

Microbial acidification and pH effects on trace element release from sewage sludge

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“Capsule”: *The primary role of microbes in the potentially substantial mobilization of trace elements from wastewater sludge appears to be that of acidification.*

Abstract

Leaching of sludge-borne trace elements has been observed in experimental and field studies. The role of microbial processes in the mobilization of trace elements from wastewater sludge is poorly defined. Our objectives were to determine trace element mobilization from sludge subjected to treatments representing microbial acidification, direct chemical acidification and no acidification, and to determine the readsorption potential of mobilized elements using calcareous sand. Triplicate columns (10-cm diameter) for incubation and leaching of sludge had a top layer of digested dewatered sludge (either untreated, acidified with H₂SO₄, or limed with CaCO₃; all mixed with glass beads to prevent ponding) and a lower glass bead support bed. Glass beads in the sludge layer, support layer or both were replaced by calcareous sand in four treatments used for testing the readsorption potential of mobilized elements. Eight sequential 8-day incubation and leaching cycles were operated, each consisting of 7.6 d of incubation at 28 °C followed by 8 h of leaching with synthetic acid rain applied at 0.25 cm/h. Leachates were analyzed for trace elements, nitrate and pH, and sludge layer microbial respiration was measured. The largest trace element, nitrate and S losses occurred in treatments with the greatest pH depression and greatest microbial respiration rates. Cumulative leaching losses from both microbial acidification and direct acidification treatments were >90% of Zn and 64–80% of Cu and Ni. Preventing acidification with sludge layer lime or sand restricted leaching for all trace elements except Mo. Results suggested that the primary microbial role in the rapid leaching of trace elements was acidification, with results from direct acidification being nearly identical to microbial acidification. Microbial activity in the presence of materials that prevented acidification mobilized far lower concentrations of trace elements, with the exception of Mo. Trace elements mobilized by acidification were readsorbed by calcareous sand when present.

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1. Introduction

Trace elements in land-applied wastewater sludge (sewage biosolids) are potentially phyto- or zoo-toxic if present in sufficient concentration and availability. Trace element mobility from land-applied sludge has been

reported or suggested in many field studies (Campbell and Beckett, 1988; Lamy et al., 1993; McBride et al., 1997; Richards et al., 1998), whereas other studies have reported little loss (Sloan et al., 1998). Incomplete analytical recovery or heavy metal dispersion by tillage practices may account for some or all of the apparent loss of applied metals in some cases (McGrath and Lane, 1989), but these factors are not applicable in all cases. Using total dissolution analysis, Baveye et al. (1999) concluded that 36–60% of applied trace metals were unaccounted for in the large experimental sludge

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application plots of Hinesly et al. (1984). Leaching losses are often ruled out due to lack of observable increases in subsoil metals concentration, but Richards et al. (1998) demonstrated that significant metal leaching may not be accompanied by detectable subsoil readsorption.

The factor most closely associated with the solubility and mobility of trace elements is soil pH (McBride, 1994; McBride et al., 2004), with most elements increasing in mobility at lower pH (Basta and Sloan, 1999; Richards et al., 2000). Mobility can also be substantial at elevated pH due to metal complexation with organic matter, which itself becomes increasingly soluble as pH increases (Richards et al., 1997; McBride, 1998). The texture and adsorption strength of the soil matrix also affects mobility and bioavailability. Richards et al. (2000) demonstrated that mobility of Ni, Cd, and Zn was greater in fine sandy loam soil than in a silt loam soil at similar pH levels.

In contrast to soil factors, less is known about the effects of microbial activity on the release of sludge-borne trace elements from the sludge matrix. Olivie-Lauquet et al. (2001) found greater concentrations of trace elements in summer months than in winter months, coupled with greater temperature and dissolved organic carbon (DOC) contents. They suggested that microorganisms that use soil iron and manganese oxyhydroxides as electron acceptors were responsible for higher concentration of trace elements and DOC. Induced microbial 'bioleaching' for removing trace metals from sludge or sediments via microbial acidification has been the focus of earlier (Couillard et al., 1994; Tyagi et al., 1994; Blais et al., 1993) and more recent work (Chen and Lin, 2001a,b; Lombardi and Garcia, 1999, 2002; Lombardi et al., 2001), focusing primarily on optimizing the process for sludge treatment. Acidification that takes place when naturally-occurring acid sulfate soils (which contain reduced sulfur compounds) are aerated is a well-documented phenomenon in soil science. Recently, Sundstrom et al. (2002) characterized the environmental significance of soil metals released during acidification of such soils. Tipping et al. (2003) predicted release of trace elements from contaminated peat soils following drought-induced episodes of aeration and acidification. Qureshi et al. (2003b) investigated the microbial role in the release and leaching of trace elements from Zn-rich peat bogs in laboratory experiments. They found that the effects of temperature and biocide on microbial respiration rates and the mobilization of S, NO₃, DOC, and trace elements were a strong indication of microbial processes facilitating and mediating the mobilization of trace elements from these materials.

Using the same incubation and leaching approach that we discuss here, we found (Qureshi et al., 2003a) that significant acidification and trace element leaching occurred from dewatered digested wastewater sludge when incubation conditions favored microbial activity (16, 28 °C). Unlike other studies that focused on process optimization, no additions of energy sources or nutrients

were made. The extent of mobilization was element-specific, with Zn, Ni, and Cu showing the greatest mobilization and loss (99, 67 and 57%, respectively). Mobilization was reduced but still substantial at 4 °C incubation. In contrast, conditions that best inhibited microbial activity (incubation at 37 °C, or the addition of silver biocide at any incubation temperature) resulted in the least mobilization of trace elements. Characterization of enrichments performed using thiosulfate as the sole energy source revealed the presence of both known *Thiobacillus* sp. and putative S oxidizing bacteria in the anaerobically digested dewatered sludge. (Most of the widely known S oxidizing bacteria have been detected (Blais et al., 1993) in indigenous sludge microflora.) The results showed that microbial acidification via S oxidation mobilized a substantial fraction of trace elements from sludge, and that inhibition of microbial activity markedly restricted release and leaching.

