Effect of chemical amendments on reduction of bioavailable heavy metals and ecotoxicity in soil

Dong-Hyun Yoon1, Won Seok Choi1, Young Kyu Hong1, Young Bok Lee2 and Sung Chul Kim1*

Abstract

Heavy metal pollution in soil has been concerned because of toxicity in ecosystem and adverse effect on human health. Main objective of this study was to examine reduction of bioavailable heavy metals and consequently, decrease of ecotoxicity to biota when chemical amendments were applied in soil. Three chemical amendments, acid mine drainage sludge (AMDS), lime stone (LS), and steel slag (SS) were applied with varied application ratio (1, 3, 5%) in heavy metal polluted soil and bioavailable fraction of heavy metal was monitored. In addition, ecotoxicity test using earthworm (Eisenia fetida) was conducted for 28 days examining mortality, weight increase, and bioaccumulation of heavy metal in the earthworm. Result showed that AMDS was the most efficient amendment for reducing bioavailable heavy metals in soil while SS showed the least efficiency. Reduction ratio of bioavailable-As, Cd, and Pb was ranged 39.0–92.0% depending on application ratio and heavy metal species for AMDS application. However, only bioavailable-Pb was reduced at the range between 39.1% and 56.5% when SS was applied in soil. In contrast, the lowest concentration of As, Cd, and Pb and ecotoxicity effect in the earthworm was observed in SS treatment indicating that exposure route of heavy metals or particle size of amendments might effect on uptake of heavy metals to the earthworm. Overall, ecotoxicity test in combination with chemical concentration monitoring is a useful tool for evaluating remediation efficiency of heavy metal polluted soil.

Keywords: Heavy metals, Amendments, Remediation, Earthworm, Ecotoxicity

Introduction

Heavy metal pollution in soil has been concerned because of adverse effect on ecosystem and human health [1, 2]. Previous study reported that heavy metal pollution in soil can have direct toxic effect on soil biota including flora and fauna and indirect effect on human health because of bio-accumulated heavy metals in crop [1, 3–5]. Rai et al. reviewed occurrence, fate, and mechanism of heavy metals in food crop and reported that level of bio-accumulated heavy metals in food crop was varied from well below 0.01 mg/kg up to over 10 mg/kg depending on heavy metal species, regions and applied materials in soil [6]. Once heavy metal was accumulated in food crop, it can affect DNA strands, alteration of genetic materials, and malfunction of normal physiological function in food crop [6, 7]. Understanding mechanism of heavy metal bioavailability from soil to living organism is a complex process. Biotic characteristics such as organism’s size, physiological properties, and growing conditions can be factors and abiotic characteristics of soil such as soil pH, redox conditions, soil texture, and organic matter contents are all impact bioavailability of heavy metals in soil [8, 9].

Heavy metal pollution in soil is especially difficult to remediate due to non-biodegradable and persistent properties when it was released into the environment [9]. Physical, chemical, and biological remediation techniques can apply for reducing bioavailable fraction of heavy metals in soil [8, 10, 11]. Among others, application of chemical amendments for soil stabilization has...
an advantage in terms of easy to control, cost effective, and eco-friendly technique compared to soil washing and thermal technique [1, 6]. Generally, alkalinity materials such as lime-stone, steel slag, and fly ash were used for immobilization of bioavailable heavy metal in soil. When soil pH is increased, bioavailable fraction of heavy metals can form a complexation or co-precipitation with hydroxide ions and might be decreased bio-accessibility in soil [12–15]. Acid mine drainage sludge (AMDS) is also used as chemical stabilization material for heavy metals including metalloide (arsenic) because high contents of Al, Fe-oxide, and gypsum compounds in AMDS has an effect on immobilization of heavy metals with oxidation, sorption, and precipitation mechanism in soil [16–18].

