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Bio-Acidification and Leaching of Metals, Nitrogen, and Phosphorus from Soil and Sludge Mixtures

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Soil and wastewater treatment sludge are commonly brought together in mixtures for a variety of beneficial purposes. The mixtures contain bioacidifying (i.e., sulfur-oxidizing) microorganisms that can easily be activated through providing the appropriate substrate and environmental conditions. In this study, contaminated soil and sludge mixtures were subjected to controlled bio-acidification and the impacts of the process on the partitioning of heavy metals, nitrogen, and phosphorus were examined. Three successive bio-acidification cycles resulted in significant leaching of metals from sludge. The leaching results, expressed as fraction of total mass of metals in the sludge, averaged 67% for Cr, 96% for Ni, 24% for Zn; 16% for Cu; 23% for Cd; and 96% for Pb. Bio-acidification of the sludge also converted 28 to 45% of the organic nitrogen into ammonia and increased the soluble orthophosphates fraction of total phosphorus by approximately 18 to 20%. Bio-acidification also resulted in significant metals leaching from the contaminated soils in the soil/sludge mixtures. Soil/sludge mixtures were prepared using six soil particle sizes (less than 0.075 mm to 2.38 mm) contaminated with 22,500 mg/kg Zn, 14,000 mg/kg Pb, 1500 mg/kg Cr, 9500 mg/kg Cu, 1000 mg/kg Ni, and 1000 mg/kg Cd. The addition of metals to the soil inhibited the sulfur-oxidizing microorganisms, preventing bio-acidification in the mixtures containing 4 to 50 g soil in 130 ml sludge, and considerably slowing bio-acidification in the mixtures containing 1 to 3 g soil. Using a mixture that contained 2-g soil samples, three successive bio-acidification cycles resulted in significant cumulative metals leaching results. The leaching results, expressed as percentage of the mass of metals added to the soil, were in the range of 56 to 98% for Cr, 77 to 95% for Zn, 33 to 66% for Ni, 64 to 82% for Cu, and 10 to 33% for Pb, with the higher results in each range belonging to the larger size soil particles. On the other hand, only Cr was leached in neutralized soil samples. The results confirmed the potential for inhibition of the sulfur-oxidizing microorganisms and bio-acidification in contaminated soil/sludge mixtures, and the significant impacts of bio-acidification on the mobility of metals, nitrogen, and phosphorus. In addition, the results confirmed the potential for using controlled bio-acidification for removing heavy metals from contaminated soil using the indigenous sulfur oxidizing microorganisms in sludge.

KEY WORDS: *contaminated soil, wastewater treatment sludge, soil/sludge mixture, bio-acidification, inhibition of bio-acidification, bioleaching, heavy metals, phosphorus, nitrogen, sulfur-oxidizing bacteria, soil particle size.*

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INTRODUCTION

BIO-ACIDIFICATION due to biological production of sulfuric acid was observed by Colmer and Hinkle (1947) while investigating the corrosion of pipes and other iron parts in contact with mine water. Soon after, bio-acidification was utilized to leach metals from the pyretic ore deposits (Bosecker and Kursten, 1978). Currently, bioleaching of metals using bio-acidification is an established technique used for extracting minerals (Lloyd, 2002) from low-grade ores and industrial waste products (Bosecker, 1986).

Bio-acidification is a natural process that results from the activity of chemo-autotrophic bacteria that oxidize sulfides and elemental sulfur and produces sulfuric acid according to Reactions 1 and 2.

Thiobacillus ferroxidans (*T. ferrooxidans*) and *Thiobacillus thiooxidans (T. thiooxidans)* act as the principal micro-organisms mediating the two reactions.

$$\mathrm{MS} + 2\mathrm{O}_2 \xrightarrow{T.\,ferrooxidans} \mathrm{M}^{2+} + \mathrm{SO}_4^{2-} \qquad \text{Reaction (1)}$$

$$\mathrm{S}^0 + 1.5\mathrm{O}_2 + \xrightarrow[T.\,ferrooxidan]{T.\,thiooxidans} \mathrm{H}_2\mathrm{SO}_4 \qquad \text{Reaction (2)}$$

The presence of toxic heavy metals is a major constraint that limits the beneficial use of sludge. As such, guidelines to control the application of sludge on land are used in many countries to minimize the risk of accumulation of heavy metals into the food chain via metal-accumulating plants and plant-grazing animals. In addition, the migration of nutrients and heavy metals from agricultural land is recognized as a significant source of off-site pollution. The acidification of soil and sludge mixtures significantly increases the potential for mobilizing the toxic heavy metals and nutrients content in both the soil and sludge.

The use of controlled bio-acidification (i.e., bioleaching) to dissolve metals from sludge using the indigenous thiobacilli bacteria in sludge was first investigated by Schonborn and Hartmann in 1978. Later, Wong and Henry (1984) and more recently (Jain and Tyagi, 1992; Sreekrishnan *et al.*, 1993; Ginige and Shanableh, 1997) renewed interest in the process as a potential sludge decontamination technology. Bio-acidification creates a highly favorable environment for metals leaching due to elevation of the oxidation-reduction potential and production of sulfuric acid. The process is recognized to proceed directly through dissolving metals sulfides (MS) according to Reaction 1, or indirectly through dissolving metals that exist as oxides, hydroxides, or other acid-leachable forms.

The alternatives for removing heavy metals from soils and sediments are limited and are generally expensive. The alternatives include: soil washing to separate the contaminated fine soil particles, and mobilization using various techniques and additives (Sims, 1990). The use of controlled bio-acidification to dissolve metals from contaminated soils and sediments was considered by researchers (Seidel *et al.*, 1998; Tichy *et al.*, 1998; Chartier *et al.*, 2001; Lloyd, 2002) and was found to be more effective than chemical extraction using sulfuric acid (Seidel *et al.*, 1998). Commercially, heap leaching is the most common method used for extracting metals from low-grade ores through bio-acidification. In heap leaching, the ore material is prepared in mounds over drainage support and the leaching solution is applied then collected for metals recovery.

Soil and wastewater treatment sludge are commonly brought together in mixtures for a variety of beneficial purposes. The application of raw or treated sludge on land for agriculture. Sludge has a considerable value as a soil conditioner/fertilizer and can satisfy the needs of most crops for nitrogen, phosphorus, and a range of other nutrients. In addition, sludge is commonly disposed-off in landfills on soils that may be contaminated with heavy metals and other pollutants. The acidifying microorganisms exist in sludge, and can be easily activated to produce sulfuric acid whenever appropriate substrate and environmental conditions are available. In this study, mixtures of contaminated soil and sludge were subjected to bio-acidification and the impacts of the process on the mobility of heavy metals, nitrogen, and phosphorus in the mixtures were evaluated.

EXPERIMENTAL EVALUATION

Sludge samples were obtained from the return sludge line of a conventional activated sludge wastewater treatment plant. The total solids content of the sludge was adjusted to approximately 2%. The characteristics of the modified, 2% solids content sludge are listed in Table 1.

