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Removal of Heavy Metals from Contaminated Soil and Sediments Using the Biosurfactant Surfactin

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The feasibility of using a biodegradable surfactant, surfactin from Bacillus subtilis, *for the removal of heavy metals from a contaminated soil (890 mg/kg zinc, 420 mg/kg copper, 12.6% oil and grease) and sediments (110 mg/kg copper, 3300 mg/kg zinc) was evaluated. Results showed that after one and five batch washings of the soil, 25 and 70% of the copper, 6 and 25% of the zinc, and 5 and 15% of the cadmium could be removed by 0.1% surfactin with 1% NaOH, respectively. From the sediment, 15% of the copper and 6% of the zinc could be removed after a single washing with 0.25% surfactin/1% NaOH. The geochemical speciation of the heavy metals among the exchangeable, oxide, carbonate, organic, and residual fractions was determined by selective sequential extraction procedure. For both matrices, the exchangeable fractions were minimal, while the carbonate and the oxide fractions accounted for over 90% of the zinc present and the organic fraction constituted over 70% of the copper. Results after washing indicated that surfactin with NaOH could remove copper from the organic fraction, zinc from the oxide, and cadmium from the carbonate fractions. The residual fraction remained untouched. These experiments indicate that the sequential extraction studies could be useful in designing soil-washing procedures.*

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INTRODUCTION

Excavation of contaminated soils was once the chosen solution. However, because of the high cost of excavation and final disposal ($600 to 1000/m^3) at hazardous waste landfills, in addition to the lack of available landfill sites, these disposal methods are becoming increasingly less popular (Clarke *et al.*, 1991). In the U.S., 1200 sites are on the National Priority (Superfund) List for the treatment of contaminated soils, indicating the extensiveness of this problem. Approximately 25% of the underground petroleum tanks are leaking, thus releasing considerable amounts of petroleum into the soil and groundwater (USEPA, 1988). Toxic heavy metals are also frequently encountered at hazardous waste sites (Hazardous Waste Consultant, 1996). For example, lead was found at 15% of the sites, followed by chromium, cadmium, and copper at 11, 8, and 7% of the sites respectively. Therefore, oil and metal contamination is a major problem.

To decrease costs, various technologies are being developed and implemented for the remediation of soils and sediments contaminated with hydrocarbons and heavy metals. However, the removal of nonvolatile hydrophobic compounds and metals bound to the soil is still a problem. Traditional pump-and-treat techniques can only remove soluble contaminants. Surfactants are surface-active compounds that can assist in the solubilization, dispersal, and desorption of hydrophobic compounds and heavy metals due to their hydrophobic and hydrophilic portions. In soil-decontamination pro-jects, surfactants can be added to washing water for contact with excavated soils in a washing unit for subsequent return of the cleaned soils to their original site or during pump-and-treat procedures where the groundwater is recovered for ground level treatment after *in situ* flushing of the contaminated soil with a surfactant-containing solution. Several synthetic surfactants have been evaluated in soil-decontamination tests (Ellis *et al.,* 1985; Texas Research Institute, 1985; Nash, 1987).

The addition of biodegradable surfactants such as biosurfactants, which are produced microbially is a potential solution for heavy metal and oil removal. In this article, the feasibility of batch washing with the biosurfactant, surfactin produced by *Bacillus subtilis,* is examined. Surfactin is one of the most effective biosurfactants that has been discovered in terms of amount of surfactant required to lower the surface tension below 30 mN/m. *Bacillus subtilis* produces a lipopeptide called surfactin containing seven amino acids bonded to the carboxyl and hydroxyl groups of a 14-carbon acid (Kakinuma *et al.,* 1969). The surfactant is very effective because it can lower the surface tension of water from 72 to 27 mN/m at concentrations as low as 0.005%. Because of the presence of two negative charges, one on the aspartate and the other on the glutamate residues of surfactin, the binding of heavy metals would be expected (Thimon *et al.*, 1992). Experiments were carried out to evaluate the feasibility of using surfactin in removing heavy metals from two different solid matrices: contaminated river sediments and an oil-contaminated soil.

Because the effectiveness of soil-washing procedures is difficult to predict, a potential aid is to obtain the information on the partitioning of the heavy metals among exchangeable, oxide, carbonate, organic, and residuals fractions in the soil via sequential extraction procedures. These methods could assist in the determination of which additives might be beneficial for soil washing. Different extractants solubilize different fractions of metals. By sequentially extracting with solutions of increasing strengths, an evaluation of the different metal fractions can be obtained (Tessier *et al.*, 1984). To understand the effectiveness of surfactin as a means of enhancing metal removal, sequential extraction procedures were used before and after surfactin washing.

MATERIALS AND METHODS

Chemicals

Surfactin was obtained as a white purified powder from Dr. Kakinuma of Japan. All other chemicals were reagent grade.

Soil and Sediment Samples

Two contaminated soil matrices were chosen for study. The first is a hydrocarbon- and metal-contaminated soil from the Toronto Harbour area (Toronto, Ontario) run by the Toronto Harbour Commission (THC). This site contained numerous leaking underground storage tanks from refineries, oil terminals, and coal storage and processing industries that released fly ash with elevated concentrations of lead, zinc, and copper and those involved in metal finishing (Richardson and Ehrenreich, 1994). Therefore, both hydrocarbon and metal contamination are present. The other matrix, sediments from the Lachine Canal (LC) (Lachine, Quebec), has been heavily contaminated with metals and some hydrocarbons by many years of industrial discharges into the canal.

Cation Exchange Capacity Determination

In the modified procedure of Chhabra *et al.* (1975), 15 g of thiourea and 0.1 *M* ammonium acetate were dissolved in 0.5 l of water that is slowly added to silver nitrate to give a final concentration of 0.01 *M*. pH was adjusted with acetic acid (0.1 *M*) or sodium hydroxide (0.1 *M*) to the desired value between 3 and 8. Approximately, 1 g of soil or sediment was added to 25 ml of solution and left for 4 h on a reciprocating shaker. The supernatant was then separated from the soil by centrifugation at 5000 × g for 10 min and analyzed for silver by atomic absorption spectrophotometry. The cation exchange capacity was then expressed as meq/100 g soil.