Conserving ecosystem in soil is important when soil is polluted with heavy metals [19]. Since bioavailable fraction of heavy metal can have a toxic effect on soil biota, earthworms have been used as a bio-indicator for evaluating heavy metal toxicity in soil [1, 20, 21]. In general, two uptake routes, dermal and gut exposure, are considered for heavy metal bioaccumulation in earthworm [22, 23]. Between two exposure route, dermal exposure occupied 82–96% of total bioavailable heavy metals for Cd, Cu, and Zn but gut exposure route was also important pathways for Cd uptake in the earthworm [24]. As shown in previous study, heavy metal toxicity and uptake route from soil to earthworm are varied depending on heavy metal species and bioavailable concentration in soil. Therefore, it is important to understand relationship between bioavailable fraction of heavy metals in soil and toxicity of heavy metals to earthworm.

Main objective of this study was to evaluate reduction efficiency of bioavailable fraction of heavy metals in soil when chemical amendments were applied in a polluted soil. Also, earthworm (*Eisenia fetida*) was used as a bio indicator to estimate toxicity of heavy metal pollution in soil.

**Materials and methods**

**Soil sampling**

Soil sample was collected from upland near at the abandoned metal mine in Korea. Previous study examined heavy metal concentration in this region and reported that concentration of As (306.7 mg/kg), Cd (11.7 mg/kg), and Pb (188.2 mg/kg) in soil was exceeded the threshold value of soil pollution in Korea for As (25 mg/kg) and Cd (4 mg/kg) respectively. Although, concentration of Pb in this field was below the criteria of Pb (200 mg/kg), still high concentration of Pb in field was observed. Soil sample used for control was collected from upland near at the Daejeon Province and verified that heavy metal concentration was well below the threshold value of soil pollution in Korea (Table 1). Soil sample was collected with shovel (Eijkelkamp, Netherlands) within the soil depth of 0–30 cm after removing organic matter and debris at the top of the soil. Soil samples from five different locations within 100 m² was collected and composited in one sample bag to make representative soil sample. Total 200 kg of soil sample was collected and homogenized in the sample bag. Homogenized soil sample was air-dried at the room temperature (25 °C) and sieved with 10-mesh to obtain less than 2 mm particle for chemical analysis and eco-toxicity test.

**Earthworm (*Eisenia fetida*) bioassay**

Earthworm (*Eisenia fetida*) was purchased from Carolina Biological Supply (CA, USA) and incubated for 2 months at the growth chamber (VS-3150Bi, Vision scientific, USA) before experiment. Container (50 × 35 × 20 cm, L × W × H) was filled with bedding mainly composed with peat and tree bark and 70% of water holding capacity (WHC) was maintained by spreading mineral water at every 2 days. Temperature and humidity of growth chamber was set to 20 ± 2 °C and 70% respectively and 16/8 h light/dark cycle was set during cultivation.

Bioassay experiment was conducted by mixing 2.5 kg of moisten soil and 1, 3, 5% of chemical amendments (w/w) in each container (25 × 20 × 15 cm, L × W × H). Application ratio of chemical amendments was determined considering alkalinity properties of each amendment (Table 2). Ten adult earthworm was added in each container and all experiment was performed in triplicate for each sample including control (total N = 30). All earthworms used in the experiment had a noticeably developed clitellum and activity was vivid. Prior to add earthworm in each container, earthworm was rinsed with distilled water to remove any particles at the surface of skin and dried with paper towel. During the experimental period (8 weeks), earthworm was fed with heavy metal free grain powder. After finishing exposure period, earthworms in each container were

| Elements | As (mg/kg) | Cd (mg/kg) | Cu (mg/kg) | Ni (mg/kg) | Pb (mg/kg) |
|----------|------------|------------|------------|------------|------------|
| Control soil | 2.77       | 0.42       | 3.02       | 10.76      | 16.75      |
| Contaminated soil | 306.71     | 11.72      | 16.79      | 27.88      | 188.25     |
| Tolerable level | 25         | 4          | 150        | 100        | 200        |
rinsed with distilled water, dried with paper towel, and purged for 48 h. Mortality of earthworm was recorded after finishing exposure period and weight increment was calculated by comparing earthworm weight before and after bioassay experiment.

Chemical and heavy metal analysis in soil and earthworm

Soil pH and electrical conductivity (EC) were measured with pH meter (MP220, Mettler Toledo, USA) and EC meter (S230, Mettler Toledo, USA) after shaking 10 g of soil and 50 mL of distilled water for 1 h. Soil organic matter, available P$_2$O$_5$, and cation exchange capacity (CEC) was measured following Walkley–Black, Bray No1, and ammonium acetate exchange method. All chemical measurement of soil was conducted before the experiment and summarized in Table 3.

