

## Comparison of Natural Humic Substances and Synthetic Ethylenediaminetetraacetic Acid and Nitrilotriacetic Acid as Washing Agents of a Heavy Metal–Polluted Soil

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Ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), and other synthetic polycarboxylic acids have been shown to possess substantial capacity as washing agents of heavy metal–polluted soils, but they are environmentally problematic. Therefore, a sample of natural soluble humic substances (HS) was tested as a possible substitute. The efficiency of HS to extract cadmium (Cd), copper (Cu), and lead (Pb) from a strongly polluted calcareous urban soil was compared with that of EDTA and NTA. The influence of extractant concentration (25–100 mmol L<sup>-1</sup> C), solution/soil ratio (5–100 L kg<sup>-1</sup>), and single-step vs. multistep extraction on heavy metal removal from the soil was investigated. The extracted pools were assessed by sequential extraction. Ethylenediaminetetraacetic acid and NTA extracted up to 86, 77, and 30% of total soil Cd, Cu, and Pb, respectively, whereas HS extracted 44, 53, and 4%. Extracted amounts of Cd, Cu, and Pb increased with increasing extractant concentration and solution/soil ratio in the range 5 to 100 L kg<sup>-1</sup>. Single-step extraction removed about the same amounts of the three metals as multiple-step extraction. The metal-extracted pools of the soil depended on the metal and on the extractant. The overall conclusion is that soluble HS can replace synthetic EDTA and NTA as washing agents for Cd- and Cu-polluted soils, whereas HS is not a promising substitute of EDTA or NTA for cleaning Pb-polluted, calcareous soils.

HEAVY METAL–POLLUTED SOILS cause serious environmental problems. Twenty-five percent of the 24,000 registered polluted sites in Denmark are polluted with heavy metals, and, together with mineral oil, heavy metals account for the most frequent soil pollution in the European Union and elsewhere (Tandy et al., 2004; European Environment Agency, 2007; Leštan et al., 2008). Because soil pollutions threaten human health and ecosystem functioning, the polluted soils should be cleaned, but cleaning of heavy metal–polluted soils is difficult because metals are nondegradable and often strongly sorbed to soil solids (Sun et al., 2001; Meers et al., 2007; Dermont et al., 2008; Jensen et al., 2009). For moderately polluted soils, in situ phytoremediation is an environmentally attractive but time-consuming solution (Evangelou et al., 2007; Jensen et al., 2009). For severely polluted soils, a possible operational solution is soil washing by ex situ extraction or in situ flushing with aqueous solutions containing a strong ligand (e.g., ethylenediaminetetraacetic acid [EDTA], nitrilotriacetic acid [NTA], or similar harsh compounds), although other cleaning or stabilization methods exist (Sun et al., 2001; Di Palma et al., 2003; Kim et al., 2003; Lim et al., 2005; Ehsan et al., 2006; Dermont et al., 2008; Leštan et al., 2008; Rao et al., 2008).

Ethylenediaminetetraacetic acid, NTA, and other polycarboxylic acids have been shown to possess substantial capacity as washing agents of polluted soils, but they are environmentally problematic (Grěman et al., 2001; Bianchi et al., 2008; Dermont et al., 2008). Therefore, replacement of these synthetic chemicals with soluble humic substances (HS) might be attractive because these natural compounds can support soil structure and stimulate plant growth (Lulakis and Petsas, 1995; Barona et al., 2001; Conte et al., 2005; Evangelou et al., 2007; Bianchi et al., 2008; Dermont et al., 2008; Leštan et al., 2008). Humic substances are mainly formed by microbial degradation of organic residues but are also formed through abiotic processes and the help of various insects. They consist of a complex mixture of small soluble molecules, e.g., organic acids, and large insoluble organic compounds (Stevenson and Cole, 1999).

Carboxylic acids and phenolic groups in HS are responsible for complex formation with heavy metals (Weng et al., 2002;

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**Abbreviations:** DOC, dissolved organic carbon; EDTA, ethylenediaminetetraacetic acid; HS, humic substances; NTA, nitrilotriacetic acid; SOM, soil organic matter.

Halim et al., 2003; Han and Thompson, 2003; Strobel et al., 2005; MacDonald and Hendershot, 2006; Zhao et al., 2007). Interaction between HS and heavy metals can change the solubility of the heavy metals in the soil. Thus, soluble HS, also termed dissolved organic carbon (DOC) or dissolved organic matter, normally forms soluble HS–heavy metal complexes, whereas solid-phase HS, such as humic acid, tend to reduce heavy metal solubility (Weng et al., 2002; Halim et al., 2003; Han and Thompson, 2003; Evangelou et al., 2004; Strobel et al., 2005; Zhao et al., 2007; Bianchi et al., 2008).

Although soluble HS seem to possess substantial capacity to extract heavy metals from soils, few studies have been published on the use of soluble HS for washing heavy metal–polluted soils (Steponkaite et al., 2008; Borggaard et al., 2009). Therefore, the aim of this paper is to present the results obtained by comparing the efficiency of soluble HS with that of the commonly used EDTA and NTA as washing agents (i.e., as extractants of cadmium [Cd], copper [Cu], and lead [Pb]) from a strongly polluted calcareous, long-term equilibrated (nonspiked) urban soil. The influence of the concentration of the washing agents and of the solution/soil ratio in multiple-step and single-step extraction mode was tested, and the soil-extracted pools of heavy metals were assessed by sequential extraction analysis.

## Materials and Methods

### Soil and Humic Substances

The used soil was a calcareous soil strongly polluted with Cd, Cu, and Pb deposited at a landfill site at Copenhagen Recycling Centre (Jensen et al., 2009). The soil was classified as an Urbic Technosol (Anthrotoxic) according to the WRB soil classification system (IUSS Working Group WRB, 2006). The soil was air-dried, sieved (2 mm), and analyzed with three replications. It contained  $10 \pm 1\%$  clay,  $16 \pm 2\%$  silt, and  $74 \pm 4\%$  sand as determined by the combined hydrometer and sieving methods (Day, 1965). The pH was  $7.5 \pm 0.2$  as determined in a 1:2.5 suspension of soil in  $0.01 \text{ mol L}^{-1} \text{ CaCl}_2$  (Allison and Moodie, 1965). The calcium carbonate content was  $5.8 \pm 0.3\%$  as determined volumetrically by a calcimeter (Allison and Moodie, 1965), and the organic carbon content was  $1.4 \pm 0.1\%$  as measured by dry combustion at  $1250^\circ\text{C}$  in oxygen (ELTRA, 1995) and corrected for the sample's content of  $\text{CaCO}_3$ . Total heavy metals were determined by inductively coupled plasma–optical emission spectrometry in the digest obtained by boiling the soil for 8 h with  $7.3 \text{ mol L}^{-1} \text{ HNO}_3$  (Tjell and Hovmand, 1978), resulting in  $15.6 \pm 2.3 \text{ mg kg}^{-1} \text{ Cd}$ ,  $1000 \pm 120 \text{ mg kg}^{-1} \text{ Cu}$ ,  $8230 \pm 580 \text{ mg kg}^{-1} \text{ Fe}$ , and  $500 \pm 70 \text{ mg kg}^{-1} \text{ Pb}$ . Total metal is based on the partial destruction with strong  $\text{HNO}_3$  as alternative to a total destruction, including the metal bound in the silicate matrices because this fraction of metal is environmentally unavailable even over many decades.

