



Mass balance of metals during the phytoremediation process using *Noccaea caerulescens*: a pot study

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Abstract

There are two widely used methods to estimate the time taken for phytoremediation for the removal of the target pollutants, i.e., using the data of metal uptake by the harvested parts of the selected plant or using the decrement in average element content between the beginning and end of the remediation. The latter not only depends on sampling points but is also determined by sampling time because even if the soil is initially perfectly homogenized, plant growth itself heterogenizes the soil as time goes by. In this study, phytoremediation was tested on one homogenized soil obtained from various soil samples taken within an e-waste dismantling and recycling site, and the remediation time for different points of bulk and rhizosphere soil was estimated using the two methods. Phytoremediation efficiency, as assessed by the change in soil metal concentrations over 100 days, widely varied depending on which of the six soil compartments of the pot was sampled, and the standard deviations of Cd, Zn, Pb, and Cu increased as the experiment proceeded, indicating the inaccuracy of this method. When applied to rhizosphere soil, this method led to a large overestimation of phytoremediation efficiency for Cd and Zn, which was 81- and 77-fold that was obtained by measuring the actual amount of metals taken up by *Noccaea caerulescens*. The significant difference between the two methods indicated that the blended soil became heterogeneous during the phytoremediation process because the species extracted metals from different soil parts, manifested by the variation in the metal content. The gap between these two estimation methods decreased when the soil was mixed thoroughly at the end of the experiment. This work shows that calculating the metal decontamination efficiency based on the measurement of the actual amount of metal taken by the plant is more robust than estimating it based on the evolution of soil metal concentration over time. In addition, our study reveals that using *N. caerulescens* may not be appropriate in Pb- or Cu-polluted soil, since this species mobilized these metals but did not extract them.

Keywords Phytoremediation · Spatial variability · Heavy metal · Remediation time · Rhizosphere soil

Introduction

With the development of urbanization and industry, different toxicants have been released into the soil, river, and atmosphere. A recent national environmental pedological and geochemical investigation showed that 16.1% of the national territorial area has been influenced by different pollutants, and heavy metal pollution is involved in 82% of such contaminated area (Wang et al. 2018). Besides the high geochemistry

background caused by geological processes including metamorphism, magmatism, and deposition, the distribution and concentration of metals were generally released from various human activities such as city sewage, industrial production, transportation, and garbage disposal (Xu et al. 2015). Among these businesses, electronic waste dismantling, which can bring economic benefit to local people by recovering valuable and reusable metals, is viewed as one of the most environmentally harmful industries (Zhang et al. 2012).

Some traditional methods including isolation, inerting, soil washing, and incineration have been exploited to dispose point source pollution with a high degree of contamination. These conventional techniques which are dependent on complex equipment or the addition of chemicals are unavailable for metal-influenced agriculture soil remediation in China, considering the extent of the polluted area (Ruiz Olivares et al. 2013). Thus, an environmentally friendly, easy to

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operate, and cost-effective alternative is urgently required. Phytoremediation is a sustainable and environmentally friendly technology (LaCoste et al. 2001). This technology comprises different subcategories to dispose various environmental media, and two major subcategories associated with heavy metal removal from soil are phytostabilization, utilizing plants to decrease bioavailability of metals in soil by changing soluble metal chemical forms to stable fractions and phytoextraction, utilizing plants to migrate metals from contaminated soil to harvestable tissues (Vyslouzilova et al. 2003).

There are generally two methods to estimate the time required for soil remediation, i.e., using the data of metal accumulation in the selected species obtained at the end of the phytoremediation or using the change in post-experiment soil metal concentrations. The calculation results obtained from these two methods may differ by orders of magnitude. For instance, Yang et al. (2012) measured the mass of As accumulated by *Pteris vittata* L. and the decrement of the metalloid after a 3-month experiment, and found that the difference in the remediation time calculated from the two methods varied significantly. In addition, Niazi et al. (2012) reported that the amounts of As accumulated in two hyperaccumulators during a 27-month remediation experiment were 42 and 48 times less than the decrement in the mass of the metalloid in the soil. They attributed this discrepancy in mass balance to the heterogeneity of As in the polluted soil, revealing that even slight differences in soil, As concentrations can result in large variations in the estimation of phytoremediation efficiency, because the estimation of phytoremediation is on a point-by-point basis rather than the mean values of the whole field (Luo et al. 2019a). This theory can explain the mass imbalance of pollutants in heterogeneous soil after phytoremediation well.

