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Natural and synthesised iron-rich amendments for As and Pb immobilisation in agricultural soil

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The immobilisation of heavy metals in contaminated soils is a promising alternative to conventional remediation techniques. Very few studies have focused on the use of iron-rich nanomaterials and natural materials for the adsorption of toxic metals in soils. Synthesised iron-rich nanomaterials (Fe and Zr–Fe oxides) and natural iron-rich materials (natural red earth; NRE) were used to immobilise As and Pb in contaminated agricultural soil. Total concentrations of As and Pb in the initial soil (as control) were 170.76 and 1945.11 mg kg⁻¹, respectively. Amendments were applied into the soil at 1, 2.5 and 5% (w/w) in triplicate and incubated for 150 days. Except for the NRE-amended soil, soil pH decreased from 5.6 to 4.9 with increasing application rates of Fe and Zr–Fe oxides. With addition of Fe and Zr–Fe oxides at 5%, the ammonium acetate (NHO₄Ac)-extractable Pb was greatly decreased by 83 and 65% compared with NRE addition (43%). All subjected amendments also led to a decrease in NHO₄Ac-extractable As in the soils, indicating the high capacity of As immobilisation. Soil amended with NRE showed a lower ratio of cy19:0 to 18:1 ω 7c, indicating decreased microbial stress. The toxicity characteristic leaching procedure produced results similar to the NHO₄Ac extraction for As and Pb. The NRE addition is recommended for immobilising heavy metals and maintaining biological soil properties.

Keywords: bioavailability; stabilisation; nanoparticle; adsorbent; remediation; soil quality

1. Introduction

Toxic metals threaten the environment due to their deleterious effects on soil biota, plants and human health.[1,2] Among the various metals in the environment, arsenic (As) and lead (Pb) are frequent and hazardous.[1,3,4] Soils are contaminated with As and Pb from different sources such as agricultural and industrial waste discharge.[5] As and Pb are listed among the priority pollutants by the US Environmental Protection Agency (EPA) due to their high toxicity. For example, the accumulation of Pb in the human body leads to harmful effects on the nervous

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system and causes blood and brain disorders,[6] whereas As is carcinogenic and very toxic to animals, plants and humans.[7,8] Hence, there is a pressing need to find cost-effective heavy metal remediation technologies using relatively economical materials such as natural red earth (NRE) and manganese oxides.[9]

Many researchers have focused on various amendments such as biochar, activated carbon and lime as heavy metal stabilisers to treat contaminated soil systems.[1,10,11] However, most of these studies were conducted in a single or binary metal system, and not with similar forms such as either anionic or cationic elements. Few studies have investigated stabilising toxic metals in different forms, i.e. both anionic and cationic in a single system at the same time.[1–3,5,6] From a practical point of view, granular iron oxide-based porous media or coagulation using iron floc shows a high affinity for arsenate.[12] Natural or synthesised Fe oxide can also be a good adsorbent for removing both cationic- and anionic-type heavy metals.[9,13–17] Effective removal of As from natural aqueous and human-induced environments using Fe oxide has been studied recently.[18] Fe oxide is a representative method to immobilise As rather than other inorganic amendments. In addition, granular zirconium (Zr)–Fe oxides showed better chemical stability, binding affinity and adsorption capacity over a wide pH range.[13,19] In batch tests, Zr–Fe oxides had high performance for removing pollutants such as fluoride from drinking water.[13] Synthetic Zr–Fe oxide adsorbent may be effective on both cationic and anionic elements because of its hybrid properties.[20]

NRE is an iron coated sand [21] comprised of Fe and Al as active surface sites. Researchers have reported that NRE can be effectively used as a low cost material to remove Pb and Cd from water.[15–17] Specifically, Pb and As show high affinity for NRE in many batch experiments.[9,16,22]

Taken together, Fe oxide, Zr–Fe oxide and NRE have the potential to clean up water polluted with Pb and As. Thus, we hypothesised that these materials might be effective in immobilising heavy metals in contaminated soils. There is an additional need to evaluate the changes in soil biochemical properties with these materials as good indicators of soil quality.[23–25] Therefore, we conjectured that Fe oxide, Zr–Fe oxide and NRE may adsorb both cationic- and anionic-type heavy metals from soil. The objectives of this study were to assess the efficacy of Fe oxide, Zr–Fe oxide, and NRE on heavy metals removal or immobilisation from a multi-metals contaminated soil and to reveal the possible mechanism involved in metal immobilisation.

