Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental Pollution 142 (2006) 191–199
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Biodegradation and speciation of residual SS-ethylenediaminedisuccinic acid (EDDS) in soil solution left after soil washing

Susan Tandy [a], Adrian Ammann [b], Rainer Schulin [a], Bernd Nowack [a,*]

[a] Institute of Terrestrial Ecology, Swiss Federal Institute of Technology (ETH), Universitätstrasse 16, CH-8092 Zürich, Switzerland
[b] Swiss Federal Institute for Environmental Science and Technology (EAWAG), CH-8600 Dübendorf, Switzerland

Received 6 July 2005; received in revised form 21 September 2005; accepted 13 October 2005

Even in polluted soils, EDDS is degraded.

Abstract

This paper aims to investigate the degradation and speciation of EDDS-complexes (SS-ethylenediaminedisuccinic acid) in soil following soil washing. The changes in soil solution metal and EDDS concentrations were investigated for three polluted soils. EDDS was degraded after a lag phase of 7–11 days with a half-life of 4.18–5.60 days. No influence of EDDS-speciation on the reaction was observed. The decrease in EDDS resulted in a corresponding decrease in solubilized metals. Changes in EDDS speciation can be related to (1) initial composition of the soil, (2) temporarily anoxic conditions in the soil slurry after soil washing, (3) exchange of EDDS complexes with Cu even in soils without elevated Cu and (4) formation of NiEDDS. Dissolved organic matter is important for metal speciation at low EDDS concentrations. Our results show that even in polluted soils EDDS is degraded from a level of several hundred micromoles to below 1 μM within 50 days.

Keywords: Chelating agents; Soil washing; Biodegradation; Metal mobilization

1. Introduction

Chelating agents are potent agents for solubilizing heavy metals from polluted soils. Two different remediation methods using chelating agents are now being investigated: chelant assisted ex-situ soil washing and chelant assisted phytoextraction. In ex-situ soil washing two methods are possible, batch washing (Tandy et al., 2004; Vandevivere et al., 2001a) and heap leaching (Hauser et al., 2005). In batch washing the soil is excavated and washed in a closed system with chelating agents and then returned to the site or used otherwise. In heap leaching the soil is also excavated but treated by sprinkling solution over it which preserves the soil structure. The percolate is collected and treated off or on-site. A variation of this procedure has been proposed where a biodegradable chelating agent is used that is degraded in a permeable reactive barrier under the soil which traps the liberated metals (Finzgar et al., 2004; Kos and Lestan, 2003a, 2004a,b).

In chelant-assisted phytoextraction chelating agents are added to the soil to increase the solubilized metals and correspondingly uptake into plants (Garbisu and Alkorta, 2001). The enormous drawback of this method is the inevitable leaching of chelants and its metal-complexes into the deeper soil layers and eventually to groundwater.

The ex-situ methods attract a lesser risk of leaching than phytoremediation as most of the chelating agent is removed from the soil before returning to the field. However some chelating agent is always left in the soil and the formation of metal complexes with this residual complexing agent is possible and in turn leaching of these metal complexes must be taken into consideration.

Previously the most used complexing agent for these methods was EDTA (Abumaizar and Khan, 1996; Peters, 1999; Thayalakumaran et al., 2003; Van Benschoten et al., 1997; Wenzel et al., 2003; Wu et al., 1999). It is however recalcitrant

* Corresponding author. Tel.: +41 44 633 6160; fax: +41 44 633 1123.
E-mail address: nowack@env.ethz.ch (B. Nowack).

0269-7491/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2005.10.013

in the environment and leaching of metal-complexes over a long time period was possible (Bucheli-Witschel and Egli, 2001; Wenzel et al., 2003; Wu et al., 2004). SS-ethylenediaminedisuccinic acid (EDDS) is a biodegradable chelating agent that is a structural isomer of EDTA (Schowanek et al., 1997; Vandevivere et al., 2001b). It is now starting to replace EDTA in soil washing and phytoextraction (Kos and Lestan, 2003b; Tandy et al., 2004). It should not be confused with the other stereo-isomers of EDDS however (RR-, RS-, SR-), which are partly or wholly non-biodegradable (Schowanek et al., 1997; Takahashi et al., 1997). Several authors recently have carried out work on EDDS assisted phytoextraction, mainly on Pb but also Zn, Cu, Cd and Ni (Grcman et al., 2003; Kos et al., 2003; Kos and Lestan, 2003a,b, 2004a,b; Luo et al., 2005; Meers et al., 2005; Tandy et al., in press). An immediate leaching risk is possible during this method until the EDDS has degraded (Kos and Lestan, 2004a). Three studies have also used EDDS for soil washing or heap leaching (Hauser et al., 2005; Tandy et al., 2004; Vandevivere et al., 2001a).

As leaching is only a risk as long as EDDS is present, it is important to look at how it degrades in soil. Most investigations into the biodegradation of EDDS have been carried out using standard degradation tests such as the Sturm test (Schowanek et al., 1997) or modifications, that use sewage sludge (Takahashi et al., 1997; Vandevivere et al., 2001b). Although these methods have shown that EDDS is degraded in sewage sludge it tells us nothing about the rate of degradation in soil. One study has carried out a degradation experiment using soil, this soil however was also spiked with sludge which might effect the degradation rate of EDDS due to more microorganisms being present in the soil (Schowanek et al., 1997). Soils remediated by washing or phytoremediation are also contaminated with heavy metals such as Cu, Zn, Pb, Cd and Ni. There is evidence that some metal-EDDS complexes with high stability constants (Cu, Ni and to a lesser extent Zn) are non-biodegradable when in isolation (Vandevivere et al., 2001b). As the soil in which EDDS was previously tested was not contaminated with heavy metals, the above might also lead to a difference in degradation rates or incomplete degradation. Another investigation looked indirectly at the degradation of EDDS after phytoextraction of heavy metal contaminated soils (Meers et al., 2005). Here no sewage sludge was added to the soil but high concentrations of EDDS were used in keeping with concentrations used in phytoremediaton. These levels are much higher than found in soil after soil washing and initial concentration of EDDS may influence the rate of degradation.

Our aim was to investigate the degradation of residual EDDS in soil following soil washing. We also wanted to investigate the solubility of metals in soil solution caused by residual EDDS and the speciation of EDDS complexes in soil solution to see if this would effect degradation. To achieve this we have washed three contaminated soils with EDDS and have investigated the changes in soil solution metal and EDDS concentrations over time.

2. Method

2.1. Chemicals

All chemicals were purchased from Merck (Switzerland) and were analytical grade or HPLC grade for the solvents unless stated. SS-EDDS (Octaquest E30, 1.092 mol kg^{-1}) was obtained from Octel (Cheshire, UK) for the experiments and from Proctor and Gamble (Belgium) as the Na_3EDDS salt for the EDDS analysis (stock solution 1 mM). Fluorenylmethyl chloroformate (FMOC-chloride) (puriss) was obtained from Fluka. All solutions were made with high purity water (Millipore, Bedford, MA).