The present study was designed to further elucidate the results of Qureshi et al. (2003a) and consisted of three objectives. The first objective was to compare the effects of microbial acidification to those of direct acidification of sludge by adding sufficient sulfuric acid (H₂SO₄) to immediately achieve pH levels observed in our earlier work. The direct acidification was thus similar to earlier sludge metal extraction processes, as summarized by Yoshizaki and Tomida (2000). The second objective was to observe the effects of preventing acidification by the addition of lime (CaCO₃) while maintaining microbial activity. The third objective was to determine the readsorption potential of mobilized trace elements by placing calcareous sand in the sludge layer, below it, or in both positions. All tests were carried out at 28 °C, the optimum temperature in our prior work.

2. Materials and methods

Columns containing anaerobically digested dewatered sludge mixed with glass beads (abbreviated G) or calcareous sand (S) were repeatedly incubated aerobically at 28 °C and leached with synthetic acid rainwater, with leachates analyzed to determine trace element mobilization. The experimental incubation and leaching techniques presented here were similar to those used in our previous studies (Qureshi et al., 2003a,b).

2.1. Experimental columns

To examine objectives 1 and 2, three treatments were prepared using sludge mixed with glass beads over a glass support bed (designated G/G to indicate [top layer]/[support bed]). Sludge treatments (Table 1) were intact sludge (G/G), sludge that was chemically acidified to achieve pH levels resulting from microbial acidification in prior work (G^{acid}/G), and sludge that was limed to

Table 1
Experimental treatments using glass beads (G) and calcareous sand (S)

Top layer (5 cm)	Support layer (3 cm)	Abbreviation
Glass + sludge	Glass	G/G
Glass + acidified sludge	Glass	G ^{acid} /G
Glass + limed sludge	Glass	G ^{lime} /G
Sand + sludge	Sand	S/S
Sand + sludge	Glass	S/G
Glass + sludge	Sand	G/S
Glass + acidified sludge	Sand	G ^{acid} /S

prevent significant pH depression (G^{lime}/G). These treatments allowed us to examine whether direct chemical acidification was as effective as microbial acidification in mobilizing trace elements and whether microbial activity in the absence of acidification (prevented with lime additions) would still be able to mobilize trace elements.

For objective 3, four treatments were prepared by replacing glass beads with calcareous sand (S) either mixed with the sludge layer (S/G) as a layer below it (G/S, G^{acid}/S) or in both positions (S/S). The presence of the sand provided a potential adsorption matrix, allowing us to determine if mobilized elements were readily reabsorbable by the sand.

Overall, seven treatments were examined with triplicate 10-cm-diameter columns (Table 1). Columns consisted of two layers (Fig. 1): in the top 5 cm, 75 g wet (16 g dry) sludge was mixed with 600 g of 1.2-mm glass beads or 830 g of moistened (to field capacity) calcareous fine sand. For acidic sludge treatments (G^{acid}/G and G^{acid}/S), 34 mL of 2 N H₂SO₄ was added to 16 g sludge solids (75 g wet mass) to reach pH 1.5. For the lime treatment (G^{lime}/G), 3.2 g of reagent-grade CaCO₃ was mixed with 16 g of sludge solids. Acid additions were determined empirically to reach sludge pH levels observed in our prior work, with lime additions being sufficient to neutralize previously observed acidification (Qureshi et al., 2003a). The sludge loading on the columns simulated an areal application rate of 20 Mg ha⁻¹.

2.2. Sludge and sand characteristics

The sludge examined was produced in 1999 at the Syracuse, NY wastewater treatment facility of the Onondaga County Drainage and Sanitation Department. The anaerobically digested sludge was dewatered in a belt press to yield approximately 22% total solids, collected from the belt press conveyors, hand mixed to ensure homogenization, and stored at 4 °C. Total trace elements determined by inductively-coupled plasma (ICP) spectroscopy (similar to Method 3125, APHA/AWWA/WEF, 1999) following nitric–perchloric acid digestion are shown in Table 2. Sludge from this source has been used in our prior studies (Richards, et al., 1997, 2000; Qureshi et al., 2003a).

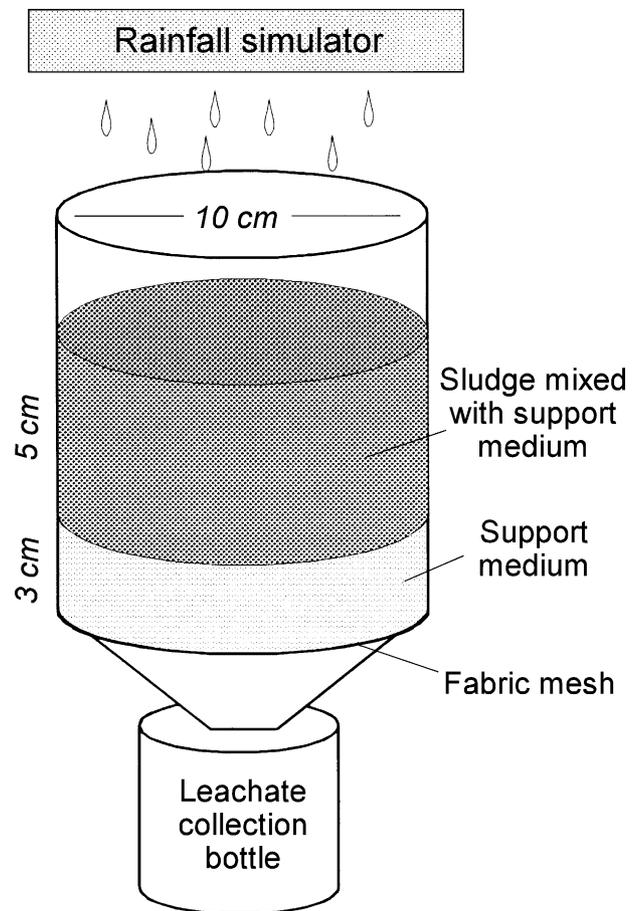


Fig. 1. Schematic diagram of incubation–leaching column. Support media in and below the sludge layer were either glass beads or sand, depending on treatment.

Commercially available “all purpose” sand (Quikrete brand, Atlanta, GA) was thoroughly washed with tap water until water was clear and then washed with distilled water. Total elemental analysis is summarized in Table 2, and particle size distribution is shown in Table 3. The calcareous nature of the sand is evident from the

Table 2

Total trace elements determined by perchloric acid digestion in digested dewatered sludge (Onondaga County, NY) and calcareous sand (mg kg⁻¹ dry wt)

Element	Sludge	Sand
Ca	29,480	25,310
Cd	5.1	0.3
Cr	112	6.3
Cu	829	9.0
Mo	23.5	0.9
Ni	82	18.0
Pb	108	7.1
S	13,620	1040
Elemental S ^o	42.9	Not determined
Zn	676	43.5
pH	6.5	8.0

Elemental sulfur (S^o) determined by methanol extraction.