2. Materials and methods

2.1. Soil collection and analysis

Agricultural soil was collected near the Poong-Jeong abandoned mine in Bonghwa-gun, Gyeongsangbuk-do Province, Korea. Soil was air-dried and sieved through a 2-mm sieve. Soil pH and electrical conductivity (EC) were measured in a 1:5 soil-to-water ratio suspension using a digital pH meter (WTW inoLab pH7110, Darmstadt, Germany) and an EC meter (EC Thermo Orion, US/555A, Thermo Electronic Corp., Rockford, IL, USA).[26] Exchangeable cations were measured using the 1 M ammonium acetate method.[27] Soil texture was determined using the procedure described by Gee and Or.[28] The collected soil was digested with Aqua Regia and microwave-assisted digestion at 200 ± 5 °C for 20 min (Mars-X, HP-500 plus, CEM Corp., Matthews, NC, USA) according to EPA Method 3051.[29] Pb and As concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 7300 DV, Perkin Elmer, Waltham, MA, USA). Selected physicochemical properties of the soil are presented in Table 1.

								NH ₄ OAc extractable bases			
Sand	Silt	Clay	Texture	pН	EC	Pb	As	Ca ²⁺	Mg^{2+}	K^+	Na ⁺
	%				$\mathrm{dS}\mathrm{m}^{-1}$	${ m mgkg^{-1}}$		$cmol(+) kg^{-1}$			
83.4	8.6	8.0	loamy sand	5.81	0.032	1945.11	170.76	7.32	1.58	0.19	0.03

Table 1. Physicochemical properties of the soil.

Notes: Pb, total Pb concentration; As, total As concentration; NH₄OAc, ammonium acetate.

2.2. Amendments

Zr–Fe oxide was synthesised according to a two-step procedure by preparing a powder and then binding it using a copolymer latex, as described by Dou et al. [13]. The dried material was ground and sieved with a 100-mesh sieve. Fe oxide was synthesised following the procedure of Cornell and Schwertman [30] using FeCl₃ and Na₂SO₄. X-Ray diffraction was conducted to understand the synthesised materials properties. NRE is a naturally occurring material that outcrops along the northwestern part of Sri Lanka (8°14′50″*N*, 79°45′45″*E*). NRE occurs as a rounded and wellsorted quartz sand in a red clay matrix with accessory ilmenite and magnetite. The brick red colour indicates the oxidising conditions due to the formation of red hematite.[17]

2.3. Incubation experiment

Zr–Fe oxide, Fe oxide and NRE were added to the soils at rates of 1, 2.5 and 5% (w/w) and placed in glass vessels (250 mL) for 150 days at 25 °C using an incubator (MIR-554, SANYO Electronic, Co., Ltd., Tokyo, Japan). Soil without amendments was used as the control and the experiment was conducted in triplicate. Distilled water was added to each amended soil to bring the soil moisture to 70% water holding capacity during the incubation period. After incubation, the soil samples were air-dried for 72 h, and the biochemical parameters and heavy metal extractability were measured.

2.4. Chemical analysis

This study measured the selected chemical properties such as pH and EC in a 1:5 soil/water extract mixture, dissolved organic carbon (DOC) in a 1:10 soil/water extract mixture and 1M NHO₄Ac-extractable As and Pb. The water-soluble anion concentrations were determined in 1:10 soil/water suspensions equilibrated for 24 h. The filtered supernatant was analysed for anions (PO_4^{3-} , SO_4^{2-} , and Cl^-) by ion chromatography (Metrohm Compact IC-861, Switzerland). Toxicity characteristic leaching procedure (TCLP)-extracted As and Pb were measured according to EPA method 1311.[31] Sequential extraction of As and Pb was conducted following the procedures by Tessier et al. [32] and Hashimoto et al. [33]. Five fractions including the exchangeable, carbonate bound, Fe/Mn bound, organic bound and residual were also determined. After each extraction step, the supernatant was analysed by ICP-OES for Pb and As.

2.5. Soil microbial analysis

Soil microbial biomass analyses have been described previously.[34,35] Briefly, soil was suspended in 0.2 M KOH and methanol, and incubated at 37 °C for 1 h. Then, 1.0 M acetic acid was added to neutralise the pH of the soil suspension. Fatty acid methyl esters (FAMEs) were extracted in hexane from the soil suspension. The separated hexane layer was then evaporated