Soluble HS was prepared by oxidative hydrolytic degradation of alkaline cow slurry heated under pressure and with limited oxygen inlet for a few hours as described by BioCorrection (2009) (Popov et al., 2002). The pH of the product was  $8.0 \pm 0.2$ , and the soluble HS contained  $0.6 \pm 0.1 \text{ mol L}^{-1} \text{ DOC}$  as quantified by a TOC-500 Total Organic Carbon Analyzer (Shimadzu, Japan). Total heavy metal concentrations were determined by inductively coupled plasma–optical emission spectrometry and

showed that the HS solution contained only  $0.3 \text{ } \mu\text{g L}^{-1} \text{ Cd}$ ,  $17 \text{ } \mu\text{g L}^{-1} \text{ Cu}$ , and  $25 \text{ } \mu\text{g L}^{-1} \text{ Pb}$ . Potentiometric titration was used to determine the contents of carboxylic acid groups (COOH) and phenolic groups (Ar-OH), where COOH was taken as titratable acidity between pH 3 and 7 and Ar-OH was taken as titratable acidity between pH 7 and 11 (Strobel et al., 2001). Accordingly, the HS contained  $100 \pm 8 \text{ mmol COOH}$  and  $70 \pm 6 \text{ mmol Ar-OH}$  per mol C. For comparison, EDTA contains  $400 \text{ mmol COOH}$  and NTA  $500 \text{ mmol COOH}$  per mol C (i.e., when concentrations are expressed as  $\text{mmol L}^{-1} \text{ DOC}$ , EDTA and NTA contain 4 and 5 times more COOH than HS, respectively, but EDTA and NTA have no Ar-OH groups).

### Extractions

The heavy metals in the soil were extracted in the batch mode by multiple-step extraction and by single-step extraction. Sequential extraction was also performed to evaluate the effectiveness of the extractions with the three extractants. In the multiple-step extraction, 2.5 g of soil was extracted with 25 mL extractant solution in 50-mL centrifuge tubes with screw caps in an end-over-end agitator ( $16 \text{ rpm min}^{-1}$ ) for 24 h. This extraction time was chosen because a preliminary investigation showed the extraction of virtually the same amount of heavy metals after 24 h agitation as after 1 wk. Furthermore, extraction for 24 h is commonly used (Barona et al., 2001; Tandy et al., 2004; Finžgar and Leštan, 2007; Dermont et al., 2008). The extractants comprised EDTA, NTA, and soluble HS at three different concentrations, including 25, 50, and  $100 \text{ mmol L}^{-1} \text{ DOC}$  (e.g.,  $25 \text{ mmol L}^{-1} \text{ DOC}$  corresponds to  $2.5 \text{ mmol L}^{-1} \text{ EDTA}$  and  $4.2 \text{ mmol L}^{-1} \text{ NTA}$  because EDTA contains 10 C atoms and NTA contains six C atoms). The extractant solutions were adjusted to pH 6.0 in  $0.05 \text{ mol L}^{-1} \text{ KNO}_3$ , which was also used as control. After centrifugation at  $3000 \times g$  for 10 min, two 10-mL portions of clear supernatant were withdrawn to two 15-mL test tubes with and without 0.5 mL concentrated  $\text{HNO}_3$  for determination of Ca, Cd, Cu, and Pb concentrations and pH and DOC concentration, respectively. Then 20 mL of fresh extractant solution was added to the centrifuge tube, and the extraction procedure with the EDTA, NTA, and soluble HS extractants was repeated nine more times as described. In the nonacidified extracts, pH and DOC was determined as described above. The concentrations of Ca, Cd, Cu, and Pb were determined in the acidified extracts by means of atomic absorption spectrometry using flame (3300; PerkinElmer, Germany) or graphite furnace (Zeeman 5100 equipped with an autosampler AS-60; PerkinElmer) atomization depending on the concentrations.

Accumulated metal releases ( $Y_n$ ) in  $\text{mg kg}^{-1}$  were calculated by the following formula:

$$Y_n = 10 \times X_n - 2 \times X_{n-1} + Y_{n-1}$$

where X is the metal concentration in  $\text{mg L}^{-1}$ , and n is number of extractions (days). The term  $2 \times X_{n-1}$  accounts for the metal content in the 5-mL solution per 2.5 g of soil left over from the previous extraction.

In the single-step extraction, 2.5 g of soil was extracted with 12.5, 25, 50, 100, 200, or 250 mL of  $50 \text{ mmol L}^{-1} \text{ DOC}$  extractant solution in an appropriate centrifuge tube in an end-over-end agitator ( $16 \text{ rpm min}^{-1}$ ) for 24 h. The extractant

solutions comprised EDTA, NTA, and soluble HS in 0.05 mol L<sup>-1</sup> KNO<sub>3</sub> as well as 0.05 mol L<sup>-1</sup> KNO<sub>3</sub> as control, all adjusted to pH 6.0. After centrifugation at 3000 × *g* for 10 min, metal concentrations, pH, and DOC were determined in the clear supernatant as described above.