However, this imbalance has also been observed in homogeneous soil. Our previous precipitation simulation experiment using thoroughly mixed soil revealed that the sum of *Noccaea caerulea* accumulated and leaching loss of Cd, Pb, Cu, and Zn was significantly lower than the decrement of these metals in the soil between the beginning and the termination of the experiment (Luo et al. 2019b). It was hypothesized that the mixed soil became a heterogeneous substrate as plants extracted metals from different parts of the soil during the phytoremediation processes (Martínez-Alcalá et al. 2016), influencing the veracity and reliability of remediation effect evaluation. In real field, one may sample the soil up to a certain depth and measure the metal content in it repeatedly during the soil remediation process to access the remediation efficiency. However, the soil sampling is a tricky task and that even if the soil is perfectly homogenized at the beginning of the remediation, plant growth itself creates a spatial heterogeneity. In addition, repeating soil sampling at the same locations may also impact the structure of the soil and the distribution of the metal.

It would take decades for hyperaccumulators to remove excessive metals from soil; thus, an inaccurate prediction, especially for real-scale fields, will result in a series of wrong decisions of land use. The major purposes of the present study were to (1) reveal the change in chemical forms of metals in different soil parts during phytoremediation using *N. caerulea* in a pot experiment, and (2) compare the metal decontamination time between the estimation based on amount of metal extracted by plants and change in post-experiment metal concentrations in this pot experiment.

Materials and methods

Soil sampling

Soil samples used in this experiment were gathered from an e-waste dismantling center located in southern China. Considering the different geological background, soil type, and terrain, 200 surface soil samples (0–20 cm) were gathered from the agricultural land. The soils were air-dried at room temperature for 2 months and sieved using 2-mm meshes to clean impurities such as artifacts, stones, and organism debris. The treated soils were mixed to form a composite soil matrix for further treatments. Several saturation and drying processes were performed to homogenize the soil matrix using an electric double conical mixer (SZH-1.0, Changzhou Desiccating Equipment Co., Ltd.). After each homogenizing cycle, 20 soil samples were collected from random positions of the composite soil for metal analysis. When the variable coefficient of each metal was lower than 10% (after 4 cycles), the initial basic parameters were measured. The electronic probe of a HANNA pH meter (HI98160, USA) was immersed in a 1:2.5 (wt:vol) soil to water suspension, and the pH was read after the stabilization of the value. A FOERSTER conductivity meter (2.070, Germany) was used to determine the EC of the filtrate of soil and water suspension (1:2, wt:vol). TOC of the soil was titrated using ferrisulfates after the soil organic matter was oxidized by adding $K_2Cr_2O_7$ (0.8 mol L⁻¹). The dried soil (4 g) was mixed with sodium acetate (30 mL, 1 M) in a 100-mL centrifugal tube, and ammonium acetate (30 mL, 1 M) was applied to the solution to replace Na⁺. The CEC value was calculated through the concentration of Na⁺ analyzed using a Perkin Elmer atomic absorption spectrometer (AA400, USA) according to the methods described by Cheng et al. (2015).

The homogenized soil matrix was slightly acidic. pH, TOC, EC, and CEC in the soil were 5.6, 15.2 cmolc kg⁻¹, 1116 μ S cm⁻¹, and 39.8 g kg⁻¹, respectively. The content of Hg, Cr, Pb, Cu, Zn, and Cd were 0.27 ± 0.05 , 135.2 ± 5.9 , 91.9 ± 3.4 , 82.5 ± 4.8 , 187.8 ± 6.7 , and 0.85 ± 0.05 mg kg⁻¹, respectively. The highest concentrations of Hg and Cr in the soil were within their admissible levels of 0.35 and

200 mg kg⁻¹, respectively, for farmland (China MEP 2008). However, content of other four metals exceeded their safe limits of 0.3 (Cd), 50 (Pb), 150 (Zn), and 50 (Cu) mg kg⁻¹ beyond which detrimental effects on agricultural products can occur. Therefore, only the metals which required decontamination are further discussed in the present study.

Experimental design

Six kilograms of homogenized composite substrate was padded in cylindrical PVC pots (100 mm in radius) to a height of 20 cm to ensure each pot had similar soil density. Two meshes (30 μm in pore diameter) which allowed metal ions and soil solution to pass through, but did not allow taproots and root hairs to penetrate, were inserted vertically into each pot to separate the rhizosphere and bulk soils. As shown in Fig. 1, the distance between the meshes was identical in all pots (5.3 cm from the circle center).

Seeds of *N. caerulea* which were offspring of Viviez ecotype were obtained from a horticultural company. The seeds with the same morphology and size were surface-sterilized using 2% NaClO for 10 min. After being cleaned using distilled water and Na₂EDTA to remove the potential ions, the seeds were cultivated on sand moistened using CaSO₄ for 20 days at 25 °C. Five seedlings of *N. caerulea* without defects were transplanted into each pot within the region of the two meshes at the beginning of the experiment and thinned to 2 after 10 days. To avoid the losses of metals during the experiment, *N. caerulea* were irrigated by pouring water to the tray put under each container. The experiment was conducted under greenhouse conditions (8/16-h dark/light photoperiod at 24/16 °C), and eight replicates were performed during the phytoremediation.