under N₂. The residue was dissolved in 1:1 hexane methyl-*tert* butyl ether and analysed by gas chromatography (Agilent 6890, Darmstadt, Germany) with a flame ionisation detector. Various groups of micro-organisms were identified according to individual fatty acids. The individual fatty acids representing total bacteria,[36] Gram-negative bacteria and Gram-positive bacteria,[37] actinomycetes,[36] fungi [38] and arbuscular mycorrhizal fungi (AMF) [38] were used as soil microbial biomass biomarkers. The ratios of cy19:0 to $18:1\omega7c$ and total monounsaturated fatty acids (MUFAs) to total saturated fatty acids (SFA) have been used as indicators of environmental stress in soil microbial communities.[39,40] The abundance of individual FAMEs is reported in absolute amounts (nmol g⁻¹ soil) for each sample. To determine total glomalin, soil was mixed with 100 mM sodium pyrophosphate and autoclaved at 121 °C for 1 h.[41] The sample was then centrifuged and the supernatant was analysed by the Bradford assay using a UV-visible spectrophotometer (UV-1650PC, Shimadzu, Kyoto, Japan).

2.6. Statistical analysis

Differences among amendments were tested with an analysis of variance and subsequent post hoc comparisons of means (Fisher's least significance difference [LSD] test at $\alpha = 0.05$). The statistical analysis was conducted using the SAS ver. 9.1.[42]

3. Results and discussion

3.1. Soil physicochemical properties

The soil was loamy sand having 83.4% sand, 8.6% silt and 8.0% clay. The EC value of the soil was 0.032 dS m⁻¹ and the pH was 5.81. Exchangeable Ca²⁺, Mg²⁺, Na⁺ and K⁺ concentrations were 7.32, 1.58, 0.03 and 0.19 cmol₍₊₎ kg⁻¹, respectively. The mean concentrations of Pb and As in the soil were 1945.11 and 170.66 mg kg⁻¹, respectively. These results indicate that the soil was highly contaminated with Pb and As according to regulatory limits of the EPA (400 mg kg⁻¹ for Pb and 0.4 mg kg⁻¹ for As), mainly due to the deposition of mine tailings that were left at the mine site without proper treatment. However, many agricultural activities are being continued without any concern about heavy metal contamination.[43–45]

3.2. Amendments' characteristics

The pH and EC values were 5.08 and 0.007 dS m⁻¹ for Zr–Fe oxide, 4.63 and 46.8 μ S cm⁻¹ for Fe oxide, and 5.83 and 19 μ S cm⁻¹ for NRE, respectively. Scanning electron microscopy (SEM) images of Fe oxide, Zr–Fe oxide and NRE showed very low porosity in the grains and amorphous nature (Figure 1). More rounded shapes were identified in Fe oxide, whereas Zr–Fe oxide grains showed some angular shapes with sharp edges. The XRD spectra are given in Figure 2. The synthesised Fe and Zr–Fe oxides clearly showed the amorphous nature of Fe. The peak characteristics of the Fe and Zr–Fe oxides were identified in the XRD spectra.[13,46] The natural material showed a crystalline behaviour dominated by SiO₂ and Fe, and Al oxides, as observed by Vithanage et al. [17]. The zero point of charge (pH_{zpc}) values for Fe and Zr–Fe oxides determined by surface titration were 5.92 and 6.61, respectively.

3.3. Soil properties in response to amendments

Figure 3 shows the effect of soil amendments on soil pH and EC after 150 days of incubation. The Fe and Zr–Fe oxides decreased pH values from 5.63 to 4.92 and 4.77, respectively, compared with



Figure 1. Scanning electron microscopy–energy dispersive X-ray (SEM–EDX) analysis: the elemental composition of (a, b) Fe oxide, (c, d) Zr–Fe oxide and (e, f) NRE.

the control soil. By contrast, Fe oxide led to the highest increase in EC followed by Zr–Fe oxide. This may be due to the remaining salts in the synthesised materials. Moreover, the EC obviously increased with increasing rates of Fe and Zr–Fe oxide application.

Fe oxide showed the highest increase in water-extractable Cl⁻ and SO₄²⁻ (25–136.5 mg kg¹⁻ for Cl and 278.7–1446 mg kg¹⁻ for SO₄²⁻), followed by Zr–Fe oxide (45.3–141.3 mg kg⁻¹ for SO₄²⁻), compared with control soil. In addition, anion concentrations increased with increasing Fe and Zr–Fe oxides application rates (Table 2). No water-extractable PO₄³⁻ was found in the soils, indicating phosphate precipitation due to P and Ca interaction in the control soil or P and Fe(III) interaction in the amended soils. It is noteworthy that the formed complexes of Fe, Ca and As in the amended soils were followed by an increase in water-extractable sulfates, contributing to a decrease in pH, and this also increased soil salinity, as indicated by the EC



Figure 2. X-Ray diffraction (XRD) spectra of (a) Fe oxide, (b) Zr-Fe oxide and (c) NRE.

values (Figure 3). Increased SO_4^{2-} availability in soil is due to the coordination of the SO_4 anion to a Fe cation. Specifically, an increase in water-extractable sulfate anions may be due to low formation of Fe-arsenosulfides through abiotic sulfidization in soils, as reported previously.[47] However, no difference in anionic concentrations was observed between the NRE-amended soils



Figure 3. (a) pH and (b) EC of agricultural soils amended with Fe oxide, Zr–Fe oxide and NRE, subjected to 1, 2.5 and 5% (w/w).