TABLE 1
Characteristics of the Sludge with Modified Solids Content

Parameter	Value
Total Solids (mg/L)	21,200
Volatile Solids (mg/L)	18,100
pH	6.8
Total Kjeldahl Nitrogen (mg/L)	1,854 (28 NH_4-N)
Total Phosphorus (mg/L)	550 (130 PO_4-P)
Sulfate in Supernatant (mg/L)	10
Zinc (mg/kg dry solids)	3,264
Copper (mg/kg dry solids)	594
Lead (mg/kg dry solids)	23
Chromium (mg/kg dry solids)	43
Nickel (mg/kg dry solids)	23
Cadmium (mg/kg dry solids)	16

Bio-acidification results from the oxidation of elemental sulfur and sulfides and the production of sulfuric acid by specialized groups of bacteria. In this study, soil and sludge bio-acidification was achieved in three cycles. For sludge, the first acidification cycle to pH <2 was achieved using 250 ml sludge samples in 500 ml sterilized Erlemneyer flasks. Sulfur (1% by weight or 2.5 g) was added to some of the flasks and the others were left without sulfur as controls. The samples were placed in a gyratory shaker (Chiltern scientific Orbital shaker model SS70) and mixed at 200 rpm inside an incubator at 28°C. The first bio-acidification cycle to pH < 2 was achieved in 5 days. The second bio-acidification cycle to pH <2 following first acidification was achieved by mixing 5% of the bio-acidified sludge from the first cycle with 250 ml raw sludge and 2.5 g sulfur. A third bio-acidification cycle was also conducted using a mixture of 5% bio-acidified sludge from the second cycle, 250 ml raw sludge and 2.5 g sulfur. Bio-acidification in each of the second and third cycles was achieved in 3 days. Daily samples, 20 ml each, were withdrawn and used for chemical analysis, and distilled water was used to compensate the daily weight of water lost by evaporation. It should be noted that all bio-acidification experiments were conducted in triplicates. The pH and sulfate results reported in this study represent averages of three measurements. On the other hand, the metals, nitrogen, and phosphorus measurements were performed on composite samples prepared by combining three samples withdrawn from the triplicate bio-acidification flasks.

A sample of clean sand used for water filtration was used as the model soil for testing. The sample was crushed and separated into six different soil sizes (Table 2). Each size was spiked with a mixture of dry metal salts, including $Zn(NO_3)_2.6H_2O$, $Cu(NO_3)_2.2.5H_2O$, $Ni(NO_3)_2.6H_2O$, $Cd(NO_3)_2.4H_2O$, $Pb(NO_3)_2$, and Na_2CrO_4. The metals contamination levels chosen for this study were based on findings reported by Esposito *et al*. (1989) on typical levels of metals found in contaminated soils. It should be emphasized that the method used for preparing the contaminated soil samples is unlikely to produce metal-soil interactions similar to those that are typically present in contaminated soil systems. Instead, the simplest form of physical interaction between the metal salts and the soil particles was used in preparing the contaminated soil samples. Clearly, metals leaching is strongly dependant on the metals mineralogy in the soil. Unfortunately, the specific metal-soil interactions and mineralogy, before and after bio-acidification, were not adequately assessed in this study.

In preparing the soil/sludge mixtures for assessing bio-acidification and partitioning of metals, 20 ml sludge samples were centrifuged at 4000 rpm for 15 min and the supernatant was decanted. The centrifuged solids (approximately 0.4 g dry basis) were mixed with soil (1 to 50 g) and 130 ml distilled water. Three types of soil/sludge mixtures were prepared: (1) samples containing contaminated soil, bio-acidified sludge, and 1 g sulfur; (2) samples containing contaminated soil, raw sludge, and no sulfur; and (3) samples containing uncontaminated soil, bio-acidified sludge, and 1 g sulfur. The samples were placed in plastic flasks, mounted on

TABLE 2
Characteristics Of The Synthetic Soil Used in the Study and Nominal Contamination Levels

Item	<0.075	0.075-0.15	0.15-0.30	0.3-0.6	0.6-1.18	1.18-2.38
Acid Soluble Fraction* (%)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Loss on Ignition @ 550°C (%)	0.7	0.6	0.6	0.6	0.6	0.6
pH (Prior to Contamination)	6.5	6	5	4.3	4.2	4
Zn (mg/kg)	22,500	22,500	22,500	22,500	22,500	22,500
Cu (mg/kg)	9,500	9,500	9,500	9,500	9,500	9,500
Ni (mg/kg)	1,000	1,000	1,000	1,000	1,000	1,000
Cd (mg/kg)	1,000	1,000	1,000	1,000	1,000	1,000
Pb (mg/kg)	14,000	14,000	14,000	14,000	14,000	14,000
Cr (mg/kg)	1,500	1,500	1,500	1,500	1,500	1,500

*Soil soaked in sulfuric acid solution at pH 1 for 15 days.

the shaker, and mixed at 200 rpm inside the incubator at 28°C. Similar to the sludge samples, bio-acidification of the soil/sludge mixtures was carried out in three cycles. Following the first acidification cycle to pH of approximately 1.5, the samples were settled and centrifuged at 4000 rpm for 15 minutes and the supernatants were carefully separated and replaced with distilled water up to the 130 ml mark. Sulfur (1 g) was added to the samples that received sulfur in the first bio-acidification cycle. The samples used in the third bio-acidification cycle following the first and second cycles were prepared in the same manner as the samples prepared in the second cycle.

The samples were prepared for heavy metals analysis as follows: each sample was centrifuged at 18,000 rpm for 30 min and the supernatant was filtered then stored at 4°C in plastic bottles. The heavy metals were measured using Plasma Emission Spectroscopy (ICP). Sample digestion was conducted according to USEPA (1986) methods for solid waste characterization. Ammonia, sulfate, TKN, and total phosphorus were measured according to Standard Methods, APHA (1992).

RESULTS AND DISCUSSION

Bio-Acidification of Sludge

The sulfur-oxidizing microorganisms in sludge were activated using three successive bio-acidification cycles. Bio-acidification to pH < 2 required 5 days during the first cycle (Figure 1) and 3 days during the two subsequent cycles. During the first cycle, approximately 7000 mg/L of SO_4 was produced, which corresponds to consumption of approximately 0.6 g (23%) of the added sulfur. The fraction of sulfur oxidized to sulfuric acid during the subsequent cycles was approximately 17% during the second cycle and 25% during the third cycle. During the first cycle, the control samples (Figure 1) demonstrated low sulfate production and the pH decreased gradually to approximately 5.1 following an initial increase on the first day. Similar behavior was experienced in the control samples during the two subsequent bio-acidification cycles.