Sequential extraction of the heavy metals was performed after multiple- or single-step extractions and washing of the soil residue in the centrifuge tube with three 20-mL portions of 0.05 mol L<sup>-1</sup> KNO<sub>3</sub>, which were discarded after centrifugation (3000 × *g* for 10 min). The sequential extraction was a slight modification of the method suggested by Tessier et al. (1979) comprising four fractions: (i) exchangeable metals determined by extraction for 1 h with 20 mL 1 mol L<sup>-1</sup> CaCl<sub>2</sub>; (ii) carbonate-bound metals determined by extraction for 4 h with 20 mL 1 mol L<sup>-1</sup>

acetate buffer at pH 5; (iii) oxide-bound metals determined by extraction for 0.5 h at 70°C with a mixture of 20 mL 0.3 mol L<sup>-1</sup> Na-citrate, 5 mL 1 mol L<sup>-1</sup> NaHCO<sub>3</sub>, and 1 g Na-dithionite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>); (iv) residual metals were determined by boiling the soil residue with 20 mL 7.3 mol L<sup>-1</sup> HNO<sub>3</sub> for 4 h. After each treatment, the suspension was centrifuged at 3000 × *g* for 10 min, the supernatant was removed by a pipette, and the metal concentrations were determined by flame atomic absorption spectrometry or graphite furnace atomic absorption spectrometry as described above. Between each treatment the soil residue was washed carefully with 0.05 mol L<sup>-1</sup> KNO<sub>3</sub>. All chemicals were of analytical grade or ultra pure. The water was triple deionized, and all glassware was acid washed before use.

## Statistical Analysis

All the extractions and soil analysis were performed as triplicates. Statistical analysis of the data was performed in Microsoft Office Excel, and comparisons between means were performed using the LSD test using SAS 9.1.2 software package at the significance level of *P* < 0.05 (SAS Institute, 2004).

## Results and Discussion

### Multistep Extraction

Although the pH of the extractants was adjusted to 6.0 before use, the pH of the extracts was about 8 (7.6–8.3) because of the high buffer capacity of the calcareous soil. The only exception was the strongest EDTA extracts (100 mmol L<sup>-1</sup> DOC), where pH gradually decreased to 6.3 during the last three extractions due to dissolution of the calcium carbonate as shown by extraction of 2.3% Ca (corresponding to 5.8% CaCO<sub>3</sub>). The DOC concentration of all extracts was very close to the initial concentration (25, 50, or 100 mmol L<sup>-1</sup>), indicating negligible precipitation of extractant DOC or dissolution of soil organic matter (SOM).

The accumulated amounts of Cu extracted during 10 extractions by EDTA, NTA, and HS are shown in Fig. 1. Similar shapes

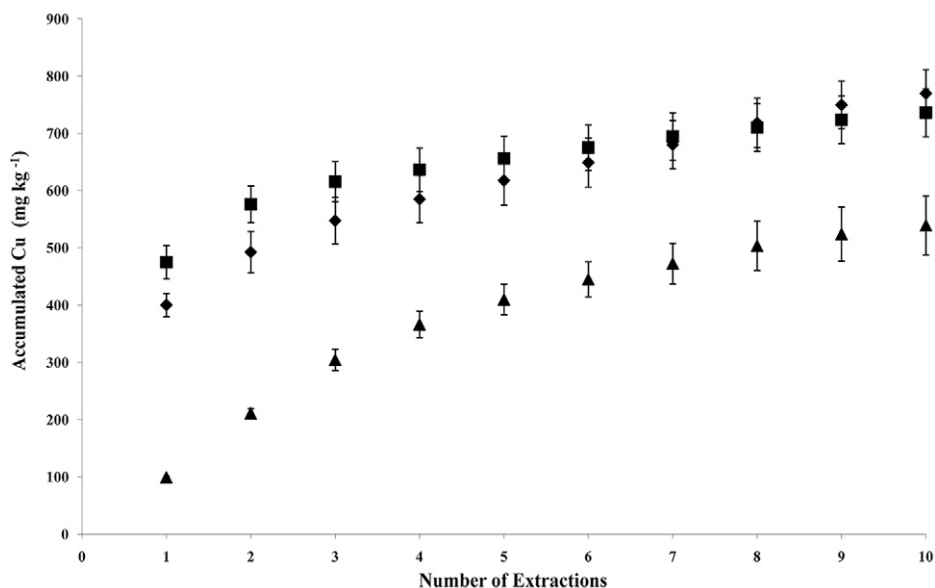


Fig. 1. Multistep extraction showing accumulated Cu extracted by 50 mmol L<sup>-1</sup> dissolved organic C as ethylenediaminetetraacetic acid (diamonds), nitrilotriacetic acid (squares), and humic substances (triangles). Standard deviations shown by vertical bars.

of the accumulated extraction curves were found for Cd and Pb (results not shown). Ethylenediaminetetraacetic acid and NTA extracted almost the same amounts of the three metals throughout the 10 extractions, and extraction by EDTA and NTA was more efficient than by HS. Nearly 50% of metals were extracted by using EDTA and NTA in the first step, whereas small amounts of the three metals were extracted by HS in the first extraction. Accumulated extractions gradually increased, resulting in HS curves that approached those for EDTA and NTA.

Accumulated amounts of Cd, Cu, and Pb extracted after 10 extractions with EDTA, NTA, and HS at different concentrations (25, 50, or 100 mmol L<sup>-1</sup> DOC) are shown in Table 1. As also indicated for Cu in Fig. 1, EDTA and NTA

Table 1. Accumulated cadmium, copper, and lead obtained after 10 extractions by ethylenediaminetetraacetic acid, nitrilotriacetic acid, and humic substances. Extractant concentrations are expressed as dissolved organic carbon.

Extractant†	Cadmium	Copper	Lead
	mg kg <sup>-1</sup>		
EDTA, mmol L <sup>-1</sup>			
25	11.3 ± 0.2 <sup>‡</sup>	531 ± 31 <sup>c</sup>	127 ± 7 <sup>de</sup>
50	12.6 ± 1.1 <sup>ab</sup>	649 ± 27 <sup>b</sup>	136 ± 8 <sup>dc</sup>
100	13.6 ± 0.6 <sup>a</sup>	770 ± 42 <sup>a</sup>	159 ± 11 <sup>ab</sup>
NTA, mmol L <sup>-1</sup>			
25	11.6 ± 0.9 <sup>bc</sup>	533 ± 21 <sup>c</sup>	115 ± 11 <sup>e</sup>
50	11.9 ± 0.6 <sup>bc</sup>	649 ± 97 <sup>b</sup>	147 ± 20 <sup>bc</sup>
100	13.2 ± 0.9 <sup>a</sup>	736 ± 42 <sup>ab</sup>	174 ± 20 <sup>a</sup>
HS, mmol L <sup>-1</sup>			
25	3.8 ± 0.1 <sup>e</sup>	412 ± 64 <sup>d</sup>	17 ± 3 <sup>f</sup>
50	5.9 ± 0.2 <sup>d</sup>	539 ± 51 <sup>c</sup>	18 ± 1 <sup>f</sup>
100	6.8 ± 0.6 <sup>d</sup>	530 ± 64 <sup>c</sup>	18 ± 4 <sup>f</sup>
Control	0.3 ± 0.1 <sup>f</sup>	10 ± 4 <sup>e</sup>	0.2 ± 0.1 <sup>f</sup>

† EDTA, ethylenediaminetetraacetic acid; HS, humic substances; NTA, nitrilotriacetic acid.