Table 3
Particle size distribution of calcareous sand (percent mass basis)

Fraction	Size (mm)	%	Fraction	Size (mm)	%
Very coarse sand	1–2	4.0	Coarse silt	0.02–0.053	0.06
Coarse sand	0.5–1	13.6	Medium silt	0.005–0.02	0.20
Medium sand	0.25–0.5	27.7	Fine silt	0.005–0.002	0.20
Fine sand	0.1–0.25	49.2	Total Silt		0.46
Very fine sand	0.053–0.1	4.4			
Total sand		98.9	Total clay		0.55

25,310 mg Ca kg⁻¹ concentration, with other notable analytes being S (1040 mg kg⁻¹) and Zn (43.5 mg kg⁻¹).

To measure the pH of sludge or sand, 5 g of solids (dry weight) were mixed with 10 mL of distilled water. After 24 h, the suspension was measured with a standardized pH meter. Elemental sulfur concentration in sludge was determined by extraction with methanol added to the sludge (1:4 v/v) followed by horizontal shaking at 120 rpm overnight at 25 °C. The suspension was centrifuged for 10 min at moderate speed (2000 × g) and the S-containing supernatant was filtered through PTFE filter membranes (0.45 μm porosity). Sulfur in the filtrate was analyzed by ICP (Wind and Conrad, 1997).

2.3. Incubation and leaching

The experiment was carried out for eight sequential 8-day incubation–leaching cycles, each consisting of 7.6 d incubation followed by leaching with synthetic acid rain. The rain was prepared by adding acid and salts to tap water (Qureshi et al., 2003a; Richards et al., 2000) resulting in rainwater pH of 4.0–4.5. The rain was applied for 8 h at 16 °C at an intensity of 0.25 cm h⁻¹ with a rainmaker apparatus (Andreini and Steenhuis, 1990). In our previous work, this amount of leaching was found to be sufficient to elute practically all mobilized analytes after each incubation period. Leachate was collected after free drainage ceased after each rainfall application, and the columns were returned immediately to the incubation chamber to begin the next cycle. During incubation, moisture in the columns was kept constant by weighing the columns every other day to determine evaporative mass losses (compared to the initial weight at a moisture content representing field capacity), which were replaced by evenly spraying an equivalent mass of water on the column surface.

Leachate pH was determined immediately after collection using a standardized pH meter. Leachate was then filtered through acid-washed cellulose filters (0.45 μm porosity). Because Zn was the most mobile element in prior studies (Qureshi et al., 2003a,b), all samples were screened for Zn levels using atomic adsorption spectrophotometry (AAS) similar to Standard Method 3111 (APHA/AWWA/WEF, 1999). This screening allowed volume-weighted composite samples

to be prepared whenever the Zn concentration in individual leachate samples showed little change over time, thus substantially reducing costs of subsequent ICP analyses. ICP analyses were carried out with a Thermo-JarrellAsh (Thermo Electron, Waltham, MA) IRIS advantage with duo-view torch. For S/S, G/S, G^{acid}/S and G^{lime}/G treatments, composite samples representing all eight cycles of leachate were analyzed. For the S/G treatment, cycle 1 and cycle 2 leachates were analyzed separately along with a composite sample representing cycles 3 to 8. For G/G and G^{acid}/G treatments, cycle 1 and cycle 2 leachates were analyzed separately, and volume-weighted composite samples were prepared for cycles 3 to 4, 5 to 6 and 7 to 8. A volume-weighted eight-cycle mean concentration was calculated from intermediate composite results for the S/G, G/G and G^{acid}/G treatments.

The synthetic acid rain was analyzed by ICP so that mass balances could be compensated for elements present therein. Substantial Ca (50.4 mg L⁻¹) and S (79.0 mg L⁻¹) were present in tap water, so those elements were in excess of target acid rainfall levels (Richards et al., 2000). Trace levels of Ni (0.011 mg L⁻¹), Cu (0.022 mg L⁻¹) and Zn (0.073 mg L⁻¹) were also found, but Cd, Cr and Pb were not detected. Leachate data presented here represent net concentrations (i.e. after subtracting rainfall input concentrations).

All leachate samples analyzed by ICP were also analyzed for nitrate, using a method modified from that described by Vendrell and Zupancic (1990). Samples (200 μL) were placed in a color development tube, and 1 mL 6% salicylic acid in concentrated H₂SO₄ was added. After 20 min, 20 mL of 2.2 M NaOH was added to each tube and tubes were allowed to cool for approximately 45 min. Samples were analyzed with a spectrophotometer at 410 nm.

2.4. Microbial respiration

The effect of different treatments on general microbial activity was assessed by using short-term glucose mineralization tests. The activities of fresh sludge–glass bead and sludge–sand mixtures (including acid and lime treatments) were measured initially and at the completion of all eight incubation–leaching cycles. Activity was determined by measuring the amounts of CO₂ released following stimulation of activity via glucose additions (Dumestre et al., 1999). Sufficient wet mass of sludge/bead or sludge/sand mixtures were measured so that 5 g sludge dry solids were present. This was placed in a 250-mL flask, to which was added 5 mL of a solution composed of 1 g L⁻¹ glucose and 20 mg L⁻¹ yeast extract. The rate of glucose amendment was thus 1 mg g⁻¹ dry sludge (0.4 mg glucose-C g⁻¹), similar to the 0.5 mg C g⁻¹ rate proposed by Dahlin et al. (1997). A 10-mL glass tube containing 8 mL of NaOH was placed in the flask to trap and measure CO₂ evolved.

The flasks were closed hermetically, placed on a rotary shaker set at 120 rpm and incubated in triplicate for 28 h at 28 °C. After each 4-h interval, a needle-sampling system was used to remove the NaOH solution and replace it with fresh solution without opening the flask. The amount of CO₂ released was measured by determining via titration the quantity of NaOH that had not reacted with CO₂ in the samples compared to a control. NaOH was titrated with standardized HCl using a pH-controlled dispenser in the presence of excess BaCl₂ added to precipitate carbonate ion as insoluble BaCO₃ (Stotzky, 1997). Risks of HCO₃⁻ remaining in the sludge solution were considered negligible at pH levels at or below 7 (Dumestre et al., 1999) but may have been greater at elevated pH.