2.2. Soils

The soils were taken from contaminated sites in northwest Switzerland. The soil characteristics can be seen in Table 1. Soils 1 and 2 were cultivated soils taken from Dornach which had been contaminated with Cu, Zn and Cd from an adjacent brass smelter. Soil 1 was a heavily contaminated topsoil of a non-calcareous Regosol and Soil 2 lightly contaminated topsoil of a Calcaric Regosol. Soil 3 was the topsoil of a Haplic Luvisol taken from in the vicinity of the village Rafz, north of Zürich, from an agricultural field contaminated with Zn, Pb and Cd from sewage sludge applications. The soils were taken from the top 20 cm, dried at 40 °C and sieved to <2 mm prior to use.

2.3. Experimental setup

Soil (12 kg DW of each) was placed in a plastic barrel with 120 l tap water and stirred with an electrical stirrer (200 rotations per minute). 20 mmol/kg Na_3EDDS was added (0.24 moles) and the solution adjusted to pH 7 if necessary with 1 M HNO_3. This equated to a EDDS:metal ratio of 1:1 for soil 1, 4:1 for soil 2 and 2:1 for soil 3. The barrels were covered and the soils were washed in this manner for 24 h. The suspension was then allowed to settle for 24 h before the supernatant was removed by suction and the soil was rinsed for 1 h with 120 l tap water. After 24 h settling the supernatant was again removed. The soil slurry was then poured into 3 l black plant pots (4 replicates) with a disc of fine mesh (60 μm) in the bottom to prevent the soil leaking out and 2 Rhizon Flex soil moisture samplers (SMS) (Rhizosphere Research Products, Wageningen, Netherlands) were installed at a 45° angle. The pots were allowed to drain over night and the clear solution present on top of the soil was removed. The pots were then transferred to a climate chamber with a 16 h (21 °C)/8 h (16 °C) day/night cycle to simulate field conditions. The first soil solution was then extracted (time 0) see Section 2.4. This corresponds to day 4 after addition of EDDS. After two days no more drainage occurred and this was then taken to be 100% water holding capacity (WHC). Soil solution was extracted every 7 days. One day prior to this the pots were made up to 100% WHC with ultra pure water and 24 h later the solution extracted. The pots were then allowed to dry until the next week. To help infiltration the surface of the soil was broken up before the addition of water. After day 35, water was added weekly for 2 weeks without extraction, then on the last week extraction was carried out again (day 56). Although the temperature in the growth chamber reached highs of between 34 and 39 °C between day 29 and 31 due to climate chamber malfunction this did not seem to affect the degradation process. At the end of the experiment the soil from the 3 replicate pots was dried at 110 °C to find the out the actual soil weight in each pot.

Table 1
Initial soil characteristics prior to soil washing

Soil	pH ($CaCl_2$)	C_{org} %	$CaCO_3$ %	Cu mg/kg	Zn mg/kg	Pb mg/kg	Cd mg/kg	Ni mg/kg	WHC MC%
1	5.65	2.73	0.48	537	786	66	2.07	46	32.75
2	6.87	1.79	1.74	111	191	39	0.53	32	27.49
3	5.07	1.24	0.49	52	515	386	0.7	21	21.35

2.4. Soil solution extraction

The solution was extracted using syringes attached to the SMS and was carried out under a cover of aluminium foil to prevent photo-degradation of EDDS during sampling. The solution was then filtered through 0.05 μm filter to remove any colloidal matter and the pH taken. 10 ml was preserved with 100 μl supra pure HNO_3 and kept refrigerated until metal analysis could be carried out. The other portion was kept refrigerated until EDDS analysis could be carried out. Dissolved organic carbon and anions were analyzed in the samples from day 56. Soil solution results were normalized to a fixed water content for each soil which was equal to the amount of water present at sampling on day 7. The amount of water present at each sampling time was measured and a correction factor calculated. In doing this we assumed that all EDDS present was in solution. This was done in order to be able to compare results over time as pot water content was slightly different at each sampling and at time 0 quite different.

2.5. Metal measurement

Filtered (0.05 μm) soil solutions were analysed for Cu, Zn, Ni, Pb, Cd and Mn by ICP-MS (ELAN 5000, Perkin-Elmer-Sciex) with a cross flow nebulizer mounted on a Scott type Ryton double pass spray chamber (RF power: 1100 W, Ar gas flows L/min: plasma 15.0, auxiliary: 1.0, nebulizer: 0.8–1.0). Mg, Ca and Fe were measured by ICP-OES (CIROS, Spectro, Germany) using a concentric nebulizer Type A mounted on a cyclonic spray chamber (AF Analysentechnik, Germany).

Total soil metals were measured by X-ray fluorescence spectroscopy (Spectro X-lab 2000, Germany). Samples from the original soil and from the 4th replicate pot taken after soil washing and dried at 40 °C were analysed.

2.6. EDDS measurement

EDDS derivatization and analysis was carried out as described in reference (Tandy et al., 2005). This method involves the derivatization of EDDS by the FMOC reagent followed by separation by HPLC (Jasco PU-980), Lichrospher 100 RP-18 5 μm column and fluorescence detection (Jasco 821-FP). For samples up to day 35 where dilution was required, the standards where made in diluted soil solution from a previously washed sample of soil 3 which had no more EDDS present. For samples from day 56 where the samples were undiluted, a calibration was made by standard addition to soil solution taken from the 4th replicate pot for each soil.

2.7. Organic carbon measurement

Non-purgeable organic carbon in soil solution was measured using a TOC-5000 (Shimadzu, Reinach, Switzerland). To calculate the soil organic matter in solution the carbon coming from EDDS was subtracted. The resulting carbon was multiplied by two as it has been found that the % C in organic matter in aquatic samples ranged from 43–50% (Abbt-Braun and Frimmel, 2002) and fulvic and humic acids from soil solution showed % C approximately 50% (Abbt-Braun and Frimmel, 2002; Frimmel and Abbt-Braun, 1999; Maie et al., 2004).

2.8. Anion measurement

Nitrate, chloride, phosphate and sulphate were measured by ion chromatography (DX–100, Dionex, Olten, Switzerland) on AS-4A-SC with 1.8 mM $NaCO_3$/1.7 mM $NaHCO_3$ buffer, using suppressed electronic conductivity detection.

2.9. Speciation modelling

ECOSAT software (Keizer and van Riemsdijk, 1999) was used to model EDDS and metal speciation in soil solution. The binding of metals to dissolved organic matter was modelled using the consistent NICCA-Donnan model (Kinniburgh et al., 1999). It was assumed all dissolved organic matter was in the form of fulvic acids (FA) as the soil solution had been filtered through 0.05 μm and FA-binding constants for generic fulvic acid were taken from (Milne et al., 2003). Input parameters were the measured soil solution pH, dissolved organic matter, the major anions (Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}), major cations (Ca, Mg) and the metals (Cu, Zn, Cd, Pb, Ni, Fe, Mn). Table 2 shows the values and the sources of stability constants for EDDS complexes. Free EDDS is the H-form of the complex and Na and K complexes have been shown by our calculations to play no role.

