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Remediation of heavy metal-contaminated soils using eco-friendly nano-scale chelators

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Abstract. Soil washing is one of the most frequently used remediation technologies for heavy metal-contaminated soils. Inorganic and organic acids and chelating agents that can enhance the removal of heavy metals from contaminated soils have been employed as soil washing agents. However, the toxicity, low removal efficiency and high cost of these chemicals limit their use. Given that humic substance (HS) can effectively chelate heavy metals, the development of an eco-friendly, performance-efficient and cost-effective soil washing agent using a nano-scale chelator composed of HS was examined in this study. Copper (Cu) and lead (Pb) were selected as target heavy metals. In soil washing experiments, HS concentration, pH, soil:washing solution ratio and extraction time were evaluated with regard to washing efficiency and the chelation effect. The highest removal rates by soil washing (69% for Cu and 56% for Pb) were achieved at an HS concentration of 1,000 mg/L and soil:washing solution ratio of 1:25. Washing with HS was found to be effective when the pH value was higher than 8, which can be attributed to the increased chelation effect between HS and heavy metals at the high pH range. In contrast, the washing efficiency decreased markedly in the low pH range due to HS precipitation. The chelation capacities for Cu and Pb in the aqueous phase were determined to be 0.547mmol-Cu/g-HS and 0.192mmol-Pb/g-HS, respectively.

Keywords: heavy metal; contaminated soil; soil washing; humic substance; chelating agent

1. Introduction

Soils contaminated with heavy metals are commonly found in the vicinities of abandoned mines, railroads, military sites and manufacturer gas plant sites. Contamination of soil with heavy metals has taken place over a long period and therefore the contamination characteristics of such soils are markedly heterogeneous, depending on contamination sources and periods (Rao *et al.* 2008). Thus, it is essential to take into consideration such contamination characteristics when selecting an appropriate remediation technology for heavy metal-contaminated soils (Bolan *et al.* 2014, Yun *et al.* 2015). Furthermore, the remediation of heavy metal-contaminated soils presents a technical challenge, because most heavy metal materials are strongly bound within the soil matrix. The currently available remediation techniques used for heavy metal-contaminated soils are as follows: physical treatments such as magnetic or gravity separation, particle separation by washing or sieving, chemical treatments such as acid or surfactant cleaning/washing and biological treatment such as phytoremediation (Goyal and Chauhan 2015, Jiang *et al.* 2011, Lestan *et al.* 2008, Li *et al.* 2014). Soil washing is one of the most frequently used technologies for remediation of heavy metal-contaminated soils. When this technique is used, it is critical to select appropriate washing agents, which play an important role in desorbing heavy metals from soils (US EPA 1996, Lim *et al.* 2004, Tejowulan and Hendershot 1988).

Several studies have been carried out on the washing agents used for extraction of heavy metals from soil matrices. Currently, strong inorganic acids, such as hydrochloric, nitric and sulfuric acids and organic acids, such as acetic, citric and oxalic acids, are typically employed as soil washing agents (Elliott and Shastri 1996, Wuana *et al.* 2010). Recently, it has been reported that washing of heavy metal-contaminated soils using chelating agents that can coordinate with heavy metals has a removal efficiency similar to that of inorganic acids, including ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic

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acid (NTA) and ethylenediamine-N, N-disuccinic acid (EDDS). However, toxicity, low removal efficiency and high cost have been noted as crucial factors limiting the use of these chemicals (Jiang *et al.* 2011, Yun *et al.* 2015, Tejowulan and Hendershot 1988, Wuana *et al.* 2010).

Humic substance (HS) is referred to as a heterogeneous mixture of carbonaceous macromolecules present in terrestrial and aqueous environments (Kim *et al.* 2012, Sparks 1998). They have evolved geologically over a long period from the debris of organisms in soils and are characterized by a high molecular weight, which is counted as nano-scale molecules and a low degree of decomposition (Kang *et al.* 2014, Stevenson 1982). HSs are classified into three fractions according to the operative definitions given by the International Humic Substances Society: fulvic acid (FA), humic acid (HA) and humin. These fractions are produced from biopolymers (BP: polysaccharides, carbohydrates, protein, fats, waxes, cellulose, lignin, etc.) derived from plant and animal cells through the humification process. FA, which has the property of dissolving at all pH values, is yellow or orange in color and its molecular structure has a high content of oxygenated functional groups. HA that dissolves only at high pH is dark brown and it has relatively smaller amounts of functional groups than FA. FA and HA have many functional groups, such as COOH, -OH and -C═O (Clapp *et al.* 2001, Lee *et al.* 2013, Tang *et al.* 2014) and these functional groups act as ligands forming coordination bonds with heavy metals (Clapp *et al.* 2001). According to Stevenson (1982), the stability of heavy metal complexes chelated by HS depends on the number of heavy metal protons, the number of ligands in the complex, the concentration of heavy metal and pH. The order of the complex stability of representative divalent heavy metals is as follows

$$Cu^{2+} > Ni^{2+} > Co^{2+} > Pb^{2+} > Zn^{2+} > Fe^{2+} > Mn^{2+}$$

The stability of most heavy metals in complexes is higher than that of Fe or Mn, which are the most commonly present metals in soils and minerals. Thus, by forming complexes with heavy metals, HS can be used as a washing agent for extracting toxic heavy metals from contaminated soils. Xiong *et al.* (2015) reported that because FA or HA molecules have a significant number of functions groups, they can coordinate with one or more heavy metal ions. This means that HS can be used as a superior chelating agent that forms complexes with heavy metals on a 1:1 basis. Kulikowska (2015) remediated soils contaminated with Cu and Cd using HS isolated from sewage sludge and the remediation efficiency was greatly increased by successive washing of soils with HS solutions. Lestan *et al.* (2008) reported that HS can be used as a chelating agent to increase the mobility of heavy metals and that this method can be applied for plant cultivation. The effect of HS on the metal uptake ability of plants was greater than that of NTA or EDTA and almost equivalent to that of EDDS, which is a well-known biodegradable chelating agent.