2.5. Statistical analysis

Statistical analysis was carried out with split–split plot design with temperature in the main plot and treatments in the sub-plot using the General Linear Model (GLM) of SAS (SAS Institute, 1996). Treatment mean leachate concentrations were separated with Duncan's Multiple Range Test.

3. Results and discussion

3.1. Microbial respiration

Initial and post-incubation cumulative respiration results of the various sludge layer treatments are summarized in Fig. 2. Cumulative respiration was greatest ($P < 0.0001$) and nearly identical for initial G^{acid} and G treatments, with results after incubation–leaching about one-third lower. These results were analogous to those in previous work (Qureshi et al., 2003a). Both G^{lime} and S treatments had initial respiration values about one-third lower than the G^{acid} and G treatments, but we saw less of a difference among the final rates. These lower respiration measurements might be attributable to CO₂ retention as bicarbonate at the elevated pH, although Dumestre et al. (1999) tested and discounted this possibility at pH 7. In any case, substantial microbial activity was maintained in all treatments, corresponding to levels in our prior work that correlated with significant acidification and trace element mobilization. In contrast, reductions in cumulative respiration of greater than 80% were measured by Qureshi et al. (2003a) for treatments that had markedly diminished acidification and mobilization.

3.2. Leachate pH, S, NO₃-N and Ca

In the G/G and G^{acid}/G treatments, leachate pH dropped to pH 1.7 for the first cycle, subsequently

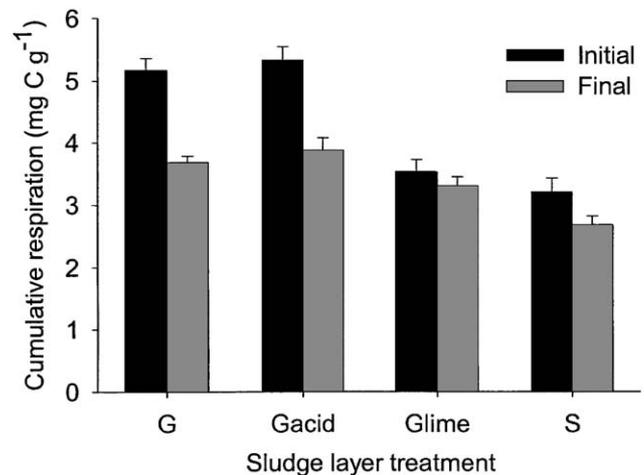


Fig. 2. Cumulative microbial respiration measured by CO₂ production following glucose addition, expressed as mg C g⁻¹. Error bars represent standard deviation. Tests performed on sludge–bead (G, G^{acid}, G^{lime} treatments) and sludge–sand (S treatment) mixtures prior to (initial) and following eight incubation–leaching cycles (final).

increasing to pH 4 (similar to the synthetic acid rain) towards the end of the experiment (Fig. 3), again mirroring prior results (Qureshi et al., 2003a). Leachate pH from G^{lime}/G and all S treatments containing calcareous sand remained near neutral, ranging from pH 6.6 to 8.

Composite leachate concentrations and elemental mass balances are shown in Table 4. The mass balances include inputs from sludge as well as from any added calcareous sand, lime, or acid. Note that applied mass values for treatment G/G represent inputs solely due to sludge, G/G being the only treatment without any other inputs of analytes, as glass beads are regarded as inert.

Treatment G/G had nearly 600 mg L⁻¹ sulfur in the first leaching, with concentrations declining rapidly with time. It should be noted that ICP analysis reports only total sulfur with no indication of specific speciation; we presume that the majority of leached sulfur was in the sulfate form. The composite leachate concentration was 140 mg L⁻¹, representing 84% of sulfur applied in sludge (Table 4). Treatments with direct acidification had predictably elevated composite sulfur concentrations, with initial G^{acid}/G leachate levels of almost 900 mg L⁻¹. Preventing acidification with CaCO₃ (G^{lime}/G treatment) resulted in the lowest composite leachate levels, representing 13.6% of sludge sulfur. Among other treatments, the composite leachate concentrations increased with the mass of calcareous sand (which contained substantial sulfur) present in the column (G/S, S/G and S/S).

Nitrate-N, analyzed only for intermediate samples of G/G, G^{acid}/G and S/G, followed trends similar to S, with initial concentrations between 200 and 250 mg L⁻¹ for G/G and G^{acid}/G and near 150 mg L⁻¹ for S/G, all of which declined to near 25 mg L⁻¹ by the end of the experiment (data not tabulated). Composite leachate Ca concentrations were lowest for the treatments containing