Table 2
EDDS complex stability constants. Calculated using source values and given at 0 M ionic strength as overall formation constant β

Complex	Log K	Source
$CaEDDS^{2-}$	6.34	(Orama et al., 2002)
$CdEDDS^{2-}$	12.70	(Martell et al., 2001)
$CuEDDS^{2-}$	20.46	(Orama et al., 2002)
$CuHEDDS^{-}$	24.39	(Orama et al., 2002)
CuH_2EDDS	26.80	(Orama et al., 2002)
$Cu(OH)EDDS^{3-}$	8.81	(Orama et al., 2002)
$Fe(III)EDDS^{-}$	23.68	(Orama et al., 2002)
$HEDDS^{3-}$	10.87	(Orama et al., 2002)
H_2EDDS^{2-}	18.33	(Orama et al., 2002)
H_3EDDS^{-}	22.5	(Orama et al., 2002)
H_4EDDS	25.66	(Orama et al., 2002)
H_5EDDS^{+}	26.95	(Orama et al., 2002)
H_6EDDS^{2+}	28.72	(Orama et al., 2002)
$MgEDDS^{2-}$	7.77	(Martell et al., 2001)
$MnEDDS^{2-}$	10.77	(Orama et al., 2002)
$NiEDDS^{2-}$	18.50	(Martell et al., 2001)
$NiHEDDS^{-}$	21.78	(Martell et al., 2001)
$PbEDDS^{2-}$	14.46	(Martell et al., 2001)
$ZnEDDS^{2-}$	15.34	(Martell et al., 2001)
$ZnHEDDS^{-}$	19.34	(Martell et al., 2001)

3. Results

3.1. Soil washing

Washing with EDDS removed significant amounts of heavy metals from the three soils. The results are presented in Table 3. Seventy four percent Cu from the most contaminated soil (soil 1) and 50% of Cu from soils 2 and 3 were extracted by EDDS. For Zn between 44–56% was extracted from soils 1 and 3, but nothing from soil 2, which was only lightly contaminated. 26% of the Pb from soil 3 (the only soil contaminated with Pb) was also extracted.

The soils used for this experiment were taken from the same sites as soils used in previous work on soil washing (Tandy et al., 2004) and column extraction (Hauser et al., 2005). In this study we used a scaled-up soil washing procedure, the soil characteristics and contamination levels were also not exactly the same. Cu and Zn extraction from soil 1 (74 and 44% respectively) was better than extraction by small-scale soil washing (61 and 34%) and column leaching (18 and 28%) for soil taken from the same site (Dornach 2). Although the extraction of Cu from soil 2 (56%) was similar to before for soil washing (53%), it was higher than for column leaching (26%) (Dornach 1). The Zn extraction from soil 2 (2%) was much worse than either previous method (19 and 20%). The level of Zn contamination in the soil 2

Table 3
Soil metal content and percentage reduction in metal after soil washing

Soil	Cu mg/kg	% Cu reduction	Zn mg/kg	% Zn reduction	Pb mg/kg	% Pb reduction	Cd mg/kg	% Cd reduction	Ni mg/kg	% Ni reduction
1	141	74	440	44	71	0	2.13	0	38	17
2	56	50	187	2	40	0	<0.5	0	32	0
3	26	50	229	56	284	26	<0.5	29	17	19

was only 30% of the previously used soil so it may be that the Zn in soil 2 was not in the soil fractions accessible to EDDS. The extraction of Zn and Pb from soil 3 by soil washing (56% and 26% respectively) was slightly better than before (Zn 48–49% and Pb 16–18%) (Rafz) but this could have been due to the lower contamination levels (about 50%) found in soil 3 compared to those previously found (Tandy et al., 2004). In conclusion we can say that the up-scaled soil washing resulted in good removal of most metals from the three used soils.

3.2. Degradation of EDDS in soil solution after soil washing

Fig. 1 shows the degradation of EDDS in the three soils as a percentage of the initial EDDS soil solution concentrations (day 0). All three soils showed a lag time before degradation got under way. Soils 2 and 3 had a lag time of about 7 days while soil 1 had a longer lag time of about 11 days. During this time the soil went from being saturated through being at water holding capacity to being at between 78 and 85% of water holding capacity.

The actual initial concentration in the soil solution of Soil 2 (647 ± 51 μM) was about twice that for Soils 1 and 3 (299 ± 42 and 286 ± 12 μM). The solution concentration during soil washing was 2000 μM, the washing of the soil slurry with tap water diluted this by about 6.5 times (assumption that the slurry contained 150% water). This would result in a solution concentration of about 300 μM, the value measured initially in soils 1 and 3.

The degradation process followed first order kinetics (after the lag phase) and the k and $t_{0.5}$ values can be seen in Table 4. The half-lives of EDDS in Soil 2 and 3 were similar (4.18 and 4.94 days respectively), however the half-life of Soil 1 was longer than the other two soils (5.60 days) showing much slower degradation. The modelled degradation curves based on the degradation rate constants calculated from the measured data (Table 4) can also be seen in Fig. 1. It can be seen that the modelled first order degradation curves fit the actual degradation curves very well for all soils. Only at day 21 for soil 1 does the actual concentration of EDDS seem higher than the modelled value.

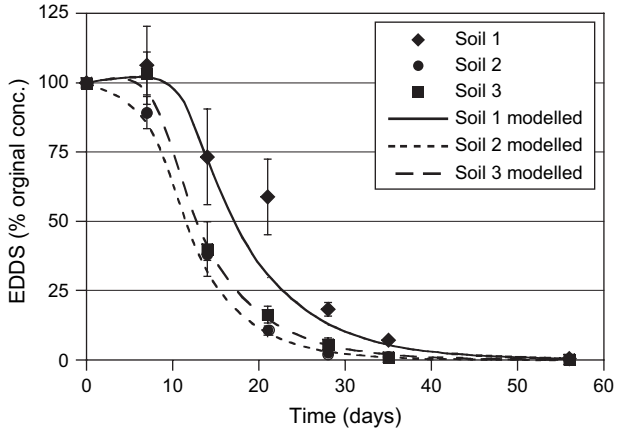

Fig. 1. Degradation of EDDS over time as percentage original EDDS concentration; actual and modelled values. Shown as mean of 3 pots ± SE.

3.3. Metal concentration in soil solution

Despite the fact the three soils have very differing concentrations of Cu it was still the heavy metal with the highest soluble concentrations in all three soils (Fig. 2). In soils 1 and 2 the initial concentrations were high, dipping to low levels at day 7, peaking again between days 7 and 21 and then reducing to trace levels. Soil 3 shows a relatively high initial concentration which peaks at day 7 then reduces to trace levels at day 28. Soluble Zn concentrations showed a similar pattern for all three soils. The concentrations started high and decreased gradually to trace levels by day 21 (Fig. 2). Soluble Ni showed fairly constant concentrations over time with a slight reduction after day 35. Soluble Pb and Cd were very low compared to the other metals (Fig. 3) but showed the same pattern in all soils of an initial high concentration reducing to very low levels by day 14.