The objective of the present study was to assess the feasibility of utilizing HS as an eco-friendly nano-scale soil washing agent for the remediation of heavy metal-contaminated soils. Given that HS can effectively chelate heavy metals, which can enhance the solubilization of heavy metals in the aqueous phase, the strategy employed herein involved optimization of HS concentration, pH control, soil:washing solution ratio and extraction time for the soil washing process. In addition, the chelation capacity of HS for heavy metals was determined. The results of this study will be potentially useful for the development of an eco-friendly, performance-efficient and cost-effective soil washing agent and its application for remediating heavy metal-contaminated soils.

2. Materials and method

2.1 Materials

To minimize the uncertainty resulting from the heterogeneous characteristics of field soil contamination, a soil that has no prior contamination history was artificially contaminated with target heavy metals in the laboratory. Surface soils were collected from the A horizon, where humus organic matter is abundantly present (a depth of 5-10 cm), in a forest region near Acha Mountain, Seoul, Korea. The collected soils were air-dried for 3 d and then passed through a 2 mm sieve to remove plant debris. The physical and chemical properties of the soils are presented in Table 1. The air-dried soil was spiked with solutions of $Cu(NO_3)_2 \cdot 3H_2O$ and $Pb(NO_3)_2$ and mixed in a rotary shaker operated at 120 strokes/min for 24 h. A 0.1 M solution of NaOH was added to neutralize the soil, followed by mixing for 24 h to prepare the contaminated seed soil. The seed soil was mixed with fresh soils at a ratio of 1:9 (w/w) and mixed thoroughly using a stainless-steel spatula followed by further mixing for 24 h using a rotary shaker. Multiple soil samples ($n = 10$) were taken from the complex contaminated soils and the levels of Cu and Pb were determined. The results indicated that the soil contamination was statistically homogeneous at a 95% confidence level ($p > 0.05$). The concentrations of Cu and Pb in the soils contaminated with Cu only and Pb only were 789 ± 31 and 968 ± 19 mg/kg, respectively and the initial concentrations of Cu and Pb were 1109 ± 34 and 999 ± 29 mg/kg in the soil contaminated with both Cu and Pb. In addition, contaminated field soil was collected at a depth of 30-50 cm from an abandoned railway site that had been used for train maintenance for more than 50 yrs. A high level of incineration of wastes as well as casting molds, which include large amounts of heavy metals, were buried at this site. The soil was pretreated in the same manner as the artificially contaminated soil. The physical and chemical properties of the soil are presented in Table 1. The initial concentrations of Cu and Pb in the samples were 259 ± 13.9 and 1332 ± 64.3 mg/kg, respectively. The HCl, HNO_3 and NaOH used for heavy metal extraction were obtained from Duksan (Daejeon, Korea). XAD-8 resin for the separation of FA and BP was purchased from Supelco (Bellefonte, PA, U.S.A.). Potassium hydrogen phthalate used as a total organic carbon (TOC) analysis standard, acetic acid, hydroxylamine hydrochloride, hydrogen peroxide (28% v/v), ammonium acetate, $Cu(NO_3)_2 \cdot 3H_2O$ and $Pb(NO_3)_2$ for artificially contaminated soil were purchased from Sigma Aldrich. (Chicago, IL, U.S.A.). Standard solution for heavy

Table 1 Properties of soil used

Soil	pH	Electrical conductivity (μS/cm)	Cation exchange capacity (meg/100 g)	Organic matter content (%, w/w)	Water content (%, w/w)	Bulk density (g/mL)	Particle density (g/mL)	Porosity
Uncontaminated soil	5.2	47.4 ± 16.6[a]	187.2 ± 7.2	7.41 ± 0.27	15.5 ± 1.1	0.87 ± 0.08	1.82 ± 0.11	0.45
Contaminated field soil	7.7	25.4 ± 7.4	7.9 ± 1.2	2.83 ± 0.13	15.2 ± 1.2	1.92 ± 0.05	2.73 ± 0.21	0.51

[a] Standard deviation (n = 3)

metal analysis (Cu and Pb = 1,000 ppm, respectively) was purchased from Kanto Chemical (Tokyo, Japan). HA, EDTA, $CuCl_2$, $PbCl_2$ and dialysis tubing (1,000 Da) for humic substance isolation and chelation tests were purchased from Sigma Aldrich. All chemicals used for instrumental analyses were obtained from Sigma Aldrich.

2.2. Isolation of humic substances

The extraction, separation and purification of HS were carried out as follows. A 10 g sample of HA, which was dried in an oven at 70°C for 72 h, was dissolved in 1 L of 0.5 M NaOH solution for 24 h. The sample was fractionated into BP, FA and HA using the following stepwise procedure: (i) acidification with 0.1 M HCl (pH = 1-2) and separation by centrifugation at 5,000 *g* for 30 min; (ii) collection of the supernatant (BP and FA fractions) and precipitates (HA fraction); (iii) separation of BP and FA by elution of their mixture using XAD-8 resin; and (iv) removal of ash residue remaining in the humic fraction, as described by Kim and Kim (2013). HS was prepared by mixing FA (500 mg/L) and HA (500 mg/L) at a ratio of 1:1 (v/v).