Amendments	Cl ⁻	NO_3^-	SO_4^{2-}	DOC				
		mg kg ⁻¹						
Control	1.11 ± 0.05	2.63 ± 0.02	1.94 ± 0.15	19.68 ± 2.40				
1% Fe oxide	24.95 ± 2.84	0.90 ± 0.12	278.70 ± 33.01	27.54 ± 4.59				
2.5% Fe oxide	61.17 ± 6.95	1.10 ± 0.14	694.48 ± 43.77	30.72 ± 4.81				
5% Fe oxide	136.49 ± 6.33	1.57 ± 0.03	1446.40 ± 93.49	40.69 ± 0.98				
1% Zr-Fe oxide	1.19 ± 0.08	3.24 ± 0.23	45.36 ± 4.00	13.77 ± 0.77				
2.5% Zr-Fe oxide	1.42 ± 0.17	2.96 ± 0.14	82.37 ± 5.59	14.66 ± 1.34				
5% Zr-Fe oxide	1.52 ± 0.07	2.94 ± 0.06	141.26 ± 13.09	18.89 ± 4.61				
1% NRE	1.32 ± 0.28	3.28 ± 0.47	2.16 ± 0.41	21.31 ± 0.97				
2.5% NRE	1.19 ± 0.13	2.39 ± 0.35	1.82 ± 0.07	20.28 ± 2.87				
5% NRE	1.26 ± 0.07	2.31 ± 0.26	1.70 ± 0.10	19.40 ± 2.81				

Table 2. Changes in soil anions and dissolved organic carbon (DOC) in response to the soil amendments.

and the control. Only Fe oxide increased DOC slightly in soils compared with the control. DOC increased with increasing Fe oxide application rate (from 1 to 5%). Regression analysis showed a negative correlation between pH and Fe oxide application rate ($R^2 = 0.88$). Conversely, on Fe oxide application, an increase in DOC was observed (from 19 to 40 mg kg⁻¹). However, no significant differences in pH, EC and DOC were found between the NRE-amended soils and the control.

3.4. As and Pb bioavailability

Iron oxide, Zr–Fe oxide and the NRE amendments immobilised Pb and As in the contaminated soil (Figure 5). The Fe oxide, Zr–Fe oxide and NRE amendments decreased NHO₄Ac-extractable As in soil by an average of 77.3, 64.7 and 65.8, respectively, compared with the control (Figure 4). Similarly, NHO₄Ac-extractable Pb decreased by 30.6-89.4% in soils amended with these amendments compared with the control. A high level of soil amendments (5%) was most effective for immobilising Pb and As in the range of 68.9–77.9% and 43.5–89.4%, respectively, compared with the control (Figure 5). These soil amendments also led to a decrease in TCLP-extracted Pb and As. Fe oxide (average 8.7%) and Zr–Fe oxide (average 9.2%) were the most effective amendments,



Figure 4. Concentrations of ammonium acetate extractable As and Pb in agricultural soils amended with Fe oxide, Zr-Fe oxide and NRE, subjected to 1, 2.5 and 5% (w/w).

followed by the NRE (average 5%; Figure 6), indicating that most of the As was stabilised well in the soil system with the soil amendments. Sequentially extracted As increased in the residual fractions of amended soils by an average of 7.9% compared with that of the control, indicating that these amendments were effective in immobilising metals in soil by transforming the available metal fractions into highly unavailable fractions.

The NRE contained little Fe²⁺ (0–1%); however, Fe³⁺ content is typically in high (> 2.0%) reaching 6%.[21] The SEM-energy dispersive X-ray results and previous data [17] showed that the NRE contained a high proportion of Al (as Al₂O₃) and Fe (as Fe₂O₃) (Figure 1), probably as an amorphous coating around silica grains. Because the pH_{zpc} of the amendments was pH 6–9, the most probable mechanism of toxic metal immobilisation was adsorption. The formation of an inner sphere surface complex of Pb(II) on the NRE through a mixture of monodentate and bidentate based on the FITEQL model was observed in a study by Mahatantila et al. [16] and the Pb adsorption onto NRE occurred by a chemisorption mechanism. Hence, the formation of inner sphere complexes with Pb may be the reason for the reduction of Pb availability in this study. Our findings are in agreement with studies of Trivedi et al. [48] and Bargar et al. [49]