The use of the sludge considered in this study for agricultural purposes would be restricted under the guidelines in many countries due to the relatively high metals content. Bio-acidification resulted in leaching significant quantities of metals from the sludge. A summary of the metals leaching results, expressed as fraction of total mass of metals, achieved using the three bio-acidification cycles is presented in Table 3. The results indicate that significant metals leaching in the range of 17 to 98% were achieved. The metals leaching trend in general followed the acidification trend as measured by pH. The retention of metals was in the following order: Cu > Cd > Zn > Cr > Ni = Pb. The leaching results in the control samples were in the range of 0 to 19%. The rates of metals leaching during the first cycle is presented in Figure 2. The data indicate gradual leaching trends for most

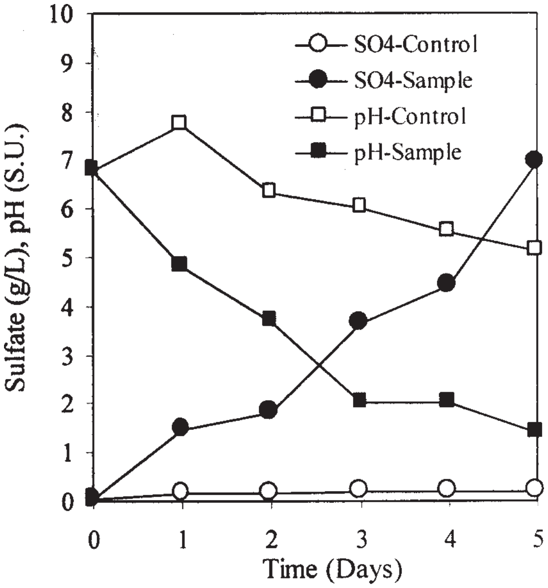

FIGURE 1

Bio-acidification of sludge during the first cycle as indicated by the pH and sulfate (sulfuric acid) production.

metals during the first 2 days. The leaching rates significantly increased between days 2 and 3 and increased gradually thereafter.

Bio-acidification of sludge had a significant impact on phosphorus and nitrogen transformations in the treated samples (Figures 3 and 4). During bio-acidification, orthophosphates and ammonia were leached from the sludge solids. The conversion of organic nitrogen into ammonia is referred to herein as mineralization of TKN. Mineralization converted approximately 45% of TKN into NH_4-N at the end of the first bio-acidification cycle. In the control samples, the NH_4-N fraction of TKN remained limited to a maximum of 5% and the pH remained above 5. Mineralization in the subsequent second and third bio-acidification cycles declined to 35 and 27%, respectively. Mineralization of TKN typically results from oxidation of organic matter, but in this case may also be attributed to the destruction of proteins from microorganisms and destruction of organic matter as a result of the newly imposed acidic environment.

During the first bio-acidification cycle (Figures 3 and 4), the soluble phosphorus fraction in the bio-acidified sludge increased over that in the control samples by approximately 18 to 20%. The extra observed leaching of phosphorus (PO_4) may be attributed in part to acidic solubilization of phosphorus, but also oxidation and destruction of microorganisms. The PO_4-P/Total-P transformation trends were

TABLE 3
Metals Leaching Results (%)* from Sludge

Item	1st Acidification		2nd Acidification		3rd Acidification	
	Acidified	Control	Acidified	Control	Acidified	Control
Ni	95.9	18.8	95.7	9.5	96.5	13.8
Pb	94.1	0.2	95.2	6.7	98.3	4.6
Cr	67.0	0.9	62.2	4.7	71.3	3.5
Zn	23.8	1.6	23.6	1.0	23.6	1.1
Cu	16.5	0.8	15.9	1.5	16.6	1.0
Cd	22.9	0.5	22.4	0.0	23.2	0.0

* percentage leaching (%) = soluble metal / total metal x 100

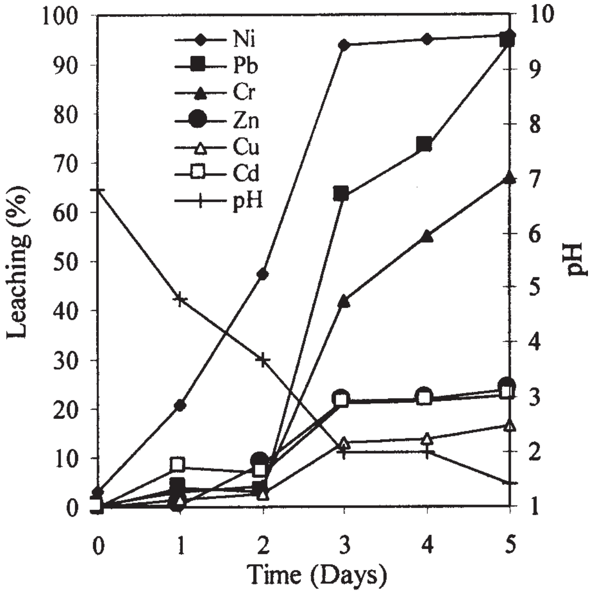

FIGURE 2

Metals leaching rates (%) during the first acidification cycle.

similar for both the bio-acidified and control samples (Figure 3). On the first day of bio-acidification, the PO_4-P/Total-P fraction declined then gradually increased. The observed phosphorus activity requires further assessment to assess the impacts of gradual digestion, increased oxidation-reduction potential, and bio-acidification on the speciation of phosphorus.

The results suggest that in addition to leaching metals, bio-acidification mobilized a significant fraction of the nitrogen and phosphorus content of sludge. As such, the use of bio-acidification to decontaminate sludge by removing metals for land application suffers from the negative consequence of potential loss of a significant fraction of the nitrogen and phosphorus content of sludge. In addition, the potential of off-site pollution from sludge due to acidification is contributed by the metals and nutrients. As such, careful considerations must be practiced when applying sludge to land, especially on acidic soils or whenever acidification may occur.

BIO-ACIDIFICATION OF THE SOIL/SLUDGE MIXTURES

The addition of metals to the soil at the levels used in this study can inhibit the bioacidifying bacteria. For example, the data in Figures 5 to 10 present a compari-