‡ Values are means ± SD. Different letters in each column represent statistical differences (*p* < 0.05) by the LSD test.

extracted almost the same amounts of the three metals, whereas less was extracted by HS. Extracted Cd, Cu, and Pb increased with increasing extractant concentration, but the increases were modest, especially for EDTA and NTA. Increased extraction with increasing EDTA and NTA concentration is in accordance with previous observations (Kim et al., 2003; Tandy et al., 2004; Zou et al., 2009). Furthermore, as shown elsewhere (Barona et al., 2001; Tandy et al., 2004; Lim et al., 2005; Meers et al., 2007; Ehsan et al., 2006; Dermont et al., 2008), the amounts of extracted heavy metals also depended on the element. Thus, EDTA and NTA extracted up to 86% of total soil Cd, 75% of total soil Cu, and 30% of total soil Pb, whereas the corresponding percentages for HS-extractable metal were 44, 53, and 4%, respectively (Table 1). This difference may indicate weaker bonding to the soil solids of Cd and Cu than of Pb because Pb is mineral bound (e.g., in  $\text{PbCO}_3$ ) or because of the higher affinity of Pb (or Pb complexes) to the soil solids as suggested by Meers et al. (2007) in their study of heavy metal mobilization by EDTA from a calcareous soil. Furthermore, as shown by Kim et al. (2003), the extractability of Pb strongly depends on the origin of the polluted sample. Accordingly, Pb extracted by EDTA or NTA is typically low in neutral to alkaline soils, such as calcareous soils that have been polluted for long time (Kim et al., 2003; Tandy et al., 2004).

### Single-Step Extraction

Extracted amounts ( $\text{mg kg}^{-1}$ ) of Cd, Cu, and Pb increased with increasing solution/soil ratio (5–100  $\text{L kg}^{-1}$ ), as illustrated for Cu in Fig. 2. Humic substances exhibited the largest relative increase from lowest to highest solution/soil ratio, but the amounts extracted by HS were lower than the amounts extracted by EDTA and NTA. Whereas HS-extracted Cu was very modest at a solution/soil ratio of 5  $\text{L kg}^{-1}$ , the amounts extracted by EDTA and NTA at this ratio were about half the amounts extracted at the highest solution/soil ratio (100  $\text{L kg}^{-1}$ ). Comparing Fig. 1 and 2 (both at 50  $\text{mmol L}^{-1}$  DOC) shows that nearly the same amount of Cu is extracted after 10 extractions (Fig. 1) and at a solution/soil ratio of 100  $\text{L kg}^{-1}$  (Fig. 2). This similarity might be expected because the overall accumulated solution/soil ratio is also 100  $\text{L kg}^{-1}$  in the multistep extractions after 10 extractions, where each step had a solution/soil ratio of 10  $\text{L kg}^{-1}$ . From a remediation point of view, this result is useful in as much as multistep extraction can be replaced by a single-step extraction, which is less laborious and time consuming. On the other hand, in a different experimental set-up, multistep extraction (leaching) was reported to result in the extraction of more Pb and Zn by EDTA than

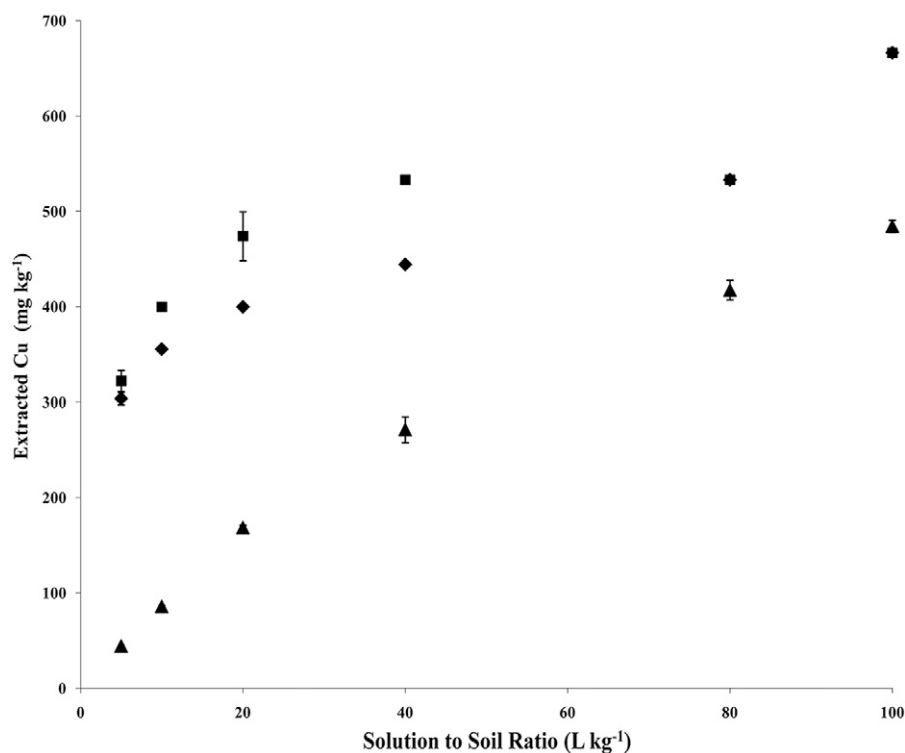


Fig. 2. Single-step extraction showing the amounts of Cu extracted by 50  $\text{mmol L}^{-1}$  dissolved organic C as ethylenediaminetetraacetic acid (diamonds), nitrilotriacetic acid (squares), and humic substances (triangles) versus the solution/soil ratio ( $\text{L kg}^{-1}$ ). Standard deviations shown by vertical bars.

single-step extraction but only from three of the four soils tested (Finžgar and Leštan, 2007). Furthermore, Poletini et al. (2009) and Zou et al. (2009) also found multistep extraction more effective than single-step extraction, but also in this case comparison with the current investigation is difficult because of different EDTA concentrations, soils, and experimental designs.