Particle Size Distribution

Grain size distribution was performed according to the sieve and hydrometer ASTM D422 method (ASTM, 1970). Approximately 300 g of soil or sediment were dried at 100°C for 24 h and then cooled to room temperature. Standard brass sieves with mesh numbers from 1 to 200 were used. After placing the sieve column in the shaker for 15 min, the dry weight retained by each sieve was determined.

Organic Matter Content

Organic matter content was determined by digestion with hydrogen peroxide (Hesse, 1971). Aliquots of 10 ml hydrogen peroxide (30%) were added to 10 g of soil at room temperature until bubbling was no longer observed. Soil was allowed to settle, the supernatant liquid poured off and the soil was then air dried. Differences in the soil weight × 100% divided by the original soil weight gave the organic matter content.

Oil and Grease Content

The Soxhlet extraction method was employed to determine the oil and grease fraction (APHA/AWWA/WPCF, 1995). Approximately 10 g of soil were added to a preweighed cellulose extraction thimble. 150 ml of clean freon were added to the Soxhlet apparatus and the heater was turned on. After repeating the extraction cycle until the solvent in the condenser was clear, the heater was turned off, the sample was removed for drying and subsequent weighing. The oil and grease content was then calculated from the difference in weight of the soil sample before and after the extract compared with the original weight × 100%.

Chemical Oxygen Demand

Chemical oxygen demand (COD) analyses were performed according to standard methods (APHA/AWWA/WPCF, 1995). A small amount (0.01 g) was weighed into borosilicate culture tubes to which 2 ml of distilled water was added. Digestion solution (1.2 ml of $K_2Cr_2O_7$ solution that included 17 g mercuric sulfate, 10.2 g potassium dichromate, 167 ml of sulfuric acid in 500 ml of distilled water) was added followed by 2.8 ml of sulfuric acid containing silver sulfate (22 g of silver sulfate in 9 lb of sulfuric acid). The tubes were capped with TFE-lined screw caps and mixed end to end. The samples were heated for 2 h at 150^oC in a Hach COD reactor. The samples were cooled and mixed. Transmittance was read with a Canlab Pye Unicam SP6-350 visible spectrophotometer (600 nm). Potassium hydrogen phthalate (0.85 g per liter corresponds to 1000 mg/l of COD) at concentrations from 0 to 1000 mg COD/l was used to make the standard curve.

Heavy Metal Content

Soil and sediment samples (0.5 g) were digested at 440^oC in a Hach Digesdahl with 7 ml of concentrated sulfuric acid. After approximately 15 min, 12 ml of 30% hydrogen peroxide was gradually added. The digestion was allowed to proceed another 15 min before the sample was removed from the heat for cooling. Once the mixture was cooled, distilled water was added so that the total volume was 100 ml. Analyses were performed using the Perkin Elmer Model 3110 atomic absorption spectrometer. Copper (324.8 nm), zinc (213.9 nm), lead (213.9 nm), and cadmium (228.0 nm) were analyzed after preparing the standards, blanks, and samples according to standard methods (APHA/AWWA/WPCF, 1995).

Amorphous Content

Amorphous iron (Fe_2O_3), aluminum (Al_2O_3), and silicon (SiO_2) were extracted from the soil and sediment by acid and base washings according to Segalen (1968). After the washings, Al and Fe were determined in the base washes, while Si and Al were determined in the acid washes by atomic absorption spectrometry. Al, Fe, and Si concentrations were then added and converted to the above oxide forms.

Soil pH and Carbonate Content

Soil pH was determined by using a 1:10 soil to water ratio using a Beckman Model ϕ12pH pH meter. In addition, total carbon (TC) and total organic content (TOC) of the soil and sediment were analyzed by an accredited laboratory (ECO Laboratories, Pointe-Claire, Quebec). Total inorganic carbon was then calculated from the difference in TC and TOC and then converted to carbonate $(CO_3)^{2-}$.

Soil Moisture Content

A 5-g sample of soil or sediment was placed in a preweighed dish and dried at 105°C overnight. It was then placed in a desiccator for cooling before weighing. The difference in the sample weight before and after drying divided by the original weight × 100% is the moisture content.

Mineralogical Analysis by X-ray Diffraction

X-ray diffraction was used to identify the crystalline components of the oil-contaminated soil and the sediment. X-rays of wavelengths of 0.01 to 100 Å allow the differentiation of crystalline structures of each mineral due to their distinct interatomic distances within their planes. A Siemens D-500 X-ray diffractometer with a Hewlett Packard D-500 AT system and a DACO-MD interface system was employed. The analyses were performed according to the procedure of Starkey *et al.* (1984). Approximately 10 to 15 g of soil and sediment were used for the analysis. The oil was removed from the soil by washing repeatedly until the solvent was clear with 10 ml of hexane or dichloromethane. Organic matter was destroyed by adding 10 to 15 ml of hydrogen peroxide (30%) with gentle heating. Samples were washed with distilled water and allowed to air dry. A 20 to 30% soil suspension was prepared and 6 ml were added to a glass slide. The slide was then scanned at 0 to 62 degrees, using Cu-K alpha radiation, 40 kV and 20 mÅ on 1^o beam slit and 0.5^o detector slit at room temperature. The scans were obtained at 0.022 degrees/min. The rate meter was set at 2×10^3 cps with a time constant of 1.2 s. The semiquantitative analysis was calculated by using the counts/s for each peak over the total counts obtained for the scan according to the data from JCPDS (1988).