In soil 1 Mn and Fe (Fig. 4) were fairly high at day 0 but increase to a maximum at day 7 before decreasing sharply to a constant low at day 21 onwards. Soils 2 and 3 show a similar trend but with a smaller increase between day 0 and 7.

3.4. Speciation of EDDS and metals in soil solution

Figs. 5–7 show soil solution speciation for one replicate for each soil 1–3 as (a) concentrations of metal-EDDS complexes, (b) metal-EDDS complexes as a percentage of the total EDDS concentration and (c) percentage of metal bound to dissolved organic matter. The first sample (day 0) was taken four days after the first contact of soil with EDDS. During this initial time the soil was being washed with EDDS so the soil was

Table 4
Rate equations for degradation of SS-EDDS (± error)

Soil	k (d^{-1})	$t_{0.5}$ (d)	Lag phase (d)
1	0.124 ± 0.006	5.60 ± 0.28	11
2	0.166 ± 0.006	4.18 ± 0.16	7
3	0.143 ± 0.007	4.94 ± 0.23	7

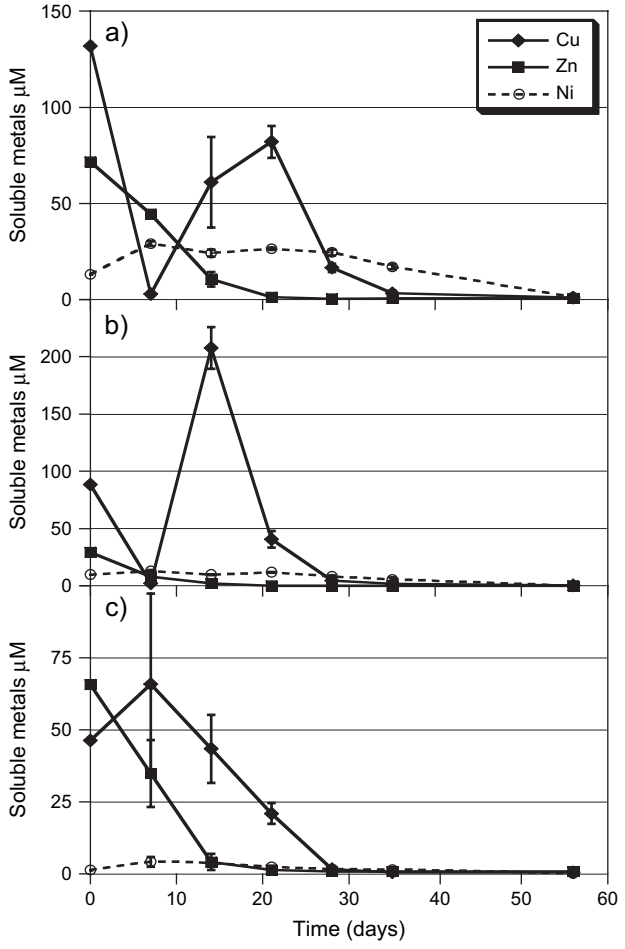

Fig. 2. Soil solution metals (a) soil 1, (b) soil 2, (c) soil 3. Cu filled diamonds, Zn filled squares and Ni open circles. Mean of 3 pots ± SE.

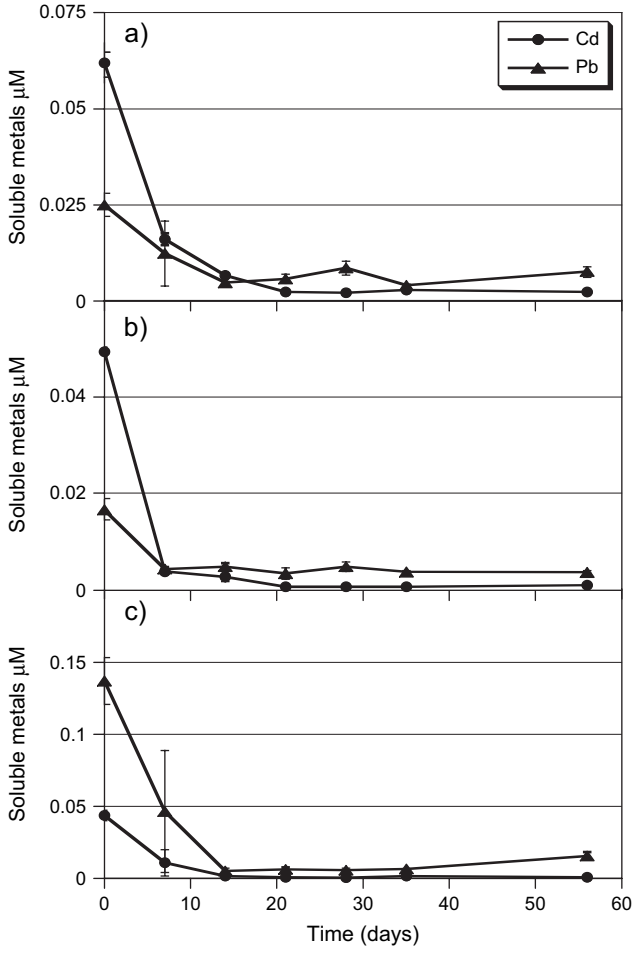

Fig. 3. Soil solution metals (a) soil 1, (b) soil 2, (c) soil 3. Cd filled circles and Pb filled triangles. Mean of 3 pots ± SE.

in contact with a bulk solution in excess to the soil. The species were calculated for soil solutions over the period when only residual water was left in the soil and real pore water could be taken. The speciation represents the situation after washed soil is returned to the field. As the total EDDS concentration in solution was reduced over time, so were the actual concentrations of metal-EDDS complexes. After day 35 the concentrations of complexes were very low. To get a clearer picture of the processes going on one must look at the metal-EDDS complexes as a percentage of the total EDDS concentration (Figs. 5b, 6b and 7b). In general for all three soils the experiment can be split into four phases with regards to the speciation; (1) initial, (2) Fe/Mn, (3) exchange and (4) final.

Initially (phase 1) Cu and Zn are the main complexes for soil 1, Cu Fe and Mn for soil 2 and Zn, Mn, Cu, Fe and Mg for soil 3. Cu and Zn are the polluting metals targeted during soil washing and Fe and Mn originate from the ligand-controlled dissolution of oxides. In the second phase (soil 1 day 0–14, soils 2 and 3 day 0–7) Fe and MnEDDS complexes peak and at the same time the heavy metal complexes go down. In the exchange phase (3) the Mn and Fe decrease giving way to CuEDDS, even for soils 2 and 3 which did not contain elevated Cu levels. For soils 1 and 2 Mg and CaEDDS appear to a small extent in this phase. In the final phase (4) CuEDDS decreases and NiEDDS increases. In all soils NiEDDS is the most important EDDS-complex at day 56. For all soils ZnEDDS decreases from its high at day 0 (not more than 35%) to a low in the exchange phase.

