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Effect of Microbial Activity on Trace Element Release from Sewage Sludge

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The microbial role in mobilization of trace elements from land-applied wastewater sludge is not well-defined. Our study examined the leachability of trace elements (Cd, Cr, Cu, Mo, Ni, P, Pb, S, and Zn) from dewatered sludge as affected by treatments designed to alter microbial activity. Different levels of microbial activity were achieved by incubating sludge columns at 4, 16, 28, and 37 °C and by the addition of $AgNO_3$ biocide at each temperature. Columns (with inert glass bead support beds) were subjected to six consecutive incubation−leaching cycles, each consisting of 7.3-d incubation followed by 16-h leaching with synthetic acid rain. Glucose mineralization tests were used to assess overall microbial activity. Significant acidification and trace element leaching occurred when conditions favored microbial activity (16 and 28 °C). Extent of mobilization was element-specific with Zn, Ni, and Cu showing the greatest mobilization (99, 67, and 57%, respectively). Mobilization was reduced but still substantial at 4 °C. Conditions that best inhibited microbial activity (37 °C or biocide at any temperature) resulted in the least mobilization. Characterization of enrichments performed using thiosulfate as the sole energy source revealed the presence of both known and putative S-oxidizing bacteria in the sludge. The results suggest that microbial acidification via S oxidation can mobilize trace elements from sludge. Elemental mobility in field situations would also be governed by other factors, including the capacity of soil to buffer acidification and to adsorb mobilized elements.

Introduction

Sewage sludge (also referred to as biosolids) is a complex mixture of organic and inorganic compounds that contains significant concentrations of nutrients. Application of sewage sludge on agricultural lands is being promoted for the benefits resulting from the recycling of organic matter and nutrients (*1*) and as a cost-effective management alternative. Unfortunately, sewage sludge also contains potentially toxic trace elements, anthropogenic organic compounds such as polychlorinated biphenyls (PCBs) and dioxins, and a range of pathogenic organisms. The general belief is that trace elements added to the soil as a result of sludge applications remain in the surface layers of the soil (*1*, *2*), based in part on packed laboratory soil column studies that have shown little or no leaching of trace elements. In field research, many investigators also assume that, in accordance with conventional convective−dispersive theory, the lack of distinguishable elemental accumulation below the application depth implies that there is no elemental loss (*3*−*5*).

Although Sloan et al. (*6*) showed complete recovery of sludge-applied trace elements, other long-term experiments have been unable to account for all the elemental mass by mass balance approaches (as summarized in refs *7*−*9*). Baveye et al. (*10*) reported that losses of sludge-borne elements from the soil profile ranged from 39% (Cu and Pb) to 60% (Ni) in the large-scale land application study of Hinesly et al. (*11*). Richards et al. (*8*) found elevated elemental concentrations in percolate from sludge-applied plots nearly 20 yr after application; nevertheless, there was little or no detectable readsorption in the subsoil to 2-m depth. On the basis of elemental deficits calculated for the soil at this same site, McBride et al. (*7*) estimated that about 40% of the sludge-applied Zn and Cu had been lost from the topsoil.

Research has shown that preferential flow phenomena result in greater mobilities of a range of contaminants than would be predicted by conventional convective−dispersive flow approaches (*12*, *13*). Camobreco et al. (*14*) found that conventionally homogenized soil columns (which force uniform water flow) were overly optimistic about soil metal retention capacity; preferential flow paths in more realistic undisturbed soil columns accelerated trace element movement through the soil. Similar results were found in the field with monolith lysimeters by Maeda and Bergstrom (*15*) and in France near smelters by Sterckeman et al. (*16*), both under transient flow conditions. These findings suggest that there may be a greater potential for groundwater contamination than originally believed, so assessment of mobilization mechanisms is warranted.

Soil pH and texture also play an important role in controlling trace element mobility; most elements in free ionic form show the greatest mobility in acidic, coarse-textured soils (*17*). Mobility can, however, also be significant at near-neutral or greater pH due to complexation with dissolved organic matter (DOM), which is more mobile at those pH levels. For example, 25−50% of total Cu, Ni, and Mo in alkaline-stabilized sludge products have been shown to be readily extractable by water or weak acid (*18*, *19*) and thus potentially mobile in the environment (*20*).