Soil extract to determine total heavy metal concentration was prepared by digestion of the soil sample with aqua regia (HNO$_3$:HCl (v/v) = 1:3) in a heating block (Block Heating Sample Preparation System, Ctrl-M Science). Bioavailable fraction of heavy metal in soil was extracted with Mehlich-3 extractant.

In order to determine heavy metal adsorbed in earthworm, five earthworms from each container were rinsed with distilled water, dried with paper towel, and left for 48 h on filter paper to depurate. After depuration, the earthworms were placed in the 15 mL test tube for lyophilization. Digestion of earthworm was conducted with heating block for 2 h after 24 h of stagnation in a HNO$_3$ solution. Heavy metal concentration was measured with ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer, ICAP 7000series, THERMO FISHER, USA). Certified reference material (BAM Germany) was used for QA/QC purpose of heavy metal measurement.

Table 2 Chemical properties and heavy metal concentration of chemical amendments

| Chemical properties | pH (1:5) | EC (dS/m) | As (mg/kg) | Cd (mg/kg) | Cu (mg/kg) | Ni (mg/kg) | Pb (mg/kg) |
|---------------------|---------|-----------|------------|------------|------------|------------|------------|
| AMDS                | 8.36    | 0.59      | 4.45       | 8.36       | 6.19       | 234.62     | 6.30       |
| LS                  | 9.09    | 0.35      | ND         | 0.36       | 1.01       | ND         | 1.89       |
| SS                  | 11.04   | 0.76      | ND         | 3.41       | 19.55      | 67.79      | 8.27       |

Table 3 Physicochemical properties of control and contaminated soil

| Soil texture (%) | Chemical properties |
|------------------|---------------------|
|                  | pH (1:5) | EC (dS/m) | Organic matter (%) | Av. P$_2$O$_5$ (mg/kg) | CEC (cmol+/kg) |
| Control soil     | 6.21     | 0.23      | 1.5                | 28.5                   | 5.8           |
| Contaminated soil| 6.06     | 0.29      | 2.7                | 22.5                   | 18.6          |

Av. P$_2$O$_5$ indicates available P$_2$O$_5$

Fig. 1 Soil pH after 8 weeks of chemical amendments application

Statistical analysis

All measurement was conducted in triplicate and statistical analysis (ANOVA test) was performed with SPSS software (Version 20.0). ANOVA test was conducted by Tukey’s method at the significance level of $p < 0.05$.

Results and discussion

Change of soil pH

Soil pH is one of the most important factor for immobilization of bioavailable heavy metal fraction in soil. After 8 weeks of chemical amendment application, soil pH of the all treatment was increased except control (Fig. 1). Compared to initial soil pH (6.06), slight increase was
observed in the control (6.10) while significantly high increase (p < 0.05) of soil pH was observed in AMDS (7.60–7.80), LS (7.90–8.20), and SS (7.60–8.00) treatment. However, no significant difference of soil pH was observed among three chemical amendments. As shown in Table 2, all three chemical amendments have alkaline properties. AMDS is produced after acid mine drainage treatment and lime or calcium hydroxide (Ca(OH)2) is generally added during the treatment process to increase pH [18]. In case of SS, lime, dolomite, and other auxiliary materials are added during the iron production process and can cause alkalinity properties of the SS [13].

**Bioavailable fraction of heavy metals in soil**

Bioavailable fraction of heavy metals in soil examined with Mehlich-3 extraction method is summarized in Fig. 2. The lowest concentration of bioavailable-As was observed in AMDS 5% treatment (0.07 mg/kg) followed by AMDS 3% (0.09 mg/kg) and AMDS 1% (0.35 mg/kg). Compared to control (0.87 mg/kg), reduction rate was 92.0%, 89.7%, and 59.8% for AMDS 5%, AMDS 3%, and AMDS 1% treated plots respectively. In case of bioavailable-Cd and Pb in soil, AMDS also showed the highest reduction rate among other chemical amendments. When compared to control, reduction rate of bioavailable-Cd and Pb in the AMDS 5% treatment was 39.0% and 87.0% respectively. The lowest reduction rate of bioavailable-As, Cd, and Pb was observed in the SS treatment. Only bioavailable-Pb concentration was reduced when SS 1% (39.1%), SS 3% (52.2%), and SS 5% (56.5%) were applied in the soil and no reduction efficiency was observed for bioavailable-As and Cd.