2.3. Soil washing

The optimum operating conditions of soil washing using HS as a washing agent were selected with regard to the pH of the washing solution, soil:washing solution ratio and extraction time. All experiments were conducted for soils contaminated with Cu only, Pb only and both Cu and Pb. For determining the optimum HS concentration of the washing solution, 1 g of soil was mixed with 25 mL of washing solution, of which the HS concentrations was 100, 500 or 1000 mg/L, in a 50 mL vial. For determining the optimum pH of the washing solution, 1 g of soil was mixed with 25 mL of washing solution with a HS concentration of 500 mg/L and the solution pH was adjusted to 4, 6, 8 or 10. For determining the optimum soil:washing solution ratio, 1 g of soil was mixed with 10, 25 or 40 mL of washing solution and the HS concentration and pH of the washing solution were 500 mg/L and 7, respectively. The vials were agitated on a shaker table operating at 250 stokes per min for 12 h. To determine the optimum extraction time, different durations of mixing were assessed: 12, 18, 24 and 48 h. The soils were then separated by centrifugation at 1,500 *g* and the concentrations of Cu and Pb remaining in the washing solutions were determined. Soil washing using EDTA was carried out in the same manner as described above.

2.4. Chelation of heavy metals by humic substances

The chelation of heavy metals by HS was evaluated by quantifying the complexation of Cu and Pb with HS. The chelation capacity was defined as the maximum amount of heavy metal forming a complex with HS (mg-heavy metal/g-HS). 5 mL of solutions containing $CuCl_2$, $PbCl_2$ or $CuCl_2$ plus $PbCl_2$ (10 mM each) were mixed with 24 mL of HS solution (500 mg/L and pH 7) in a dialysis tube and then each dialysis tube was placed in a beaker containing Milli-Q water for 2 d. Free metal that does not form a complex with HS can move to the distilled water phase by passing through the dialysis membrane. The concentrations of heavy metals inside and outside of dialysis tube were measured.

2.5. BCR sequential extraction

A 0.5 g sample of air-dried soil was transferred to a 50 mL centrifuge tube and the sequential extraction of heavy metals from soil was carried as follows:

(1) Exchangeable fraction

To the soil sample, 20 mL of 0.11 M acetic acid was added and mixed vigorously on a shaker table operating at 150 strokes per min for 16 h at 22 ± 5°C. The soil was separated by centrifugation at 1,000 *g* for 5 min. The supernatant was filtered and acidified with 1 M HCl. The concentrations of heavy metals in the solution were subsequently measured (exchangeable fraction). Milli-Q water (10 mL) was added to the soil and stirred for 15 min. The soil was separated by centrifugation and the supernatant was discarded.

(2) Reducible fraction

To the soil recovered from Step 1, 20 ml of 0.5 M $NH_2OH{\cdot}HCl$ (pH was adjusted to 1.5 with 2 M nitric acid) was added and mixed as described for Step 1. After centrifugation at 1,000 *g* for 5 min, the reducible fraction in the supernatant was determined as for Step 1. The soil was washed and recovered as in Step 1.

(3) Oxidizable fraction

To the soil recovered from Step 2, 5 mL of 8.8 M H_2O_2 (pH 2-3) was carefully added in small portions and stirred as described for Step 1. The centrifuge tube was shaken occasionally at room temperature for 1 h. The centrifuge tube cap was then opened and the soil suspension was digested in a water bath at 85 ± 2°C for 1 h. A 5mL of 8.8 M H_2O_2 (pH 2-3) was added to the suspension when the volume of the suspension had decreased to 2-3 mL and was thereafter continuously digested until the suspension was completely dry. 25 mL of 1.0 M ammonium acetate (adjusted to pH 2) was then added followed by mixing at room temperature for 16 h. After centrifugation at 1,000 *g* for 5 min, the oxidizable fraction in the supernatant was determined as described for Step 1. The soil was washed and recovered as in Step 1.

(4) Residual fraction

A mixture of HNO_3 and HCl (9:3, v/v) was added to the soil recovered from Step 3 and digested at 80°C for 2 h. After centrifugation at 1,000 *g* for 5 min, the residual fraction in the supernatant was determined as described for Step 1.

2.6 Adsorption of HS

Uncontaminated soil washed with Milli-Q water three times and air-dried prior to use was transferred to a 50 mL centrifuge tube (Fisher Scientific, Chicago, IL, U.S.A.) containing a buffer solution and sorbate (HS, FA or HA). The buffer solution was composed of 0.555 g/L of $CaCl_2$, 5 mg/L of $NaHCO_3$ and 100 mg/L of NaN_3 (pH 7). Each tube was sealed using a Teflon-coated silicon septum and a screw cap (Fisher Scientific) and mixed on a shaker table operated at 120 stokes/min and 25°C in the dark. A sorption equilibration time of 24 h was chosen, as it proved sufficient to approach sorption equilibrium in preliminary experiments (data not presented). Then, the soil was separated by centrifugation at 3,000 g for 20 min and the supernatant was isolated. The concentration of sorbate in the supernatants was measured to determine the amounts of sorbate on the soil. The Langmuir and Freundlich isotherm models were employed to describe the sorption equilibria for all the systems studied. These models have the forms

$$q_e = \frac{Q_{\max} b C_e}{1 + b C_e} \qquad \text{(Langmuir model)} \qquad (1)$$

$$q_e = K_F C_e{}^n \qquad \text{(Freundlich model)} \qquad (2)$$

where q_e is the solid-phase sorbate concentration at equilibrium, Q is the maximum sorption capacity, b is the sorption enthalpy-related coefficient, C_e is the liquid phase sorbate concentration at equilibrium, K_F is a concentration-specific sorption capacity term and the exponent n is a joint measure of the cumulative magnitude and distribution of energies associated with a particular sorption reaction.