It is generally believed that microorganisms play an important role in the solubilization, mobilization, and leaching of trace elements in soils, processes that are still poorly understood. For example, experiments with soil suspensions (*21*) indicate that, although Cd is initially sorbed by soil, it is subsequently mobilized into the soil solution by microbial activity. Recently, Leita et al. (*22*) reported that the concentrations of DTPA-extractable Cd and Ni were strongly correlated with microbial biomass and activity, suggesting that the mobilization equilibria of both metals were mediated by microbial biomass. Blais et al. (*23*) investigated the biological mobilization of trace elements from sewage sludge in batch experiments with added ferrous iron and elemental sulfur at a range of temperatures, concluding that bacterial growth was the rate-limiting step for metal solubilization from the sludge.

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TABLE 1. Total Trace Elements in Dewatered Sludge Determined by Nitric–Perchloric Digestion and ICP Spectroscopy

element	concn (mg kg^{-1})	element	concn (mg kg^{-1})
Cd	5.1	P	2 361
Cr	112	Pb	108
Cu	829	S	13 620
Mo	23.5	S^0 (elemental)[a]	42.9
Ni	82	Zn	675.5

[a] Elemental S was determined by methanol extraction and ICP spectroscopy.

In the experiments described here, we purposed to observe the rate and extent of trace element release from sludge under controlled laboratory conditions. We used two different types of treatments designed to alter microbial activity (various incubation temperatures and the presence of a biocide), hypothesizing that this would alter the rate and extent of trace element release. The experiments described here were designed to test this hypothesis. Microbial characterization was carried out to confirm the presence of S-oxidizing bacteria.

Experimental Section

Anaerobically digested dewatered sludge, mixed with glass beads to promote aeration and to prevent ponding, was incubated at several temperatures to alter microbial activity. Addition of $AgNO_3$ as a biocide was the other treatment for limiting microbial activity. The release of trace elements was determined by periodically leaching with synthetic acid rainfall. The effects of temperature and Ag biocide on overall microbial activity were assessed by using short-term glucose mineralization tests. Enrichments were used to culture S-metabolizing bacteria for purposes of identification.

Sludge. The sludge tested was collected in 1999 from the Onondaga County Drainage and Sanitation Department wastewater treatment facility in Syracuse, NY, which produces anaerobically digested dewatered sludge that is dewatered to approximately 21% total solids. Sludge was mixed to ensure homogenization and stored at 4 °C. The pH of a sludge suspension (20 g wet plus 10 mL of distilled water) was measured with a standardized pH meter 24 h after mixing. Sludge trace elements and total S were determined both initially (Table 1) and at the end of the experiment via nitric–perchloric acid digestion and inductively coupled argon plasma (ICP) spectroscopy using a Thermo-Jarrell-Ash model 975 ICP unit. Elemental sulfur (S^0) was analyzed by the method used by Wind and Conrad (*24*), wherein methanol was added to the sludge (1:4 v/v) and shaken overnight at 120 rpm at 25 °C. The suspension was centrifuged for 10 min at 4000 rpm, and the S-containing supernatant was filtered through a 0.45-μm PTFE filter paper prior to analysis by ICP–IRS.

Incubation and Leaching. Sludge (16 g dry weight, 75 g wet weight) was mixed with 600 g of 1.2 mm diameter glass beads. The beads were used as a nonadsorptive matrix to prevent ponding during leaching. The mixture was placed as a 5-cm layer on top of a 3-cm layer of 2-mm glass beads in a 10 cm diameter column (Figure 1). Three replicate sludge columns were incubated at each temperature in addition to one additional column at each temperature that had been treated with Ag biocide (sprayed on as a solution of $AgNO_3$ at the rate of 0.635 g of Ag/kg of dry sludge). The sludge loading on the columns simulated an areal application rate of 20 Mg ha^{-1}, midway between typical agronomic loading rates and application rates used for soil reclamation.

Columns were incubated at 4, 16, 28, and 37 ± 1 °C. During incubation, moisture in the column was kept constant by replacing evaporative losses by spraying equivalent amounts of distilled water every other day (every day for 37 °C). The observed extent of drying was slight and was confined to the surface of the columns. Each incubation-leaching cycle consisted of 7.3 d of incubation followed by 16 h of leaching. Leaching was carried out in a rainmaker apparatus at 16 °C using synthetic acid rain (Table 2) (*20*). Rain was applied at 0.35 cm h^{-1} for 16 h; rates and durations were chosen to allow significant washing of the sludge (5.6 cm total rainfall depth per cycle) but without resulting in ponding. Leachate was collected for 16 h until free drainage ceased. The columns were immediately returned to the temperature chambers for the next incubation cycle.