Batch Soil Washing Studies

Soil-washing studies were performed by varying the surfactant concentrations and pH values in centrifuge tubes containing 10:1 w/w solution to soil ratios (Ellis *et al.,* 1988). Initial pH was modified either by the addition of hydrochloric acid or sodium hydroxide. Samples were taken after 24 h shaking to ensure that equilibrium had been reached and then centrifuged (5000 × g, 10 min). The supernatant was analyzed for metal concentration by atomic absorption spectrophotometry. The percentage metal removal was determined by the equation:

$$\text{Percent metal removed} = \frac{(\text{the amount of heavy metal in solution})}{\text{the amount of metal originally in the soil}} \times 100\%$$

Surfactin solutions at different concentrations and with different co-additives were evaluated for their capability in removing heavy metal from the soil. The use of distilled water alone was used to account for removal of contaminants by physical mixing. Controls included the same additives as for the biosurfactant studies without the presence of the biosurfactant. All experiments were performed in duplicate and the average results are presented.

Experiments using a series of washings were performed by the addition of fresh solution (surfactant or control) after shaking for 24 h on a reciprocating shaker, and centrifugation (5000 × g, 10 min) to remove the old supernatant. Each of the supernatants was collected for subsequent heavy metal analysis.

Soil-Spiking Procedure

Soil from the Toronto Harbour was spiked with a solution containing 1 m*M* of each $Cu(SO_4)_2$, $CdCl_2$, $Pb(NO_3)_2$, and $Zn(SO_4)_2$. A ratio of 1 g of soil per 16 ml of solution was used. The soil was shaken for 3 days on the rotary shaker then removed by centrifugation (5000 × g, 20 min) and air dried. The sample was analyzed for heavy metal content after acid digestion.

Surfactin Concentration

A Fisher Tensiomat was used to measure the surface tension using the Du Nouy method. The critical micelle concentration (CMC) was determined by measuring the surface tension at various dilutions (Mulligan and Gibbs, 1990). The CMC is the point at which the surface tension abruptly increases. In the case of surfactin, a specific method for determining concentration has been developed based on amino acid analysis (de Roubin *et al.*, 1989). A 10-ml aliquot was dried and acid hydrolyzed for 2.5 h at 150°C in a PICO-TAG amino acid analysis system. The residue was redissolved in 200 ml of sodium buffer and injected on a Beckman System 6300 high-performance analyzer equipped with a Beckman Model 7000 data station. All buffers and ninhydrin reagents were purchased from Beckman. The concentration of surfactin was calculated by multiplying the lipopeptide concentration by the molecular weight (1036).

Sequential Extraction Studies

The procedure used for the sequential extraction was similar to that of Yong *et al.* (1993). Soil or sediment samples (1.5 g) were washed with the surfactant solutions and controls and then subsequently dried prior to sequential extraction. The soil was then extracted with the solutions, as shown in Table 1 and each of the supernatants were collected and the concentrations of heavy metals were determined by atomic absorption spectrometry. The amount of metals extracted from the soil by each of the extractants was then calculated as a percentage of the original soil content.

RESULTS AND DISCUSSION

Organic Characterization of the THC Soil and Lachine Canal Sediments

Characterization of the organic content was performed on the untreated THC soil and sediment samples (Table 1). The COD, oil and grease, and organic matter contents were determined. As indicated, the THC soil contains substantially higher levels of petroleum contamination than the sediment. COD levels of the sediments were approximately 25 g/kg, considerably less than the THC soil. The COD gives an indication of the organic content and cannot differentiate between oil and grease and natural organic matter. The levels of organic matter in the sediment are, however, quite substantial compared with its oil and grease content, indicating that the carbon originated mainly from nonpetroleum sources such as algae.

Inorganic Characterization of the THC Soil and Sediments

Table 1 contains the results of the analyses for inorganic components of the soil and sediment samples. The air-dried moisture content of the samples was

TABLE 1
Analysis of Organic and Inorganic Components of Soil and Sediment Samples

Parameter	Concentration in soil (mg/kg)	Concentration in sediment (mg/kg)
Total oil and grease (%)	12.6	1.4
COD (g/kg)	360	25
Organic matter (%)	20.7	13.4
Moisture content (%)	6.6	7.0
Inorganic carbon as carbonate (%)	0.25	5.5
Al_2O_3 (%)	1.8	4.0
Boron	12	8
Cadmium	3	5
Calcium (%)	9.6	1.1
Chromium	844	100
Copper	414	109
Fe_2O_3 (%)	9.0	4.9
Magnesium	8000	10,000
Manganese	534	456
Molybdenum	8	< 2
Nickel	71	49
Lead	320	410
Potassium	1650	3700
SiO_2 (%)	25.1	48.8
Sodium	5900	500
Zinc	870	3300

approximately the same. The carbonate content of the sediment was approximately 20 times higher than in the soil. Although potassium levels were comparable for both matrices, calcium and sodium contents were 10 times higher, and magnesium twice as high in the soil sample. The soil contained significant levels of lead, copper, and zinc. In the sediment, the concentration of copper was lower, zinc was substantially higher, and lead was comparable. Other heavy metals such as chromium and nickel were found in concentrations less than 10 mg/kg soil. Subsequently, the THC soil was used as an oil- and metal-contaminated matrix and the Lachine Canal sediments for its heavy metal contamination. Zinc and copper were retained for further study for both samples because they were considered contamination levels for both the soil and the sediment. The CEC of the soil and the sediment (pH 7) were 7.1 meq/100 mg and 17.2 meq/100 g, respectively.

Analyses were also performed on the sample of THC soil that was spiked with 1 m*M* of heavy metals. The analyses showed a high concentration of cadmium (2000 mg/kg), followed by zinc (1200 mg/kg) and copper (550 mg/kg). This sample was retained to study cadmium contamination.