Soil 3 shows a slightly different pattern with regards to the other two soils. In this soil H-EDDS shows two high peaks at day 14 and 28 while in the other soils percentage concentrations of uncomplexed EDDS are low through out the experimental time. This is brought about due to the much lower soil solution pH in soil 3. In Soil 3 ZnEDDS and CuEDDS also increases again after day 28 to a certain extent while in the other soils they remain low. In all soils Cd and Pb in solution are not important in the speciation of EDDS, even in soils where they are contaminants. Likewise despite the high concentrations of Ca and Mg in solution they play a limited role in EDDS speciation as the majority is in the free form through out the experimental time.

In all soils the absorption of metals to dissolved soil organic matter (DOM) is important in the speciation of metals and EDDS especially at the end of the experiment when EDDS and metal concentrations are low (Figs. 5c, 6c and 7c). For all soils most Fe is complexed to DOM after day 14. High

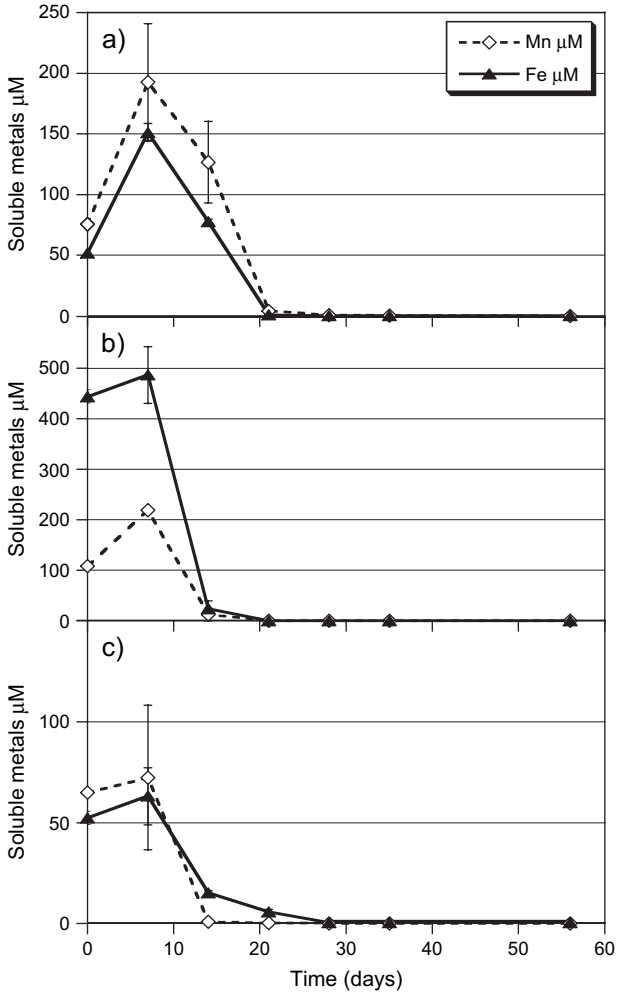

Fig. 4. Soil solution metals (a) soil 1, (b) soil 2, (c) soil 3. Mn open diamonds and Fe filled triangles. Mean of 3 pots ± SE.

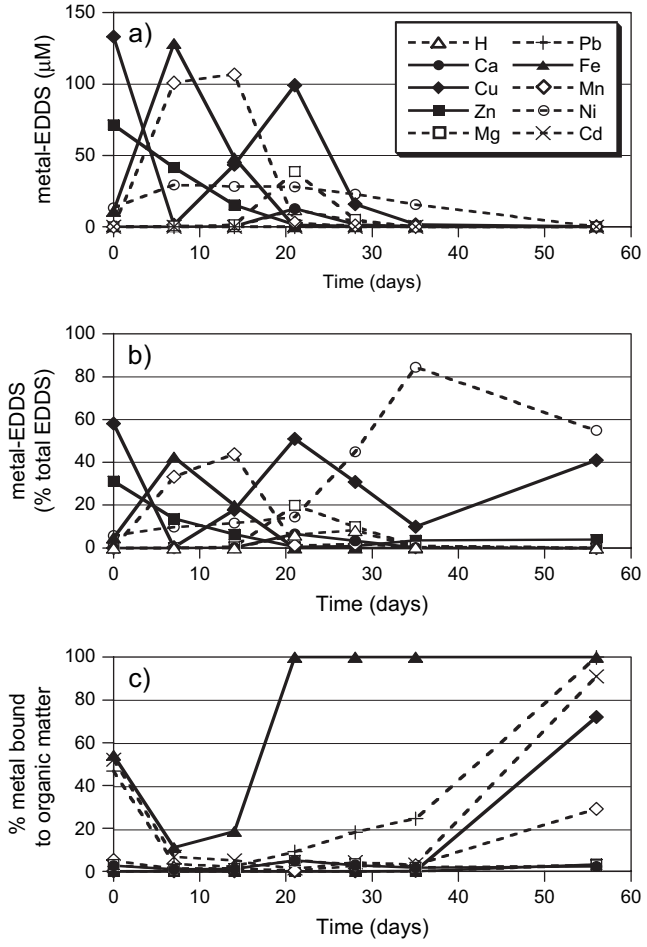

Fig. 5. Soil 1 solution speciation. (a) concentration of metal-EDDS complexes, (b) metal-EDDS complexes as a percentage of the total EDDS concentration, (c) percentage metal bound to DOM.

percentages of the total Pb, Cd and Cu are also complexed in the latter part of the experiment. If this were not taken into account the EDDS speciation results would be quite different as both Cu and Fe form strong complexes with EDDS. A speciation calculation without DOM would predict that at the end almost all EDDS be complexed with Cu.

4. Discussion

4.1. Degradation of EDDS in soil solution

The soils showed a lag phase of between 7 and 11 days before degradation of EDDS started. Most biodegradation work carried out on SS-EDDS has used acclimatized sludge, where the sludge has been fed EDDS for a period prior to the experiment, so no lag phase was found (Schowanek et al., 1997; Vandevivere et al., 2001b). Where unacclimatized sludge has been used a lag phase of between 5–16 days has been found (Jaworska et al., 1999; Schowanek et al., 1997; Takahashi et al., 1997). A lag phase of 3 days was also found in river water amended with sewage sludge, but not for sewage sludge amended soil (Schowanek et al., 1997). Soils unammended with sludge showed a lag time of 4 days however (Meers et al., 2005). It may also be the longer lag phase seen in our work was caused by the waterlogged conditions at the start of the experiment. Especially as the soil with the longest lag time (soil 1) had the greatest water holding capacity.