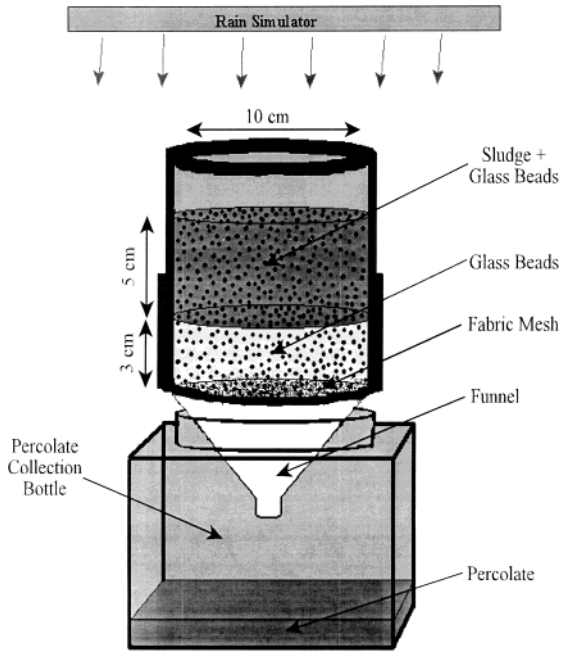

FIGURE 1. Schematic of sludge incubation/leaching column.

TABLE 2. Synthetic Acid Rainwater Formulation (*20*) of Ions Added to Tap Water, Resulting pH 4.0–4.5

ion	concn (mg/L)	ion	concn (mg/L)
Na^+	0.15	Ca^{2+}	0.83
NH_4^+	0.32	NO_3^-	2.88
K^+	0.09	Mg^{2+}	0.08
SO_4^{2-}	3.96[a]	Cl^-	0.47

[a] Sulfate formulation used in ref *20* was lower than 4.96 mg/L target concentration.

Leachate pH was determined immediately after collection using a standardized pH meter and then filtered through acid-washed cellulose filters (0.45 μm porosity). As Zn was the most mobile element, a preliminary screening analysis of all leachates collected over the 6 weeks was carried out by flame atomic absorption spectroscopy (AA). After screening with AA, selected leachate samples were analyzed for trace elements and total S with a ThermoJarrell Ash IRIS advantage ICP with duo-view torch. The results of AA and ICP were in good agreement up to 5 mg L^{-1}, but a bias was evident for higher concentrations. Because screening testing showed little trends in later leachates, samples of the last 2 weeks were composited into a final sample for ICP analysis.

Microbial Respiration. As an indicator of microbial respiration, glucose mineralization was measured at all temperatures (37, 28, 16, and 4 °C) for fresh sludge, fresh Ag-treated sludge, and aged sludge (after six incubation–

leaching cycles). The method described by Dumestre et al. (*25*) involved measuring the amounts of CO_2 released following glucose addition. Five grams of sludge (dry weight) was added to a 250-mL flask to which 5 mL of a solution made of 1 g L^{-1} glucose and 20 mg L^{-1} yeast extract was added. A 10-mL glass tube containing 8 mL of NaOH was placed in the flask to trap the evolved CO_2. The rate of glucose-C amendment was 500 μg of C/g of dry sludge, as proposed by Dahlin et al. (*26*). The flasks were sealed, placed on a rotary shaker set at 120 rpm, and incubated in triplicate for 28 h at each temperature. After each 4-h interval, the NaOH solution was replaced with a fresh solution by using a needle-sampling system. The amount of CO_2 released was measured by determining the quantity of NaOH in solution that had not reacted with CO_2 as compared to a control. NaOH was neutralized with HCl using a pH-controlled dispenser in the presence of excess $BaCl_2$ added to precipitate carbonate as $BaCO_3$ (*27*). The error caused by HCO_3^- remaining in the sludge solution was considered in these experiments, but previous measurements of evolved CO_2, after identical incubation times in flasks acidified just before the NaOH removal, showed this error to be negligible (*25*).