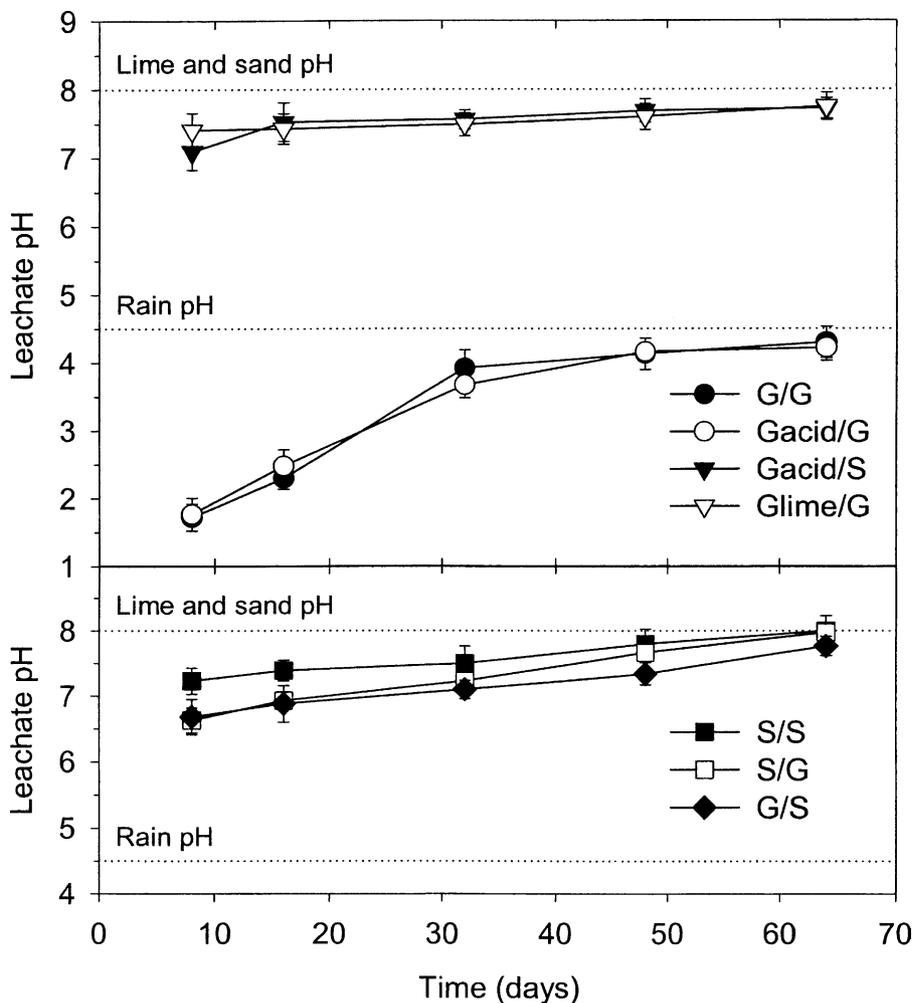


Fig. 3. Leachate pH trends for all treatments, separated into upper and lower graphs for clarity. Dotted reference lines indicate the pH levels of lime and calcareous sand (8.0) and synthetic acid rain (4.5). Sludge pH was 6.5.

only sludge (G^{acid}/G and G/G), representing just over one-third the Ca mass applied. Conversely, concentrations were greatest for G^{acid}/S followed by G^{lime}/G . Percent recoveries for Ca were low in view of substantial inputs from lime and, even more notably, calcareous sand.

3.3. Trace element leaching

Composite leachate concentrations and mass balances are presented in Table 4. Direct and microbial acidification were almost equally effective for mobilizing Zn, representing losses of 91 (G/G) to 95% (G^{acid}/G) of sludge-borne Zn in the leachate. In contrast, the presence of CaCO_3 or calcareous sand in the sludge layer restricted leaching for the S/G and G^{lime}/G treatments, representing losses of 1.6–2.5% of sludge-borne Zn. Support layer calcareous sand (G/S , G^{acid}/S , S/S) virtually eliminated all Zn leaching from the system, even from treatments that mobilized significant Zn in the sludge layer. Note that none of the substantial Zn contribution from the

calcareous sand was mobile, with treatment S/S showing no detectable Zn in leachate (Table 4).

Trends in Cu concentrations were almost identical to those of Zn, although the degree of mobilization was somewhat less. Treatments G^{acid}/G and G/G had the greatest initial and mean concentrations, representing losses of 81% for G^{acid}/G and 74% for G/G (Table 4). Preventing sludge layer acidification (G^{lime}/G , S/G) restricted leachate concentrations by a factor of 100, whereas calcareous sand in the support layer (G/S , S/S) resulted in no detectable leaching, with only a trace of leachate Cu from treatment G^{acid}/S . As with Zn, none of the Cu contribution from the calcareous sand appeared to be mobile, with treatment S/S showing no detectable Cu in leachate.

Leachate trends and percent recovery of Ni were very similar to Cu, with maximum losses of 81% (G^{acid}/G) and 64% (G/G). The addition of lime again restricted losses to 4.6% for G^{lime}/G columns, whereas calcareous sand in the sludge layer (S/G) limited losses even further to 1.4% when expressed as percent of sludge Cu. Calcareous sand

Table 4

Elemental mass applied (mg), volume-weighted composite leachate concentration (mg L^{-1}) and total mass recovered in leachate (mg and as percent of total applied)

Treatment	Total applied ^a		Recovered in leachate ^b			
	mg	mg L^{-1}	mg	%		
Ca	G/G	472	123.9	g	162.5	34.44
	G ^{acid} /G	472	136.9	f	178.7	37.88
	G ^{lime} /G	3675	259.2	b	339.8	9.25
	S/S	26,794	142.5	e	186.8	0.70
	S/G	15,658	145.6	d	181.3	1.16
	G/S	11,608	176.6	c	231.5	1.99
	G ^{acid} /S	11,608	314.8	a	412.6	3.55
	Cd	G/G	0.08	0.0074	b	0.0098
G ^{acid} /G		0.08	0.0089	a	0.0116	14.15
G ^{lime} /G		0.08	0.0029	d	0.0038	4.63
S/S		0.38	0.0014	e	0.0020	0.52
S/G		0.26	0.0007	f	0.0009	0.35
G/S		0.21	0.0001	g	0.0001	0.05
G ^{acid} /S		0.21	0.0047	c	0.0007	0.33
Cr		G/G	1.79	0.2961	b	0.3880
	G ^{acid} /G	1.79	0.3835	a	0.5000	28.00
	G ^{lime} /G	1.79	0.0040	c	0.0052	0.29
	S/S	8.30	0.0020	c	0.0027	0.03
	S/G	5.54	0.0005	c	0.0010	0.02
	G/S	4.54	0.0035	c	0.0046	0.10
	G ^{acid} /S	4.54	0.0048	c	0.0060	0.13
	Cu	G/G	13.29	7.46	b	9.80
G ^{acid} /G		13.29	8.28	a	10.80	81.28
G ^{lime} /G		13.29	0.07	c	0.09	0.68
S/S		22.62	0.00	c	0.00	0.00
S/G		18.67	0.03	c	0.04	0.21
G/S		17.24	0.00	c	0.00	0.00
G ^{acid} /S		17.24	0.01	c	0.01	0.08
Mo		G/G	0.38	0.012	f	0.015
	G ^{acid} /G	0.38	0.014	f	0.018	4.80
	G ^{lime} /G	0.38	0.104	a	0.1365	36.40
	S/S	1.29	0.025	d	0.033	2.56
	S/G	0.90	0.070	b	0.090	9.97
	G/S	0.76	0.018	e	0.020	2.62
	G ^{acid} /S	0.76	0.037	c	0.048	6.30
	Ni	G/G	1.31	0.639	b	0.84
G ^{acid} /G		1.31	0.813	a	1.06	80.85
G ^{lime} /G		1.31	0.044	c	0.06	4.58
S/S		20.05	0.011	de	0.02	0.07
S/G		12.12	0.019	de	0.02	0.19
G/S		9.24	0.001	e	0.00	0.01
G ^{acid} /S		9.24	0.023	d	0.03	0.32
Pb		G/G	1.73	0.07	b	0.09
	G ^{acid} /G	1.73	0.08	a	0.10	5.80
	G ^{lime} /G	1.73	0.00	c	0.00	0.00
	S/S	9.12	0.00	c	0.00	0.00
	S/G	5.99	0.00	c	0.00	0.00
	G/S	4.85	0.00	c	0.00	0.00
	G ^{acid} /S	4.85	0.00	c	0.00	0.00
	S	G/G	218	140.4	c	184.0
G ^{acid} /G		1242	495.9	a	647.1	52.10
G ^{lime} /G		218	22.6	g	29.6	13.60
S/S		1300	98.2	d	128.8	9.91
S/G		842.0	82.7	e	103.0	12.23
G/S		675.6	60.0	f	78.7	11.65
G ^{acid} /S		1700	323.4	b	424.0	24.94