Amorphous Content

The contents of amorphous iron, silicon, and aluminum were determined for the soil and sediment. The total amorphous content was 13.3% for the soil (1.8% Al_2O_3, 9.0% Fe_2O_3 and 2.5% SiO_2) and 12.4% (4.0% Al_2O_3, 4.9% Fe_2O_3, and 3.5% SiO_2) for the sediment.

Particle Size Distribution

The grain size distribution of the THC soil indicates a sandy soil. Approximately 74.5% was finer than 2 mm, and only 5% passed through a 0.075-mm sieve (10% silt and 90% sand). This distribution reflects agglomerated soil particles due to the hydrocarbon contamination, which is not the natural distribution. In contrast, approximately 85% of the sediment particles passed through the 0.075-mm sieve (10% sand, 70% silt, and 20% clay). Based on the Shepard classification (1954), the sediments are classified as fine grain clayey. The organic and the carbonate contents can interfere with the sedimentology test and were removed according to ASTM (1984) procedures. The values obtained thus represent the original mineralogical content.

Mineralogical Contents of THC Soil and LC Sediments

The X-ray diffraction results are summarized in Table 2. Because the oil contamination interfered with the analysis for the THC soil, the oil was removed by hexane prior to analysis. The main primary minerals in all scans were quartz (SiO_2) and feldspar ($KAlSi_3O_8$). Quartz was identified by the peaks at 4.25, 3.33, and 2.28 Å and feldspar at 3.68, 3.18, and 3.02 Å. The quantified values for quartz agree fairly closely with the total SiO_2 analyses in Table 1. The carbonate contents for both the soil and sediment in Table 1 also agree with values determined by the X-ray scans.

Kaolinite ($Al_4Si_4O_{10}(OH)_8$) was the main clay mineral found in the sediment and soil samples. Peaks were found at 4.46, 2.10, and 1.81 Å. Illite $(Na,K)Al_{12}(Si_3AlO_{10})$ was also found in the soil and the sediment at peaks

TABLE 2
Semi-Quantitative Analysis of the Mineralogical Composition of Soil and Sediment

Component	Soil (hexane-extracted) concentration (% of total mineral identified)	Sediment concentration (% of total mineral identified)
Quartz	30	56
Feldspar	36	17
Illite	2	7
Kaolinite	27	13
Chlorite	3	ND
Carbonate	1	3
Others	1	4

Note: ND, not detected.

2.59, 1.99, and 1.98 Å. Small amounts of chlorite ($(Mg,Fe,Al)\ (Al,Si)_4O_{10}(OH)_8$) were also seen in addition to mica.

Soil Batch Washing Studies with Surfactin

A purified white powder (62%) was received from Dr. Kakinuma of Japan and thus was used for all studies. To solubilize this powder, the pH was increased to 8 by addition of NaOH. The effect of surfactin concentration on metal removal was determined for between 0.13 and 4.0% surfactin. Without any other additives, only 2 and 4% surfactin showed any copper (0.8 and 4.1%, respectively) or zinc (1.3 and 2.9%, respectively) removals (data not shown). The addition of another 1% NaOH (w/v), however, substantially increased metal removal (Figure 1). It is particularly interesting that the removal at the value of 0.25% surfactin was the highest for both metals. Acid addition, which dropped the pH below 5.5 (not shown), inactivated the surfactin by precipitation of the surfactin. These results were duplicated and were reproducible within 10%.

Surfactin concentrations were measured in the supernatant after the soil-washing experiments, as shown in Table 3. Several trends were found. At higher concentrations, the losses of surfactin are less. Also, the co-addition of NaOH decreases the losses of the surfactin at the lower concentrations.

Soil-washing experiments were performed at two surfactin concentrations with and without NaOH for two different matrices, spiked soil and sediment.

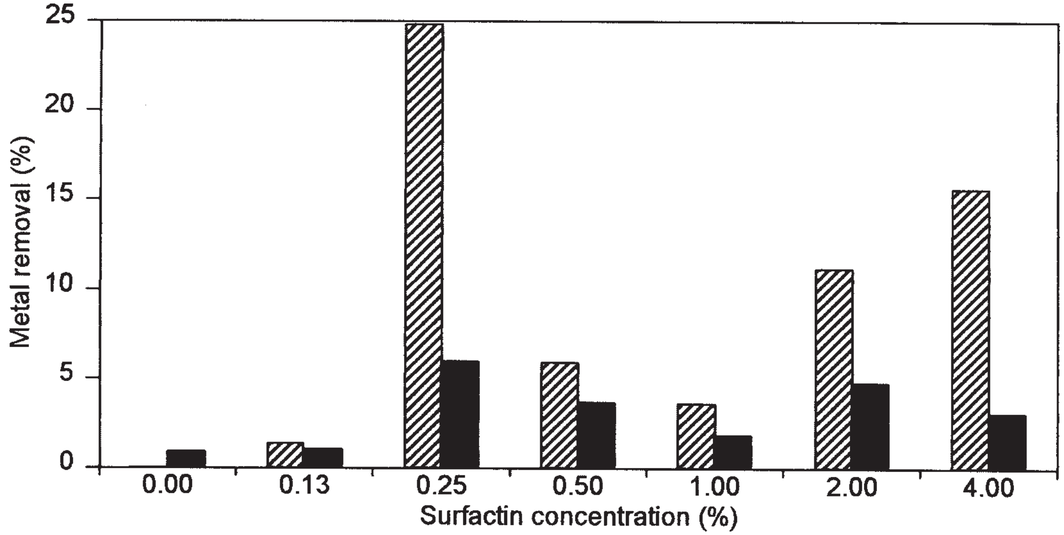

FIGURE 1

Removal of copper (▨) and zinc (■) with surfactin from the THC soil.

TABLE 3
Concentration of Surfactin in the Supernatant Before and After Soil Washing

Additive	Initial surfactin concentration (%)	Final surfactin concentration (%)
Surfactin	4.0	3.0
	2.0	1.5
	1.0	0.5
	0.5	ND
	0.25	0.15
	0.13	ND
Surfactin and 1% NaOH	4.0	3.5
	2.0	1.0
	1.0	0.3
	0.5	0.28
	0.25	0.1
	0.13	0.02

Note: ND, not detected.