Microbial Characterization. Enrichments and subsequent cultivation were done in a defined medium containing 3.0 g/L KH_2PO_4, 0.4 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.25 g/L $CaCl_2 \cdot 2H_2O$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 10 g/L $Na_2S_2O_3 \cdot 5H_2O$. Sludge (2.5 g dry weight) was incubated in 100 mL of thiosulfate medium at 28 °C while being shaken at 120 rpm. The reduction in pH of the medium was used as an indicator of activity of sulfur-oxidizing bacteria. After the pH dropped to 3.5, the enrichment culture was transferred (10% v/v) to a fresh thiosulfate medium. To determine if known thiosulfate-oxidizing bacteria was present in the sludge, a crude lysate of the enrichment culture was generated using a bead-beater (Biospec Products, Bartlesville, OK) set at medium speed for 30 s. Genomic DNA from the enrichment culture was purified from lysates using Miniprep Express Matrix from Bio101 (Carlsbad, CA) according to the manufacturer's instructions to be used as a template for PCR amplification.

Ribosomal intergenic spacer analysis (RISA) was applied to the enrichment culture DNA using primers 1054F (ATGGCTGTCGTCAGCT) and 23SR (GGGTTBCCCCATTCRG) (*28*). Thermal cycler conditions were based on a modified touchdown protocol (*29*). The initial denaturation step for the first primer set was 5 min at 95 °C followed by 10 cycles of denaturation at 95 °C for 15 s, annealing at 65 °C for 45 s, and extension at 70 °C for 60 s. For touchdown PCR, the annealing temperature was decreased by 1 °C $cycle^{-1}$ for the first 10 cycles, followed by 20 cycles of annealing at 55 °C. A 3-min extension at 70 °C was included after completion of the 30 cycles of touchdown amplification. PCR products amplified from the ribosomal intergenic spacer region were separated in a 1% agarose gel by electrophoresis for 1 h at 100 mV.

PCR products amplified with the 1054F/23sR primer pair were also ligated into pGemT-Easy (Promega Corp., Madison, WI) following the manufacturer's instructions. The ligation mixture was electroporated into DH5α electrocompetent cells and plated onto LB containing 50 mg of ampicillin and 40 mg mL^{-1} X-gal. Transformants were screened for inserts by PCR using primers T7F and M13R and the same thermocycler conditions described above. PCR products from 80 clones were digested with *Hae*III and analyzed on a 2% agarose gel. Identical banding patterns were considered to represent the same operational taxonomic unit (OTU), and 74 unique patterns were found. A representative of each of the 74 unique OTUs was sequenced at Cornell University, and sequences were analyzed using the BLASTn program available on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

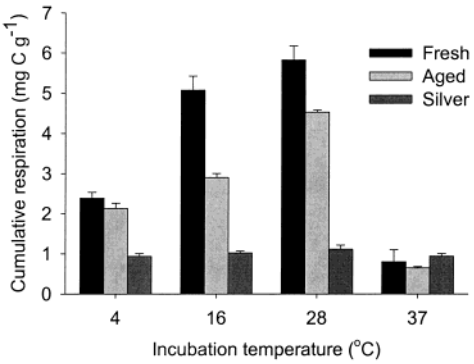

FIGURE 2. Microbial respiration results for fresh sludge, aged sludge, and silver biocide-treated sludge.

Statistical Analysis. The effects of three factors (i.e., temperature, time, and treatment) were analyzed using SAS/IML (*30*) to compute orthogonal polynomials and SAS/GLM to run the analyses (*31*). The three-factor interaction has been used as an error term, but it is not appropriate here as some parameters of the three-factor interaction represent real effects. We used the treatment by linear and quadratic effects of time and temperature. An exploratory model procedure presented by Bozivich et al. (*32*) and applied by Federer (*33*) was used to determine which of these three-factor interaction parameters to retain in the model. Individual treatment means were then compared with Duncan's Multiple Range Test (*31*).

Results and Discussion

Microbial Activity. Glucose mineralization tests were used to assess the impacts of incubation temperature and biocide treatments on general microbial activity. Cumulative 28-h respiration results are shown in Figure 2. Respiration was significantly greater ($P < 0.0001$) at 28 °C, with only slightly lower values at 16 °C. Respiration at 4 °C was still greater than the silver biocide treatments (all temperatures). Interestingly, the 37 °C treatment had the lowest respiration. C mineralization in the fresh sludge was significantly greater ($P < 0.0001$) than aged leached sludge (Figure 2), especially at 28 and 16 °C. There was little difference in microbial activity between aged and fresh sludges incubated at 4 and 37 °C. The $AgNO_3$ biocide was so uniformly effective at limiting microbial activity that there was no temperature effect among the biocide treatments ($P < 0.0001$).