2.7 Analytical procedures

The concentrations of Cu and Pb in the soil phase were determined as follows: a 3 g sample of soil was placed in a 50 mL-centrifuge tube containing 28 mL of a mixture of HNO_3 and HCl (7:21, v/v). To this mixture was added 15 mL of 0.5 M HNO_3 and this was allowed to stand at room temperature for 2 h to facilitate slow oxidation of organic matter in the soil. The tube was then placed in a water bath at 80°C for 2 h and subsequently slowly cooled. To precipitate insoluble residues from the suspension, 10 ml of 0.5 M HNO_3 was added and then the supernatant was filtered through a 0.7 μm GF/C filter (Millipore, Darmstadt, Germany). The filtrate was diluted 10-fold with Milli-Q water and then the concentrations of Cu and Pb in the diluted solution were analyzed using inductively coupled plasma-optical emission spectroscopy (ICP-OES; ICAP 6000 series, Thermo Scientific, Bartlesville, OK, U.S.A.). The organic carbon content in each fraction was quantified as the TOC concentration using a TOC analyzer (Sievers 900, GE Power and Water, Schenectady, NY, U.S.A.). The structure, surface and composition of the contaminated field soil were analyzed by X-ray diffraction (XRD; D8 Advance Eco, Bruker, Madison, WI, U.S.A.) and using a field emission scanning electron microscope (FE-SEM; SIGMA VP, Carl Zeiss, Munich, Germany) equipped with an energy dispersive X-ray spectrometer (EDS; Carl Zeiss).

3. Results and discussion

3.1 Optimization of the soil washing process

3.1.1 HS concentration of the washing solution

The HS concentration of the washing solution was varied to evaluate the efficiency of the soil washing treatment for the heavy metal-contaminated soils. The concentrations of each heavy metal removed as a function of the HS concentration of the washing solution are presented in Fig. 1 The heavy metal removal efficiency of the washing solution increased as the HS concentration of the washing solution increased. In all cases, the highest removal efficiencies were achieved with washing solutions containing 1,000 mg/L HS. The removal efficiencies for Cu-, Pb- and Cu+Pb-contaminated soils were 69%, 56% and 44%, respectively. The removal efficiency for soil contaminated with both Cu and Pb was lower than that for either Cu- or Pb-contaminated soil because the contamination level of the former was higher than that of the latter two soils. The removal of heavy metals from soils was attributed to the chelation capabilities of HS. In the cases of soils contaminated with Cu and Pb only, the amounts of heavy metals removed by HS were 0.371 mmol-Cu/g-HS and 0.140 mmol-Pb/g-HS, respectively. In the case of soil contaminated with both Cu and Pb, the amounts of heavy metals removed by HS were 0.484 mmol-Cu/g-HS and 0.142 mmol-Pb/g-HS. Thus, the removal of heavy metals by chelation with HS was higher for Cu than for Pb, which is ascribed to the more stable complex formation between Cu and HS than that between Pb and HS (Stevenson 1982).

3.1.2 pH of the washing solution

Fig. 2 shows the results of the experiment in which the pH of the washing solution was varied to determine the optimum pH conditions for soil washing. The efficiency of heavy metal removal increased with increasing pH. The stability of HS complexes with heavy metals is high at high pH (Fan *et al*. 2009). In addition, the HS used in the present study was mainly composed of HA, which can dissolve at high pH and precipitates at low pH. Therefore, the chelation attributable to HS became more significant with an increase in pH, resulting in higher heavy metal removal efficiency. Note that the removal efficiency for the soils contaminated with Cu only and with both Cu and Pb was very low at pH 4, which is attributable to the precipitating characteristics of HA at low pH. Such precipitation may indeed have stimulated an increase in the heavy metal bound to the soil matrix (solid phase). The main removal mechanism of conventional soil washing using inorganic and/or organic acids involves an increase in the solubility of heavy metals in the aqueous phase by lowering pH, which is subject to operation at low pH conditions followed by post-treatment neutralization in the soil washing process. It is worth noting that such post-treatment is unnecessary in soil washing using HS since the appropriate pH is in the range 6 to 10.

3.1.3 Soil:Washing solution ratio

When using the soil washing method, the soil:washing solution ratio is a critical factor for determining the operational cost of the process. Thus, the optimal soil:washing ratio (w/v, g/mL) for soil washing with HS