Among 3 chemical amendments, AMDS showed the highest reduction efficiency for bioavailable fraction of As, Cd, and Pb in soil while SS showed the least reduction efficiency. Lee et al. (2018) studied heavy metal sorption including arsenic on granular polyurethane impregnated with coal mine drainage sludge (PUcdms) and reported that arsenic can be sorbed on the surface of PUcdms with anionic exchange mechanism since surface of PUcdms is negatively charged at pH < 9 with dominant species of FeOH2+SO42− [17]. Since soil pH of the AMDS treatment in our study was maintained at the range of 7.0–8.1, dominant As species could be As(V) that exists mainly as negative forms such as HSO−4 and HAsO−42 in soil and anion exchange mechanism might be the reason for As reduction in AMDS treatment. In case of bioavailable-Cd and Pb reduction mechanism, AMDS mainly contains iron oxide or iron sulfate hydroxide that can sequester cations such as Cd and Pb in soil with adsorption or co-precipitation [25]. Although, exact mechanism for reduction of bioavailable-As, Cd, and Pb was not verified in this study, combined reduction mechanisms including anion exchange, adsorption on the surface of AMDS and co-precipitation with iron bearing hydroxide could contribute the reduction of bioavailable-As, Cd, and Pb.

Another possibility for high reduction efficiency of bioavailable-As, Cd, and Pb with AMDS treatment can be a smaller particle size of AMDS than LS and SS. As shown in Table 4, particle size of AMDS was mainly distributed at the range 0.15–1.0 mm (46.8%) and < 0.15 mm (22.4%).
In case of SS, 82.7% of particle was greater than 2 mm and only 0.8% of the SS particle was smaller than 1.0 mm. The effect of particle size on reduction of bioavailable heavy metals in soil were studied with various materials. Rock phosphate with a smaller size (< 35 μm) showed more high efficiency than a larger grain size (133–266 μm) on decreasing bioavailability of metals in soil [26]. Cui et al. examined various size of hydroxyapatite for decrease of bioavailable heavy metal concentration and change of microbial community composition [27]. This study reported that micro-hydroxyapatite (< 12 μm) was the most effective particle size on reducing bioavailable heavy metal concentration in soil compared to normal hydroxyapatite (> 100 μm) and even nano-hydroxyapatite (< 35 nm). The reason was that smaller particle size of micro-hydroxyapatite has larger surface area and greater reactivity compared with normal size and coagulation of nano size-hydroxyapatite reduce the efficiency of sorption or co-precipitation of bioavailable heavy metals.

### Bioaccumulation of heavy metals in earthworm

Bioaccumulation of heavy metals in earthworm after 8 weeks of exposure is summarized in Fig. 3. Heavy metal concentration of the earthworm was lower than control (As: 123.0 mg/kg, Cd: 9.2 mg/kg, Pb: 29.5 mg/kg) at the range from 3.3 to 89.5% when chemical amendments was applied. Bioaccumulation of As, Cd, and Pb in the earthworm was ordered LS (82.0–116.0 mg/kg) > AMDS (69.0–94.0 mg/kg) > SS (32.0–75.0 mg/kg), AMDS (6.2–8.9 mg/kg) > LS (6.2–7.8 mg/kg) > SS (3.7–5.4 mg/kg) and AMDS (15.6–24.3 mg/kg) > LS (3.1–20.1 mg/kg) > SS (4.2–10.8 mg/kg) respectively. The lowest heavy metal concentration in earthworm was observed at the treatment of SS 1% for As (32.0 mg/kg), SS 1% for Cd (3.7 mg/kg), and LS 5% for Pb (3.1 mg/kg).