Leachate pH and S. Figure 3 illustrates temporal trends in leachate pH and S concentrations, with elemental mass balances presented in Table 3. Because microbial activities among all biocide treatments were identical and there were no observable differences in leachate characteristics, the results reported for the biocide treatment are averages for all temperatures.

Figure 3a summarizes leachate pH trends (this and similar figures plot the cumulative incubation/leaching time on the *x*-axis). Acidification was substantial, particularly during the initial incubation cycle, with first-cycle leachate pH levels of 2.0 (28 and 16 °C) and 3.0 (4 °C). Leachate pH subsequently moderated to remain between 3.0 and 4.4 by the end of the experiment as acidification diminished. The 37 °C and Ag-treated sludge treatments showed less acidification, with initial leachate pH levels near 4.5, increasing to approximately 6.0 near the end of the experiment.

First-cycle leachate total S concentrations (Figure 3b) were greatest at 28 and 16 °C, exceeding 300 mg L^{-1}, with the 4 °C treatment near 250 mg L^{-1}. The 37 °C and Ag treatments leachate S concentrations were an order of magnitude lower. Cumulative total S leached from the sludge was equal to or greater than 90% of initial S for 16 and 28 °C (Table 3;

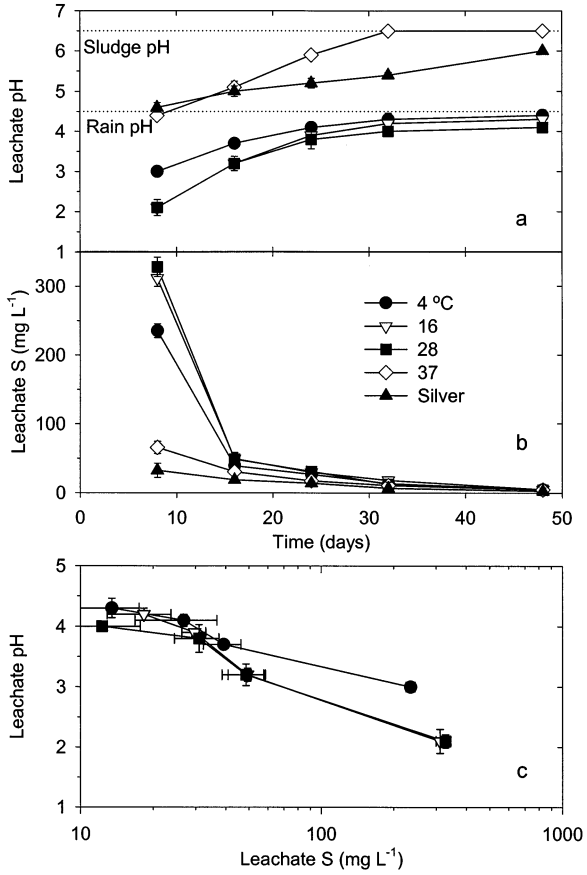

FIGURE 3. Temporal trends in leachate pH (a) and leachate total S (b), and the correlation between leachate pH and S (c). Dotted lines in panel a denote initial sludge pH and the input synthetic acid rain pH.

treatments are listed in the table so that those with greatest losses appear first). Leachate S losses (as percentage of initial sludge content) were 69% at 4 °C, 28% at 37 °C, and 16.5% for the Ag-treated sludge (Table 3). Note that the mass balances comparing initial contents with total recovered in leachate and remaining in the sludge at the end of the experiment were very good, with total recoveries typically in excess of 98% for all analytes (Table 3). Methanol extracted elemental sulfur in the initial sludge was 43 mg kg^{-1} (Table 1), indicating that elemental S was only a small fraction of the total S present in the sludge.

Leachate S correlated well with leachate pH levels ($R^2 =$ 0.89 for leaching cycles 1−4 leachates from the 4, 16, and 28 °C treatments, as shown in Figure 3c), suggesting that microbial S oxidation was a major source of acidification (*34*, *35*). Nitrification is also an acidifying microbial process, but its contribution could not be confirmed in the current experiment because leachate nitrate was not measured. In a subsequent experiment (unpublished data), we found that elevated nitrate leachate concentrations indeed coincided with elevated leachate S and depressed pH.