A regression analysis clearly indicated a positive relationship between pH and Pb or As leached by NHO₄Ac for two synthetic amendments except the NRE. The decrease in pH was clear with increasing application rate. Zr–Fe oxide showed a strong relationship with Pb and As (R^2 values were 0.995 and 0.982, respectively), whereas it was slightly lower for Fe oxide (R^2 values for Pb and As were 0.885 and 0.978, respectively). A decrease in pH from 5.63 to 4.77 with increasing the application rate of Zr–Fe oxide from 0 to 5% led to a reduction in Pb and As extractability from 125 to 13 mg kg⁻¹ and 1.11 to 0.25 mg kg⁻¹, respectively. However, no such relationship was observed for NRE. Fe oxide increased DOC in soils (Table 2), which may be due to the cations input and the pH decrease. The DOC may promote the formation of soluble As–Fe organic complexes and prevent As from being adsorbed and precipitated onto Fe oxides by inhibiting the formation of Fe oxide complexes.[8,50] Specifically, the additions of Fe and Zr–Fe oxides form As–Fe organic



Figure 5. Toxicity characteristic leaching procedure (TCLP)-extracted (a) Pb and (b) As in agricultural soils amended with Fe oxide, Zr–Fe oxide and NRE, subjected to 1, 2.5 and 5% (w/w).

complexes, which may be adsorbed in the presence of Ca^{2+} ions on DOC at lower soil $pH \le 5$ (Figure 3). Therefore, the increase in DOC concentration may be responsible for the increase in As mobility, as reported by Jackson et al. [51]. However, no considerable increase in As mobility was seen even with higher DOC in the Fe-oxide-amended soils. This may be a reason for postulating adsorption as the prominent toxic metal immobilisation mechanism in this soil.



Figure 6. Sequential-extracted (a) As and (b) Pb in agricultural soils amended with Fe oxide, Zr–Fe oxide and NRE, subjected to 1, 2.5 and 5% (w/w).

3.5. Soil microbial concentrations

Microbial concentrations and their ratios in the amended soils and the control are presented in Table 3. Only Zr–Fe oxide and NRE resulted in an abundance of actinomycetes compared with the control, with the exception for 1% Zr–Fe oxide and 2.5% NRE (Table 3). However, Fe oxide (1-5% application rates) and NRE (1%) decreased the glomaline concentrations compared with the control. No significant differences in fungi, total bacteria, Gram-negative bacteria, Gram-positive bacteria, AMF, FAME, SFA and MUFA were observed compared with the control.

AMF biomass must be determined to understand soil nutrient dynamics, [52] and AMF in NRE-amended soils may play a major role in soil functioning, [53] indicating that NRE is an effective amendment for removing Pb and As via maintenance of the soil's biological qualities. The 5% NRE soil showed a lower average ratio of cy19:0 to $18:1\omega7c$ and a higher average ratio of MUFAs to SFAs than the control, indicating an decrease of microbial stress in the OF plot.[39,54]

	Control	Fe oxide			Zr–Fe oxide			NRE		
Microbial biomass		1%	2.5%	5%	1%	2.5%	5%	1%	2.5%	5%
Total FAMEs	50.48 a	49.45 a	52.26 a	46.56 a	53.02 a	56.11 a	69.71 a	46.97 a	46.25 a	52.20 a
Total bacterial	7.55 a	8.73 a	7.39 a	6.08 a	8.36 a	7.86 a	11.42 a	7.99 a	7.70 a	8.59 a
Gram-negative bacteria	3.34 a	3.53 a	2.75 a	2.07 a	3.56 a	3.32 a	4.79 a	3.38 a	3.66 a	4.2 a
Gram-positive bacteria	4.14 a	5.20 a	4.64 a	4.02 a	4.8 a	4.54 a	6.27 a	4.61 a	4.04 a	4.32 a
Actinomycetes	0.00 c	0.42 abc	0.81 a	0.14 bc	0.00 c	0.66 ab				
Fungi	5.10 a	5.38 a	6.40 a	6.81 a	5.38 a	5.44 a	10.67 a	5.07 a	6.07 a	5.98 a
Arbuscular mycorrhizal fungi	0.91 a	1.17 a	0.67 a	0.79 a	0.87 a	0.74 a	1.31 a	1.01 a	1.08 a	0.63 a
G-/G+	0.81 a	0.70 a	0.61 a	0.52 a	0.74 a	0.73 a	0.8 a	0.74 a	0.91 a	0.98 a
Fungi/bacteria	0.68 ab	0.62 b	0.88 ab	1.12 a	0.68 b	0.69 ab	0.85 ab	0.66 b	0.80 ab	0.71 ab
cy17:0:16:1w7c cy19:0:18:1w7c	0.13 a 0.39 a	0.00 a 0.27 a	0.60 a 0.32 a	0.46 a 0.43 a	0.16 a 0.48 a	0.22 a 0.45 a	0.21 a 0.44 a	0.08 a 0.35 a	0.29 a 0.28 a	0.30 a 0.37 a
MUFA:SFA Glomalin	0.59 a 323 a	0.67 a 252 b	0.52 a 246 b	0.50 a 249 b	0.53 a 297 c	0.54 a 305 bc	0.65 a 344 a	0.54 a 261 b	0.81 a 311 a	0.69 a 307 ab