The observed dependency between the amount of extracted heavy metal and the solution/soil ratio indicates the influence of the chelant to heavy metal concentration ratio rather than to a volume-to-weight effect. Kim et al. (2003) found no effect of the solution/soil ratio in the range of 3:1 to 10:1  $\text{L kg}^{-1}$  on EDTA extraction of Pb. However, the importance of the molar ratio between chelant and metal has frequently been shown for extraction of various heavy metals by EDTA, NTA, and other synthetic chelants; the higher chelant/metal ratio, the more metal is extracted (Kim et al., 2003; Tandy et al., 2004; Meers et al., 2007; Finžgar and Leštan, 2007; Dermont et al., 2008; Zou et al., 2009).

In the present study, the molar ratio between the chelant taken as  $\text{mmol DOC}$  (50  $\text{mmol L}^{-1}$ ) to  $\text{mmol Cd} + \text{Cu} + \text{Pb}$  (18.28  $\text{mmol kg}^{-1}$ ) ranges from 14 to 270 (solution/soil ratio = 5–100  $\text{L kg}^{-1}$ ), indicating a substantial chelant surplus. However, for EDTA and NTA, a 50  $\text{mmol L}^{-1}$  DOC solution corresponds to 5  $\text{mmol L}^{-1}$  EDTA and 8.3  $\text{mmol L}^{-1}$  NTA, respectively, and as EDTA and NTA form 1:1 complexes with divalent metal cations (Martell et al., 2004), the stoichiometric chelant/metal ratio is 1.4–27 for EDTA and 2.3–45 for NTA, still indicating chelant surplus. This surplus vanishes when taking complexation between the chelants and  $\text{Ca}^{2+}$  from the



calcium carbonate into consideration because it affects heavy metal extraction (Sun et al., 2001; Kim et al., 2003; Meers et al., 2007; Dermont et al., 2008). Thus, 5.8% CaCO<sub>3</sub> corresponds to 580 mmol kg<sup>-1</sup> Ca, resulting in the following stoichiometric ratios:

$$\frac{\text{EDTA}}{\text{Ca} + \text{Cd} + \text{Cu} + \text{Pb}} = 0.04 \text{ to } 0.84$$

and

$$\frac{\text{NTA}}{\text{Ca} + \text{Cd} + \text{Cu} + \text{Pb}} = 0.07 \text{ to } 1.4$$

These ratios indicate chelant shortage, except for the largest NTA solution/soil ratio. Furthermore, EDTA and NTA form stronger complexes with Fe than with any of the other four metals (Table 2), indicating even lower chelant/metal ratios. However, total Fe in the soil is modest at 8230 mg kg<sup>-1</sup>, corresponding to 147 mmol kg<sup>-1</sup>, and only a small part will dissolve by extraction of this calcareous soil where Fe occurs as heavily soluble oxides even at equilibrium (Borggaard, 1976). Despite chelant shortage, the EDTA and NTA solution extracts Cd, Cu, and Pb from the soil because EDTA and NTA form more stable complexes with the heavy metals than with Ca (Table 2). The extraction efficiency was, however, reduced in the calcareous soil in accordance with previous observations (Barona et al., 2001; Tandy et al., 2004; Dermont et al., 2008).

Stoichiometric ratios cannot be calculated for HS due to lack of knowledge about HS structure (Stevenson and Cole, 1999). The smaller content of carboxylic groups in HS (100 mmol COOH per mol C) compared with 400 and 500 mmol COOH mol C<sup>-1</sup>, in EDTA and NTA, respectively, indicates that the stoichiometric ratios are well below 1 in all HS extractions. Accordingly, less heavy metal is extracted by HS than by EDTA and NTA (discussed below).

## Sequential Extraction

By the sequential extraction, the Cd, Cu, and Pb contents of the soil were partitioned into specific but operationally defined pools with increasing bonding strength: exchangeable < carbonate-bonded < oxide-bonded < residual metal (Tessier et al., 1979; Barona et al., 2001; Sun et al., 2001; Rao et al., 2008; Borggaard et al., 2009). In relation to soil washing, sequential extraction can be used to assess the fraction(s) removed by the extraction; ideally the weakest bonded metal fraction(s), which are the most available/mobile pools, should be removed by the

extraction (Barona et al., 2001; Tandy et al., 2004; Borggaard et al., 2009).

According to the sequential extraction results after extraction with 0.05 mol L<sup>-1</sup> KNO<sub>3</sub> (control), containing virtually the original heavy metal content (Table 1), the distribution of Cd, Cu, and Pb between the four fractions is different, which is in agreement with earlier observations (Barona et al., 2001; Sun et al., 2001; Ehsan et al., 2006; Dermont et al., 2008). Thus, total Cu is almost equally distributed between the carbonate-bound, oxide-bound, and residual fractions (i.e., exchangeable Cu is small). In contrast, exchangeable Cd is the largest pool, followed by carbonate-bound Cd, and the residual fraction dominates for Pb (Table 3). The relatively high carbonate-bound fractions may be ascribed to the calcium carbonate content and high pH of the soil, in accordance with previous studies (Barona et al., 2001; Ehsan et al., 2006; Meers et al., 2007).

The results of the sequential extraction of the residues after multistep extraction of Cu by 25, 50, and 100 mmol L<sup>-1</sup> DOC of the three extractants are shown in Table 4, and the partitioning of the three metals after the single-step extraction with 50 mmol L<sup>-1</sup> DOC as EDTA, NTA, and HS is shown in Table 3. From a soil washing point of view, the results in Tables 3 and 4 are not very informative (i.e., encouraging) except for Cu (Table 4), where the exchangeable fraction remains very low and both the carbonate-bound and oxide-bound fractions are strongly reduced by the three extractants compared with the control, in agreement with previous results (Borggaard et al., 2009). In contrast, exchangeable Cd and Pb remain high after extraction with EDTA, NTA, and HS, and, although the other Cd and Pb fractions are lower after extraction, substantial carbonate-bound and oxide-bound fractions persist. These rather complex findings are in agreement with similarly inconclusive results obtained by applying sequential extraction after EDTA extraction of various soils (Barona et al., 2001; Sun et al., 2001), indicating the difficulty in generalizing about the relative mobility (extractability) of the different fractions resulting from sequential extraction.