Trace Element Leaching. Figure 4 depicts the temporal trends in mean leachate concentrations for Cu, Zn, and Mo (leaching cycles 5 and 6 composited into a single sample). Aside from Mo, all analytes followed the same general pattern seen for Cu and Zn in Figure 4 where, as with S, leachate concentrations were greatest when acidification was greatest and decreased with successive leachings as pH moderated. As can be seen in the elemental mass balances in Table 3, Cd, Cu, Ni, P, and Zn had similar treatment trends for losses to leachate: the greatest losses to leachate were found at

TABLE 3. Elemental Mass Balances for Treatments after Six Incubation/Leaching Cycles[a]

element	initial mass (mg)	treatment	elemental recovery (% of applied) in leachate	residual	total
Cd	0.08	28 °C	29.3[a]	65.8	95.1
		16	12.2[b]	80.5	92.7
		4	11.6[bc]	77.6	89.2
		37	1.2[d]	96.2	97.4
		Ag	5.7[c]	91.0	96.8
Cr	1.79	28	3.6[a]	94.4	98.0
		16	3.2[a]	95.0	98.2
		4	2.9[ab]	93.4	96.4
		37	0.5[b]	97.3	97.8
		Ag	0.9[b]	97.8	98.6
Cu	13.3	28	56.8[a]	42.4	99.2
		16	55.6[a]	41.7	97.3
		4	35.4[b]	58.6	94.1
		37	1.8[c]	97.7	99.5
		Ag	1.6[d]	98.1	99.7
Mo	0.38	28	12.3[a]	87.0	99.3
		16	9.9[a]	80.0	89.9
		4	5.3[b]	89.1	94.4
		37	6.1[b]	86.9	93.0
		Ag	6.1[b]	93.8	99.9
Ni	1.3	28	67.4[a]	32.3	99.7
		16	59.1[a]	37.1	96.2
		4	47.0[b]	53.0	100.0
		37	12.9[d]	85.7	98.5
		Ag	18.9[c]	80.8	99.8
P	38.1	28	5.6[a]	93.1	98.6
		16	4.4[ab]	94.8	96.2
		4	3.9[b]	96.1	100.0
		37	1.7[c]	97.9	99.5
		Ag	1.6[c]	98.3	99.9
Pb	1.73	28	4.1[a]	94.6	98.8
		16	3.5[ab]	93.4	96.9
		4	1.6[bc]	98.0	99.6
		37	nd	na	na
		Ag	0.4[c]	99.3	99.7
S	218	28	91.7[a]	6.7	98.5
		16	90.0[a]	8.6	98.2
		4	69.2[b]	29.8	99.1
		37	28.4[c]	67.4	95.9
		Ag	16.5[d]	82.6	99.1
Zn	10.8	28	98.8[a]	1.1	99.9
		16	89.5[a]	10.4	99.9
		4	78.6[b]	20.5	99.1
		37	10.7[d]	89.3	100.0
		Ag	27.8[c]	72.2	100.0

[a] Initial mass present in sludge was identical for all treatments. Elemental recovery in leachate and residual in sludge at end of experiment are expressed as percent of initial elemental mass. For each analyte, leachate recovery values followed by similar letters are not significantly different ($P = 0.05$). Ag biocide treatments are averaged for all temperatures due to similar leachate concentrations. nd, not determined; na, not available.

28 °C but typically were not significantly greater than those at 16 °C. The losses at 4 °C were again lower but still substantial, while the Ag biocide and 37 °C treatments resulted in the lowest losses to leachate, always significantly lower ($P < 0.0001$) than the 16 and 28 °C treatments. Even so, these treatments had Zn and Ni losses of 10−13%. As shown in Figure 5, the release of sulfur and metals into leachates was highly correlated.

Mo leachate concentrations were unique in that while concentrations decreased with time in the most acidified treatments, concentrations from the 37 °C and biocide treatments (with the greater pH levels) actually increased with time (Figure 4) as pH levels moderated. Mo in sludge has been observed to have greater mobility under neutral to alkaline conditions (*18*−*20*).