Most degradation work has been carried out in sludge in line with traditional biodegradation tests. For acclimatized sludge k values range from 0.21–0.9 d^{-1} with $t_{0.5}$ ranging from 0.77–3.3 days (Schowanek et al., 1997; Vandevivere et al., 2001b). In unacclimatized sludge degradation rates seem to be slower ($k = 0.08$–$0.4\ d^{-1}$, $t_{0.5} = 1.73$–8.63 d) (Schowanek et al., 1997). Degradation in river water seemed to be slowest of all ($k = 0.11\ d^{-1}$, $t_{0.5} = 6.27$ d) (Schowanek et al., 1997). Only two soil experiments for the degradation of EDDS have been carried out. The first was in soil amended with sewage sludge (Schowanek et al., 1997). The degradation rate constant was $0.27\ d^{-1}$, and $t_{0.5} = 2.55$ d. The second investigation only indirectly followed EDDS degradation by using the decrease in the mobilization of metals as a pseudonym (Meers et al., 2005). As the concentrations of EDDS used were very high, the use of solublized metals instead of EDDS could

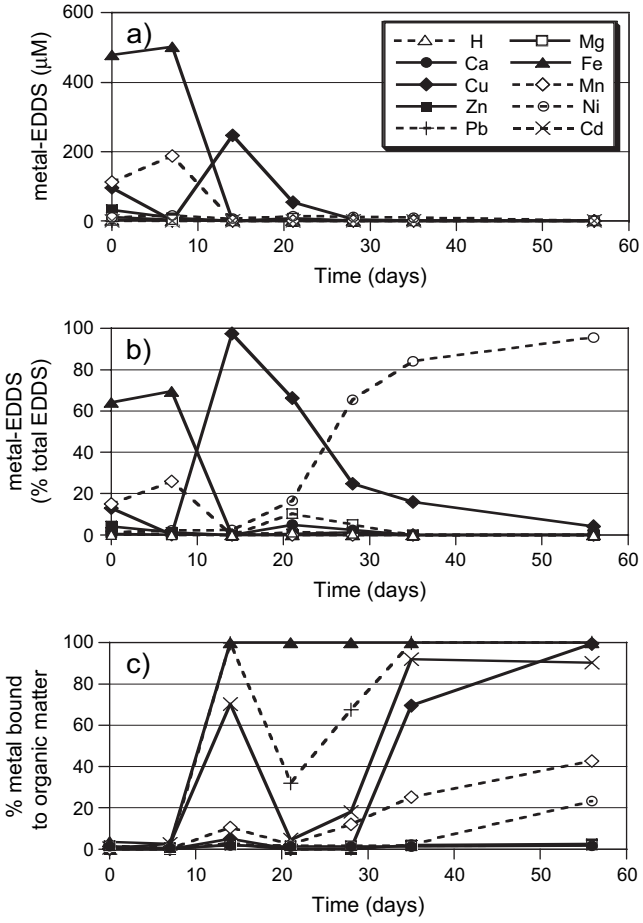

Fig. 6. Soil 2 solution speciation. (a) concentration of metal-EDDS complexes, (b) metal-EDDS complexes as a percentage of the total EDDS concentration, (c) percentage metal bound to DOM.

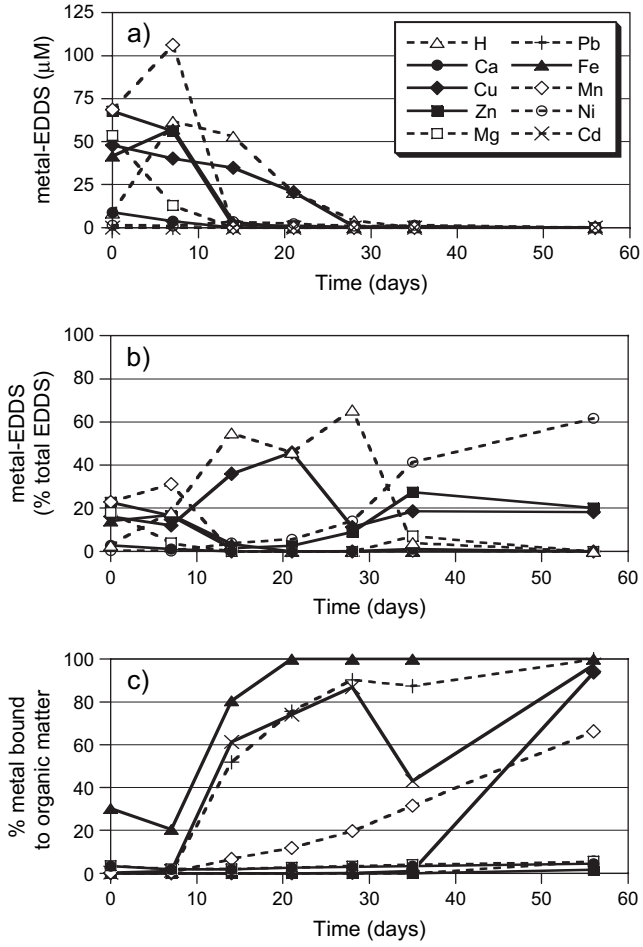

Fig. 7. Soil 3 solution speciation. (a) concentration of metal-EDDS complexes, (b) metal-EDDS complexes as a percentage of the total EDDS concentration, (c) percentage metal bound to DOM.

give false results as at high EDDS concentrations often free EDDS (H-EDDS) is present which would not be accounted for. The degradation rate constants ranged from 0.09–0.18 d^{-1} and the half lives from 3.8–7.5 d depending on the initial EDDS concentration. The degradation rates in our soils (Table 3) seem to fit in the range of unacclimatized sludge and the soils unamended with sludge and seem to be lower than for the sludge amended soil. Our soils were unamended with sludge which would explain the lower degradation rate compared to the sludge amended soil, as usually sludge brings with it high levels of microorganisms so increasing biodegradation. Also our soils had initial EDDS concentrations of one to two orders of magnitude higher than the sludge amended soil discussed previously. It has been found, at least for unacclimatized sludge, the lower the initial EDDS concentration the faster the degradation (Schowanek et al., 1997). Having said this however although the degradation rate increased with decreasing EDDS concentration in the unamended soils and the initial EDDS concentrations where at least an order of magnitude greater than ours the degradation rates in Meer's experiment were similar to ours (Meers et al., 2005).

Degradation of EDDS followed a first order rate for the whole degradation period. Thus there appeared to be no influence of the changing speciation of EDDS complexes on degradation. This is despite the finding that some metal-EDDS complexes when isolated are non-biodegradable depending on the strength of their stability constants (Vandevivere et al., 2001b). It was found that this dependence was actually related to the amount of free EDDS present in solution. When metal-EDDS complexes were in equilibrium or pseudo-equilibrium with other metals, compounds and anions, this recalcitrance of complexes with high stability constants could be overcome (Vandevivere et al., 2001b). This appeared to be the case in soil solution as although NiEDDS increased as a percentage of total EDDS concentration at the end of the experiment, the actual NiEDDS concentration in all soils decreased showing that NiEDDS must have been degraded. This is in disagreement to the work of Vandevivere et al. (2001b) who showed that NiEDDS is not degraded by sewage sludge. Degradation can either be achieved by direct degradation of NiEDDS or by exchange of Ni with another metal and degradation of this metal-EDDS complex. However Ni is known to exchange very slowly. Our results clearly show that in soils low NiEDDS concentrations are not recalcitrant.

4.2. Speciation of EDDS and metals in soil solution

The speciation of the metals and EDDS in soil solution was influenced by the constantly decreasing EDDS concentration, the precipitation or adsorption of metals that were in surplus of the dissolved EDDS concentration, and the presence of DOM. Fig. 8 shows a plot of the sum of soluble Cu, Cd, Pb, Ni, Zn and Fe versus EDDS in solution. The decrease in metal concentrations with time is clearly coupled to the degradation of EDDS. It can be seen at high concentrations EDDS is in excess of soluble metals. Only at low concentrations of EDDS at the end of the experiment where DOM is playing a role are the soluble metals in excess to EDDS.