## Comparison of Extractants

According to Table 1, the efficiency of the three extractants to extract Cd, Cu, and Pb decreases in the order: EDTA ≈ NTA > HS, with a very great difference between the efficiency of EDTA and NTA to extract Pb compared with HS,

**Table 2. Stability constants (logK<sub>f</sub>) and conditional constants at pH 7 (logK<sub>7</sub>) for the formation of relevant metals complexes with ethylenediamine-tetraacetic acid, nitrilotriacetic acid, and humic substances.**

Chelant†	Ca <sup>2+</sup>		Cd <sup>2+</sup>		Cu <sup>2+</sup>		Fe <sup>3+</sup>		Pb <sup>2+</sup>	
	logK <sub>f</sub>	logK <sub>7</sub>	logK <sub>f</sub>	logK <sub>7</sub>	logK <sub>f</sub>	logK <sub>7</sub>	logK <sub>f</sub>	logK <sub>7</sub>	logK <sub>f</sub>	logK <sub>7</sub>
EDTA‡	10.7	5.1	16.5	11.0	18.8	13.2	25.1	19.6	18.0	12.5
NTA‡	6.4	5.9	9.8	11.8	13.0	14.0	16.0	20.4	11.5	13.3
HS§	2.9	-0.1	3.3	0.3	4.9	1.9	7.7	-	5.2	2.2

† EDTA, ethylenediaminetetraacetic acid; HS, humic substances; NTA, nitrilotriacetic acid.

‡ The stability constants and the acid dissociation constants used for calculating the conditional constants at pH 7 and 50 mmol L<sup>-1</sup> dissolved organic C (DOC) are for 0.1 mol L<sup>-1</sup> ionic strength and 25°C from Martell et al. (2004). The conditional constants K<sub>7</sub> = K<sub>f</sub> × [Y<sup>n-</sup>] = [MeY<sup>-(n-m)}</sup>]/[Me<sup>m+</sup>], where [Y<sup>n-</sup>] was calculated by means of the total concentration of EDTA (0.005 mol L<sup>-1</sup>) or NTA (0.0083 mol L<sup>-1</sup>) and the acid dissociation constants for EDTA or NTA.

§ The HS stability constants are derived from and the conditional constants at pH 7 and 50 mmol L<sup>-1</sup> DOC calculated by Visual Minteq using the Gaussian DOC model (Gustafsson, 2007; Grimm et al., 1991).

**Table 3. Percentages of total cadmium, copper, and lead obtained by single-step extraction at a solution/soil ratio of 100 by 50 mmol L<sup>-1</sup> dissolved organic carbon of ethylenediaminetetraacetic acid, nitrilotriacetic acid, and humic substances and by the control (0.05 mol L<sup>-1</sup> KNO<sub>3</sub>) followed by sequential extraction partitioning the remaining heavy metals in four fractions: exchangeable, carbonate-bound, oxide-bound and residual. Removed metal expressed as percentage of total cadmium (15.6 mg kg<sup>-1</sup>), total copper (1000 mg kg<sup>-1</sup>), and total lead (500 mg kg<sup>-1</sup>). Coefficients of variation on sequential extraction results are 5 to 10%.**

Metal/ extractant†	Extracted	Exchangeable	Carbonate- bound	Oxide- bound	Residual
<b>Cd</b>					
EDTA	71	16	5.7	4.4	10
NTA	71	17	6.6	5.2	11
HS	34	26	20	5.2	17
Control	1.2	40	36	4.4	18
<b>Cu</b>					
EDTA	63	0.7	7.2	1.7	32
NTA	63	0.7	8.7	1.3	38
HS	46	0.6	13	3.7	41
Control	0.2	0.5	38	19	42
<b>Pb</b>					
EDTA	31	6.2	2.7	2.8	51
NTA	32	6.4	3.8	2.9	55
HS	3.9	7.8	15	4.2	68
Control	0.0	6.6	14	3.2	79

† EDTA, ethylenediaminetetraacetic acid; HS, humic substances; NTA, nitrilotriacetic acid.

**Table 4. Accumulated copper obtained after 10 extractions by ethylenediaminetetraacetic acid, nitrilotriacetic acid, and humic substances at various concentrations and control (0.05 mol L<sup>-1</sup> KNO<sub>3</sub>) followed by sequential extraction partitioning the remaining copper in four fractions: exchangeable, carbonate-bound, oxide-bound and residual. Removed copper expressed as percentage of total soil copper (1000 mg kg<sup>-1</sup>). Coefficients of variation on sequential extraction results are 5 to 10%.**

Extractant†	Extracted	Exchangeable	Carbonate- bound	Oxide-bound	Residual
<b>EDTA, mmol L<sup>-1</sup></b>					
25	53	0.46	5.0	1.72	37
50	65	0.41	4.4	1.41	25
100	77	0.46	2.7	0.99	23
<b>NTA, mmol L<sup>-1</sup></b>					
25	53	0.64	8.3	1.61	36
50	65	0.58	6.8	1.56	30
100	74	0.47	3.1	0.87	25
<b>HS, mmol L<sup>-1</sup></b>					
25	41	0.91	13.5	0.76	43
50	54	0.71	9.7	0.88	40
100	53	0.63	7.3	0.65	43
Control	0.97	0.46	40	30	34

† EDTA, ethylenediaminetetraacetic acid; HS, humic substances; NTA, nitrilotriacetic acid.

which possesses very limited capacity to remove this element. Therefore, HS cannot be recommended for cleaning Pb-polluted soil, at least not if it is a calcareous soil that has been polluted over a long time because removal of the pollutant from such soils is especially difficult (Heil et al., 1999; Kim et al., 2003; Meers et al., 2007). On the other hand, because substantial amounts of Cd and Cu can be removed by HS (Tables 1, 3, 4) (Bianchi et al., 2008; Steponkaite et al., 2008; Borggaard et al., 2009), HS may replace EDTA and NTA as a cleaning agent for Cd- and Cu-polluted soils.

Replacement of the synthetic EDTA and NTA by the natural HS is environmentally very attractive. Ethylenediaminetetraacetic acid is toxic and persistent and NTA is hazardous to human health, whereas HS is non-toxic and can stimulate plant growth (Lulakis and Petsas, 1995; Grėman et al., 2001; Bianchi et al., 2008; Dermont et al., 2008). Furthermore, HS stimulates soil microbial activity and improves soil structure, whereas EDTA can reduce soil porosity (Heil et al., 1999; Halim et al., 2003; Conte et al., 2005).