For the spiked soil (Figure 2), 2% surfactin and NaOH provided the most copper removal followed by 0.25% surfactin with NaOH. The 2% surfactin alone removed the highest percentage of cadmium, lead, and zinc. For the sediment (Figure 3), increased zinc removal was obtained at 2% surfactin while 2% surfactin, with NaOH and 0.25% surfactin with NaOH removed equal amounts of copper. Lead removal was quite significant at 0.25% surfactin with NaOH. The soil residues from these experiments were used for subsequent sequential extraction studies. As in Figure 2, these results were duplicated and were reproducible within 10%.

A series of washings was performed on the spiked soil using 0.25% surfactin and 1% NaOH. Copper (Figure 4A), zinc (Figure 4B) and cadmium (Figure 4C) removals were followed for 5 days. The control was washed with 1% NaOH. For copper, the control showed a final cumulative removal of 20%, while approximately 70% was removed by the surfactin. For zinc, removal rates were lower, 10% for the control and 25% for surfactin. The control showed minimal cadmium removal, while surfactin removed approximately a total of 15% of the cadmium. For all metals, the rates of removal were highest before 3 days.

Sequential Extraction Studies

Once the feasibility of metal removal with the biosurfactants was established, an investigation was undertaken to determine the speciation of metals that

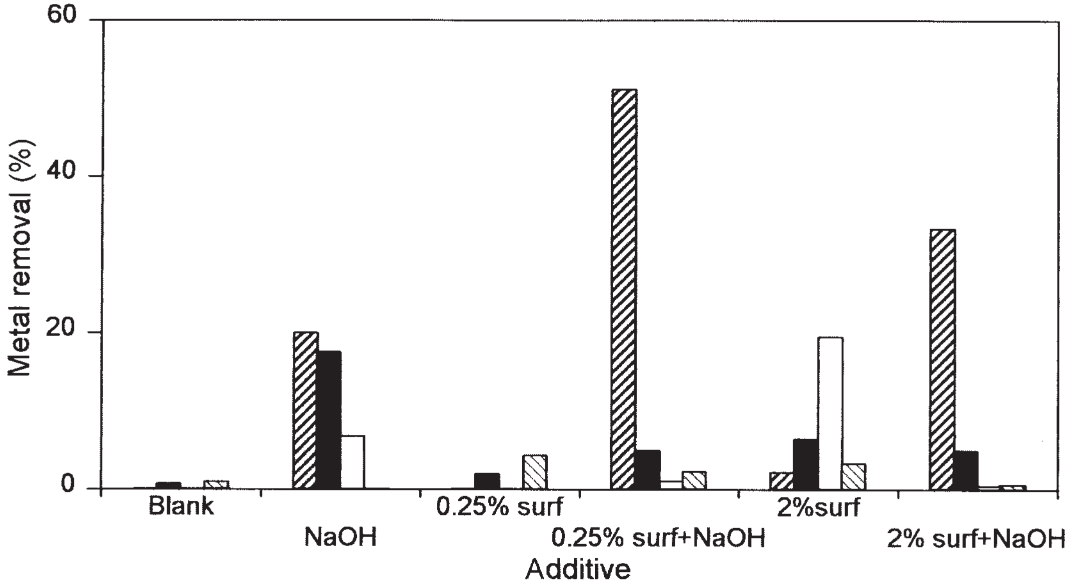

FIGURE 2

Copper (▨), zinc (■), lead (□) and cadmium (▧) removal from the spiked soil by surfactin.

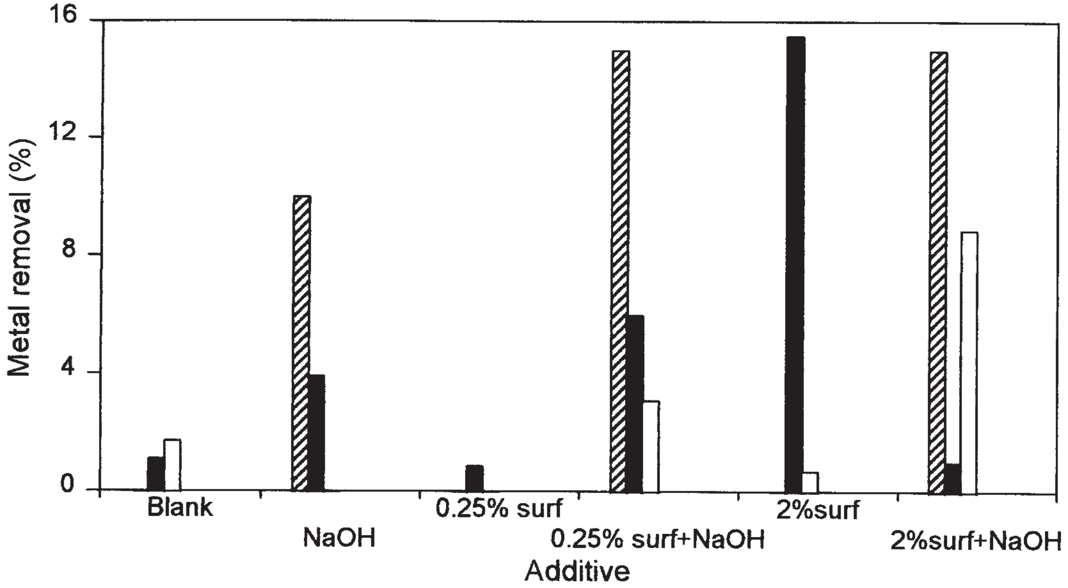

FIGURE 3

Copper (▨), zinc (■), and lead (□) removal from the sediment by surfactin.