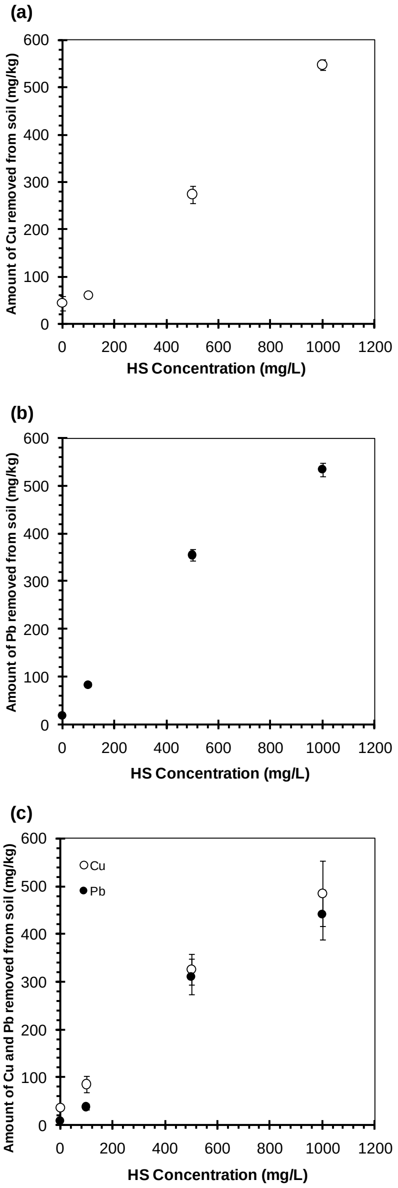


Fig. 1 Effect of HS concentration on heavy metal extraction from (a) soil contaminated with Cu, (b) soil contaminated with Pb and (c) soil contaminated with Cu+Pb. Error bars denote standard deviations (*n* = 3)

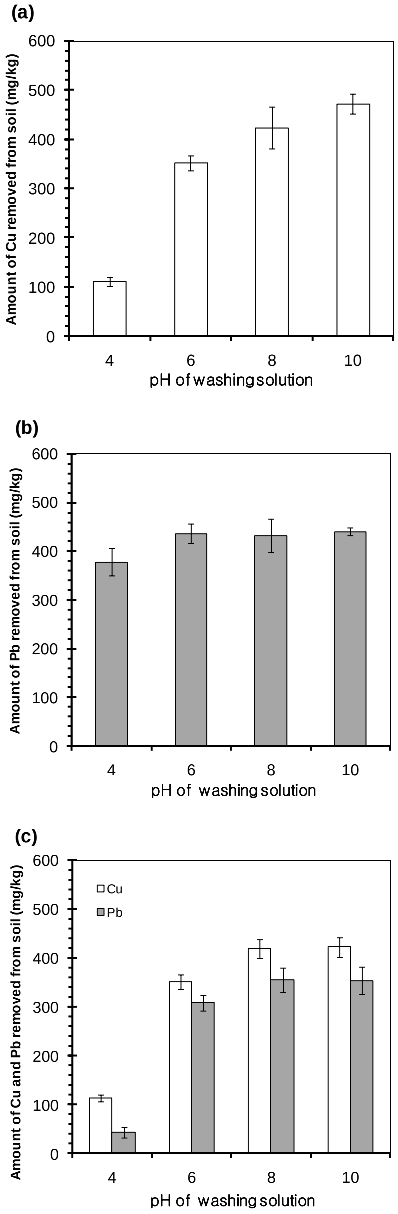


Fig. 2 Effect of pH on heavy metal extraction from (a) soil contaminated with Cu, (b) soil contaminated with Pb and (c) soil contaminated with Cu+Pb

was determined using the following ratios: 1:10, 1:25 and 1:40. The removal of heavy metals as a function of the soil:washing solution ratio in each case is shown in Fig. 3 The removal efficiency was found to increase as the ratio increased. The highest removal efficiencies were obtained using a ratio of 1:40: 60% and 38% for the soils contaminated only with either Cu or Pb, respectively and 38% for the soil contaminated with both Cu and Pb. In the case of the soil contaminated with Cu only, the removal efficiency of Cu increased linearly with an increasing ratio. In contrast, for the soil contaminated with Pb only, the removal efficiency of Pb at a ratio of 1:25 was almost same as that at a ratio of 1:40, which is indicative of the desorption equilibrium of Pb from the soil. The removal amount of heavy metals by HS was analyzed and the results are provided in Table 2. As expected, more HS needs to be added to the soil when the ratio increases. Interestingly, the amount of heavy metal removed did not increase or even decrease when the ratio was varied from 1:25 to 1:40. Thus, 1:25 was selected as the optimal soil:washing solution ratio in this study.

3.1.4 Extraction time

Soil washing was carried out using various extraction times of 6, 9, 12, 18, 24 and 48 h. Typically, soil washing processes involve several steps such as separation, crushing, washing and sludge dehydration and the residence time in the washing tank is a key factor in determining the overall operational cost. The concentration of heavy metal removed as a function of extraction time is shown in Fig. 4. In all cases, the removal of heavy metals from contaminated soils was somewhat increased with increasing extraction time.

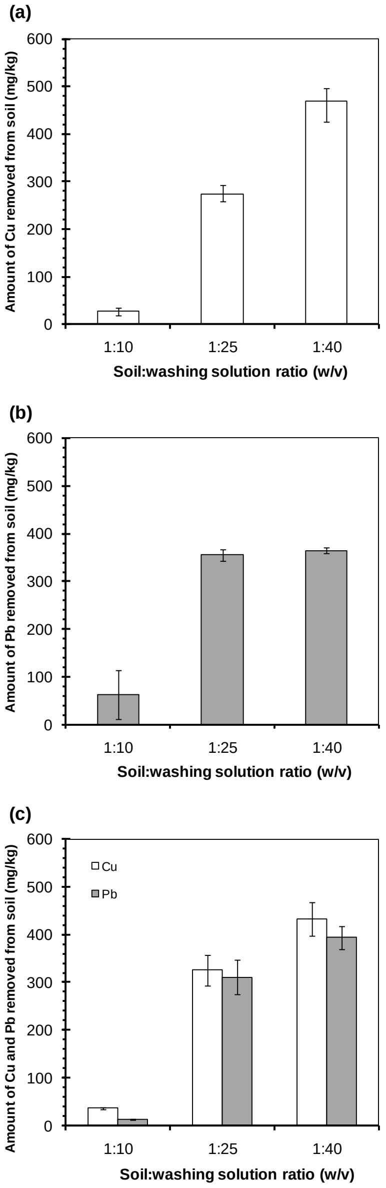


Fig. 3 Effect of soil:washing solution ratio on heavy metal extraction from (a) soil contaminated with Cu, (b) soil contaminated with Pb and (c) soil contaminated with Cu+Pb

