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Journal of Hazardous Materials B94 (2002) 253–272

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Feasibility of metal recovery from soil using DTPA and its biostability

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Received 29 June 2001; received in revised form 19 March 2002; accepted 20 March 2002

Abstract

Removal of heavy metals from contaminated soil by chelation can be a valid remediation method. Important properties of the chelating agent used are: strength of the chelation bonding, reusability, and biostability during the remediation operation. This work tested the extraction, recovery, and biostability of diethylenetriaminepentaacetate (DTPA) as a remediation agent for soils contaminated with metals. Reported here are effects of parameters such as DTPA concentration, precipitant type and concentration, and pH relative to extraction and recovery efficiencies of the chelator, as well as workable recovery conditions. The assessment of biostability was determined at different DTPA concentrations, in aqueous and soil slurry systems, and in presence of lead using acclimated and unacclimated activated sludge cultures. The results showed that DTPA was capable of extracting lead from the tested contaminated soils and could be recovered by the use of cationic and anionic precipitants in alkaline pH conditions. It was biostable to some extent especially with unacclimated cultures. Thus, DTPA proved to be a strong and reusable chelating agent for some metals in soils, and it was relatively biostable, which makes it a valid remediation agent for soil metal extraction.

Keywords: Remediation; Soil; Metal; Chelating; Stability

1. Introduction

In many industrial societies today heavy metal contamination of soil and groundwater is not at all uncommon. Elevated levels of lead, chromium, cadmium, zinc and mercury

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0304-3894/02/$ – see front matter
PII: S0304-3894(02)00067-5

have been reported at many sites in the US National Priority List. These reported contaminations come from industrial, mining and smelting operations. Additionally, heavy metal contamination also has been found at several US army installations [1].

Heavy metals are toxic to human as well as to other organisms. These contaminated sites also pose a threat to groundwater supplies if the metals are not properly contained and treated. Heavy metals interact with soil matrix and may persist for a long period of time creating long-term hazards to the environment and human health. In addition, unlike organic compounds that can biodegrade with time, or can be incinerated, heavy metals are robust and remain a potential threat to the environment or human health for long time.

A promising method for removing heavy metals from soil is chelation extraction. The metal has a higher affinity for the chelating agent than the soil, thus the metal contaminant goes into the liquid phase during the extraction procedure. Afterward the soil is separated from the metal–chelator complex solution, the chelating agent can then be recovered for reuse by appropriate manipulation. The extracted metal can be precipitated for safe disposal or recovered [2–6]. The chelating extraction process for contaminated soil remediation can be economically successful only if the chelating agent can be recovered and reused at least several times. Furthermore, chelating agents used for soil remediation should be biostable, and not biodegraded during repeated extractions and reuses.

As strong chelating agents effect more complete extraction, their use in soil remediation is advantageous provided that they can be recovered and reused. Earlier we presented a paper showing the use of a strong chelating agent, EDTA, for remediation of heavy metal-contaminated soil [7]. In the present paper the use of an even stronger chelating agent for soil metal extraction is presented. The chelating agent used for this study is diethylenetriaminpentaacetic acid (DTPA).

2. Materials and methods

2.1. Extraction and recovery experiments

Distilled-deionized water (18 MΩ cm) was obtained from a heat distiller (Barnstead Glass Bi-Distiller, Barnstead) with a four-stage Milli-Q Plus system (Millipore) and was used throughout. Chelating agent DTPA (Sigma) was used as received. Soils used in the metal extraction part of the study were taken from a contaminated site in Salt Lake City, air-dried for 1 month, then passed through a 2 mm sieve. Soil properties were characterized and shown in Table 1. Typical experiments were conducted in 125 ml glass Erlenmeyer flasks using a batch solution volume (V) of 100 ml. All flasks were sealed with stoppers to reduce gas exchange with the atmosphere during experiment. All pH adjustments were performed by addition of either a 5 M HNO_3 or 5 M NaOH solution. pH measurements were made with an Orion model SA 720 pH meter. Stock metal solutions (1000 mg/l) were prepared for instrument calibrations according to ASTM Methods (ASTM Annual Standards D3559, D1688, D3557, and D1691 for Pb, Cu, Cd, and Zn, respectively). A gyratory shaker table (New Brunswick Scientific Co., Model G-2) provided agitation during all extraction procedures. The soil was kept in suspension by operating the shaker table at 260 rpm. All experiments were conducted at the room temperature of $23 \pm 1\,^{\circ}C$. Total dissolved metal

Table 1
Characteristics of the studied sandy loam

Sand	Silt (%)	Clay (%)	VC (%)	C (%)	M (%)	F (%)	VF (%)	OM (%)	OC (%)	Cu (mg/kg)	Pb (mg/kg)	Ni (mg/kg)	Zn (mg/kg)	As (mg/kg)
68	21	11	9.0	27.9	14.5	20.3	0.0	2.72	1.58	675 700[a]	12,000 15,500[a]	8 18.3[a]	1410 1810[a]	762

Note: Analyzed by Utah State University Soil Testing Laboratory. Abbreviations: VC, very coarse; C, coarse; M, medium; F, fine; VF, very fine; OM, organic matter; OC, organic carbon.

[a] Obtained by shaking in 10% HNO_3 at 60 °C for 8 h.

concentrations (Me_T) were measured after aliquots were withdrawn from the reaction mixtures and filtered through a 0.45 μm filter (Gelman Sciences sterile aerodisc) and then acidified with nitric acid. Metal analyses were determined by atomic absorption (AA) spectrometry (Perkin-Elmer Model 280) using ASTM methods. Whenever available, standard procedures were followed for other laboratory procedures [8,9].

Metal extraction and recovery experiments were conducted with varying DTPA concentrations (3–20 mM), precipitant concentrations (0–10 mM Na_2S with and without the addition of $Ca(OH)_2$), and pH (recovery at 7–12). For extraction, 5% by weight of soil was added to 100 ml of DTPA solution of which the pH was initially adjusted to between 7.0 and 7.5. The slurry was continuously maintained in suspension by a shaker table. After the extraction period of 4 h, the slurry was centrifuged, decanted, filtered, and the resulting aqueous solution analyzed for various metal contents (e.g. Pb, Cu, Zn). For recovery, i.e. separation of metals from DTPA after extraction, to the complex solution was added with 5 mM each of $Ca(OH)_2$ and Na_2S in solid forms and the solution pH elevated to pH 12 as necessary. The solution was continually agitated and allowed to precipitate for 1 h. It was then centrifuged, decanted, filtered, and analyzed for various metal contents remaining in the aqueous phase. At this point, the aqueous part contained the chelator DTPA, which could be reused. Recovery operations were also performed with varying amounts of Na_2S at various pH without the use of $Ca(OH)_2$. For experiments using reclaimed DTPA, the pH of the recovered solution was adjusted to about seven and was added with contaminated soil to start another extraction and recovery cycle.