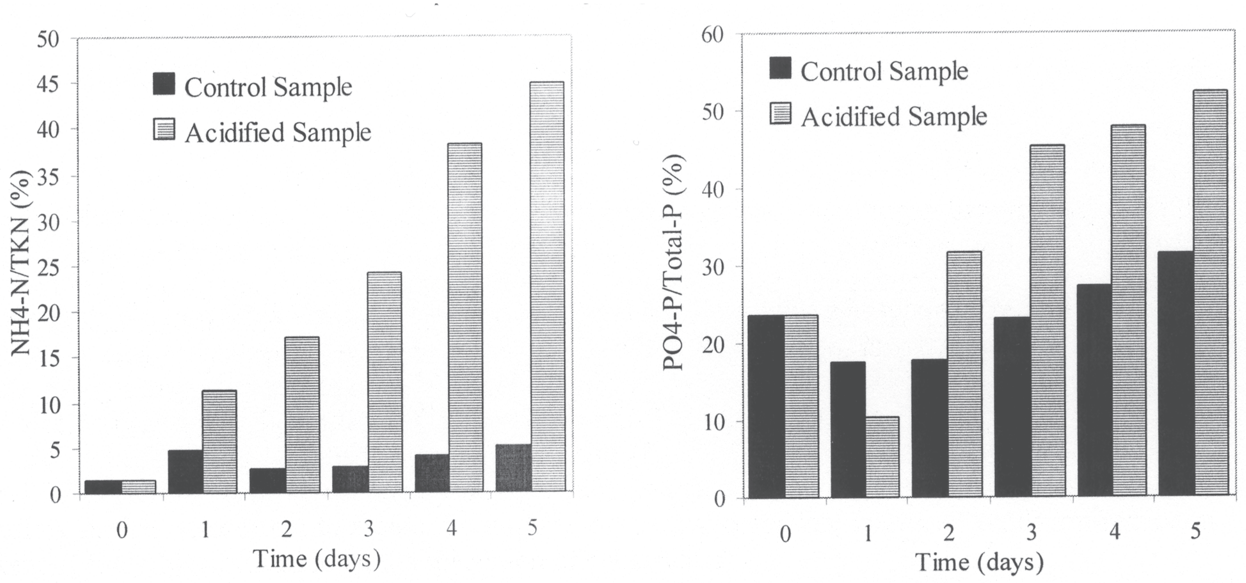

FIGURE 3

Rates of mineralization of TKN (as NH_4-N/TKN) and leaching of phosphorus (as PO_4-P/Total-P) from sludge during the first bio-acidification cycle.

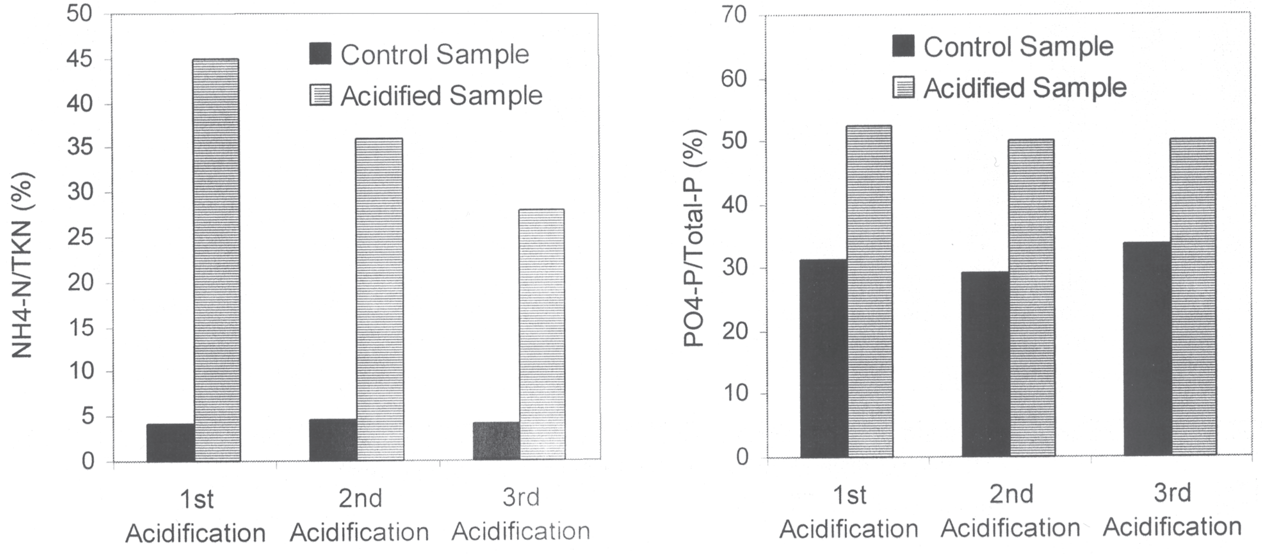

FIGURE 4

Mineralization of TKN (as NH_4-N/TKN) and leaching of phosphorus (as PO_4-P/Total-P) during three successive bio-acidification cycles of the sludge.

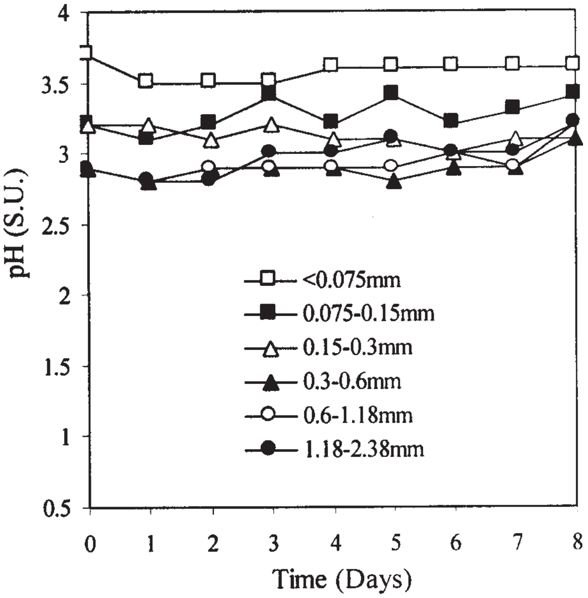

FIGURE 5

Inhibition of bio-acidification in the experiments conducted using 50-g contaminated soil samples, 1 g sulfur and acidified sludge.

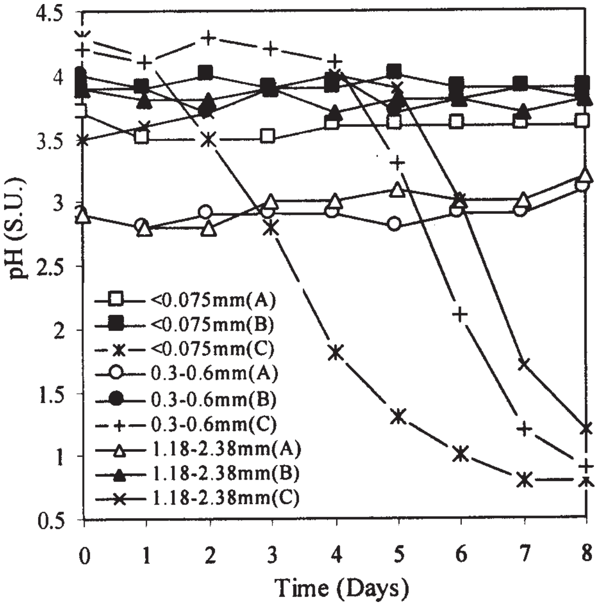

FIGURE 6

Inhibition of bio-acidification in the experiments conducted using 50-g soil samples. (A) contaminated soil+bio-acidified sludge+sulfur; (B) contaminated soil+raw sludge+no sulfur; and (C) uncontaminated soil+bio-acidified sludge+sulfur.