The lowest bioaccumulation of As, Cd, and Pb in the earthworm was observed in SS treatment while AMDS showed the highest reduction efficiency of bioavailable heavy metals in soil. The reason for showing contradictory result can be assumed that exposure route of heavy metal to the earthworm could be different. According to previous study, heavy metals in soil can be exposed to earthworm via either dermal or gut exposure route. If bioavailable concentration of heavy metal is low in soil and high in the earthworm, gut exposure of heavy metal

| Particle size | AMDS | LS | SS |
|---------------|------|----|----|
| > 2 mm        | 7.1  | 1.4| 82.7|
| 1 mm < PS < 2 mm | 23.7 | 50.1| 16.5|
| 0.15 mm < PS < 1 mm | 46.8 | 44.4| 0.8|
| < 0.15 mm     | 22.4 | 4.1 | 0.0|
| Total         | 100.0| 100.0| 100.0|
is dominant. Meanwhile, bioavailable concentration of heavy metal is low in both soil and earthworm, dermal exposure route can be dominant [22]. Since bioavailable concentration of heavy metal was low in soil and high in earthworm when AMDS was treated in soil, main exposure route of heavy metal in AMDS treatment could be gut exposure. In addition, small particle of AMDS that heavy metal was adsorbed on the surface can be digested in the earthworm and cause high concentration of heavy metal in the earthworm.

**Mortality and growth change of earthworm in toxicity test**

Mortality and growth change of earthworm in each treatment are summarized in Table 5 and Fig. 4. Among varied chemical amendments and application rate, mortality of earthworm was only observed in control (6.7%), AMDS 3% (3.3%), and AMDS 5% (10.0%). In terms of growth change determined by measuring body weight of the earthworm prior to and after experiment, weight of earthworm in all treatment was increased except AMDS 3% treatment (−2.3%). The highest growth increase of earthworm was observed in SS 5% (50.6%) followed by LS 5% (50.3%), SS 1% (31.3%) and SS 3% (31.1%). Compared heavy metal concentration in earthworm to the result of toxicity test, higher heavy metal concentration in earthworm showed higher mortality and more slow body weight increase (Fig. 4). Since higher concentration of heavy metal was observed in AMDS treatment, high mortality and low growth increase rate were measured. Meanwhile, SS treatment showed no mortality and higher weight increase compared to AMDS and LS treatment. Previous studies also reported that mortality and growth of earthworm is highly correlated with bioaccumulated concentration of heavy metals showing that higher mortality rate and poor or negative weight increase when higher bioavailable heavy metal was accumulated in the earthworm [1, 28–30].

Three chemical amendments, AMDS, LS, and SS were applied in the heavy metal polluted soil with 1, 3, 5% application ratio. Among three amendments, AMDS showed the highest efficiency for reducing bioavailable heavy metals followed by LS and SS. Reduction ratio was ranged 24.0–92.0% for As and Cd when AMDS and LS were applied. All three chemical amendments showed 2.0–87.0% of reduction efficiency for bioavailable-Pb in soil. The reason for showing high reduction efficiency of bioavailable heavy metal concentration in soil with AMDS could be assumed that AMDS mainly contains iron oxide or iron sulfate hydroxide that can sequester cations such as Cd and Pb in soil with adsorption or co-precipitation [25] and smaller particle size might contribute more sorption or co-precipitation of bioavailable heavy metals [27].

Heavy metal concentration in the earthworm was lower than control when three chemical amendments were applied at the range of 3.3–89.5%. However, ecotoxicity test revealed that SS treatment showed less toxicity in terms of mortality, weight increase, and heavy metal concentration in the earthworm compared to AMDS and LS. This result could be interpreted that gut exposure route was dominant for uptake of heavy metals from soil to the earthworm since smaller particle size of AMDS had more chance to be ingested in the earthworm [22].

Ecotoxicity test should be conducted in combination with chemical monitoring for better understanding efficiency of heavy metal remediation and toxicity effect on soil biota.

**Acknowledgements**

This work was supported by research fund of Chungnam National University.