Experimental results have been presented by plots of the extracted metal concentrations in the aqueous solution, i.e. the remaining metal concentrations in the aqueous solution after the recovery process. Extraction efficiencies were calculated by taking the ratio of the extracted metal amount in solution to the total available (as characterized by strong digestion conditions using 10% HNO_3 at 60 °C for 8 h) in the amount of soil used. For example, the complete removal (100%) of Pb in one run was calculated by the ratio of extracted Pb in solution to the total amount of Pb in the 5 g of soil used in 100 ml (i.e. 3.78/3.74 mM = 100%). It should be noted that 5 g of the sandy loam (15,500 mg Pb/kg soil, Table 1) amounted to 3.74 mM Pb in 100 ml. Separation (metal recovery) efficiencies were calculated using the metal amounts in the extraction solution before and after the recovery process.

2.2. Biodegradation experiments

2.2.1. Materials

Chelator DTPA (99% pure free acid form) was purchased from Aldrich Chemical Company, Milwaukee, WI. The DTPA measuring indicator 1-(2-pyridylazo)-2-naphthol (PAN) was also obtained from this company. The ingredients for microbial growth media PTYG (peptone, tryptone, yeast extract and glucose) were purchased from Fisher Scientific, St. Louis, MO. The basal salt media for the microorganism growth consisted of a mixture of following chemicals: NH_4NO_3, K_2HPO_4, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, $MnCl_2{\cdot}4H_2O$, $CaCl_2{\cdot}2H_2O$, $FeSO_4$. These salts were of reagent grade and also purchased from Fisher Scientific.

The recipe for the PTYG growth media was as follows: peptone −0.25 g, tryptone −0.25 g, yeast extract −0.5 g, glucose −0.5 g, $MgSO_4{\cdot}7H_2O$ −0.6 g, and $CaCl_2{\cdot}2H_2O$

−0.6 g in 1 l of distilled water. The recipe for the basal salt media was as follows: NH_4NO_3 −0.4 g, K_2HPO_4 −0.1 g, KH_2PO_4 −0.05 g, $MgSO_4{\cdot}7H_2O$ −0.005 g, $MnCl_2{\cdot}4H_2O$ −0.02 g, $CaCl_2{\cdot}2H_2O$ −0.2g, $FeSO_4$ −0.005 g in 1-l distilled water. Lead in the form of lead nitrate [$Pb(NO_3)_2$]was used as a typical heavy metal for the experiments. It was also purchased from Fisher Scientific.

2.2.2. Microorganisms

The seed microorganisms used in the experiments were originally obtained from the Wastewater Treatment Plant in Columbia, MO. The microorganisms (sludge) were obtained from the recycle line of the activated sludge treatment unit. They were used as a seed to develop a microbial culture in the laboratory in a 5 l batch reactor using the PTYG and basal salt growth media. The initial chemical oxygen demand (COD) of the media was about 500 mg/l with COD:N:P = 100:8:1. The pH of the reactor liquid was kept between 7.0 and 7.5 by the addition of 5N HCl or 5N NaOH. The temperature was maintained at $22 \pm 2\,^{\circ}C$. The reactor was aerated using filtered compressed air through air diffusers. Daily the aeration in the reactor was stopped for an hour, the biomass (sludge) were allowed to settle down, about 2 l of supernatant was discarded, and fresh media was added. After a few days when the biomass in the reactor had grown to sufficient levels, a fixed amount of biomass (sludge) was wasted from the unit in order to maintain a constant initial mixed liquor suspended solids (MLSS). The wasted sludge from this unit was used for experimentation or for developing cultures acclimated to DTPA.

The acclimation process for the microorganisms to DTPA was started in a 4 l batch reactor. Progressively higher amounts of DTPA were added to the reactor in place of PTYG media. The total COD of the reactor content (PTYG + DTPA) was maintained at 500 mg/l level with basal salts added to meet the nutrient requirements. At the start the COD loading from PTYG was 450 mg/l and 50 mg/l was from DTPA. This loading was continued on a daily basis with sludge and supernatant wastage, and feed addition for 2 weeks. The carbon source was gradually switched from PTYG to DTPA, allowing the bacteria to acclimate to the new molecule. The concentration of DTPA was increased in successive weeks from 50, 100, 200, 300, 400 and 500 mg/l as COD with corresponding decreases in PTYG concentrations to maintain a total COD of 500 mg/l. It was observed that at concentrations of DTPA greater than 100 mg/l there was some inhibition of the microbes present. So the experiments were conducted only up to a DTPA concentrations of 300 mg/l as COD (with PTYG added to maintain a total COD of 500 mg/l).

2.2.3. Soils

The soil used for experiments simulating metal contamination was Menfro series silty loam. This soil consists of deep, well drained soils on upland areas. The soil was sieved through 2 mm sieve prior to use. The physical and chemical properties are given in the Table 2 [10].

2.3. Analytical methods

Biomass measurements in the batch reactors were made by determining the total suspended solids (TSS) using procedures outlined in Standard Methods [9]. COD test was

Table 2
Properties of the Menfro series silt loam

Depth	Size distribution (%)			pH		Organic content (%)
	Clay (<0.002 mm)	Silt (0.002–0.05 mm)	Sand (0.05–2.0 mm)	$CaCl_2$ (0.01 M)	H_2O	
0–5 cm	10.9	80.4	8.7	5.5	6.1	1.38

conducted using HACH's procedure (HACH Co., Loveland, CO). Their method is very similar to Method 5220D in the Standard Methods [9].

The undegraded DTPA was determined by a complexation titration at pH 2.5 with copper sulfate using (PAN) as an indicator. Ten millilitre sample of a solution containing DTPA was filtered through a 0.45 μm filter paper then it was diluted to 50 ml. Three millilitre of buffer solution and three drops of PAN indicator were added to the sample. At the end point of titration, the color of the solution turns dark violet from initial yellow color [11].