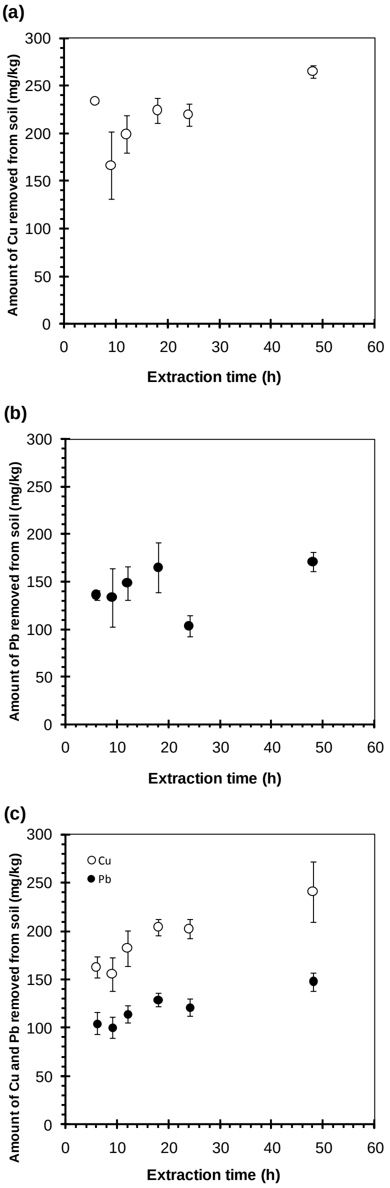


Fig. 4 Effect of extraction time on heavy metal extraction from (a) soil contaminated with Cu, (b) soil contaminated with Pb and (c) soil contaminated with Cu+Pb

Table 2 Extraction capacity of HS as a function of soil:washing solution ratio

Soil:washing solution ratio (w:v)	Amount of heavy metal removed from soil (mmol-metal/g-HS)			
	Soil contaminated with Cu	Soil contaminated with Pb	Soil contaminated with Cu+Pb	
	Cu	Pb	Cu	Pb
1:10	0.084	0.060	0.114	0.012
1:25	0.344	0.137	0.410	0.120
1:40	0.369	0.088	0.341	0.095

However, although removal efficiencies increased with extractions times greater than 12h, the differences were found to be statistically insignificant ($p > 0.05$) at a 95% confidence level. Therefore, 12 h was selected as the optimal extraction time.

3.2 Soil washing of contaminated field soil

3.2.1 Characteristics of contaminated field soil

Soil and heavy metal contamination characteristics were examined for soil washing of contaminated field soil using HS. The results of XRD and SEM are presented in Figs. 5 and 6. The contaminated field soil primarily comprised limestone and the major constituents were silicate, carbonates, iron oxide and organic matter. The heavy metals present in contaminated field soil were extracted using the BCR sequential extraction method and the heavy metals in the BCR fractions were analyzed (Fig. 7). It was found that the exchangeable fraction was less than 1% and that most heavy metals were strongly bound in the soil matrix,