Table 4 (continued)

Treatment	Total applied ^a		Recovered in leachate ^b			
	mg	mg L^{-1}	mg	%		
Zn	G/G	10.80	7.51	b	9.80	90.74
	G ^{acid} /G	10.80	7.85	a	10.23	94.72
	G ^{lime} /G	10.80	0.20	c	0.27	2.50
	S/S	56.04	0.00	e	0.00	0.00
	S/G	36.90	0.13	d	0.17	0.46
	G/S	29.94	0.00	e	0.00	0.00
G ^{acid} /S	29.94	0.01	e	0.01	0.03	

^a Total mass applied includes sludge, calcareous sand and any acid or lime additions; italicized G/G values reflect contributions from sludge only.

^b Leachate concentrations followed by similar letters are not significantly different from others within the same element ($P = 0.05$).

in the support layers of treatments G/S and G^{acid}/S resulted in much lower leachate concentrations, with similar levels seen for treatment S/S, all in spite of substantial sand Ni contributions relative to sludge.

Unlike other trace elements examined, the mobility of molybdenum increases with increases in pH. Trends observed were consistent with this fact, with leachate concentrations gradually increasing as pH levels rose to neutral (data not shown). Treatment G^{lime}/G had the greatest concentration, corresponding to a 36% loss of sludge-applied Mo (Table 4). The lowest concentrations were from the most acidic treatments G/G and G^{acid}/G. Another distinction was that the range in observed composite leachate concentrations was relatively narrow, spanning only one order of magnitude.

Mass loadings and removals of Cd were the least of any analyte (Table 4), although trends were similar to Cu and Ni. Losses of sludge-borne Cd were 12–14% for G^{acid}/G and G/G, and 4.6% for G^{lime}/G. Calcareous sand in the support layer restricted losses (G/S compared to G/G), but was far less effective in doing so when the sludge layer was acidified: treatment G^{acid}/S had a loss representing 5.8% of sludge-borne Cd. This result may be related to the contribution by sand of up to 80% of Cd present (treatment S/S). Treatment S/S and S/G leachates were well in excess of the ICP detection limit of 0.0001 mg L^{-1} .

Leachate Pb losses were between 5 and 6% for G^{acid}/G and G/G treatments (Table 4) but were below detection for other treatments. Leaching of Cr followed a similar pattern, although relative losses were greater, representing 28 and 22% of sludge Cr for G^{acid}/G and G/G treatments, respectively. All other treatments were statistically indistinguishable, with the greatest loss among them amounting to 0.3% of applied Cr.

3.4. Treatment comparisons

The results from the G/G microbial acidification treatment are in agreement with our previous studies

(Qureshi et al., 2003a), where optimum conditions supported the activity of nitrifying and S oxidizing bacteria, leading to acidification, as reflected in the leachate pH, S, and $\text{NO}_3\text{-N}$ levels. Leachate pH varied inversely with S and $\text{NO}_3\text{-N}$ molar concentrations, summed in Fig. 4 as acidification products (r^2 values shown are empirical linear fits; actual leachate buffer curves not determined). This relationship supports the conclusion that the observed acidification was due to oxidation of S and N (Couillard et al., 1994; Blais et al., 1993). The relative contribution of S to the total of S plus $\text{NO}_3\text{-N}$ is shown as the S mole fraction plot inset in Fig. 4a.

As in our prior work, microbial acidification in treatment G/G resulted in substantial (>50%) mobilization

for Cu, Ni, and Zn, in addition to notable mobilization (5–50%) of Cd, Cr, and Pb. Leachate concentrations of trace elements were strongly correlated to pH (Zn and Cu shown in Fig. 4b and c, with respective linear fit r^2 values of 0.95 and 0.89).

The direct acidification of $\text{G}^{\text{acid}}/\text{G}$ did not alter the potential for microbial activities (Fig. 2) compared to the G/G treatment. As intended, treatment $\text{G}^{\text{acid}}/\text{G}$ had identical leachate pH levels (Fig. 3), even though the leachate S concentration in the $\text{G}^{\text{acid}}/\text{G}$ treatment was significantly greater (Fig. 4). This was likely due to the presence of leachable but non-oxidized S present in the leachate, which is also suggested by the plot in Fig. 4a. (As noted previously, S speciation was not available). Patterns of