2.4. Biodegradation test procedures

These tests were performed in 250 ml shake flasks. In each 250 ml shake flask, PTYG growth media, basal salts, acclimated and unacclimated microorganisms, and different concentrations of DTPA solutions were added. The COD:N:P ratios in the flasks were adjusted to 100:8:1 as mentioned earlier. All experiments were carried out at room temperature (20–25 °C). The pH of the media was adjusted to 7.0–7.5 by adding 0.1N HCl or 0.1N NaOH solution. Duplicate flasks at a specific DTPA concentration were placed on a shaker and shaken at 200 rpm. A blank and an abiotic control with 5 g/l of Hg_2Cl_2 were also included in each experimental set. The incubation of the flasks were carried out in the dark to avoid any photodegradation of the chelator. Ten millilitre of aliquots were withdrawn from the flasks at different times and filtered through 0.45 μm filter. The filtrate COD and DTPA concentrations were determined for each sample. In addition the amounts of biomass in the flasks were measured by determining the TSS of the samples.

For determining the effect of chelator biodegradation in presence of soil slurry (5%) and in presence of Pb-complexed chelate in the soil slurry, respirometric tests were conducted. The BODTrak respirometer (HACH Co.) was used to determine the oxygen uptake during the biodegradation of the chelate under different conditions.

In these tests acclimated microorganisms culture were obtained from the stock unit. Sufficient acclimated microorganisms (800–1200 mg/l MLSS) were added to the flasks to allow reasonable oxygen uptake rates. The flasks had an initial concentration of DTPA varying from 50 to 600 mg/l and basal salts. No PTYG media was added in these experiments. A control flask was used to account for any background oxygen uptake by the seed culture. It had all other ingredients but no chelator compound. Since an earlier study had shown that unacclimated seed microorganisms showed no biodegradation or removal of the DTPA compound from the mixture, no abiotic control was used in the respirometry studies.

In the study of Pb-complexed chelate biodegradation in presence of soil slurry, lead nitrate solution was added to DTPA solution at a metal-to-chelator ratio of 1:2. The mixture was shaken at 220 rpm for 2 days to assure complete complexation. The complexed chelate was

then added to the respirometer flask together with acclimated bacteria, 5% soil slurry and basal salts. The pH of the flask content was adjusted to 7.0. The oxygen uptake rate in this flask was measured.

3. Results

3.1. Chelation extraction and recovery

The effects of DTPA concentration, precipitant concentration (Na_2S), and pH on the extraction and recovery of several prevalent metal contaminants were experimentally studied. Fig. 1 shows extraction of Pb, Zn, and Cu metals from a contaminated sandy loam soil using 3–20 mM DTPA solutions. The results showed that 100, 74, and 55% of Pb, Zn, and Cu, respectively, were extracted from the soil under neutral pH condition after an extraction period of 4 h. In general, the extracted amounts increased with increasing concentration of DTPA employed, and the removal was complete in the case of Pb, the major contaminant that was an order of magnitude more abundant than others.

DTPA is a strong chelating agent, slightly stronger than EDTA (e.g. pK_s of Pb complexation are 19 and 18 for DTPA and EDTA, respectively). Thus, in order for DTPA to be reclaimed and reused after soil extraction of metals, it must be amenable to release and be separated from the extracted metals. Experiments were conducted to demonstrate the

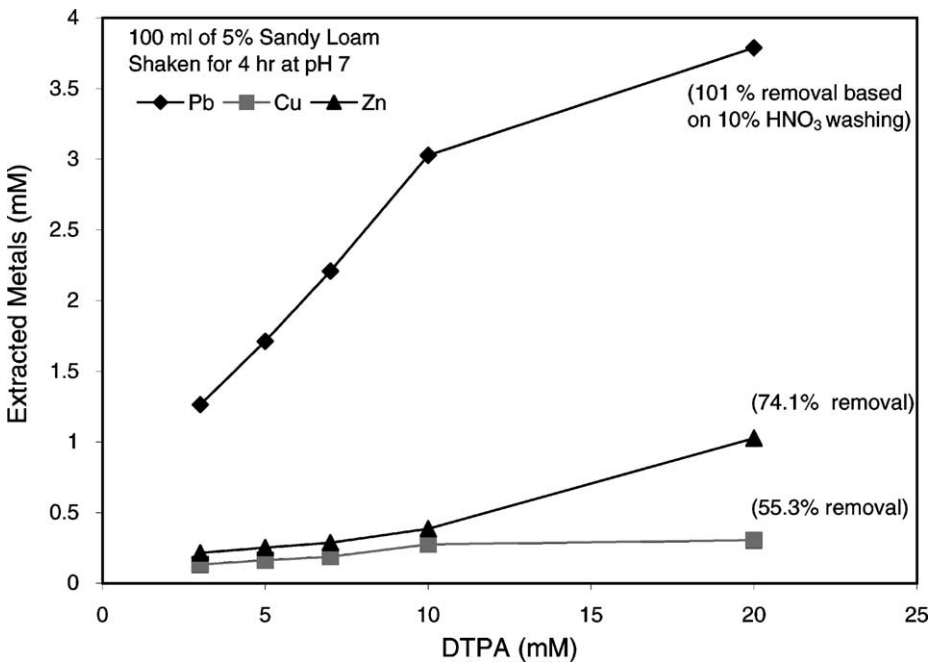

Fig. 1. Extraction of contaminant metals from a sandy loam soil using different DTPA concentrations. (Metal concentrations in this and all other figures indicated metal contents in the aqueous phase.)

effectiveness of recovering mixed metals and chelator from their complex solution. Fig. 2 shows results of recovering mixed metals (Pb, Cd, and Cu) and DTPA at different pH conditions (7–10) and Na_2S dosages (0–10 mM). The mixed complex solutions were prepared by mixing 1 mM each of Pb, Cd, and Cu, and 5 mM DTPA (i.e. [metal]:[DTPA] = 1:5 for