**Authors’ contributions**

DH was the first author who mainly write this manuscript. WS and YK organized the experiment, collect a data, and summarize a main finding for the

---

**Table 5 Mortality number of earthworm after 8 weeks of chemical application**

| Amendments | Application rate (%) | # of mortality | Mortality rate (%) |
|------------|----------------------|----------------|-------------------|
| Control    | 0                    | 2              | 6.7               |
| AMDS       | 1                    | 0              | 0.0               |
|            | 3                    | 1              | 3.3               |
|            | 5                    | 3              | 10.0              |
| LS         | 1                    | 0              | 0.0               |
|            | 3                    | 0              | 0.0               |
|            | 5                    | 0              | 0.0               |
| SS         | 1                    | 0              | 0.0               |
|            | 3                    | 0              | 0.0               |
|            | 5                    | 0              | 0.0               |

*Fig. 4 Change of earthworm weight in each treatments*
manuscript. YB interpreted a data and gave a comment for improving the manuscript. SC manage and organize a full manuscript as a corresponding author. All authors read and approved the final manuscript.

Funding
This work was supported by research fund of Chungnam National University.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Bio Environmental Chemistry, Chungnam National University, Daejeon 34134, South Korea. 2 Department of Agricultural Chemistry and Food Science & Technology, Gyeongsang National University, Jinju 52828, South Korea.

Received: 31 July 2019 Accepted: 18 September 2019 Published online: 14 October 2019

