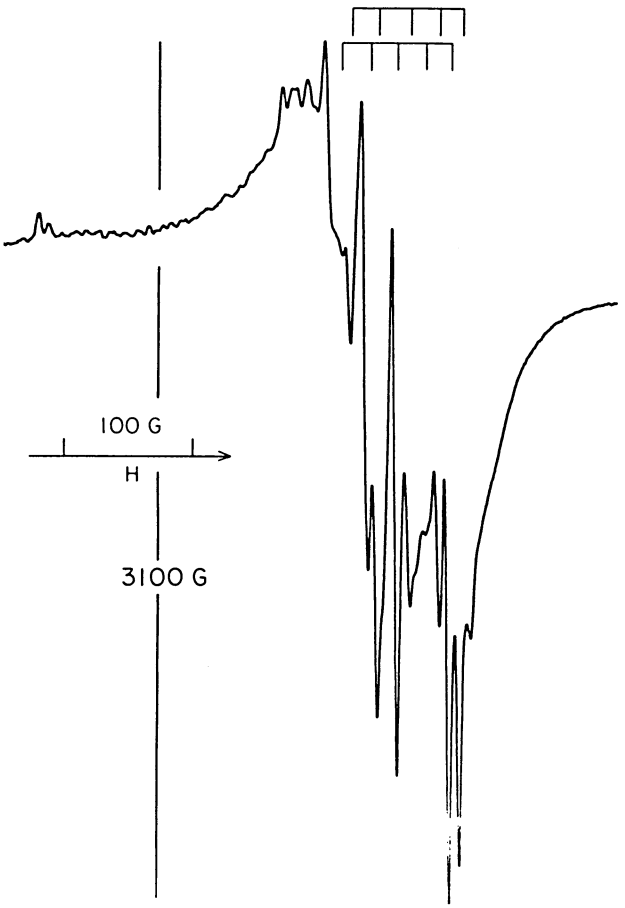


Figure IV-12. ESR spectrum (scan range, 1 kG) at 77 K of a Cu-Rialto FA air-dried sample from a resin-treated solution at Cu/FA = 0.8 (gain, 1.25×10^4; time constant, 100 ms; scan time, 500 s)

(5) No saturation of the Cu(II) complexation capacity was observed, but increasing the Cu content involves less and less reactive external functional groups of FA in the coordination, thereby shifting the complexation equilibria more and more towards the formation of weaker complexes; in agreement with the results of electrochemical studies of Cu complexation by sludge FA (Sposito et al., 1979).

(6) Rialto FA appears to possess a greater overall reactivity to Cu(II) than does Ontario FA.

TABLE IV-12

SPECTRAL PARAMETERS DERIVED FROM ESR SPECTRA (RT) OF SAMPLES DRIED AFTER RESIN TREATMENT

Cu/FA Ratio	$g_{\parallel}$ ($\pm$0.001)	$\lvert A_{\parallel} \rvert$ † ($\pm$2 $cm^{-1} \times 10^{4}$)	$g_{\perp}$ ($\pm$0.001)	Number of lines at $g_{\perp}$ ‡	FA Ligand atoms
Rialto FA					
0.08:1	2.291 §	174	2.052	3 lines	4 O/3 O,1 N
	2.273	181	2.039	5 lines	2 O,2 N
0.8:1	2.294 ¶	166	2.056	3 lines	4 O/3 O,1 N
	2.276 ¶	175/187	2.040	5 lines	2 O,2 N
8:1	2.291 ¶	171	2.057	3 lines	4 O/3 O,1 N
	2.273 ¶	175/190	2.043	5 lines	2 O,2 N
80:1	2.277	176/190	2.056	2x5 lines	2 O,2 N
			2.042		
Ontario FA					
0.08:1	2.289 ¶	171	2.053	3 lines	4 O/3 O,1 N
	2.273 ¶	178	2.041	5 lines	2 O,2 N
0.8:1	2.273	178/192	2.053	2x5 lines	2 O,2 N
			2.041		
8:1	2.301 ¶	167	2.056	--	4 O
	2.275 ¶	175/190	2.041	5 lines	2 O,2 N
80:1	2.299	166	2.057	--	4 O
	2.289 ¶	--		--	3 O,1 N
	2.275 ¶	178/190	2.043	5 lines	2 O,2 N

† two values for $\lvert A_{\parallel} \rvert$ refer to couples of peaks at the same $g_{\parallel}$

‡ $\lvert A_{\perp}^{14}N \rvert = (20 \pm 2\ cm^{-1}) \times 10^{-4}$

§ very low absorption

¶ main absorption

V. CONCLUSIONS AND RECOMMENDATIONS

The sequential extraction procedure developed and applied in Sec. II appears to provide reliable indicators of trace metal solubility and plant-availability in both light- and heavy-textured arid-zone soils. The data obtained in the present study lead to the conclusions that: (1) the labile, bioavailable forms of trace metals are exchangeable and organically-bound species that can be extracted in sequence by KNO_3, water, and NaOH, and (2) the labile forms transform gradually into inorganic precipitates (probably carbonates) and resistant minerals that are not readily available to plants. It is recommended that either the sequential KNO_3-H_2O-NaOH procedure or a single DTPA extraction be used to estimate plant-available trace metals in sludge-amended soils. Soluble trace metals can be estimated with the results of a sequential KNO_3-H_2O extraction.

Trace metal sorption by the five representative arid-zone soils included in this study was significant and produced trace metal concentrations that were comparable to those observed in sludge-amended soils when the soil solution concentrations were at or below 0.1 g m^{-3} (100 ppb). At these low concentrations, the sorption isotherms often took on a sigmoid shape that precluded conventional interpretation in terms of a distribution coefficient. Additional, detailed research on very low-level sorption is required in order to establish the causes of the sigmoid isotherm shape and a reliable interpretive model of the sorption process.

The soluble organic fraction of sludge-amended soil appears to retain many of the chemical properties of the applied sludge, but is more reactive toward trace metals because of oxidation in the irrigated soil environment. The persistence of supposedly biodegradable anionic surfactant compounds (detergents) in sludge-amended field soils was observed even after several years of cropping and tillage. Further research on the microbiological and phytotoxicity aspects of these compounds in irrigated soils is needed to determine the water quality hazards they may pose.

Strong organic complexes of sewage sludge with copper were found in this study. The method of electron spin resonance spectroscopy was effective in clarifying the structure and lability of these complexes in relation to different kinds of sewage sludge and to synthetic copper chelate compounds. More research on the copper organic forms in sludge-amended soils is needed to assess the relationship of these forms to potential copper phytotoxicity.

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