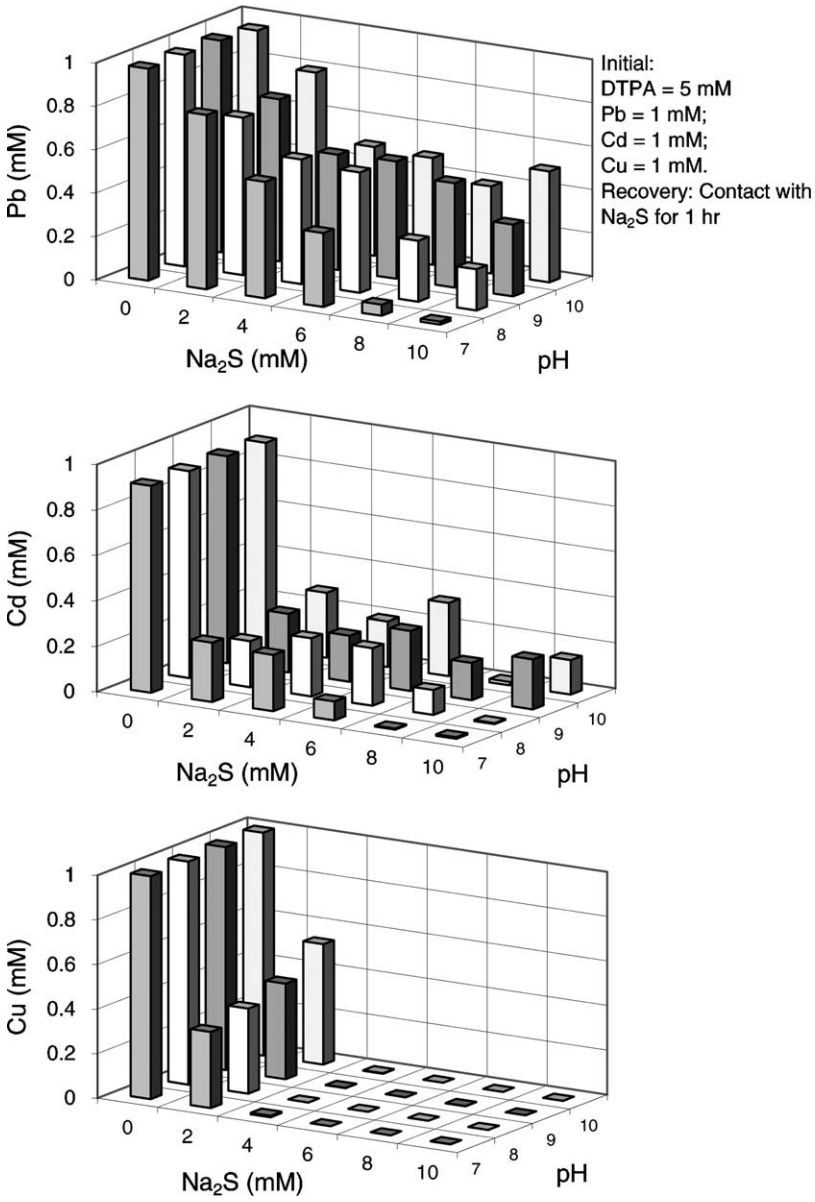

Fig. 2. Recovery of metals using different pH and Na_2S concentrations. Metal concentrations indicated those remaining solubilized in the aqueous phase after the recovery process.

each metal in the solution). The solutions were added with specified amounts of Na_2S and their pH adjusted with drops of concentrated acid or base to 7–10. The results were plotted as metal concentrations remaining in the aqueous solution after the Na_2S treatment at different pHs. Thus, a lower metal content in the supernatant indicated better metal recovery efficiency. The metals were most readily recovered in this decreasing order: Cu, Cd, and Pb, and they all were almost entirely recoverable given appropriate Na_2S and pH conditions.

Fig. 3 shows results of extracting and recovering Pb, Zn, and Cu contaminants from the sandy loam using reclaimed DTPA solutions (3–10 mM initial DTPA concentrations) in successive washing cycles (using a fresh soil sample in each cycle). The extraction was performed at pH 7 for 4 h and recovery performed at pH 12 for 1 h. The separation of DTPA from metals was effected by use of 5 mM each of $Ca(OH)_2$ and Na_2S, resulting in a wide range of separation depending on the metal type and chelating agent concentrations. In general, the contaminant metals were recovered in this decreasing order: Cu, Pb, and Zn. The recovery was more effective (exceeding 90%) under appropriate precipitants-to-chelator ratios (e.g. [precipitants]:[DTPA] $\sim$ 1:1 for the test conditions). It should be noted that all extraction and recovery efficiencies were determined based on aqueous concentrations before and after an extraction or recovery step. There appeared to be a small to modest loss of removal efficiency during successive application of reclaimed DTPA, as also observed in our previous study of EDTA [7].

3.2. Biodegradation experiments

3.2.1. DTPA biodegradation by unacclimated microorganisms

In these experiments microorganisms from natural soil and stream water were used as unacclimated seed cultures in shake flask tests. In the first experiment natural soil was mixed with some distilled water in a 250 ml flask and shaken for 2 days. The shaker was stopped and soil was allowed to settle. The supernatant containing some soil particles and microorganisms was withdrawn for use in the biodegradation study. In the test shake flasks, DTPA concentration was varied from 100 to 200 mg/l, 50 ml of soil microbial seed and basal salt media were also added. No PTYG was added to these flasks. The pH was adjusted to 7.0 ± 0.2.

A similar experiment was conducted with microorganism seed from a local stream water in place of soil microorganisms. Fifty millilitre stream water was added to the shake flask containing DTPA and basal salts, and the experiment was conducted in the same manner as with the soil microorganism. The volatile suspended solids (VSS) for the soil microbial seed and the stream water were low about 50 mg/l.

The results of DTPA biodegradation with unacclimated microorganisms from natural soil are shown in Fig. 4. It can be seen that there was no degradation of DTPA in 9 days. The slight increase in the COD with time was perhaps due to input of organic matter from the lysis of cells present. The results with the stream water samples were practically identical to that observed with the soil microorganisms.

3.2.2. DTPA biodegradation by acclimated microorganisms

The microorganisms used in these experiments were grown on PTYG media supplemented with DTPA and thus were acclimated to this substrate under aerobic conditions. Fig. 5 and Table 3 show the results of the experiment where the initial COD of the reactor