Table 3. Soil microbial biomass concentrations (nmol g^{-1}) and ratios in response to the soil amendments.

Notes: G-/G+, Gram-negative/Gram-positive bacteria. Means followed by different letters within the same row are significantly different at a 0.05 significance level.

4. Conclusions

Iron oxide, Zr–Fe oxide and NRE effectively immobilised Pb and As in a contaminated soil. All subjected soil amendments decreased the concentrations of NHO₄Ac-extractable As and Pb in the soils compared with the soil with no addition. The highest rate of all soil amendments (at 5%) was most effective for Pb and As immobilisation, according to the result of decreasing TCLP-extracted Pb and As. The sequential extraction results revealed that all amendments altered the initial forms of Pb and As in soil. Soil amendments also increased residual fractions of Pb and As. Moreover, the addition of NRE effectively maintained soil biota, especially AMF. Compared with Fe and Zr–Fe oxides, NRE is recommended as a low-cost natural material for Pb and As immobilisation, and soil quality maintenance, especially pH, EC, anions and biota. NRE not only immobilises As in a contaminated soil but also decreases As mobility through the reduction of soluble As–Fe organic complexes when compared with the soils amended with Fe and Zr–Fe oxides.

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References

- Ahmad M, Hashimoto Y, Moon HD, Lee SS, Ok SY. Immobilization of lead in a Korean military shooting range soil using eggshell waste: an integrated mechanistic approach. J Hazard Mater. 2012;209–210:392–401.
- [2] Ahmad M, Lee SS, Yang EJ, Ro HM, Han Lee Y, Ok SY. Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. Ecotoxicol Environ Saf. 2012;79:225–231.