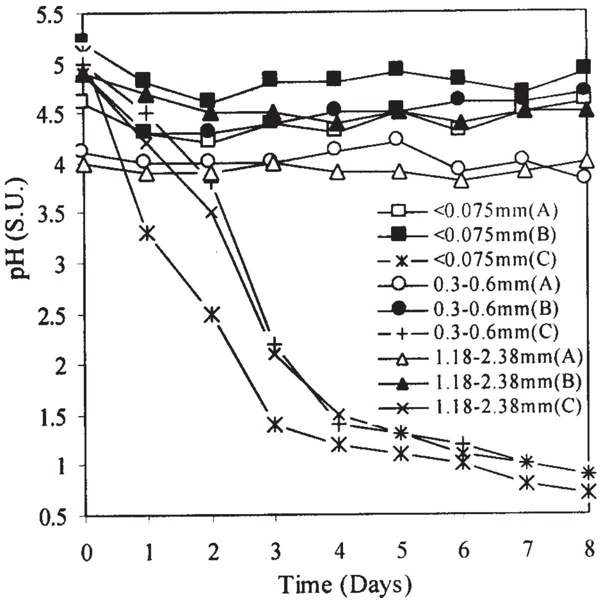

FIGURE 7

Inhibition of bio-acidification in the experiments conducted using 10-g soil samples. (A) contaminated soil+bio-acidified sludge+sulfur; (B) contaminated soil+raw sludge+no sulfur; and (C) uncontaminated soil,+bio-acidified sludge+sulfur.

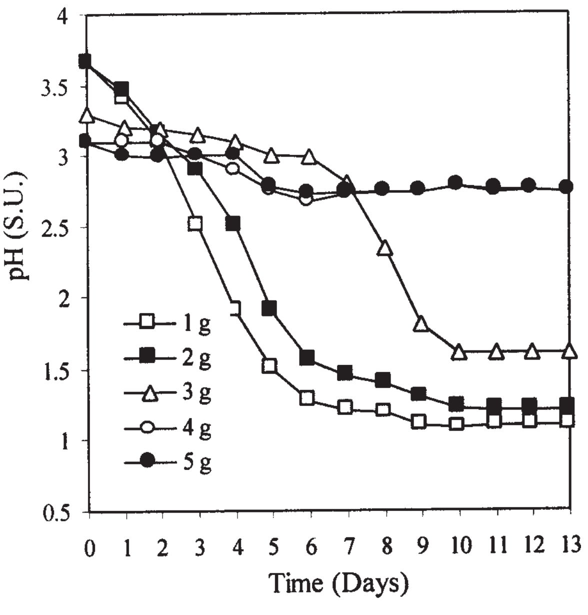

FIGURE 8

Bio-acidification of the 0.3- to 0.6-mm contaminated soil samples mixed with 1 g sulfur and acidified sludge.

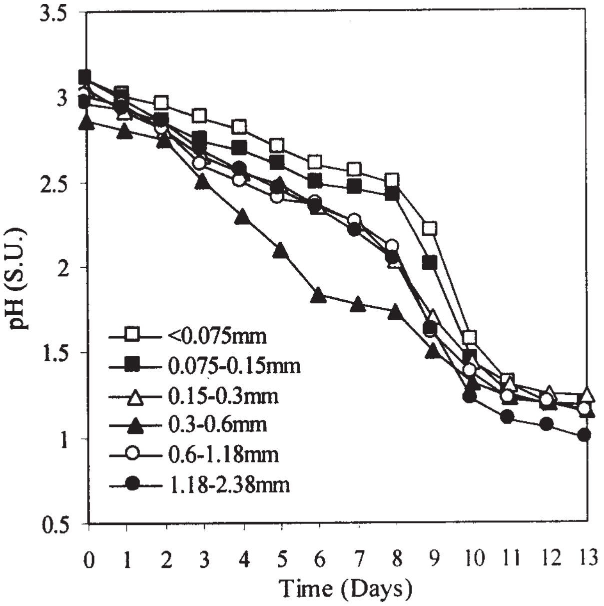

FIGURE 9

Bio-acidification during experiments conducted using 2-g contaminated soil samples, 1 g sulfur, and acidified sludge.

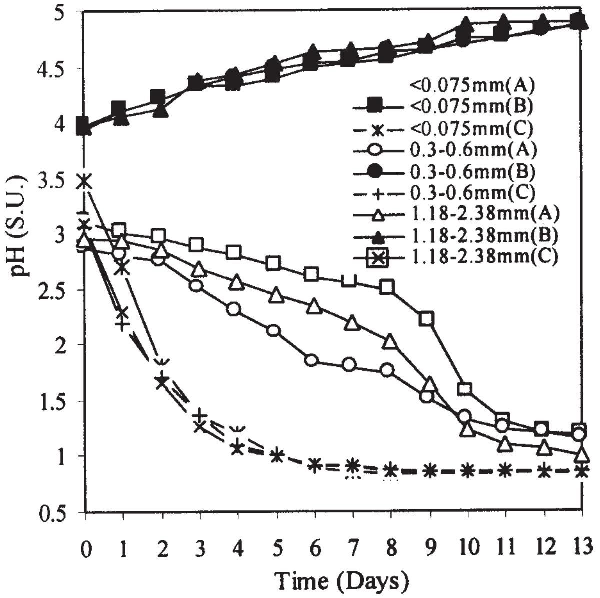

FIGURE 10

Bio-acidification in the experiments conducted using 2-g soil samples. (A) contaminated soil+bio-acidified sludge+sulfur; (B) contaminated soil+raw sludge+no sulfur; and (C) uncontaminated soil+bio-acidified sludge+sulfur.

son of the acidification trends in a variety of samples containing contaminated and uncontaminated soils. In the first set of experiments in which 50 g of the various contaminated soil sizes were mixed with bio-acidified sludge (Figure 5), the pH remained relatively unchanged. In the second set of experiments, which involved 50 g of three contaminated and uncontaminated soil sizes mixed with sludge (Figure 6), acidification occurred only in the uncontaminated samples. Similarly, acidification occurred only in the uncontaminated samples in the experiments that involved a reduced quantity (10 g) of three contaminated and uncontaminated soil sizes mixed with sludge (Figure 7).

The data in Figures 6 and 7 indicate that in the experiments involving uncontaminated soil samples, the pH declined faster in the mixtures that contained the smaller size soil particles. In addition, the data in Figures 6 and 7 indicate that the resistance to pH decline, or capacity to neutralize acids, was higher in the mixtures containing 50 g soil compared with the mixtures containing 10 g soil.

Additional experiments were conducted to determine the level of contamination that allowed bio-acidification to proceed without inhibition. A set of 1 to 5 g contaminated soil samples of the same size (0.3 to 0.6 mm) were tested for this purpose and the acidification results are presented in Figure 8. The data indicate that bio-acidification proceeded in the samples containing 1 to 3 g soil samples and was largely inhibited in the samples containing 4- and 5-g soil samples. The bio-acidification activity was related to the amount of contaminated soil used in the various samples, with the highest pH decline being in the sample that contained 1 g of soil.