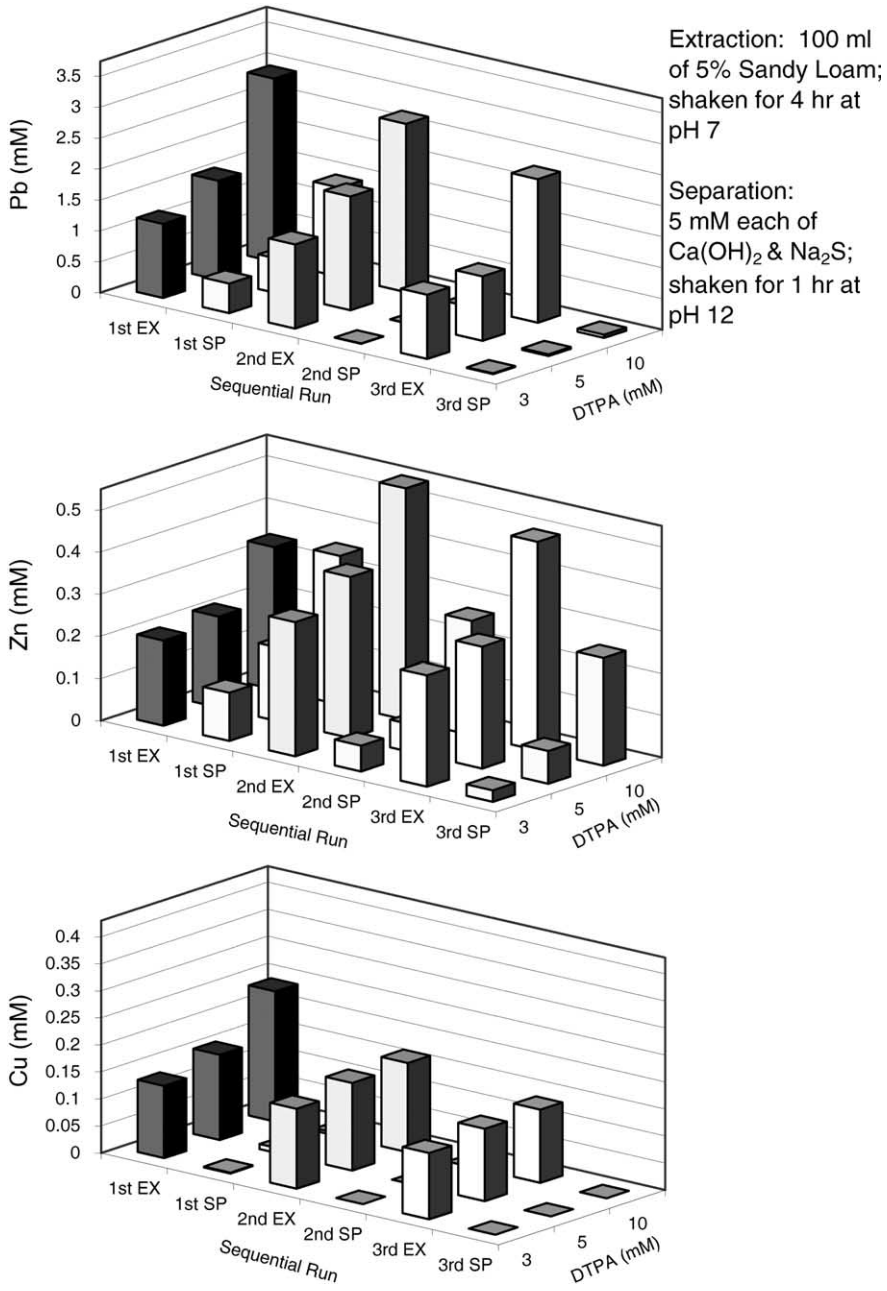

Fig. 3. Extraction and recovery cycles of metals using reclaimed DTPA during successive runs with different DTPA concentrations. Metal concentrations indicated those remaining in the aqueous phase after the extraction or recovery process. (1st EX = after the first extraction process; 1st SP = after the fist separation process, likewise for 2nd EX, 2nd EP, etc.).

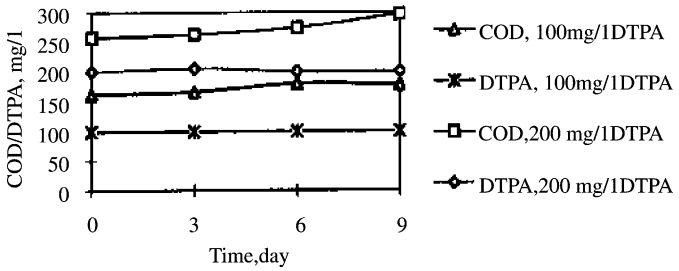

Fig. 4. Biodegradation of DTPA with unacclimated microorganisms from natural soil.

was adjusted to 500 mg/l with PTYG and varying amounts of DTPA. The DTPA concentration in different flasks varied from 50 to 300 mg/l. In 6 days some amount of DTPA was being removed from the liquid phase in all the systems except the abiotic control containing Hg_2Cl_2. The percentage removals of DTPA varied from 20 to 48%. The removals were higher for the system with lower amounts of DTPA concentration while the lowest removal was at the highest concentration of DTPA. The COD removals varied from 32 to 54%. It should be noted that the substrate consisted of easily degradable PTYG media and DTPA, so some COD removal was due to the utilization of the PTYG substrate. It appears that DTPA at higher doses caused some inhibition to the microorganisms present. As mentioned earlier in the Section 2 section that similar toxicity of DTPA was observed during acclimation experiments when DTPA concentrations were >100 mg/l.

The growth of the microorganisms in these systems occurred at the expense of the substrate (PTYG and DTPA) as indicated by the increase of SS and decrease of substrate COD. The first datum for SS after the start was measured after 12 h, which may have missed the peak growth of the microorganisms. But at the end of 6 days there was some substrate (COD and DTPA) left, which would also indicate some inhibitory effects. The abiotic flask showed no degradation of the DTPA present and there was a continuous decrease of SS in the system presumably due to endogenous respiration of the cellular components.

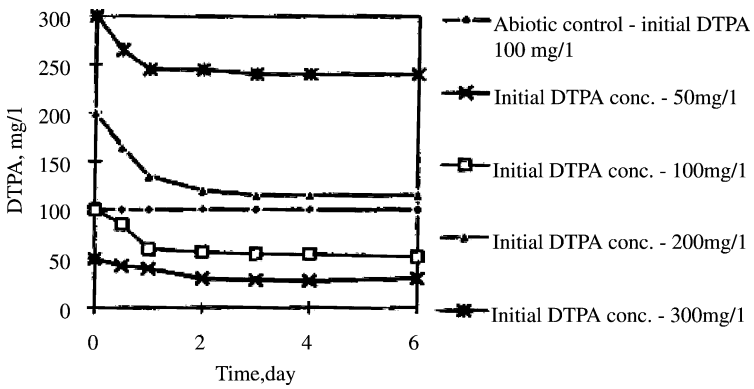

Fig. 5. Biodegradation of DTPA in shake flasks with acclimated microorganisms in aqueous systems.