According to the stability constants in Table 2, the extraction efficiency should decrease in the order: EDTA > NTA > HS. However, when looking at the conditional constants, which show the complexing affinity under the actual conditions (pH 7 and 50 mmol L<sup>-1</sup> DOC), the extraction efficiencies of EDTA and NTA are comparable. Accordingly, EDTA and NTA extract almost the same amounts of the three metals (Fig. 1 and 2; Table 1). Tandy et al. (2004) found NTA to be more efficient than EDTA for Cu but not Pb extraction at pH 7, which was explained by reduced efficiency of EDTA due to coextraction of Ca from calcium carbonate. We also found more Ca extracted by EDTA than NTA, but the difference was small, and the similar amounts of Cd, Cu, and Pb extracted (Table 1) can be readily explained by means of the conditional constants as demonstrated in Table 2. For comparison, the stability constants and conditional constants for HS are included in Table 2. The stability constants are derived from Visual Minteq (Gustafsson, 2007), and the conditional constants are calculated by this model as [MeHS]/[Me<sup>m+</sup>] ratios at pH 7 and 50 mmol L<sup>-1</sup> DOC. Because the necessary parameters for the used HS (processed cow slurry) are not known, the Gaussian DOC model described by Grimm et al. (1991) was used. Accordingly, HS consists of many different molecules with different protolytic and complexing properties, in contrast to the well defined EDTA and NTA. Despite these limitations, the data in Table 2 indicate that HS is a less efficient extractant than EDTA and NTA, in accordance with the results in Table 1. The constants cannot explain the discrepancy between the substantial amounts of Cu (~54%) extracted by HS compared with the very limited extractability of Pb (<4% of total content) in as much as the Cu-HS and Pb-HS constants are similar. Despite this, the present investigation has, in accordance with previous studies (Conte et al., 2005; Bianchi et al., 2008; Steponkaite et al., 2008; Borggaard et al., 2009), demonstrated that HS is an environmentally

friendly alternative to synthetic chelating agents.

friendly and fairly effective washing agent for soils polluted with Cd, Cu, and other pollutants.

By soil washing, the metals are transferred from the soil to the water phase, irrespective of the extractant. However, several methods exist for cleaning heavy metal-polluted water, such as (i) electrochemical reduction with deposition of the heavy metals at the cathode; (ii) precipitation of heavy metals by adding strong bases ( $\text{Ca}(\text{OH})_2$ ,  $\text{NaOH}$ ), phosphate, or  $\text{Na}_2\text{S}$ ; (iii) formation of heavy metal slag by water evaporation and burning of the organic extractant; and (iv) removal of the organic chelant by oxidation (mineralization) (Di Palma et al., 2003; Lim et al., 2005; Ehsan et al., 2006; Leštan et al., 2008; Borggaard et al., 2009). From an environmental point of view, electrochemical reduction and precipitation seem most attractive because the water and chelant can be reused. However, the choice of method for treating the heavy metal-polluted wash water (extract) also depends on economic and technical factors and opportunities, the discussion of which is not part of this study.

## Conclusions

The extraction of Cd, Cu, and Pb from this strongly polluted calcareous soil depended on characteristics and concentration of the extractant, the metal, and the solution/soil ratio. Thus, increased extractant concentration and solution/soil ratio augmented extracted metal, and up to 86, 77, and 30% of total soil Cd, Cu, and Pb were extracted by EDTA and NTA, whereas the corresponding percentages for HS-extractable metal were 44% Cd, 53% Cu, and 4% Pb. Single-step extraction removed about the same amounts of the three metals as multiple-step extraction. The metal-extracted pools of the soil varied with the extractant and the element, but in general the most weakly bonded Cu pools (exchangeable together with carbonate- and oxide-bound) were largely removed, whereas considerable amounts of exchangeable Cd and Pb remained unextracted, especially after HS extraction. However, the investigation has shown that soluble HS can replace EDTA and NTA as a washing agent for Cd- and Cu-polluted soils, whereas for strongly Pb-polluted calcareous soils, HS does not seem to be a recommendable alternative to EDTA and NTA.

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## References

Allison, L.E., and C.D. Moodie. 1965. Carbonate. p. 1379–1396. *In* C.A. Black et al. (ed.) *Methods of soil analysis*. ASA and SSSA, Madison, WI.

Barona, A., I. Aranguiz, and A. Elias. 2001. Metal associations in soils before and after EDTA extractive decontamination: Implications for the effectiveness of further clean-up procedures. *Environ. Pollut.* 113:79–85.

Bianchi, V., G. Masciandaro, D. Giraldo, B. Ceccanti, and R. Iannelli. 2008. Enhanced heavy metal phytoextraction from marine dredged sediments comparing conventional chelating agents (citric acid and EDTA) with humic substances. *Water Air Soil Pollut.* 193:323–333.

BioCorrection. 2009. Humic substances, BioCorrection A/S. Available at <http://www.biocorrection.com> (verified 23 Feb. 2010).

Borggaard, O.K. 1976. The use of EDTA in soil analysis. *Acta Agric. Scand.* 26:144–150.

Borggaard, O.K., H.C.B. Hansen, P.E. Holm, J.K. Jensen, S.B. Rasmussen, N. Sabiene, L. Steponkaite, and B.W. Strobel. 2009. Experimental assessment of using soluble humic substances for remediation of heavy metal polluted soils. *Soil Sediment Contam.* 18:369–382.

Conte, P., A. Agretto, R. Spaccini, and A. Piccolo. 2005. Soil remediation: Humic acids as natural surfactants in the washings of highly contaminated soils. *Environ. Pollut.* 135:515–522.

Day, P.R. 1965. Particle fractionation and particle-size analysis. p. 545–567. *In* C.A. Black et al. (ed.) *Methods of soil analysis*. ASA and SSSA, Madison, WI.

Dermont, G., M. Bergeron, G. Mercier, and M. Richer-Lafleche. 2008. Soil washing for metal removal: A review of physical/chemical technologies and field applications. *J. Hazard. Mater.* 152:1–31.

Di Palma, L., P. Ferrantelli, C. Merli, and F. Biancifiore. 2003. Recovery of EDTA and metal precipitation from soil flushing solutions. *J. Hazard. Mater.* B103:153–168.

Ehsan, S., S.O. Prasher, and W.D. Marshall. 2006. A washing procedure to mobilize mixed contaminants from soil: II. Heavy metals. *J. Environ. Qual.* 35:2084–2091.

ELTRA. 1995. CS500 simultaneous carbon/sulphur determinator. ELTRA GmbH, Neuss, Germany. Available at <http://www.eltragmbh.com> (verified 23 Feb. 2010).

European Environment Agency. 2007. Progress in management of contaminated sites (CSI 015). Available at <http://www.eea.europa.eu> (verified 2 Mar. 2010).

Evangelou, M.W.H., H. Daghan, and A. Schaeffer. 2004. The influence of humic acids on the phytoextraction of cadmium from soil. *Chemosphere* 57:207–213.