Based on the bio-acidification results in Figure 8, 2-g soil samples were used in the remaining bio-acidification experiments to assess the leaching of metals from the soil/sludge mixtures. Three bio-acidification cycles were carried out for this purpose. The bio-acidification results during the first cycle are presented in Figures 9 and 10. The acidification trends for all soil sizes are presented in Figure 9. For selected soil sizes, two types of control samples were used; one containing uncontaminated soil and the other containing contaminated soil and raw sludge. The pH trends in Figures 10 indicate that the pH increased gradually in the control samples containing contaminated soils, raw sludge and no sulfur. Compared with the pH changes in the control samples in Figure 10, the pH increase may be related to the addition of metals in the contaminated soil. In Figure 10, the pH decreased rapidly in the control samples containing clean soils, acidified sludge and sulfur. In comparison, the pH decreased gradually in the samples containing contaminated soil, acidified sludge and sulfur. The difference in the pH reduction trends in the contaminated and uncontaminated soil samples may be attributed to inhibition of the bio-acidification activity and to any differences in alkalinity between the contaminated and clean soil samples.

The causes of inhibition of the bio-acidification activity were not thoroughly investigated in this study. The presence of heavy metals in the various contaminated soil samples is a likely cause. Nevertheless, the tolerance of the bio-acidifying microorganisms to the presence of a mixture of metals was quite high as indicated in Table 4, which shows the concentrations of metals associated with the different soil quantities used in the experiments. The data in Table 4 also differentiates among the samples that achieved bio-acidification to $pH < 2$ and those that did not. For example, bio-acidification to $pH < 2$ or less occurred only in the samples that contained 3 g of contaminated soil or less.

Bio-acidification due to the activity of the indigenous sulfur oxidizing microorganisms in sludge resulted in partitioning of metals among the liquid, sludge, and soil in the soil/sludge mixtures. The partitioning results for Cr, Zn, Ni, Cu, and Pb are briefly described in the following subsections. Three control soil samples of sizes <0.075 mm, 0.3 to 0.6 mm, and 1.18 to 2.38 mm were prepared and used alongside the six bio-acidified samples. The control samples contained contaminated soil and raw sludge but no sulfur. The pH was adjusted in the control samples to approximately 7 to 8, and the soluble metals were measured. The data in Table 5 present a comparison of the leaching results achieved in the bio-acidified and control samples. The data show that Cr was the only metal that was leached in appreciable amounts from the control samples.

Leaching of Cr

The mechanisms governing Cr leaching from soil were not investigated in this study. However, the size of the soil particles appeared to affect the Cr solubilization results. The data in Figure 11 indicate that solubilization of Cr decreased as the size of the soil particles decreased. Solubilization was in the range of 20 to 69% after the first acidification cycle, increased to 43 to 78% after the second acidification cycle, and reached 56 to 98% following the third cycle.

Hexavalent chromium exits as an anion that is generally soluble in aqueous solutions. Generally, the solubility, adsorption-desorption reactions, and reduction (i.e., conversion of Cr^{6+} to the less soluble Cr^{3+}) of the chromate anion govern its mobility. The presence of organic matter and colloids that develop a positive charge at low pH favor retention of Cr by sorption. The presence of certain cations, such as Ca^{2+}

and Pb^{2+} (which was added to the soil), can precipitate the chromate anion and significantly reduce its mobility, especially in the neutral pH range (Nieboer and Jusys, 1988). Calcium chromate ($CaCrO_4$) is moderately soluble and lead chromate ($PbCrO_4$) has low solubility. The solubility of both $CaCrO_4$ and $PbCrO_4$ significantly increases in acidic environments. The reduction of hexavalent Cr^{6+} to the less soluble Cr^{3+} can significantly alter the mobility of chromium.

Leaching of Zn

The first bio-acidification cycle resulted in 51 to 60% Zn leaching (Figure 12) from the various soil sizes. The second bio-acidification cycle increased Zn leaching to

TABLE 4
Nominal Concentrations* of Metals (mg/l) and Bio-Acidification Results in the Various Experiments

Added Soil	Zn	Pb	Cu	Cr	Ni	Cd	Bioacidification to pH < 2 Achieved
1 g	173	108	73	12	8	8	Yes
2 g	346	215	146	23	15	15	Yes
3 g	519	323	219	35	23	23	Yes
4 g	692	431	292	46	31	31	No
5 g	865	538	365	58	38	38	No
10 g	1731	1077	731	115	77	77	No
50 g	8654	5385	3654	577	385	385	No

* Nominal concentration = contamination level (from Table 2) x added soil (g) / 130 ml water

TABLE 5
Comparison of Metals Leaching (%)* from Selected Bio-Acidified and Control Soil Samples

Metal	Bio-Acidified <0.075 mm	Control <0.075 mm	Bio-Acidified 0.3-0.6 mm	Control 0.3-0.6 mm	Bio-Acidified 1.18-2.38 mm	Control 1.18-2.38 mm
Cr	55.9	3.6	75.0	3.2	98.0	6.4
Ni	32.5	<1.0	46.8	<1.0	66.3	1.2
Zn	77.4	1.3	78.3	<1.0	95.0	1.6
Cu	63.6	1.6	78.0	1.3	82.1	1.7
Pb	10.5	<1.0	15.6	<1.0	32.5	<1.0

*Relative to the mass of metals added to the soil.

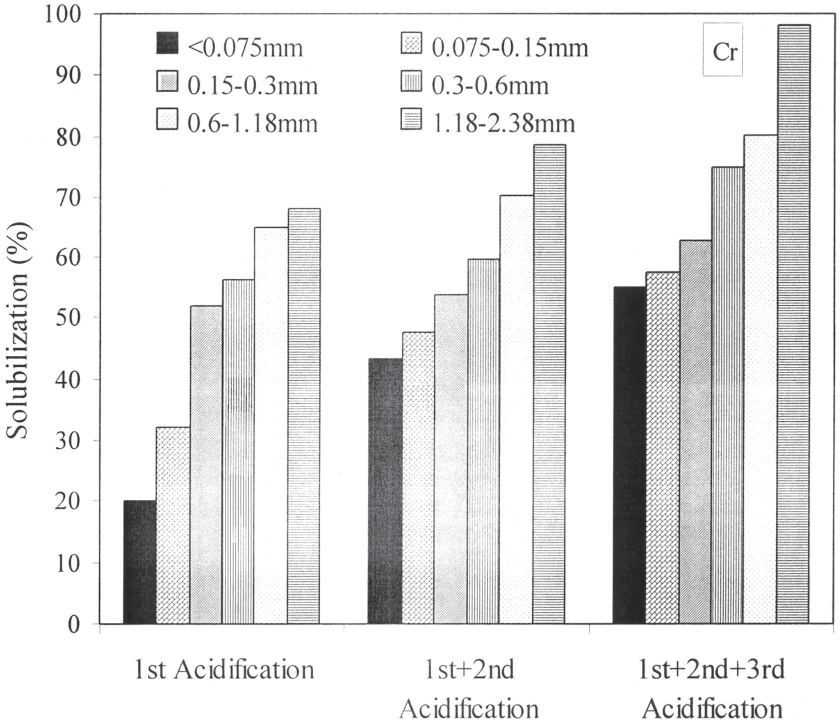

FIGURE 11

Cumulative leaching of Cr from the different soil sizes in the soil/sludge mixtures as a result of three successive bio-acidification cycles.