Table 3
Biodegradation of DTPA by acclimated microorganisms in aqueous systems

Time (days)	Abiotic control		Initial DTPA concentration −50 mg/l			Initial DTPA concentration −100 mg/l			Initial DTPA concentration −200 mg/l			Initial DTPA concentration −300 mg/l		
	SS (mg/l)	DTPA (mg/l)	SS (mg/l)	COD (mg/l)	DTPA (mg/l)	SS (mg/l)	COD (mg/l)	DTPA (mg/l)	SS (mg/l)	COD (mg/l)	DTPA (mg/l)	SS (mg/l)	COD (mg/l)	DTPA (mg/l)
0	945	100	677	500	50	865	500	100	834	500	200	951	500	300
0.5	521	100	738	3l0	43	936	353	85	897	245	165	982	415	265
1	410	100	730	306	40	864	362	60	874	230	135	1020	394	245
2	300	100	728	303	30	852	345	57	863	228	120	938	385	245
3	142	100	665	298	29	848	328	55	856	230	115	915	350	240
4	102	100	616	274	28	811	323	55	841	225	115	888	345	240
6	80	100	591	283	30	782	330	52	823	230	115	848	341	240

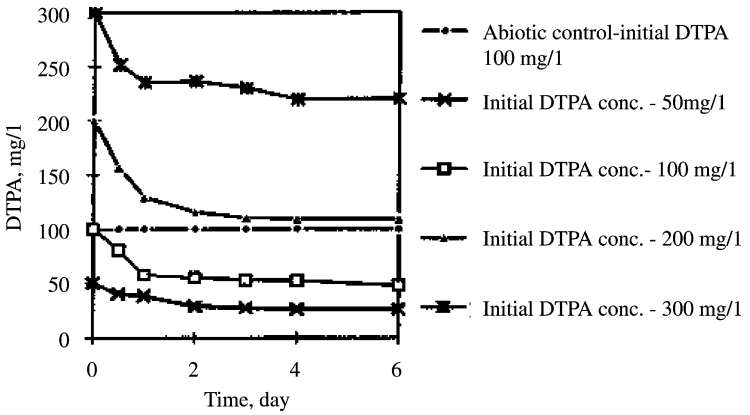

Fig. 6. Biodegradation of DTPA in shake flasks with acclimated microorganisms with 5% soil slurry.

3.2.3. DTPA biodegradation by acclimated microorganisms in 5% soil slurry

In this experiment the effects of the presence of soil slurry on the aerobic biodegradation of DTPA by acclimated microorganisms was evaluated in batch reactors. The DTPA concentrations varied from 50 to 300 mg/l. Fig. 6 and Table 4 show the results. The removal rate of DTPA in presence of 5% soil slurry was somewhat greater than that observed in aqueous system presented earlier. However, the trends of DTPA removals were very similar. The percentage removals of DTPA varied from 26.7 to 52%, while the corresponding COD removals varied from 37.4 to 56.2%. The highest and lowest percentage of DTPA removals occurred at concentrations of 100 and 300 mg/l, respectively. Again there was no DTPA biodegradation observed in the abiotic system. At the end of 6 days considerable amount of substrate COD was left in the flask indicating some reluctance of the microorganisms to biodegrade not only the DTPA molecule but also the organic compounds present in the PTYG media. It should be noted that the experiment reported in Table 4 determined the biodegradation of DTPA in presence of 5% soil slurry. The initial biomass suspended solids (SS) were measured before the addition of soil and reported. But, with time, no biomass growth could be determined by gravimetric methods because of presence of high amount of soil (5%). Thus, no SS measurements were made.

The initial biodegradation rate of DTPA in the aqueous and soil slurry system was evaluated assuming a linear removal rate over the initial 1-day period. Table 5 shows these results in millimole DTPA removed per milligram MLSS present per day. Generally, the soil system had a better removal rate for the chelate than the aqueous system, and higher DTPA concentrations (>200 mg/l) caused a retardation of the removal rate possibly due to some inhibition.

3.2.4. DTPA biodegradation in a respirometer with acclimated microorganisms

Two experiments were conducted with a respirometer to confirm the aerobic biodegradation of the DTPA molecule. In both experiments, the seed cultures used were acclimated to DTPA with basal salts but no PTYG media was added. The concentrations of DTPA varied from 50 to 600 mg/l. The first experiment was carried out in aqueous phase, while the second experiment with 5% soil slurry.

Table 4
Biodegradation of DTPA by acclimated microorganisms in 5% soil slurry

Time (days)	Abiotic control		Initial DTPA concentration −50 mg/l			Initial DTPA concentration −100 mg/l			Initial DTPA concentration −200 mg/l			Initial DTPA concentration −300 mg/l		
	SS (mg/l)	DTPA (mg/l)	SS (mg/l)	COD (mg/l)	DTPA (mg/l)	SS (mg/l)	COD (mg/l)	DTPA (mg/l)	SS (mg/l)	COD (mg/l)	DTPA (mg/l)	SS (mg/l)	COD (mg/l)	DTPA (mg/l)
0	945	100	984	500	50	895	500	100	902	500	200	980	500	300
0.5	–	100	–	295	40	–	335	81	–	233	157	–	394	252
1	–	100	–	294	38	–	348	58	–	220	130	–	378	235
2	–	100	–	292	29	–	333	55	–	220	116	–	372	236
3	–	100	–	286	27	–	315	53	–	220	110	–	336	230
4	–	100	–	260	26	–	307	52	–	214	109	–	315	219
6	–	100	–	246	26	–	304	48	–	219	109	–	313	220

Table 5
Aerobic biodegradation rate of DTPA in batch reactors (in mM chelate/mg MLSS per day)

Type of system	DTPA concentration			
	50 mg/l	100 mg/l	200 mg/l	300 mg/l
Aqueous system	0.038	0.118	0.199	0.148
Soil slurry system	0.03	0.121	0.199	0.169

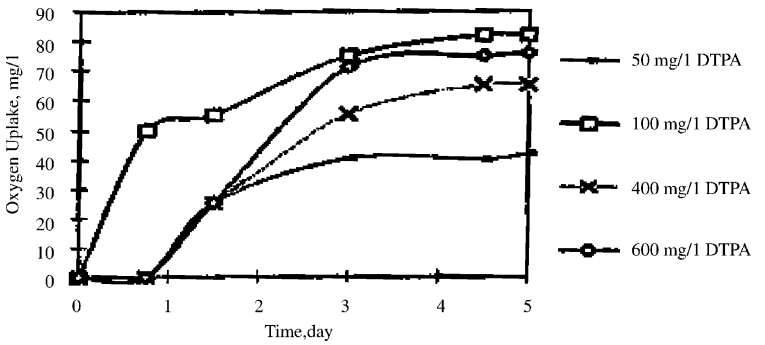

Fig. 7. Net oxygen uptake by acclimated microorganisms degrading DTPA in aqueous systems.