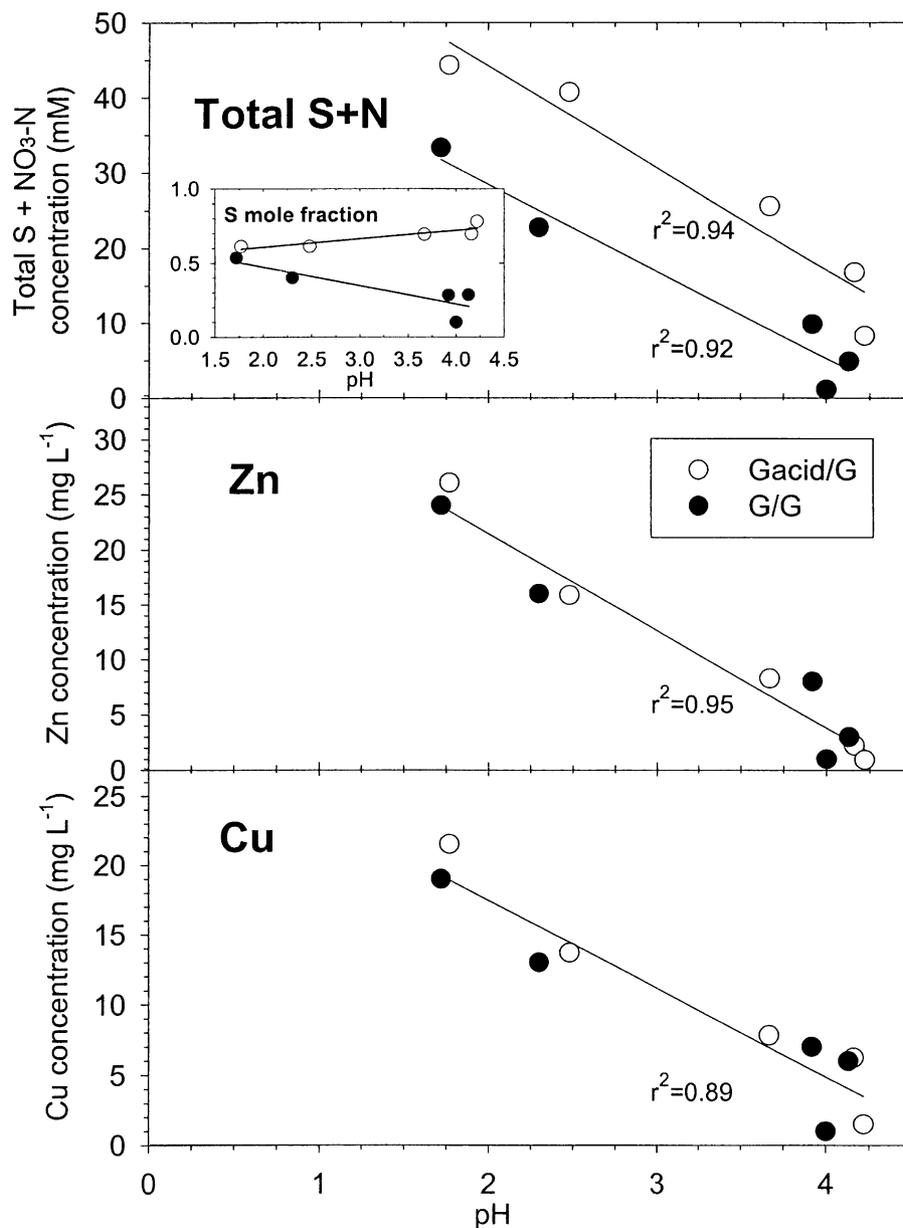


Fig. 4. Treatment G/G and $\text{G}^{\text{acid}}/\text{G}$ leachate pH correlated with: (a) the sum of leachate total S and nitrate-N for treatments (inset shows the mole fraction of S in the sum of S and $\text{NO}_3\text{-N}$); (b) leachate Zn; and (c) leachate Cu. Coefficients of linear regression shown as r^2 values.

trace element release and leaching were very similar for G^{acid}/G and G/G treatments and were comparable to bioleaching and acid extraction studies (Lombardi and Garcia, 1999; Yoshizaki and Tomida, 2000). It is noteworthy that trace element concentrations in the composited G^{acid}/G leachate were consistently greater than G/G for all elements except Mo (Table 4); the difference was most notable for Ni. In Fig. 4b and c, the leachate trace metal vs. pH relationships are indistinguishable for the two treatments. From the results of the G/G and G^{acid}/G treatments we may conclude that the direct acidification was at least as efficient as microbial acidification, and that the observed pH depression alone (apart from any other form of microbial mineralization) could likely explain the leaching results observed for the G/G microbial acidification treatment.

We next turn to consideration of the G^{lime}/G treatment, where acidification was prevented by presence of lime ($CaCO_3$) in the sludge layer. As shown in Table 4, this resulted in substantial reductions of trace element mobility for Cr, Cu, Ni, Pb and Zn. Although diminished slightly (Fig. 2), the general microbial activity continued at levels shown earlier (Qureshi et al., 2003a) to be correlated with substantial trace element mobilization. Nevertheless, this microbial activity—occurring in the absence of acidification—did not result in substantial mobilization of trace elements. The fact that there was still low but observable leaching of Cd, Cu, and Ni may be attributable to complexation with organic matter which itself would be mobile to some extent at pH levels measured for treatment G^{lime}/G (Richards et al., 1998, 2000). Reductions in sulfur leaching may be attributable to complexation with Ca, but in the absence of sulfur speciation this cannot be determined. Unlike other elements, Mo mobility increased in the G^{lime}/G treatment, with 36% of applied Mo recovered in the leachate compared to 4–5% from G/G and G^{acid}/G treatments. Enhanced Mo mobility and/or availability at near- and above-neutral pH has been observed (Richards et al., 2000; McBride et al., 2000; Basta and Sloan, 1999; McBride, 1998).

Treatment S/G, with the presence of calcareous sand in the sludge layer, was in many ways functionally similar to G^{lime}/G . Initial leachate pH levels (Fig. 3) were somewhat lower than G^{lime}/G , but microbial activities were similar. Trace element concentrations were below the G^{lime}/G levels in three cases (Cd, Ni, Zn) or were ranked statistically similar (Cr, Cu, Pb). Mo leaching was slightly lower than G^{lime}/G , although the sand itself contained detectable Mo. Taken together, the G^{lime}/G and S/G treatments both indicate that microbial activity apart from acidification did not result in substantial mobilization of most trace elements. This finding suggests that the primary microbial role in mobilizing metals (in both this and our prior experiments) was acidification of the sludge layer and not other mechanisms of mineralization.