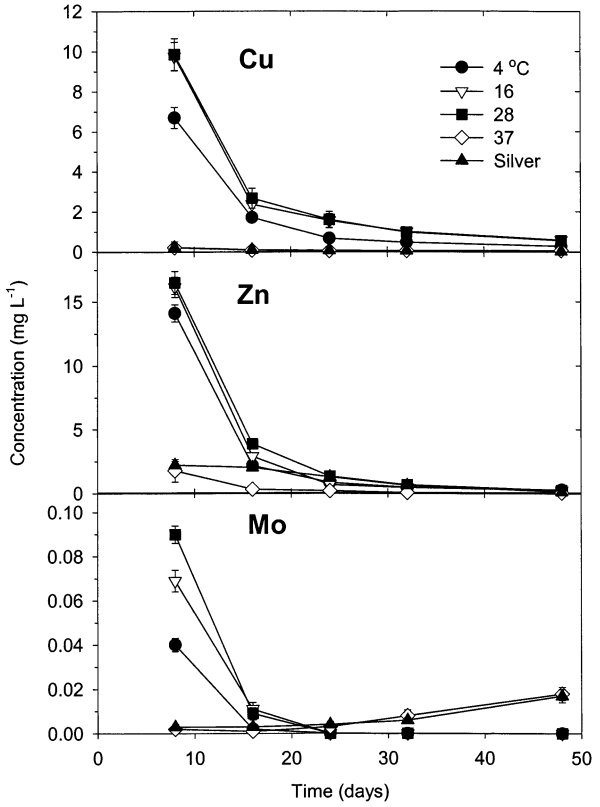

FIGURE 4. Temporal trends in leachate trace element concentrations: (a) Cu, (b) Zn, and (c) Mo.

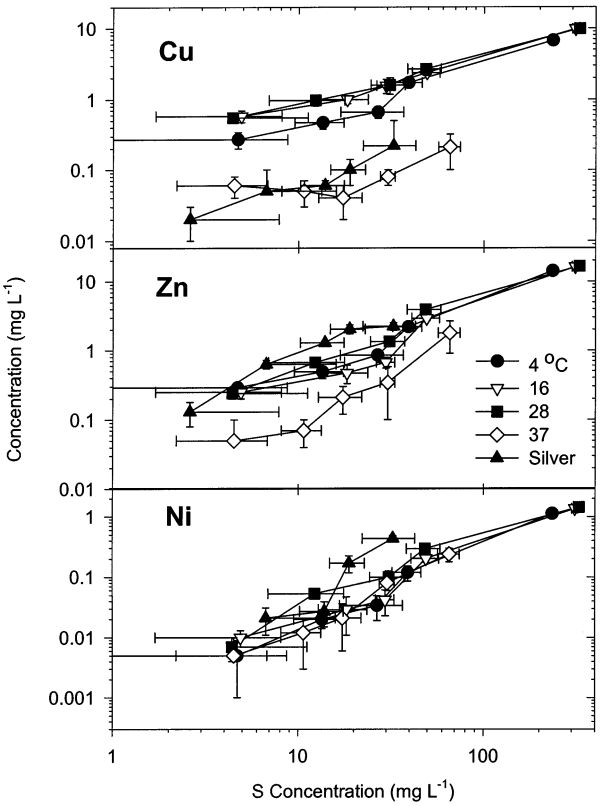

FIGURE 5. Correlation between leachate S and trace element concentrations: (a) Cu, (b) Zn, and (c) Ni.

At 28 and 16 °C, relative rankings of trace element loss were as follows: Zn ≈ S > Ni > Cu ≫ Cd ≫ Mo > P ≈ Pb ≈ Cr (Table 3). S losses have already been discussed above. Zn losses were the greatest, ranging between 90 and 99% with Ni losses between 59 and 68%, Cu losses of approximately 56%, and Cd losses nearly 30%. All other analytes experienced leaching losses at or below 6%. The relative rankings of losses by leaching at 4 °C were essentially identical. Although the magnitudes were somewhat lower, losses were still notable, ranging from 78% for Zn, 47% for Ni, 35% for Cu, and to nearly 12% for Cd, while all other analytes were below 4%. The 37 °C and biocide treatments that had the least microbial activity also had the least elemental leaching. Leaching losses were all under 10% aside from S (28% at 37 °C, 16% for Ag), Zn (11% at 37 °C, 28% for Ag) and Ni (13% at 37 °C, 19% for Ag). Relative rankings were S > Ni > Zn > Mo > Cu ≈ P ≈ Cd > Cr for 37 °C (sludge Pb results not available) and Zn > Ni > S > Mo ≈ Cd > P ≈ Cu ≈ Cr ≈ Pb for the Ag biocide treatment.