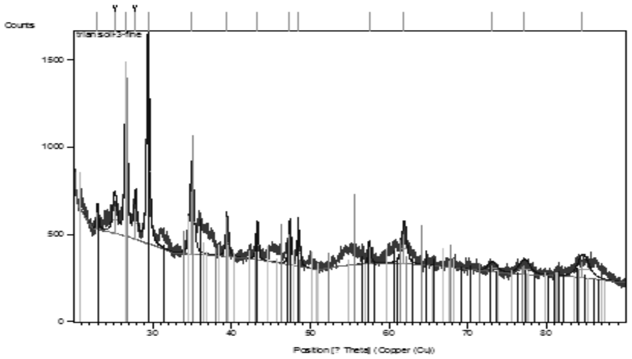


Fig. 5 XRD profile of contaminated field soil

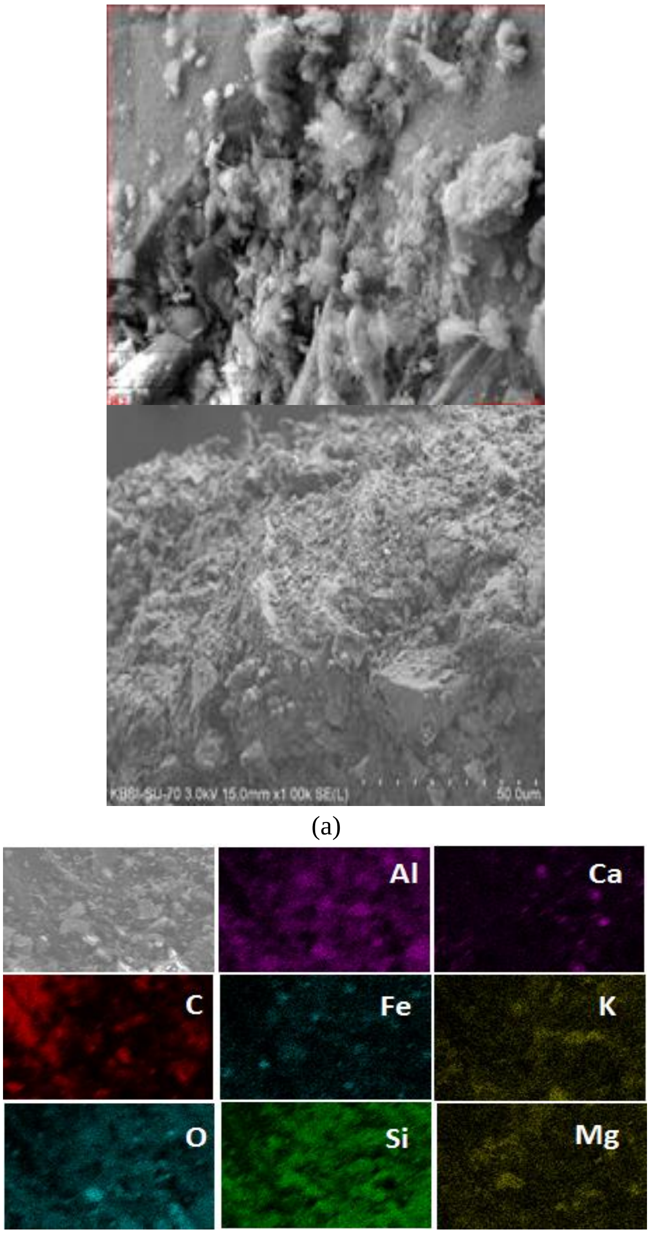
which indicates that this soil has been contaminated with heavy metals for a long period (Ure *et al*. 1993).

3.2.2 Soil washing of contaminated field soil

Fig. 8 presents the results of heavy metal removal from the contaminated field soil, by soil washing using either HS or EDTA, the latter of which is a well-known chemical chelator. The highest removal efficiency was achieved at an EDTA concentration of 500 mg/L and no further enhancement was observed even if the EDTA concentration was increased. In contrast, the removal efficiency steadily increased with an increase in HS concentration. These observations are consistent with the results obtained for laboratory-contaminated soil, although the removal efficiency was considerably lower than that for the laboratory-contaminated soil. This difference in efficiency can be attributed to the strong binding of heavy metals within the soil matrix as a result of an aging effect (i.e., prolonged contamination over a long period, Yun *et al.* 2015). This supposition is supported by the high level of the oxidizable and residual fractions of heavy metals, which was determined by the BCR sequential extraction. Interestingly, whereas a high removal efficiency was achieved at low pH when EDTA was used, a high removal efficiency was achieved at high pH when HS was used. This is because the complexation capacity of EDTA increases as pH decreases, but the aqueous solubility of HS increases as pH increases, which facilitate an increase in complex formation with HS. It should therefore be noted that when EDTA is used as a washing agent, the pH needs to be lowered, so that the solubility of heavy metals also increases. This may be an additional benefit in terms of heavy metal removal from soils; however, this is subject to post-treatment such as pH adjustment for the acid-treated soils.

3.3 HS sorption to soil

HS can extract heavy metals by formation of complexes and at the same time, it can be bound to soils (Liang *et al.* 2011). This sorbed HS does not contribute to the release of heavy metals to the aqueous phase (Arias *et al*. 2002, Ashley 1996). Therefore, in order to accurately quantify the HS available for the formation of complexes in the aqueous phase, the amount of HS sorbed to the soil (i.e., HS_{sorbed})



(a)

(b)

Fig. 6 (a) SEM images of contaminated field soil and (b) the results of EDX mapping showing the elemental distribution on the soil surface

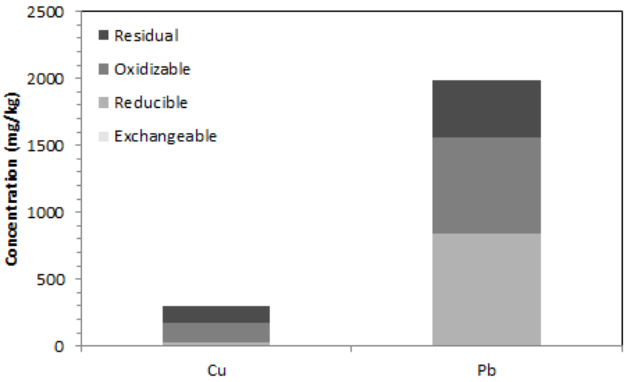
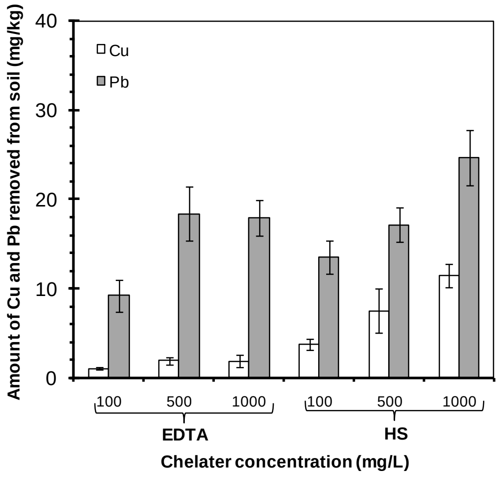
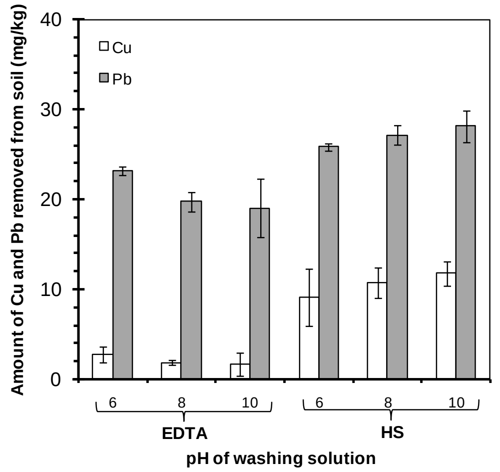