The polluting metals determined the initial EDDS speciation in soil solution after soil washing. Where Cu and Zn were high in the initial soil (soil 1) they dominated in the initial soil solution. Where Cu and Zn were lower or Zn was found with Pb which forms a much weaker complex with EDDS, Fe and Mn started to play a role in the soil solution (soils 2 and 3).

Regardless of the contaminating metals Fe and Mn played an important role in the second phase of speciation. Manganese's role may be an artefact of our experiment however. It seems strange that Mn is important in phase 2 while it's stability constant is much less than for Cu, Zn or most heavy metal EDDS complexes (Martell et al., 2001). This can be explained by the fact at the start of the experiment the soils were waterlogged and in turn may have become anaerobic. Mn concentrations increased two or more times between day 0 and day 7 for soils 1 and 2 and remained high for this time for soil 3. These concentrations were at least an order of magnitude higher than the concentrations in the following phase.

During this phase where Fe and Mn dominate, soluble Cu drops greatly but Zn does not. As CuEDDS is stronger than ZnEDDS it seems there must be an alternative explanation for this. Under anoxic conditions Cu and Zn sulphides can be formed (Dyrssen and Kremling, 1990). As CuS has a much lower solubility constant than ZnS (Dyrssen and Kremling, 1990) the formation of sulphides may explain why Zn remains in solution and Cu does not. As the biggest drop in Cu during the phase dominated by Fe and Mn is found for soil 1 which also has the largest water holding capacity and therefore has the potential to be more anoxic this also seems to back up this theory.

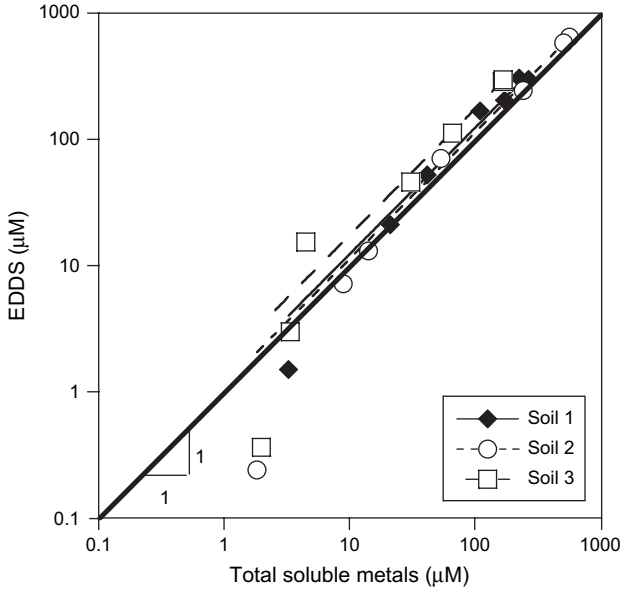

Fig. 8. Sum of soluble Cu, Cd, Ni, Pb, Zn and Fe verses soluble EDDS. Thick solid line is 1:1 line. Solid line soil 1 ($y = 1.2272x$, $R^2 = 0.972$), small dashed line soil 2 ($y = 1.1246x$, $R^2 = 0.998$), large dashed line soil 3 ($y = 1.7344x$, $R^2 = 0.999$) regression. All values are mean of 3 replicates.

The soil structure after soil washing was destroyed and initially slurry was present rather than a soil. With time the water evaporated and the soil became aerobic again so Mn(II) was oxidized again to Mn-oxide and precipitated. By the time this occurred the EDDS concentration was already much lower than the initial concentration due to biodegradation and the CuEDDS complex out-competed most other complexes. In this phase even when Cu in the initial soil was not high, CuEDDS dominated the speciation.

In the final phase NiEDDS dominated the speciation although Ni concentrations in the initial soils and throughout the experimental time were low. This final exchange was due to the DOM binding of Cu, making it less available for complexation with EDDS. In this phase the EDDS concentrations were very low and DOM became a competitor for heavy metals. Because Cu-binding by DOM is very strong, it out-competed EDDS. If binding by DOM were not taken into account during speciation then the majority of EDDS at the end of the experimental time would be thought to be CuEDDS and not NiEDDS. This shows it is very important to include DOM in speciation calculations especially at low EDDS and metal concentrations.

The high peaks of H-EDDS in Soil 3 over days 14–28 were a result of a large pH change in solution. Soil 3 is the most acid soil of the three soils investigated. Initially the soil solution was mildly alkaline due to the pH of the washing solution. Gradually it decreased to acid pH's between day 14 and 28 giving a huge rise in H-EDDS. At the same time there was an increase in CuEDDS as in the other soils. Following this time period the pH again changed to neutral so reducing H-EDDS.

Pb and Cd played a very limited role in EDDS speciation even in Soil 3 which had Pb contamination. This was primarily due to the low concentrations of these metals in solution which in turn was due to the low stability constants for these EDDS complexes (Martell et al., 2001). On the other hand EDDS played an important role in Pb and Cd speciation because in the early part of the experimental time most of the Pb and Cd were bound to EDDS. Only in the latter part of the experiment were most of the Cd and Pb bound to organic matter. Ca and Mg played a limited role through out the experimental time being mainly in the free form due to their weak complexes which they form with EDDS.

5. Conclusions

Residual EDDS from soil washing was degraded after a lag phase of 7–11 days with a half-life of 4.18–5.60 days. No influence of EDDS-speciation on the reaction rate was observed. The decrease in EDDS resulted in a corresponding decrease in

solubilized metals. Changes in EDDS speciation can be related to (1) initial composition of the soil, (2) temporarily anoxic conditions in the soil slurry after soil washing, (3) exchange of EDDS complexes with Cu even in soils without elevated Cu content and (4) finally formation of NiEDDS. DOM is important for metal speciation at low EDDS concentrations. Although considered recalcitrant, NiEDDS is also degraded with time. Our results show that even in polluted soils EDDS is degraded from a level of several hundred micromoles to below 1 μM within 50 days.

Acknowledgements

We thank Diederik Schowanek from Procter & Gamble and Raj Patel from Octel, U.K. for providing the S,S-EDDS. This work was funded in part by the Federal Office for Education and Science within COST Action 837 and the Swiss National Science Foundation in the framework of the Swiss Priority Program Environment. We thank Iso Christl for his help with ECOSAT and Werner Attinger and Anna Grünwald for their technical assistance.

References

Abbt-Braun, G., Frimmel, F.H., 2002. The relevance of reference materials – isolation and general characterization. In: Frimmel, F.H., Abbt-Braun, G., Heumann, K.G., Hock, B., Ludemann, H.D., Spiteller, M. (Eds.), Refractory Organic Substances in the Environment. Wiley-VCH, pp. 1–38.

Abumaizar, R., Khan, L.I., 1996. Laboratory investigation of heavy metal removal by soil washing. J. Air Waste Manag. Assoc. 46, 765–768.