References
1. Abd Aziz A, Lee BT, Han HJ, Kim KW (2019) Assessment of the stabilization of heavy metal contaminants in soils using chemical leaching and an earthworm bioassay. Environ Geochem Health 41:447–460
2. Saljnikov E, Mrvic V, Cakmak D, Jaramaz D, Perovic V, Antic‑Mladenovic S, Pavlovic P (2019) Pollution indices and sources appointment of heavy metal pollution of agricultural soils near the thermal power plant. Environ Geochem Health. https://doi.org/10.1007/s10653‑019‑00281‑y
3. Ning D, Liang Y, Song A, Duan A, Liu Z (2016) In situ stabilization of heavy metals in multiple‑metal contaminated paddy soil using different steel slag‑based silicon fertilizer. Environ Sci Pollut Res Int 23:23638–23647
4. Lee SB, Kang DW, Yoo JH, Park SW, Oh KS, Lee JH, Cho IK, Moon BC, Kim WM (2017) Determination of bioconcentration factor of heavy metal (loid)s in rice grown on soils vulnerable to heavy metal (loid) contamination. Korean J Soil Sci Fertil 50:106–114 (in Korean)
5. Yoo JH, Park WM, Lee SB, Oh KS, Moon BC, Kim SC (2017) Effects of soil amendments application on growth of rice cultivated in soils polluted with heavy metal (loid) and on the as and cd content in brown rice. Korean J Soil Sci Fertil 50:663–673 (in Korean)
6. Rai PK, Lee SS, Zhang M, Tsang YF, Kim KH (2019) Heavy metals in food crops: health risks, fate, mechanisms, and management. Environ Int 125:365–385
7. Yang Y, Chang AC, Wang M, Chen W, Peng C (2018) Assessing cadmium exposure risks of vegetables with plant uptake factor and soil property. Environ Pollut 238:263–269
8. Ashraf S, Ali Q, Zahir ZA, Ashraf S, Asghar NH (2019) Phytoremediation: environmentally sustainable way for reclamation of heavy metal polluted soils. Ecotoxicol Environ Saf 106:53–63
9. Zhao T, Zhang K, Chen J, Shi X, Li X, Ma Y, Fang G, Xu S (2019) Changes in heavy metal mobility and availability in contaminated wet‑land soil remediated using lignin‑based poly(acrylic acid). J Hazard Mater 368:459–467
10. Zhan T, Zhang K, Chen J, Shi X, Li X, Ma Y, Fang G, Xu S (2019) Changes in heavy metal mobility and availability in contaminated wet‑land soil remediated using lignin‑based poly(acrylic acid). J Hazard Mater 368:459–467
11. Zhan T, Zhang K, Chen J, Shi X, Li X, Ma Y, Fang G, Xu S (2019) Changes in heavy metal mobility and availability in contaminated wet‑land soil remediated using lignin‑based poly(acrylic acid). J Hazard Mater 368:459–467
12. Dermatas D, Meng X (2003) Utilization of fly ash for stabilization/solidification of heavy metal contaminated soils. Eng Geol 70:377–394
13. Guo J, Bao Y, Wang M (2018) Steel slag in china: treatment, recycling, and management. Waste Manag 78:318–330
14. Kumpiene J, Lagerkvist A, Maurice C (2008) Stabilization of as, cu, pb and zn in soil using amendments—a review. Waste Manag. 28:215–225
15. Leclercq‑Dranart J, Demuynck S, Bidar G, Douay F, Grumiaux F, Louvel B, Perrin C, Lepretre A (2019) Does adding fly ash to metal‑contaminated soils play a role in soil functionality regarding metal availability, litter quality, microbial activity and the community structure of diptera larvae? Appl Soil Ecol 138:99–111
16. Ko M‑S, Kim J‑Y, Park H‑S, Kim K‑W (2015) Field assessment of arsenic immobilization in soil amended with iron rich acid mine drainage sludge. J Clean Prod 108:1073–1080
17. Lee G, Cui M, Yoon Y, Kim J, Jang M (2018) Passive treatment of arsenic and heavy metals contaminated circumneutral mine drainage using granular polyurethane impregnated by coal mine drainage sludge. J Clean Prod 186:292–292
18. Sephton MG, Webb JA, McKnight S (2019) Applications of Portland cement blended with fly ash and acid mine drainage treatment sludge to control acid mine drainage generation from waste rocks. Appl Geochem 103:1–14
19. Chai WS, Hong YK, Min JI, Kim KJ, Kim SC (2017) Evaluating soil respiration as an indicator of heavy metal pollution in agricultural field. Korean J Soil Sci Fertil 50:472–481 (in Korean)
20. Bigorgne E, Cossu‑Leguille C, Bonnard M, Nahmiani J (2010) Genotoxic effects of nickel, trivalent and hexavalent chromium on the Eisenia fetida earthworm. Chemosphere 80:1109–1112
21. Cigerci IH, Ali KM, Kaygisiz SI, Luman R (2016) Genotoxicity assessment of cobalt chloride in Eisenia fetida earthworms coelomocytes by comet assay and micronucleus test. Chemosphere 144:754–757
22. Hobbelen PH, Koolhaas JE, van Gestel CA (2006) Bioaccumulation of heavy metals in the earthworms Lumbricus rubellus and Aporrectodea caliginosa in relation to total and available metal concentrations in field soils. Environ Pollut 144:639–646
23. Lanno R, Wells J, Conder J, Bradham K, Basta N (2004) The bioavailability of chemicals in soil for earthworms. Ecotoxicol Environ Saf 57:39–47
24. Morgan AJ, Stürzenbaum SR, Winters C, Grime GW, Aziz NAA, Kille P (2004) Differential metallothionein expression in earthworm (Lumbricus rubellus) tissues. Ecotoxicol Environ Saf 57:11–19
25. Rakotonirainao TV, Neculita OM, Bussière B, Benzazzaoua M, Zagury GJ (2017) Recovery and reuse of sludge from active and passive treatment of mine drainage‑impacted waters: a review. Environ Sci Pollut Res 24:73–91
26. Chen SB, Zhu YG, Ma YB (2006) The effect of grain size of rock phosphate amendment on metal immobilization in contaminated soils. J Hazard Mater 134:74–79
27. Cui H, Shi Y, Zhou J, Chu H, Cang L, Zhou D (2018) Effect of different grain sizes of hydroxyapatite on soil heavy metal bioavailability and microbial community composition. Agric Ecosyst Environ 267:165–173
28. Latif R, Malek M, Mirmonsef H (2013) Cadmium and lead accumulation in three endogeic earthworm species. Bull Environ Contam Toxicol 90:456–459
29. Hirano T, Tamae K (2011) Earthworms and soil pollutants. Sensors 11:11157–11167
30. Köpki-Koski J, Perttinen OP, Vaisanen AO, van Gestel CAM (2019) An uptake and elimination kinetics approach to assess the bioavailability of chromium, copper, and arsenic to earthworms (Eisenia fetida) in contaminated field soils. Environ Sci Pollut Res Int 26:15095–15104

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.