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- [3] Almaroai AY, Usman AAR, Ahmad M, Kim KR, Moon HD, Lee SS, Ok SY. Effects of synthetic chelators and low-molecular-weight organic acids on Chromium, Copper, and Arsenic uptake and translocation in Maize (Zea mays L.). Soil Sci. 2012;177:655–663.
- [4] Almaroai AY, Usman AAR, Ahmad M, Kim K-R, Vithanage M, Ok SY. Role of chelating agents on release kinetics of metals and their uptake by maize from chromated copper arsenate-contaminated soil. Environ Technol. 2013;34(6):747–755.
- [5] Usman ARA, Lee SS, Awad MY, Lim JK, Yang EJ, Ok SY. Soil pollution assessment and identification of hyperaccumulating plants in chromated copper arsenate (CCA) contaminated sites, Korea. Chemosphere. 2012;87:872–878.
- [6] Dwivedi PC, Sahu NJ, Mohanty RC, Mohan RB, Meikap CB. Column performance of granular activated carbon packed bed for Pb(II) removal. J Hazard Mater. 2008;156:596–603.
- [7] Gratão PL, Polle A, Lea JP, Azevedo AR. Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol. 2005;32:481–494.
- [8] Hartley W, Lepp WN. Effect of in situ soil amendments on arsenic uptake in successive harvests of ryegrass (Lolium perenne cv Elka) grown in amended As-polluted soils. Environ Pollut. 2008;156:1030–1040.
- [9] Vithanage M, Senevirathna W, Chandrajith R, Weerasooriya R. Arsenic binding mechanisms on natural red earth: a potential substrate for pollution control. Sci Total Environ. 2007;379:244–248.
- [10] Lim EJ, Ahmad M, Lee SS, Shope LC, Hashimoto Y, Kim RK, Usman AAR, Yang EJ, Ok SY. Effects of lime-based waste materials on immobilization and phytoavailability of Cd and Pb in contaminated soil. CLEAN - Soil, Air, Water. doi:2013.10.1002/clen.201200169.
- [11] Ok SY, Kim SC, Kim DK, Skousen JG, Lee JS, Cheong YW, Kim SJ, Yang JE. Ameliorants to immobilize Cd in rice paddy soils contaminated by abandoned metal mines in Korea. Environ Geochem Health. 2011;33:23–30.
- [12] Sylvester P, Westerhoff P, Möller T, Badruzzaman M, Boyd O. A hybrid sorbent utilizing nanoparticles of hydrous iron oxide for arsenic removal from drinking water. Environ Eng Sci. 2007;24:104–112.
- [13] Dou X, Zhang Y, Wang H, Wang T, Wang Y. Performance of granular zirconium–iron oxide in the removal of fluoride from drinking water. Water Res. 2011;45:3571–3578.
- [14] Koo N, Jo HJ, Lee SH, Kim JG. Using response surface methodology to assess the effects of iron and spent mushroom substrate on arsenic phytotoxicity in lettuce (Lactuca sativa L.). J Hazard Mater. 2011;192:381–387.
- [15] Mahatantila K, Vithanage M, Seike Y, Okumura M. Adsorptive removal of cadmium by natural red earth: equilibrium and kinetic studies. Environ Technol. 2011;33:597–606.
- [16] Mahatantila K, Seike Y, Okumura M. Adsorptive removal of lead(II) ion using natural red earth from its iron and aluminum oxide forms. Int J Eng Sci Health. 2011;3(2):1655–1666.
- [17] Vithanage M, Chandrajith R, Bandara A, Weerasooriya R. Mechanistic modeling of arsenic retention on natural red earth in simulated environmental systems. J Colloid Interface Sci. 2006;294:265–272.
- [18] Gallegos-Garcia M, Ramírez-Muñiz K, Song S. Arsenic removal from water by adsorption using iron oxide minerals as adsorbents: a review. Miner Process Extr Metall Rev. 2011;33:301–315.
- Blackwell AJ, Carr WP. Study of the fluoride adsorption characteristics of porous microparticulate zirconium oxide, J. Chromatogr. 1991;549:43–57.
- [20] Biswas K, Bandhoyapadhyay D, Ghosh U. Adsorption kinetics of fluoride on iron(III)-zirconium(IV) hybrid oxide. Adsorption. 2007;13:83–94.
- [21] Dahanayake JKS. Study of red and brown earth deposits of north-west Sri Lanka. J Geol Soc India. 1979;20:433-440.
- [22] Rajapaksha UA, Vithanage M, Jayarathna L, Kumara KC. Natural red earth as a low cost material for arsenic removal: kinetics and the effect of competing ions. Applied Geochem. 2011;26:648–654.
- [23] Friedel KJ, Langer T, Siebe C, Stahr K. Effects of long-term waste water irrigation on soil organic matter, soil microbial biomass and its activities in central Mexico. Biol Fertil Soils. 2000;31:414–421.
- [24] Usman ARA, Kuzyakov Y, Stahr K. Effect of clay minerals on immobilization of heavy metals and microbial activity in a sewage sludge-contaminated soil. J Soils Sed. 2005;5:245–252.
- [25] Usman ARA, Kuzyakov Y, Stahr K. Effect of clay minerals on extractability of heavy metals and sewage sludge mineralization in soil. Chem Ecol. 2004;20:123–135.
- [26] Yoon Y, Ok SY, Kim YD, Kim GJ. Agricultural recycling of the by-product concentrate of livestock wastewater treatment plant processed with VSEP RO and bio-ceramic SBR. Water Sci Technol. 2004;49:405–412.
- [27] Sumner EM, Miller PW. Cation exchange capacity and exchange coefficients. In: Sparks ALPDL, Helmke AP, Loeppert HR, Soltanpour NP, Tabatabai AM, Johnston TC, Sumner EM, editors. Methods of soil analysis, Part 3 chemical methods. Madison, Wisconsin, USA: American Society of Agronomy-Soil Sci. Soc. Am. Inc.; 1996. p. 1201–1230.
- [28] Gee WG, Orr D. Particle size analysis. In: Dick WA, editor. Methods of soil analysis part 4, Physical methods. Soil Science Society of America, Inc; 2002, p. 255–293.
- [29] US Environmental Protection Agency (EPA). Soil screening guidance: user's guidance. Washington, DC: Office of Solid Waste and Emergency Response, USEPA; 1996. p. 30.
- [30] Cornell MR, Schwertmann U. The iron oxides. In: The Iron Oxides Structure, Properties, Reactions, Occurrence and Uses. Weinheim, Germany: Willey-VCH VerlagGmbH & Co. KGaA, ProSatz Unger; 2003, p. 345–363.
- [31] EPA. Toxicity characteristics leaching procedure. US Environmental Protection Agency, Fed. Reg; 1999, p. 11798.[32] Tessier A, Campbell CGP, Bisson M. Sequential extraction procedure for the speciation of particulate trace metals.
- Anal Chem. 1979;51:844–851.
- [33] Hashimoto Y, Taki T, Sato T. Sorption of dissolved lead from shooting range soils using hydroxyapatite amendments synthesized from industrial byproducts as affected by varying pH conditions. J Environ Manage. 2009;90:1782–1789.