Evangelou, M.W.H., M. Ebel, and A. Schaeffer. 2007. Chelate assisted phytoextraction of heavy metals from soil: Effect, mechanism, toxicity, and fate of chelating agents. *Chemosphere* 69:989–1003.

Finžgar, N., and D. Leštan. 2007. Multi-step leaching of Pb and Zn contaminated soils with EDTA. *Chemosphere* 66:824–832.

Grěman, H., Š. Velikonja-Bolta, D. Vodnik, B. Kos, and D. Leštan. 2001. EDTA enhanced heavy metal phytoextraction: Metal accumulation, leaching and toxicity. *Plant Soil* 235:105–114.

Grimm, D.M., L.V. Azarraga, L.A. Carreira, and W. Susetyo. 1991. Continuous multiligand distribution model used to predict the stability constant of Cu(II) metal complexation with humic material from fluorescence quenching data. *Environ. Sci. Technol.* 25:1427–1431.

Gustafsson, J.P. 2007. Visual Minteq. Available at <http://www.lwr.kth.se/english/OurSoftWare/Vminteq/> (verified 23 Feb. 2010).

Halim, M., P. Conte, and A. Piccolo. 2003. Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. *Chemosphere* 52:265–276.

Han, N., and M.L. Thompson. 2003. Impact of dissolved organic matter on copper mobility in aquifer material. *J. Environ. Qual.* 32:1829–1836.

Heil, D.M., Z. Samani, A.T. Hanson, and B. Rudd. 1999. Remediation of lead contaminated soil by EDTA. I. Batch and column studies. *Water Air Soil Pollut.* 113:77–95.

IUSS Working Group WRB. 2006. World reference base for soil resources 2006. World soil resources reports no. 103. FAO, Rome, Italy.

Jensen, J.K., P.E. Holm, J. Nejrup, M.B. Larsen, and O.K. Borggaard. 2009. The potential of willow for remediation of heavy metal polluted calcareous urban soils. *Environ. Pollut.* 157:931–937.

Kim, C., Y. Lee, and S.K. Ong. 2003. Factors affecting EDTA extraction of lead from lead contaminated soils. *Chemosphere* 51:845–853.

Leštan, D., C.L. Luo, and X.D. Li. 2008. The use of chelating agents in the remediation of metal contaminated soils: A review. *Environ. Pollut.* 153:3–13.

Lim, T.T., P.C. Chui, and K.H. Goh. 2005. Process evaluation for optimization of EDTA use and recovery of heavy metal removal from a contaminated soil. *Chemosphere* 58:1031–1040.

Lulakis, M.D., and S.I. Petsas. 1995. Effect of humic substances from vinecanes mature compost on tomato seedling growth. *Bioresour. Technol.* 54:179–182.

MacDonald, J.D., and W.H. Hendershot. 2006. Modelling trace metal partitioning in forest floors of northern soils near metal smelters. *Environ. Pollut.* 143:228–240.

Martell, A.E., R.M. Smith, and R.J. Motekaitis. 2004. NIST critical stability constants of metal complexes. Version 8, NIST Standard Reference Data, Gaithersburg, MD.

Meers, E., E. Lesage, S. Lamsal, M. Hopgood, P. Vervaeke, F.M.G. Tack, and M.G. Verloo. 2007. Enhanced phytoextraction: I. Effect of EDTA and

- citric acid on heavy metal mobility in a calcareous soil. *Int. J. Phytorem.* 7:129–142.
- Polettini, A., R. Pomi, and G. Calcagnoli. 2009. Assisted washing for heavy metal and metalloid removal from contaminated dredged materials. *Water Air Soil Pollut.* 196:183–198.
- Popov, A.I., V.P. Shipov, V.A. Trofimov, E.S. Pigarev, N.E. Nikolayenkova, S.I. Gorskhov, and E.I. Fedoros. 2002. Method of isolating humic substances from natural raw material. [Russia] Patent RU2178777-C1, 21 July 2001.
- Rao, C.R.M., A. Sahuquillo, and J.F. Lopez Sanchez. 2008. A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials. *Water Air Soil Pollut.* 189:291–333.
- SAS Institute. 2004. SAS 9.1.2 qualification tools user's guide. SAS Inst., Cary, NC.
- Steponkaite, L., N. Sabiene, H.C.B. Hansen, and O.K. Borggaard. 2008. Mobilisation of Cu from soils by dissolved natural and processed humic substances. *Ekologija* 54:88–92.
- Stevenson, F.J., and M.A. Cole. 1999. Cycles of soil carbon, nitrogen, phosphorus, sulfur, micronutrients. 2nd ed. John Wiley & Sons, New York.
- Strobel, B.W., O.K. Borggaard, H.C.B. Hansen, M.K. Andersen, and K. Raulund-Rasmussen. 2001. Cadmium and copper release kinetics in relation to afforestation of cultivated soils. *Geochim. Cosmochim. Acta* 65:1233–1242.
- Strobel, B.W., O.K. Borggaard, H.C.B. Hansen, M.K. Andersen, and K. Raulund-Rasmussen. 2005. Dissolved organic carbon and decreasing pH mobilize cadmium and copper in soil. *Eur. J. Soil Sci.* 56:189–196.
- Sun, B., F.J. Zhao, E. Lombi, and S.P. McGrath. 2001. Leaching of heavy metals from contaminated soils using EDTA. *Environ. Pollut.* 113:111–120.
- Tandy, S., K. Bossart, R. Mueller, J. Ritschel, L. Hauser, R. Schulin, and B. Nowack. 2004. Extraction of heavy metals from soils using biodegradable chelating agents. *Environ. Sci. Technol.* 38:937–944.
- Tessier, A., P.G.C. Campbell, and M. Bisson. 1979. Sequential extraction procedure for speciation of particulate trace metals. *Anal. Chem.* 51:844–851.
- Tjell, J.C., and M.F. Hovmand. 1978. Metal concentrations in Danish arable soils. *Acta Agric. Scand.* 28:81–89.
- Weng, L., E.J.M. Temminghoff, S. Lofts, E. Tipping, and W. Van Riemsdijk. 2002. Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. *Environ. Sci. Technol.* 36:4804–4810.
- Zhao, L.Y.L., R. Schulin, L. Weng, and B. Norwack. 2007. Coupled mobilization of dissolved organic matter and metals (Cu and Zn) in soil columns. *Geochim. Cosmochim. Acta* 71:3407–3418.
- Zou, Z., R. Qiu, W. Zhang, H. Dong, Z. Zhao, T. Zhang, X. Wei, and X. Cai. 2009. The study of operating variables in soil washing with EDTA. *Environ. Pollut.* 157:229–236.