Fig. 7 BCR fractions of heavy metals present in the contaminated field soil



(a)



(b)

Fig. 8 Comparison of the efficiencies of heavy metal removal by EDTA and HS as a function of (a) chelator concentration and (b) pH of washing solution

was determined by conducting an adsorption experiment. It was reasonably assumed that in the aqueous phase there are HS that do not chelate with heavy metals (HS_{free}) and HS that form complexes with heavy metals (HS-Me). The total HS in the aqueous phase, which is equal to the sum of HS_{free} and HS-Me, can be calculated by the difference between HS added and HS_{sorbed}. The adsorption isotherms for HS and its major components FA and HA in the uncontaminated soil were analyzed using the Langmuir and Freundlich models and the model parameters. The results are presented in Table 3 and Fig. 10.

3.4 Chelation of heavy metals by HA

In general, the equilibrium between heavy metals and chelators is described by the complex formation constant (K_f). For the estimation of K_f, the precise quantification of HS_{free} and Me HS is required. It was assumed that HS_{free} is negligible considering the very high complex formation capacity of HS (i.e., HS_{free} << HS). Thus, the chelation capacity of HS can be defined as the amount of heavy metal

Table 3 Adsorption isotherm model parameters for HS, FA and HA in uncontaminated soil

Langmuir model parameter	Q_{max}	b	r^2
HS	13,145	0.0006	0.93
FA	1,615	0.0045	0.89
HA	11,201	0.0013	0.91
Freundlich model parameter	n	K_F	r^2
HS	0.76	26.2	0.92
FA	0.41	86.1	0.85
HA	0.62	91.3	0.89

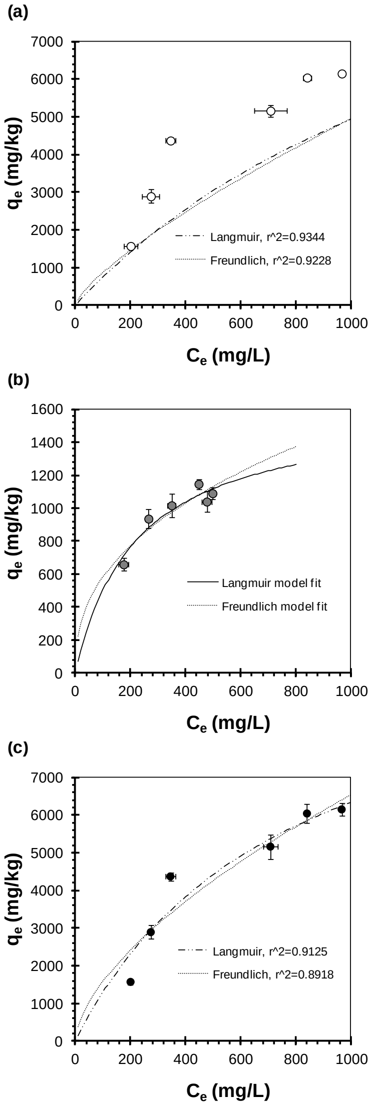


Fig. 10 Adsorption isotherms of (a) HS, (b) FA and (c) HA in uncontaminated soil

Table 4 Chelation capacity of HS for heavy metals (mmol-metal/g-HS)

In water		For soil contaminated with Cu	For soil contaminated with Pb	For soil contaminated with Cu+Pb	
Cu	Pb	Cu	Pb	Cu	Pb
0.547	0.192	0.484	0.142	0.371	0.140

that can chelate with HS per weight of HS and this value can be used for evaluating the chelation effect of HS with heavy metals (Table 4). The chelation capacity of HS for Cu was higher than that for Pb, which is in accordance with Stevenson (1982). Dube *et al*. (2001) and Senesi *et al*. (1991) reported that HS can be strongly associated with heavy metals via complex formation and that the maximum chelation capacity is as high as 0.52 mmol-heavy metal/g-HS. The chelating capacity of HS for Cu and Pb observed in the present study was considerably higher than that recorded in previous studies, which indicates the superior washing capability compared with the levels reported previously. This difference can be attributed to the high purity of HS and that fact that HS contain a high level of functional groups that can serve as ligands in complex formation with heavy metals.

4. Conclusions

The purpose of this study was to develop an eco-friendly soil washing method based on the chelation effect of nano-scale humic substances which are natural-origin organic materials for the treatment of heavy metal-contaminated soil. The highest removal rates of soil washing (69% for Cu and 56% for Pb) were achieved at 1,000 mg/L HS with a soil:washing solution ratio of 1:25. When the pH value was higher than 8, the washing with HS was found to be effective, which can be attributed to the strong chelation between HS and heavy metals in the high pH range. In contrast, the washing efficiency decreased markedly in the low pH range due to HS precipitation. The chelation capacities for Cu and Pb in the aqueous phase were determined to be 0.547mmol-Cu/g-HS and 0.192 mmol-Pb/g-HS, respectively. The results of this study are expected to provide fundamental information that could contribute to the design and operation of heavy metal-contaminated soil remediation via soil washing of heavy metals using eco-friendly nano-scale HS as a chelator.

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