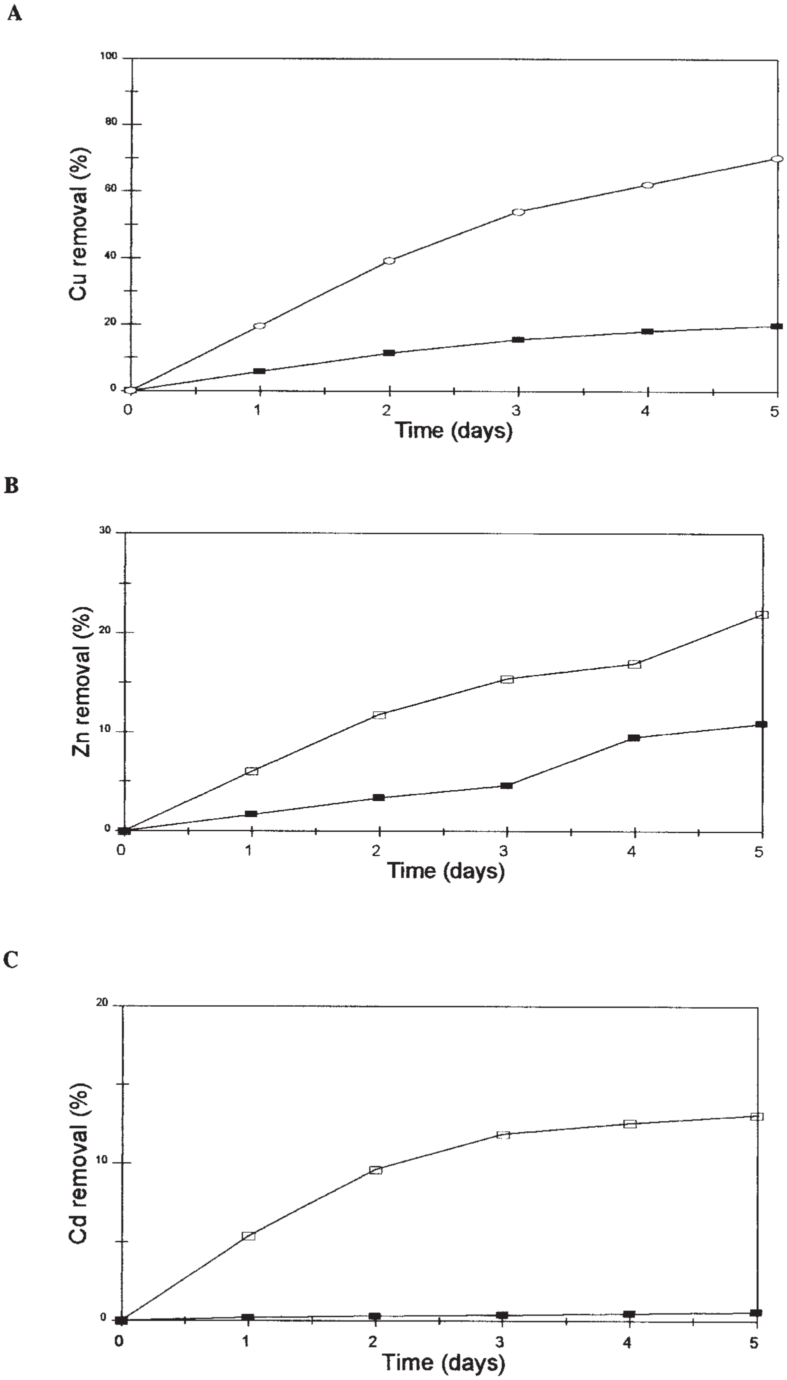

FIGURE 4

Series of washings for copper (A), zinc (B), and cadmium (C) by surfactin (□) and control (■) using the spiked soil. The control was 1% NaOH.

were removed by surfactin. Based on the speciation, it could then be possible to predict the removal of the metals by the surfactin during soil washing procedures.

Sequential extractions were performed on the spiked THC soil and the sediment without washing. These results are indicated in Figures 5 and 6 as the bar with no treatment prior to sequential extractions. As can be seen from Figure 5 for the spiked THC soil, the exchangeable fractions of copper and zinc were very low. The carbonate fraction was significant only for zinc, whereas the oxide fractions accounted for over 70% of the zinc present in the soil. The organic fraction constituted over 70% of the copper. Residual amounts made up about 20% of the copper and approximately 10% of the zinc. In the spiked THC soil (Figure 5), exchangeable cadmium was found. More than 30% of the cadmium was present in the carbonate form, while the remaining amount was mostly in the oxide form. Less than 10% of the cadmium was in the organic phase and insignificant amounts (less than 1%) in the residual.

In the sediment (Figure 6), copper was found mainly in the organic fraction. For zinc, approximately 5% was exchangeable, 20% was carbonate, 20% organic, and 5% residual, with the largest amount being in the oxide fraction. Lead (not shown) was similar to zinc with smaller exchangeable and carbonate fractions.

For both the sediment and the spiked soil, copper has the most affinity for the organic fraction, followed by the residual, the oxide, carbonate, and exchangeable fractions. Despite the fact that the sediment and the soil are quite different in composition and that the organic content of the THC soil is in the form of oil, whereas the sediment is composed of organic matter, the affinities of metals for the various fractions are very similar. The affinity of zinc was the highest for the oxide fraction, and then the carbonate, residual, organic, and exchangeable fractions in the soil. In the sediment, the results for zinc were also the highest for the oxide and carbonate fractions, but the organic, exchangeable, and residual fractions followed, which is a slightly different order than for the soil. In the spiked soil, most of the cadmium was found in the oxide fraction, followed by the carbonate, exchangeable, organic, and residual fractions.