The differences among trace elements (within a given treatment) in the degree of leachability may be attributed to two factors: solubility product and the form of element in the sludge (*8*, *20*, *36*). For example, the Zn recovery close to 99% was notable and correlates to the greater solubility of ZnS relative to other metal sulfides such as CuS and CdS. In addition, chemical speciation in the sludge is important because Cr and Pb are likely to exist in sewage sludges in forms other than sulfides, which may partially resist dissolution even at pH 3.

Our observations of microbial mobilization of trace elements from sludge are similar to elemental recovery from low-grade ores and wastes using a process termed bioleaching, which occurs in nature wherever suitable conditions are found for the growth of ubiquitous bioleaching microorganisms (*37*). In a metal bioleaching study using slag and ash from municipal waste incineration, Krebs et al. (*38*) reported that the pH had dropped to approximately 1 at the end of the growth phase of microorganisms and that more than 80% of several elements (such as Cd, Cu, and Zn) were mobilized.

Microbial Characterization. As stated above, the correlation of pH and leachate sulfur suggested that the reduction in pH was caused at least in part by the microbial oxidation of sulfur. Several OTUs having phylogenetic similarities to known or putative sulfur-oxidizing organisms were identified from sequence analysis of approximately 500 base pairs of the 16S gene that was cloned along with the intergenic spacer fragment after amplification from the enrichment DNA. Approximately one-third of the 74 clones sequenced had sequence similarity with known or putative S oxidizers. These included known sulfur chemoautolithotrophic bacteria such as *Thiobacillus thioparus* and *T. denitrificans* (97−99% sequence similarity, Accession Nos. AF005628.1 and AJ243144), which were represented by 12% (9 of 74) clones. On the basis of pH declines and sulfate production, Tyagi et al. (*39*) speculated that *Thiobacillus* species are present in aerobically digested sewage sludges obtained from sewage treatment plants.

Interestingly, 20% (15 of 74) of OTUs recovered from the enrichment DNA corresponded to the putative sulfur chemoautolithotrophic bacterium *Ralstonia solanacearum* (99% sequence identity, Accession No. AF441313). Although no studies could be found demonstrating sulfur oxidation by *R. solanacearum*, the recently completed genome sequence (http://sequence.toulouse.inra.fr/R.solanacearum.html) reveals the presence of putative sulfur oxidation genes, with open reading frames similar to the *sox* genes found in organisms that utilize a paracoccus-type sulfur oxidation pathway. That this organism is found in sludge and persists in the harsh acid environment associated with S oxidation may also be of agronomic concern, as it is widely known as a plant pathogen (*40*).

As noted earlier, general microbial activity was inferred from carbon dioxide evolution. Although growth of obligate chemoautolithotrophs results in a net consumption of carbon dioxide, the presence of metabolically versatile organisms (which may grow both chemautolithotrophically as well as heterotrophically) strongly suggests that both mixotrophy and heterotrophy by acid tolerant organisms may be prevalent in organic-rich matrixes such as sludge. Thus, carbon dioxide evolution would be an appropriate measure of microbial activity and correlates well with acid production.

Treatments where the microbial activity was limited by either silver nitrate or temperature (4 and 37 °C) did not experience the same degree of pH depression. Reduced microbial activity was expected for the Ag treatment but not for the 37 °C treatment. However, this unanticipated result is supported by the findings of Blais et al. (*35*) and van Elsas et al. (*41*), who reported no *Thiobacilli* and *Ralstonia* growth at temperatures above 35 °C. Additional evidence for a reduced rate of bacterial activity at 37 °C on artificial media and in humic soil has been reported (*23, 42*−*44*). The reduced microbial activity may also have been contributed to by thermal release of toxicants present in the sludge, with thermal stress rendering the microorganisms more susceptible at 37 °C than at other temperatures.

Finally, our results show that incubation and leaching can lead to significant mobilization of sludge trace elements under a range of conditions that favor significant (16 and 28 °C) or even moderate (4 °C) microbial activity. These findings should not be construed as a predicted degree of leachability through soil to groundwater. The actual degree of mobilization in field situations will be governed by many factors, including the ability of the soil matrix to buffer pH changes and to readsorb the released elements as well as the occurrence and extent of preferential flow paths through the soil.

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