Fig. 7 and Table 6 show the net oxygen uptake for the four different aqueous DTPA concentrations. The cumulative 5-day oxygen uptake amounts for DTPA concentrations of 50 and 100 mg/l were approximately proportional to the initial DTPA and COD concentrations. About >70% of the initial COD was exerted in the 5-day period, which clearly showed that DTPA was biodegradable at these concentrations. However, this was not the case with higher DTPA levels (i.e. 400 and 600 mg/l systems). There was clear evidence that these higher levels were inhibitory to the microbes present. In addition, most of the systems also showed a marked lag initially, even though the microorganisms used were acclimated to the substrate. The system with 100 mg/l DTPA had no lag, which was possibly due to the fact that these cultures were acclimated at DTPA concentration of 100 mg/l.

The respirometer data from the experiment where soil slurry was included with the DTPA substrate, basal salt solution and acclimated microorganisms are shown in Fig. 8 and Table 7.

Table 6
Respirometric study of DTPA biodegradation in aqueous system net oxygen uptake (mg/l)

Time, days	50 mg/l DTPA	100 mg/l DTPA	400 mg/l DTPA	600 mg/l DTPA
0	0	0	0	0
0.75	0	50	0	0
1.5	25	55	25	25
3	40	75	55	71
4.5	40	82	65	75
5	42	82	65	76
Initial COD, mg/l	59	109	409	609

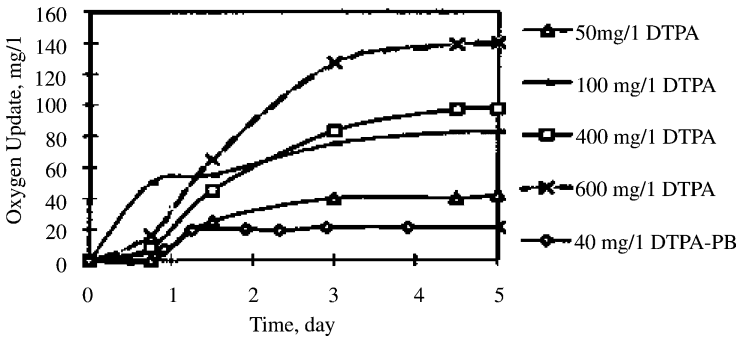

Fig. 8. Net oxygen uptake by acclimated microorganisms degrading DTPA in 5% soil slurry.

The oxygen uptake data for 50 and 100 mg/l DTPA concentrations in presence of soil were very similar to that observed in the aqueous system. There was an initial lag in most of the systems studied except in the flask containing 100 mg/l DTPA, as was observed in the aqueous systems reported. The presence of soil had no negative effect on the biodegradation of DTPA at these concentrations. The main effect of the soil presence was manifested at higher DTPA concentrations (400 and 600 mg/l). At these higher DTPA concentrations, there was a marked increase of oxygen uptake values at the 5-day period compared with the aqueous systems. However, the overall net oxygen uptake amount was still much less than the initial COD values for these systems. Typically for domestic wastewater, the 5-day BOD values (same as the oxygen uptake value) are about 65–70% of the ultimate BOD (BOD_u) value. The BOD_u value is often approximated to be equal to the COD value. It should be noted that the COD values for municipal wastewater are about 1.6 BOD_5 [12], and BOD_5 is related to BOD_u by the degradation rate constant k. If k is about 0.2 per day (natural base e), then BOD_u/BOD_5 is 1.58. The relationship is used as an approximation here as the k value was not determined for DTPA. The 5-day oxygen uptake values for the system with DTPA concentrations of 50 and 100 mg/l were proportional to the initial COD concentration and varied from 71 to 75% of the initial substrate concentration as was observed with the aqueous system. But at higher DTPA concentrations (400 and 600 mg/l), the 5-day oxygen uptake values were only about 23% of the initial COD due to some inhibitory effects.

Table 7
Respirometric study of DTPA biodegradation in 5% soil slurry net oxygen uptake (mg/l)

Time (days)	50 mg/l DTPA	100 mg/l DTPA	400 mg/l DTPA	600 mg/l DTPA	Time (days)	40 mg/l DTPA-Pb
0	0	0	0	0	0	0
0.75	0	50	8	16	0.91	7
1.5	25	55	45	65	1.25	19
3	40	75	83	127	1.91	20
4.5	40	82	97	139	2.33	19
5	42	82	97	140	2.91	21
Initial COD (mg/l)	59	109	409	609	3.91	21
					5.08	21

In one test DTPA was complexed with lead (2:1 molar ratio) and added to the respirometer flask at a concentration of 40 mg/l together with the acclimated seed culture and basal salts. Fig. 8 shows the oxygen uptake rate for acclimated microorganisms with 50 mg/1 DTPA and 40 mg/l DTPA plus Pb. The oxygen uptake at 5-days was about 50% less when lead was present (Table 7). Granted the substrate concentration was 40 mg/l in the sample with lead rather than 50 mg/l so the expected decrease should have been 20, not 50%. Thus, the presence of lead in the system reduced the DTPA biodegradation to some extent possibly due to lead toxicity.

4. Discussion

The removals of Zn and Cu (74 and 55%, respectively; Fig. 1) by DTPA complexation were less than complete, and were similar to previous results reported with EDTA [7] in which the removals were 60 and 56% for Zn and Cu, respectively. The extraction of Pb from this contaminated soil sample using DTPA or EDTA was apparently much more complete than of Zn or Cu. Chen and Hong [13] studied the chelation extraction of Pb and Cu from soil using two other chelators and observed similarly high extraction effectiveness for these metals. They also performed equilibrium modeling and computation, and found the technique useful in determining the extraction potential of different chelators toward different metals. In the present case, chemical solubility data alone apparently cannot account for the discrepancy in extraction. Equilibrium modeling cannot predict the extraction differences without a sufficient understanding of the complex solution–soil–chelator processes that require mineralogical details of the soil as well as mechanistic details regarding the metal–soil, complex–soil, chelator–soil, and metal–chelator interactions. The metals appear to be bound to different degrees that may have resulted from age, mineral content, and other physical and chemical factors.Fig. 3 identifies experimental conditions favorable to the separation of metals as sulfide solids and the recovery of DTPA. It appears from this figure that an increasing sulfide dose has a positive effect on precipitation of the metal sulfides, thus on the recovery of DTPA, whereas pH has negligible effects. These results suggest that in complex solution with excess chelating agent (i.e. DTPA concentration:total metal concentration = 5:3), an excess amount of sulfide (i.e. sulfide:DTPA = 2:1) is needed for it to successfully compete with DTPA to result in significant precipitation of metal sulfides, and thus the separation and recovery of DTPA for reuse.