Few studies have been performed on the sequential extraction of soils and sediments contaminated with copper, zinc, and cadmium. Cesium, cobalt, and uranium fractions were examined by Gombert (1994). Others such as the study by Yong *et al.* (1993) have determined the distribution of zinc and copper in kaolinite, illite, montmorillonite, and clay soils, which have organic contents of less than 2%. However, comparisons with illite and natural clay soil can be made for zinc and cadmium at pH values around 7 because the organic phase does not play a large role in the retention of these metals. For both the THC

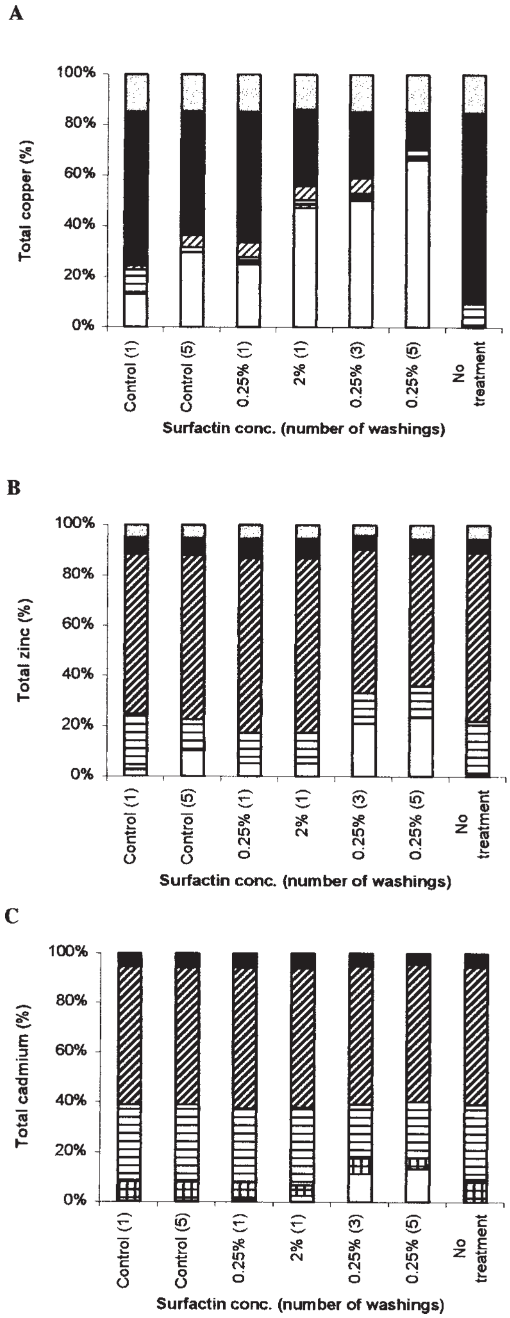

FIGURE 5

Sequential extraction of copper (A), zinc (B), and cadmium (C) after surfactin washing of THC spiked soil. The following are represented. Residual (▒); organic fraction (■); oxide (▨); carbonate (▬); exchangeable (▦); soluble (□).

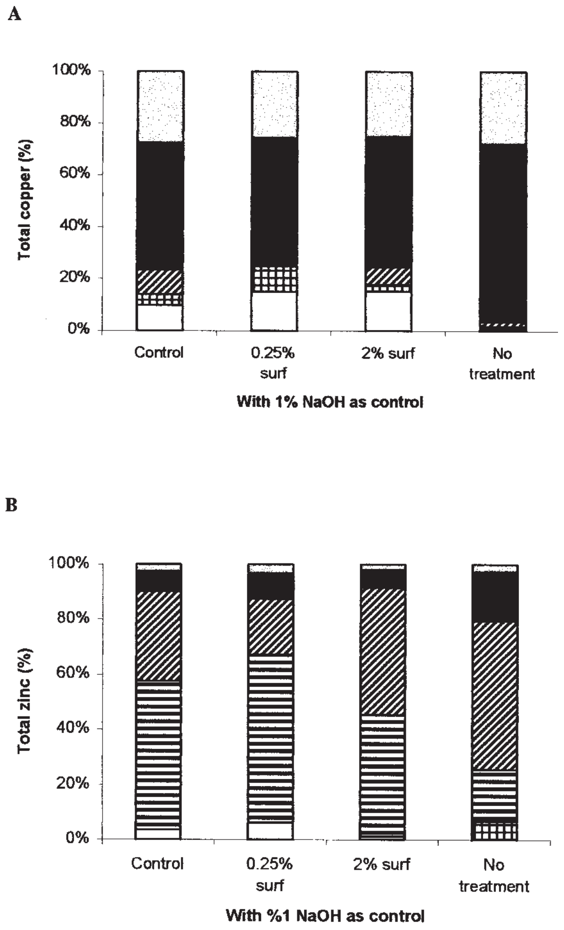

FIGURE 6

Sequential extraction of copper (A) and zinc (B) after washing of the sediments with surfactin. The following are represented. Residual (▒); organic fraction (■); oxide (▨); carbonate (▤); exchangeable (▦); soluble (□).

soil and the sediment, the results for zinc, were similar, indicating preference for the oxide phase followed by the carbonate. Both the natural clay and the illite showed equal contributions of the oxide and carbonate fractions like the spiked soil for cadmium. The exchangeable fraction, however, was higher. In this study, the carbonate content of both the sediments and the soil was much lower (5 and 0.5%, respectively) compared with the 15 and 10% for the illite and natural clay soil.

In a study by Ramos *et al.* (1994), copper, cadmium, and zinc were sequentially extracted from soils with organic contents of approximately 15%. Similar to this research, they found that copper was associated with the organic matter or residual fractions and zinc was primarily found in the oxide fraction. Cadmium, however, was found mainly in the exchangeable and carbonate fractions. Contrary to our findings, they determined that a significant amount of cadmium was present in the residual fraction. This was most likely because of the fact that their soil was contaminated for a much longer period of time compared with the 3-day spiking procedure used in this study.