61 to 78% and the third cycle increased Zn leaching to 77 to 95%. In general, the leaching results for Zn were not highly dependant on the size of the soil particles.

The mechanisms governing Zn leaching from soil were not investigated in this study. In general, the solubility of Zn is related to the solubility of Zn oxides, hydroxides, and carbonates and is strongly pH dependent. Zinc also adsorbs favorably on iron and manganese oxide surfaces and organic matter (Chartier *et al*., 2001). However, zinc is relatively soluble and mobile, especially in acidic environments, and the high leaching results clearly demonstrate this behavior.

Leaching of Ni

The leaching of Ni from the different soil sizes at the end of the first acidification cycle was in the range of 26 to 46% (Figure 13). The following two bio-acidification cycles increased Ni leaching only slightly to 26 to 59% and 33 to 66%.

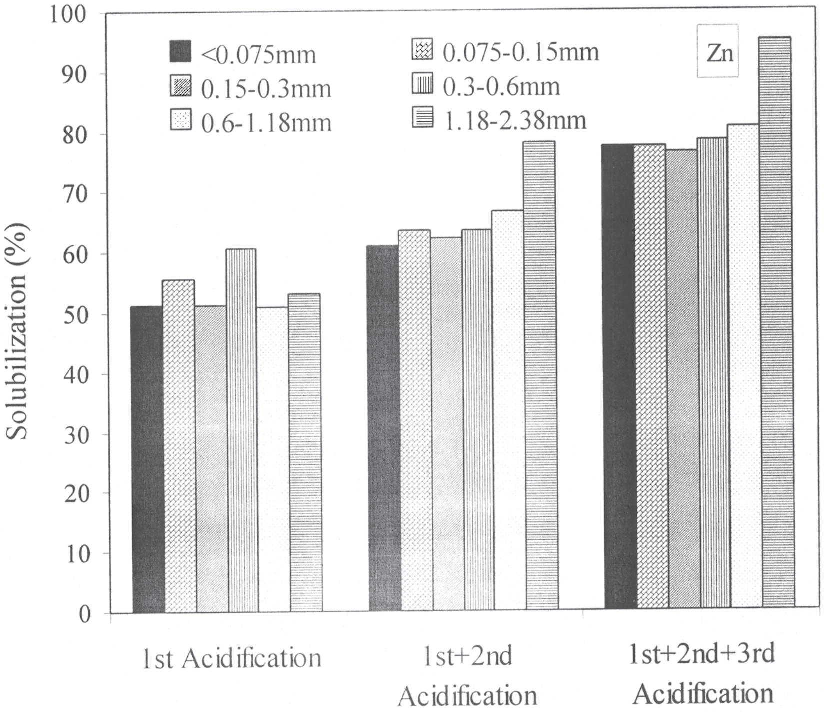

FIGURE 12

Cumulative leaching of Zn from the different soil sizes in the soil/sludge mixtures as a result of three successive bio-acidification cycles.

The mechanisms governing Ni leaching from soil were not investigated in this study. However, the soil particle size had a significant impact on the leaching of Ni. The solubility of Ni evolves around the solubility of nickel oxides, hydroxides, and phosphates and thus increases as the pH decreases within the acidic pH range. Chartier *et al*. (2001) reported that most of the leached Ni from sediments came from Ni associated with the carbonates, and Fe/Mn oxides. Leaching also depends on other mobilization mechanisms, such as Ni uptake by soluble organic legands and immobilization reactions such as sorption onto soil and organic particles surfaces.

Leaching of Cu

The data presented in Figure 14 indicate that the first acidification of the soil samples resulted in 35 to 53% Cu leaching results. The second acidification cycle increased the leaching efficiency to 58 to 65% and the third acidification cycle

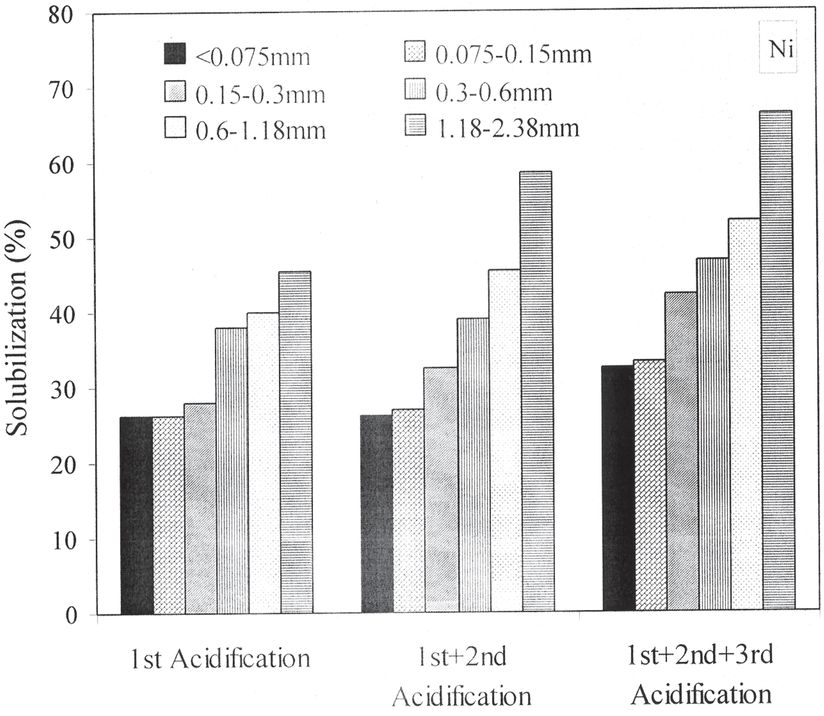

FIGURE 13

Cumulative leaching of Ni from the different soil sizes in the soil/sludge mixtures as a result of three successive bio-acidification cycles.

increased Cu leaching to 64 to 82%. The soil particle size appeared to have some impact on the removal of Cu.

The mechanisms governing Cu leaching from soil were not investigated in this study. Copper may exist in numerous forms, including soluble organic and inorganic complexes and precipitated oxides, hydroxides, carbonates, and other salts. In general, Cu is favorably immobilized by organic and inorganic colloids. Chartier *et al.* (2001) found that most of the Cu in contaminated sediments was associated with the organic matter.

Leaching of Pb

Lead leaching (Figure 15) was in the range of 10 to 23% as a result of the first acidification cycle. The following two bio-acidification cycles did not significantly improve Pb leaching except in the largest size soil sample. The results suggest that the residual lead was stabilized in the soil and sludge matrix.