Bucheli-Witschel, M., Egli, T., 2001. Environmental fate and microbial degradation of aminopolycarboxylic acids. FEMS Microbiol. Rev. 25, 69–106.

Dyrssen, D., Kremling, K., 1990. Increasing hydrogen-sulfide concentration and trace-metal behaviour in the anoxic baltic waters. Marine Chem. 30, 193–204.

Finzgar, N., Kos, B., Lestan, D., 2004. Washing of Pb contaminated soil using [S,S] ethylenediamine disuccinate and horizontal permeable barriers. Chemosphere 57, 655–661.

Frimmel, F.H., Abbt-Braun, G., 1999. Basic characterization of reference NOM from Central Europe – similarities and differences. Environ. Internat. 25, 191–207.

Garbisu, C., Alkorta, I., 2001. Phytoextraction: a cost-effective plant based technology for the removal of metals from the environment. Biores. Technol. 77, 229–236.

Grcman, H., Vodnik, D., Velikonja-Bolta, S., Lestan, D., 2003. Ethylenediamine dissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. J. Environ. Qual. 32, 500–506.

Hauser, L., Tandy, S., Schulin, R., Nowack, B., 2005. Column extraction of heavy metals from soils using the biodegradable chelating agent EDDS. Environ. Sci. Technol. 39, 6819–6824.

Jaworska, J.S., Schowanek, D., Feijtel, T.C.J., 1999. Environmental risk assessment for trisodium [SS]-ethylene diamine disuccinate, a biodegradable chelator used in detergent applications. Chemosphere 38, 3597–3625.

Keizer, M.G., van Riemsdijk, W.H., 1999. ECOSAT 4.7. Department of Environmental Sciences, Subdepartment of Soil Sciences and Plant Nutrition, Wageningen Agricultural University, Wageningen, Netherlands.

Kinniburgh, D.G., van Riemsdijk, W.H., Koopal, L.K., Borkovec, M., Benedetti, M.F., Avena, M.J., 1999. Ion binding to natural organic matter: competition, heterogeneity, stoichiometry and thermodynamic consistency. Colloids Surf. A 151, 147–166.

Kos, B., Lestan, D., 2003a. Induced phytoextraction/soil washing of Lead using biodegradable chelate and permeable barriers. Environ. Sci. Technol. 37, 624–629.

Kos, B., Lestan, D., 2003b. Influence of biodegradable (SS-EDDS) and non-degradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextracton and leaching. Plant Soil 253, 403–411.

Kos, B., Lestan, D., 2004a. Chelator induced phytoextraction and in situ soil washing of Cu. Environ. Pollut. 132, 333–339.

Kos, B., Lestan, D., 2004b. Soil washing of Pb, Zn and Cd using biodegradable chelator and permeable barriers and induced phytoextraction by *Cannabis sativa*. Plant Soil 263, 43–51.

Kos, B., Grcman, H., Lestan, D., 2003. Phytoextraction of lead, zinc and cadmium from soil by selected plants. Plant Soil Environ. 49, 548–553.

Luo, C., Shen, Z., Lia, X., 2005. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. Chemosphere 59, 1–11.

Maie, N., Watanabe, A., Taki, K., Yano, H., Kimura, M., 2004. Comparison of humus composition in the subsoil of Japanese paddy and upland fields. Geoderma 120, 309–323.

Martell, A.E., Smith, R.M., Motekaitis, R.J., 2001. NIST Critically Selected Stability Constants of Metal Complexes V6.0. NIST, Gaithersburg, USA.

Meers, E., Ruttens, A., Hopgood, M.J., Samson, D., Tack, F.M.G., 2005. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. Chemosphere 58, 1011–1022.

Milne, C.J., Kinniburgh, D.G., Van Riemsdijk, W.H., Tipping, E., 2003. Generic NICA-Donnan model parameters for metal-ion binding by humic substances. Environ. Sci. Technol. 37, 958–971.

Orama, M., Hyvonen, H., Saarinen, H., Aksela, R., 2002. Complexation of [S,S] and mixed stereoisomers of *N,N′*-ethylenediaminedisuccinic acid (EDDS) with Fe(III), Cu(II), Zn(II) and Mn (II) ions in aqueous solution. J. Chem. Soc. Dalton Trans. 24, 4644–4648.

Peters, R.W., 1999. Chelate extraction of heavy metals from contaminated soils. J. Hazard. Mater. 66, 151–210.

Schowanek, D., Feijtel, T.C.J., Perkins, C.M., Hartman, F.A., Federle, T.W., Larson, R.J., 1997. Biodegradation of [S,S], [R,R] and mixed stereoisomers of ethylene diamine disuccinic acid (EDDS), a transition metal chelator. Chemosphere 34, 2375–2391.

Takahashi, R., Fujimoto, N., Suzuki, M., Endo, T., 1997. Biodegradabilities of ethylenediamine-*N*, *N′*-disuccunic acid (EDDS) and other chelating agents. Biosci. Biotechnol. Biochem. 61, 1957–1959.

Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R., Nowack, B., 2004. Extraction of heavy metals from soils using biodegradable chelating agents. Environ. Sci. Technol. 38, 937–944.

Tandy, S., Schulin, R., Nowack, B. The influence of SS-EDDS on the uptake of heavy metals in hydroponically grown sunflowers. Chemosphere, in press, doi:10.1016/j.chemosphere.2005.06.005.

Tandy, S., Schulin, R., Suter, M.J.F., Nowack, B., 2005. Determination of [S,S′]-ethylenediamine disuccinic acid (EDDS) by high performance liquid chromatography after derivatization with FMOC. J. Chromatogr. A 1077, 37–43.

Thayalakumaran, T., Robinson, B., Vogeler, I., Scotter, D., Clothier, B., Percival, H., 2003. Plant uptake and leaching of copper during EDTA-enhanced phytoremediation of repacked and undisturbed soil. Plant Soil 254, 415–423.

Van Benschoten, J.E., Matsumoto, M.R., Young, W.H., 1997. Evaluation and analysis of soil washing for seven lead-contaminated soils. J. Environ. Eng. 123, 217–224.

Vandevivere, P., Hammes, F., Verstraete, W., Feijtel, W., Schowanek, D., 2001a. Metal decontamination of soil, sediment and sewage sludge by means of transition metal chelate [S,S]-EDDS. J. Environ. Eng. 127, 802–811.

Vandevivere, P., Saveyn, H., Verstraete, W., Feijtel, W., Schowanek, D., 2001b. Biodegradation of metal-[S,S]-EDDS complexes. Environ. Sci. Technol. 35, 1765–1770.

Wenzel, W.W., Unterbrunner, R., Sommer, P., Sacco, P., 2003. Chelate-assisted phytoextraction using canola (*Brassica napus* L.) in outdoors pot and lysimeter experiments. Plant Soil 249, 83–96.

Wu, J., Hsu, F., Cunningham, S.D., 1999. Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints. Environ. Sci. Technol. 33, 1898–1904.

Wu, L.H., Luo, Y.M., Xing, X.R., Christie, P., 2004. EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. Agric. Ecosyst. Environ. 102, 307–318.