Sequential Extraction of Soil after Surfactin Washing

Experiments with sequential extraction were performed on the spiked THC soil and the sediments after washing with surfactin. Figure 5A shows the sequential extraction of copper with 2% surfactin with 1% NaOH after one washing and for the 0.25% surfactin with 1% NaOH after one, three, and five washes. The controls (1% NaOH) are also shown after one and five washes. The oxide, carbonate, and exchangeable fractions were fairly consistent until the fifth washing, where the oxide fraction was finally removed. The organic fraction, however, decreased with increasing copper removal. For zinc (Figure 5B), the carbonate and organic fractions decreased insignificantly. Removal was mainly from the oxide fraction. Because cadmium removal by 1% NaOH was minimal, the sequential extraction showed similar characteristics to the untreated soil. After the addition of one wash of surfactin (0.25% with NaOH), some removal occurred in the exchangeable fraction. Further washings indicated removal from the carbonate fraction. The others (organic and oxide fractions) did not change appreciably. The wash with 2% surfactin showed mainly removal from the exchangeable fraction.

A few experiments were also run with the sediment to examine washing of the sediments. Figure 6A shows a decrease in the oxide and organic fractions of copper by 0.25% surfactin/1% NaOH. For zinc, the experiments with 1% NaOH (Figure 6B) showed a decrease in the oxide phase after washing with 0.25% surfactin, while there was little difference with the control for the 2% surfactin. Without addition of NaOH (not shown), a slight decrease of zinc in the oxide and carbonate fraction was noted for 2% surfactin.

A few studies have tried to correlate sequential extraction results with soil-washing results. Gombert II (1994) examined the sequential extraction of cesium, cobalt, and chromium to determine if soil washing could be used to treat the soil. In our study, it was determined that any fraction other than the residual could be removed by chemical enhanced soil washing. Therefore, cesium could not be removed because it occurred mainly in the residual phase

(80%). However, no detail is given for what types of additives can be used for effective soil washing from the different phases.

In our study, only the residual metal fraction was untouched. All other fractions could be decreased. Under basic conditions (co-addition of surfactin with NaOH) copper could be released as it was found mainly in the organic phase. In conclusion, the results clearly indicated the feasibility of removing the metals from the various fractions with the anionic biosurfactant surfactin, even though the exchangeable metal fractions are low.

CONCLUSIONS

Surfactin was able to remove copper more easily than the other heavy metals. As seen from the sequential extraction studies, the copper was found mainly in the organic fraction in the soil and sediment samples. Usually, oxidative conditions are required to release metals in this form. Copper has been found to bind strongly to organic matter by forming complexes (Cameron, 1992). The use of a surfactant and hydroxide is a combination that has been used for enhanced oil recovery known as alkaline flooding. The presence of acidic components in the petroleum or organic matter such as carboxylic acid, phenolics, and asphaltene can hydrolyze under basic conditions to form surfactant products (de Zabala *et al.*, 1982; Peru and Lorenz, 1990). These surfactants thus are negatively charged and have been postulated to be responsible for enhanced oil recovery and probably contributes to metal removal. They lower the interfacial tension between the oil and the rock These surfactants could also enable metal removal due to their negative charge and the interfacial surface tension lowering (Surkalo, 1990). It has been shown that the chemical reactions of the NaOH with the oil forms surfactants that lower the interfacial between water and oil from 18 to 2 mN/m. The addition of surfactants with the NaOH can further reduce the interfacial tension to 0.020 mN/m in the case of petroleum sulfonate (Lin *et al.*, 1987).

The combination of the added surfactant and the base (NaOH) in this study has been shown to enhance copper removal and therefore is a good strategy to remediate soils containing copper in the organic fraction. Zinc also forms complexes with organic matter (Cameron, 1992), and some removal from the organic fraction was also noted using the biosurfactant with NaOH. The initial content of zinc in the organic matter in the soil and sediments was significantly less (10 and 15% for zinc compared with 80 and 85% for copper in soil and sediments, respectively).

Zinc was associated with carbonates in the soil and the sediments, while the spiked cadmium was found in the same fraction in the spiked THC soil sample. Illite, a component shown in the X-ray diffractograms of the soil and sediment, contains carbonates (Yong et al., 1993). It was able to retain cadmium in this fraction. Surfactin (pH 9) seems to enhance the solubility of the carbonate phase of zinc from the sediments. High pH conditions after multiple washings can also decrease carbonate fraction.

The benefit of using sequential extractions is that it can be used to design a strategy for soil-washing procedures. The results can show if the heavy metals are exchangeable cations, associated with oxides and hydroxides, or carbonates and organic matter or considered within the lattice of the lattice materials. Based on these results, an appropriate washing fluid can be chosen. The addition of surfactant and hydroxide is a good strategy to use for copper removal, which was bound mainly in the organic fraction. A combination of these procedures (a series of washings with different additives) could then be used to remediate the soils and sediments to adequate levels that contain high levels of copper, zinc, and cadmium. Acidic conditions would be more useful for oxide and carbonate fractions.

The following conclusions can be made:

- The exchangeable fractions of copper and zinc were very low.
- The carbonate fraction was significant only for zinc (11%), whereas the oxide fractions accounted for over 74% of the zinc present in the soil.
- The organic fraction in the THC soil constituted over 77% of the copper.
- In the sediment, copper was found mainly in the organic (59%) and residual (28%) and zinc was found in the carbonate (52%), oxide (44%), and organic (12%) fractions.
- A series of washings with low concentrations of surfactin enhanced metal removal significantly (up to 70% for copper and 23% for zinc).
- From both matrices, removal of copper by surfactin was mostly from the organic phase. The oxide fraction only decreased in the soil after the fifth wash.
- From both matrices, zinc removal was from the oxide fraction. However, without NaOH, surfactin could remove zinc from the sediment in the carbonate fraction.
- Cadmium removal from the soil was mainly from the carbonate phase.

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