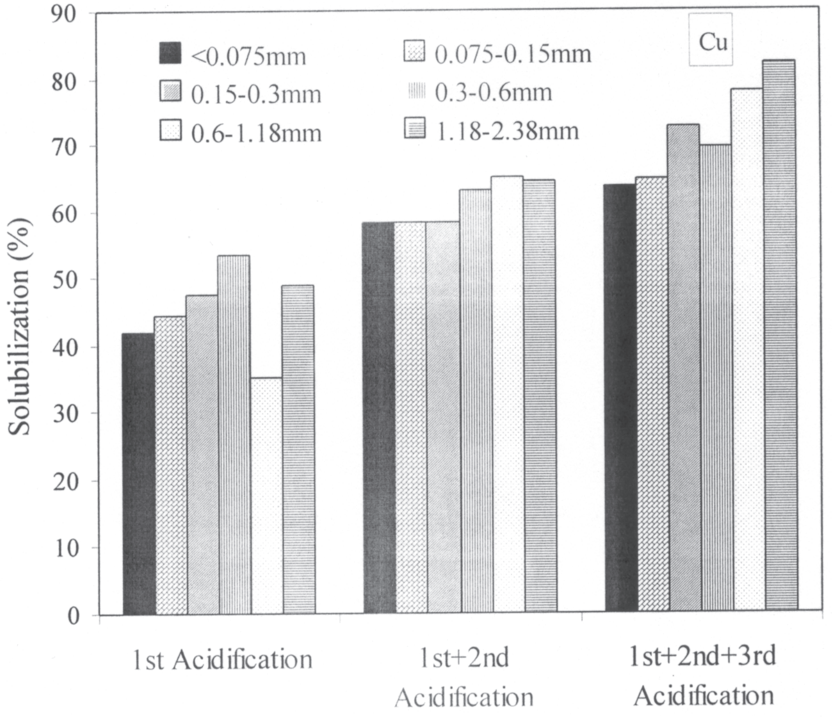

FIGURE 14

Cumulative leaching of Cu from the different soil sizes in the soil/sludge mixtures as a result of three successive bio-acidification cycles.

The mechanisms governing Pb leaching from soil were not investigated in this study. Generally, the solubility of lead oxides, carbonates, and phosphates increases as the pH decreases. Lead is known to favorably adsorb onto fine soil and organic particles and as such becomes highly immobilized. In addition, the phosphates released from sludge may have played a role in modifying the solubility of Pb in the soil/sludge mixture. The presence of the chromate anion can also influence the solubility of lead through the formation of lead chromate ($PbCrO_4$), which has low solubility in the neutral pH range (Nieboer and Jusys, 1988).

The results suggest that bio-acidification of soil and sludge mixtures, especially soils contaminated with heavy metals, have serious consequences on the mobility of minerals. In this case, accelerated bio-acidification experiments demonstrated that minerals such as toxic heavy metals, phosphorus, and nitrogen were leached to a great extent and mobilized. As such, controlled bio-acidification can be used to partially clean soils contaminated with heavy metals through leaching. Clearly, bioleaching of metals is expected to be more effective for large-grained soils with

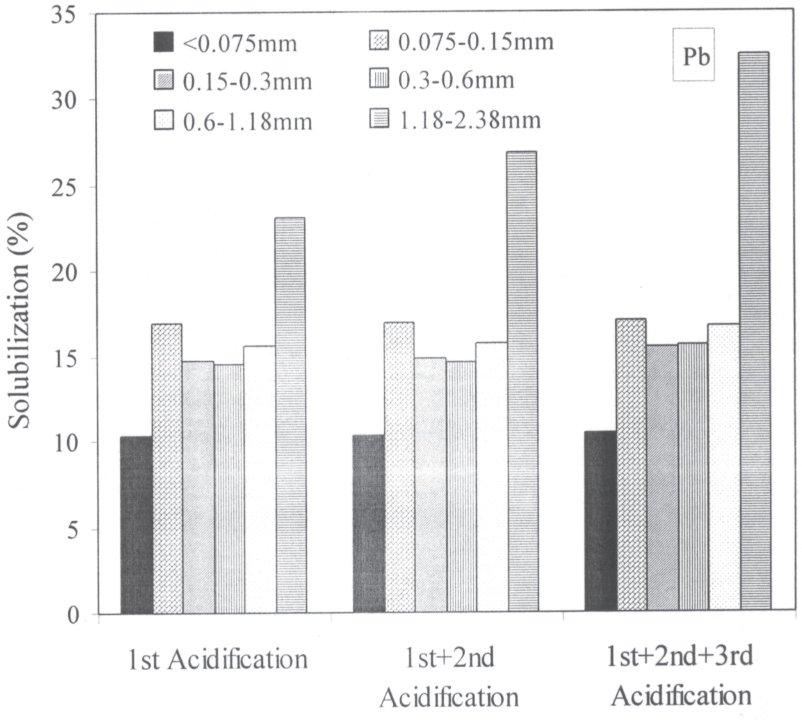

FIGURE 15

Cumulative leaching of Pb from the different soil sizes in the soil/sludge mixtures as a result of three successive bio-acidification cycles.

low organic content than for fine-grained soils. In addition, the process is expected to be more effective for some metals than others, especially in a mixture of metals.

CONCLUSIONS

The results presented in this paper confirmed that bio-acidification had significant impacts on the soil and sludge mixtures in terms of leaching of ammonia, phosphorus, and heavy metals. In this case, the bio-acidification of sludge converted 28 to 45% of the organic nitrogen into ammonia and increased the soluble orthophosphate fraction of total phosphorus by approximately 18 to 20%. The process also resulted in significant leaching of metals from the sludge that reached 67% for Cr, 96% for Ni, 24% for Zn; 16% for Cu; 23% for Cd; and 96% for Pb. The addition of metals to the soil prevented bio-acidification in the samples containing 4 to 50 g soil in 130 ml sludge, and considerably slowed bio-acidification in the samples containing 1 to 3 g soil. Nevertheless, bio-acidification in the 2 g soil/sludge mixtures created conditions that generally favored metals leaching from the six different soil sizes considered in this study. The cumulative leaching results from the three bio-acidification cycles were in the range of 56 to 98% for Cr, 77 to 95% for Zn, 33 to 66% for Ni, 64 to 82% for Cu, and 10 to 33% for Pb, with the higher results in each range generally belonging to the larger size soil particles.

The results confirmed the potential for inhibition of the sulfur-oxidizing microorganisms and bio-acidification in contaminated soil/sludge mixtures, and the significant impacts of bio-acidification on the mobility of metals, nitrogen, and phosphorus in bio-acidified soil/sludge mixtures. In addition, the results confirmed the potential for using controlled bio-acidification for removing heavy metals from contaminated soil using the indigenous sulfur-oxidizing microorganisms in sludge.

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