It should be noted that corresponding to DTPA being a stronger chelating agent than EDTA, a larger dose of total sulfide ($S_T = [H_2S] + [HS^-] + [S^{2-}]$), i.e. twice that of DTPA concentration, is necessary for the recovery of DTPA, compared with an equal amount of total sulfide needed for EDTA [7].

The combined use of precipitating agents such as Na_2S and $Ca(OH)_2$ was shown (Fig. 3) to be useful in circumventing the difficulty in recovering strong chelating agents such as DTPA. The role of $Ca(OH)_2$, a "cationic precipitant", was to provide Ca^{2+} ions to compete with contaminant metals for DTPA, thus replacing the chelated contaminant metals and encouraging their release from the chelator. Working concertedly was Na_2S, an "anionic precipitant", which provided HS^-/S^{2-} anions to compete with DTPA for the precipitation of the contaminant metals as metal sulfides.

The extraction and recovery results show that DTPA, being a very strong chelating agent, is capable of removing metal contaminants from soil; the results further show that even for a strong chelating agent such as DTPA, effective recovery and reuse can be implemented by proper selection of precipitant concentrations (both cationic and anionic precipitants) as well as the pH condition. When the obstacle of recovering strong chelating agent such as DTPA is overcome, its strong extraction potential can then be best harnessed and multiplied by reclaiming and reusing over repeated cycles, thereby increasing its economic appeal for applications in soil remediation.

The data presented here show that DTPA can be biodegraded to some extent following a period of acclimation of the microorganisms. The removal of the chelate coincided with an increase of biomass in the batch systems, clearly indicating biodegradation of the chelator. Additional evidence of biodegradation of the chelator was obtained from respirometric studies where DTPA was the only substrate present in the flasks. There were some differences in biodegradation rates in the batch and the respirometer data. One of the reason for the difference was that in the batch system the substrates included both PTYG and DTPA compared with the respirometer case that had only DTPA. But the trends of the results were quite similar, i.e. DTPA at lower concentrations were degraded more easily compared with at higher concentrations. The lag in the respirometer experiments was not observed in the batch tests. It was not clear why this was so.

The biodegradation rates presented should be treated as maximum for the aqueous and soil environment systems. Laboratory conditions were very conducive to biodegradation with plenty of nutrients, acclimated biomass, oxidizing conditions and neutral pH. In actual field conditions the biodegradation rates for DTPA would perhaps be much less as mixing conditions, availability of nutrients and acclimated microorganisms would be far from optimal.

The presence of soil in comparison to aqueous systems had no effects on the biodegradation of DTPA up to a concentration of 100 mg/l, but at higher DTPA concentrations (200 and 400 mg/l) there was a reduced inhibition both in batch experiments as well as in the respirometer studies. The reduced inhibition could be due to a favorable environment on the soil surface for the biodegradation process, or it could be due to sorption and removal from aqueous phase of some intermediate product that could have been inhibitory to the microorganisms. In contrast to these findings Regmi [10] found that chelators pyridine dicarboxylic acid (PDA) and S-carboxymethyl-1-cysteine (SCMC) had lower biodegradation rates in presence of soil than in aqueous system. The difference in behavior must be in the biochemical make-up of the microorganisms involved in the biodegradation process.

The presence of chelator–metal (Pb) complex (2:1 molar ratio) in presence of soil caused reduced biodegradation of the chelate, The reduced biodegradation rate was probably due to the lead present in the system, which could have caused some toxic reaction to the microorganisms. Regmi [10] reported that the biodegradation of PDA in presence of lead (2:1 molar ratio) was not affected in presence of soil. But the biodegradation of SCMC was somewhat reduced in the presence of lead–chelator complex.

The biodegradation of metal–chelator complex in soil system is quite complex as other metals present in the soil may also form complexes with the chelator. In addition, the added metal (Pb in this case) can interact with the soil by cation exchange or sorption or soil-mediated precipitation. It is expected that these factors will reduce the toxic effects

of the metal. Also the dominant chelator complexation may be with Ca(II) or Fe(II) ions present in the soil. Firestone et al. [14] found that the complex of copper and NTA was converted to Fe–NTA complex prior to active biodegradation period in soil environment. Similar competing ion effects on metal–chelate biodegradation were reported by Swisher [15] who found that the addition of Fe(II) in excess amounts stimulated the biodegradation of NTA in presence of other metal ions. These findings suggest that the biodegradation of metal complex in soil media causes a reequilibration of the chelators with other cations present in the soil.

It can be seen from the results that DTPA used to remediate soils containing metals can be reused many times as the microorganisms present in soil will not be able to biodegrade it much as they will not be acclimated to this molecule. Further, the conditions during the remediation process will not be optimal for biodegradation process (improper mixing or nutrient deficiency, lack of aeration, etc.).

5. Conclusions

The experiments with DTPA performed in this study with respect to its extraction, recovery, and biostability relevant to soil metal remediation have led us to conclude the following:

1. DTPA is a strong and effective chelating agent similar to EDTA in extraction of contaminant metals from soil.
2. DTPA can be made amenable to recovery by use of $Ca(OH)_2$ and Na_2S, or Na_2S alone in proper dosages.
3. DTPA is capable of being reused at least several times with some loss of extraction activity.
4. In batch studies, unacclimated microorganisms could not biodegrade DTPA molecules in the concentration range 50–300 mg/l.
5. DTPA could be biodegraded by acclimated microorganisms up to a concentration of 300 mg/l. However, the biodegradation of DTPA at concentrations >100 mg/l was inhibited to some extent.
6. Presence of 5% soil slurry allowed less inhibition of DTPA biodegradation at concentrations >100 mg/l.
7. Presence of Pb–chelate complex retarded the biodegradation rate of the chelator in soil slurry system.
8. Even though DTPA molecule could be biodegraded to some extent under well acclimated laboratory conditions, in soil remediation applications it is expected that DTPA will be quite biostable as the microorganisms in the soil and site conditions will not be favorable for the biodegradation process.

Acknowledgements

We thank the USEPA Regions 7 and 8 Great Plains/Rocky Mountain Hazardous Substances Research Center for funding of this collaborative work.

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