- [34] Lee Y, Kim H. Response of soil microbial communities to different farming systems for upland soybean cultivation. J Korean Soc Appl Biol Chem. 2011;54:423–433.
- [35] Yang KS, Kim KM, Seo WY, Choi JK, Lee TS, Kwak SY, Lee HY. Soil microbial community analysis of between No-till and tillage in a controlled horticultural field. Acad J. 2012;28(4):1797–1801.
- [36] Schutter EM, Dick PR. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Sci Soc Am J. 2000;64:1659–1668.
- [37] Zelles L. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere. 1997;35:275–294.
- [38] Frostegård Å, Tunlid A, Bååth E. Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl Environ Microb. 1993;59:3605–3617.
- [39] Bossio AD, Scow MK. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol. 1998;35:265–278.
- [40] Guckert BJ, Hood AM, White CD. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of vibrio cholerae: increases in cis/trans ratio and proportions of cyclopropyl fatty acid. Appl Environ Microb. 1986;52:94–801.
- [41] Wright FS, Nichols AK, Schmidt FW. Comparison of efficacy of three extractants to solubilize glomalin on hyphae and in soil. Chemosphere. 2006;64:1219–1224.
- [42] SAS Institute. SAS/STAT User's Guide, Release 9.1. Cary, NC, USA: SAS Institute Inc.; 2004.
- [43] Ok SY, Lee SS, Jeon WT, Oh SE, Usman ARA, Moon HD. Application of eggshell waste for the immobilization of cadmium and lead in a contaminated soil. Environ Geochem Health. 2011b;33:31–39.
- [44] Ok SY, Lim EJ, Moon HD. Stabilization of Pb and Cd contaminated soils and soil quality improvements using waste oyster shells. Environ Geochem Health. 2011c;33:83–91.
- [45] Ok SY, Oh ES, Ahmad M, Hyun S, Kim KR, Moon HD, Lee SS, Lim JK, Jeon WT, Yang EJ. Effects of natural and calcined oyster shells on Cd and Pb immobilization in contaminated soils. Environ Earth Sci. 2010;61:1301–1308.
- [46] Jayarathne L, Ng JW, Bandara A, Vitanage M, Dissanayake BC, Weerasooriya R. Fabrication of succinic acid-γ-Fe2O3 nano core–shells. Colloids Surf Physicochem Eng Aspects. 2012;403:96–102.
- [47] Kumpiene J, Desogus P, Schulenburg S, Arenella M, Renella G, Brannval E, Largerkvist A, Andreas L, Sjoblom R. Utilisation of chemically stabilized arsenic-contaminated soil in a landfill cover. Environ. Sci Pollut Res. 2013;20(12):8649–8662.
- [48] Trivedi P, Dyer AJ, Sparks LD. Lead sorption onto ferrihydrite. 1. A macroscopic and spectroscopic assessment. Environ Sci Technol. 2003;37:908–914.
- [49] Bargar RJ, Brown EG, Jr, Parks AG. Surface complexation of Pb(II) at oxide-water interfaces: I. XAFS and bond-valence determination of mononuclear and polynuclear Pb(II) sorption products on aluminum oxides, Geochim. Cosmochim Acta. 1997;61:2617–2637.
- [50] Wang S, Mulligan NC. Enhanced mobilization of arsenic and heavy metals from mine tailings by humic acid. Chemosphere. 2009;74:274–279.
- [51] Jackson PB, Seaman CJ, Bertsch MP. Fate of arsenic compounds in poultry litter upon land application. Chemosphere. 2006;65:2028–2034.
- [52] Balser CT, Treseder KK, Ekenler M. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biol Biochem. 2005;37:601–604.
- [53] Van Der Heijden MGA, Wiemken A. Sanders RI, Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. New Phytol. 2003;157:569–578.
- [54] Mechri B, Chehab H, Attia F, Mariem BF, Braham M, Hammami M. Olive mill wastewater effects on the microbial communities as studied in the field of olive trees by analysis of fatty acid signatures. Eur J Soil Biol. 2010;46:312–318.