Fig. 5, which places this experiment in the context of our earlier work (Qureshi et al., 2003a), shows that the extent of trace element losses correlated strongly with our measures of microbial activity (in the absence of other factors). Results from our earlier work include incubation temperatures of 4 °C, 16 °C, 28 °C (connected by lines to other temperature data) and 37 °C and with silver biocide (all temperatures). These treatments were in all other respects equivalent to the G/G treatment in this experiment. Cumulative respiration was normalized as a fraction of the values measured at 28 °C in each experiment. G^{acid}/G treatment results are included in the figure for comparison, although it could be argued that those losses would have occurred independent of respiration results due to the direct acidification treatment. The observed differences in the cumulative losses at 28 °C between the first (Qureshi et al., 2003a) and present experiment were not surprising due to storage of the sludge. The figure highlights the drop in losses from the treatments which prevented acidification (G^{lime}/G and S/G) despite substantial microbial respiration levels.

The experimental context—with optimum temperature, moisture and aeration—favored rapid acidification that may not be directly applicable to field conditions, especially if incorporation and mixing with soil occurs following application at typical agronomic rates. Nevertheless, some acidification has been observed, particularly with heavy sludge applications typical of “reclamation” situations. Logan et al. (1997) observed a one unit decrease in pH in a well-buffered soil following heavy sludge loadings, and Harrison et al. (1996) reported depression of soil pH from 5.4 to 4.5 following a 300 T ha⁻¹ sludge loading. Richards et al. (2000) saw significant and long-term depression of leachate and soil pH to 4 following sludge loadings totaling 230 T ha⁻¹ to intact soil columns, with large leachate S and nitrate-N concentrations coinciding with leaching of a range of trace elements. Questions of long-term mineralization, where long-term microbial breakdown of stable organic matter could be expected to mobilize complexed elements, were beyond the scope of this experiment.

Comparisons of treatment pairs where the glass bead support layer was replaced by sand (G/G vs. G/S , G^{acid}/G vs. G^{acid}/S , and S/G vs. S/S) indicate that the calcareous sand layer virtually eliminated trace element leaching even when trace elements had been substantially mobilized in the sludge layer (Table 4), which finding shows that the mobilized elements were in a readily re-sorbable form. Mo again was the only exception, with treatments with sand support layers producing leachate Mo concentrations that were intermediate between the high levels of G^{lime}/G and S/G (0.07–0.10 mg L⁻¹) and the 90% lower concentrations from the strongly acid G/G and G^{acid}/G treatments. Even though calcareous, the sand support layer nevertheless reduced Mo leachate concentrations

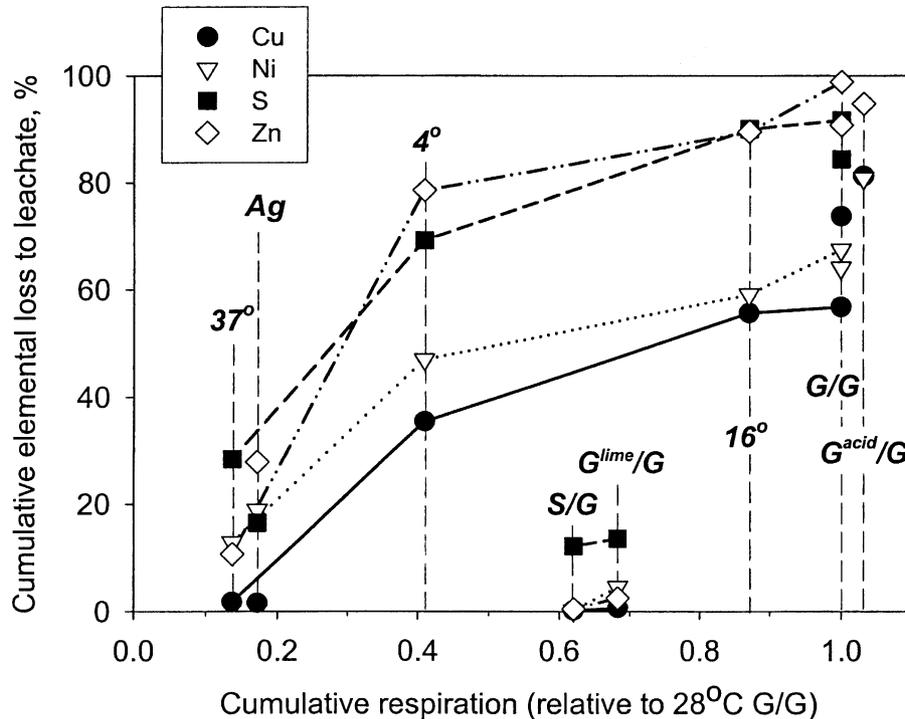


Fig. 5. Cumulative elemental loss of Cu, Ni, S and Zn as a function of relative microbial respiration and treatments in this study and in Qureshi et al. (2003a). Loss is expressed as percent of that initially present in the sludge. Relative cumulative respiration values are normalized to respiration in a sludge only (G) treatment at 28 °C = 1.0. Vertical dashed lines serve to group the results for a given incubation temperature or amendment treatment. Treatments shown from Qureshi et al. (2003a) include (left to right) 37 °C, silver (Ag) biocide, 4 °C, 16 °C, and a 28 °C G/G treatment. Treatments shown from the present study include S/G, G^{lime}/G, G/G and G^{acid}/G, all at 28 °C.

by two-thirds when comparing treatments S/G and S/S. Interestingly, treatment S/S had greater concentrations than the minima observed for Cd and Ni. For both these analytes, the sand was a significant contributor to the total analyte mass present in columns (although the labile fraction of analytes in the sand was unknown), and the S/S treatment had the greatest mass of sand of any treatment.

These calcareous sand results are consistent with findings of strong adsorption of trace elements in calcareous soils (Sterckeman et al., 2000; Wong et al., 2000). Although the mechanisms of readsorption were not determined in these experiments, the results indicate that the trace elements mobilized by acidification were in a form easily subject to readsorption. In contrast, metals leaching through an old sludge field site—some complexed with organic ligands and all flowing along preferential flow paths in the strongly structured soil—showed no detectable evidence of being accumulated in calcareous subsoil horizons (Richards et al., 1998; McBride et al., 1997).

4. Conclusions

Our results suggest that the primary microbial role in the rapid release and leaching of trace elements from sludge is that of acidification. Direct acidification

produced nearly identical results to microbial acidification, whereas microbial activity in the absence of acidification mobilized far lower concentrations of trace elements. The only exception was Mo, which was more efficiently mobilized at neutral pH. Trace elements mobilized by acidification were in a form that was readily readsorbed when calcareous sand was present.

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