All plants were harvested just after the last soil sampling (100 days after the start of the experiment, DAS), and the

species did not grow beyond the rosette stage during this study.

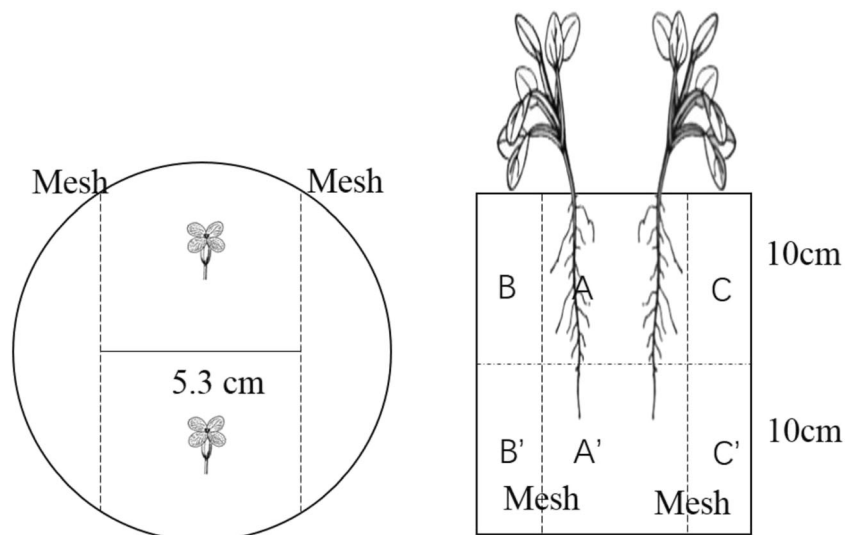
As shown in Fig. 1, soil in each pot was partitioned into six compartments, and one rhizosphere and two bulk soil samples were collected at fixed positions (in the center of each compartment) in each pot every 20 days using a cylinder stainless steel soil sampler (0.5 cm in diameter). The soil columns were divided into surface (0–10 cm) and deep layer (10–20 cm) samples to observe the change in metal concentrations, fractions, and distribution. The holes created by soil sampling naturally disappeared within the 20-day interval. Owing to the small area of the pot, only one soil column was sampled in each compartment. After the last soil sampling, the soil in each pot was mixed several times to homogenize the metals in it. Twenty soils were collected randomly in the composite substrate after each blending procedure until the variable coefficient of each metal was lower than 10%.

The harvested plants were divided into below ground and aerial parts. The shoots were washed using running water to eliminate adhered materials and soaked in CaCl₂ (10 mmol/L) for half an hour to clean out adsorbates. After washing with distilled water several times, the roots were immersed in Na₂EDTA (15 mM) for 30 min to remove the metal ions adhering to the root surface. Plant tissues were oven-dried at 70 °C until no mass variation was observed, and then ground into a fine powder using a crushing machine and stored in plastic bottles for chemical analysis. Metal concentrations in soil adhered on the root surface and the Na₂EDTA solution were analyzed for further mass balance evaluation.

Sample analysis

One gram of soil and plant samples was ground in to fine powders, sieved using 74-μm membrane, and dissolved using 10 mL aqua regia in a 50-mL tube, for 3 h. After being mixed

Fig. 1 Diagram of the experimental. A represents surface rhizosphere soil, B and C represent surface bulk soil, A' represents deep rhizosphere soil, and B' and C' represent deep bulk soil



with H_2O_2 , the solution was heated to nearly dry. HNO_3 (20 mL, 1%) was applied into the tube to dissolve the residue for 1 h at 100 °C in a water bath. The digestion protocol is the same for plant roots and shoots. The cooled digestion was diluted with deionized water to 50 mL and filtered through a 0.45- μm polyethersulfone membrane filter (Kenker, USA). The metal contents in the supernate were analyzed by ICP-MS (Agilent 7700, USA).

The roots soaked Na_2EDTA solution was filtered using Whatman filter paper (2.5 μm , UK) after shaking for 1 h. The metal contents in the digestion were determined using the ICP-MS.

Water-soluble and exchangeable forms of above elements were measured at the beginning and end of the experiment. For water-soluble and exchangeable metal analysis, 1.2 g dried soil and 25 mL CaCl_2 (10 mmol/L) were blended in a 50-mL tube, shaken for 30 min at a constant velocity, and then centrifuged for 15 min at 5000 rpm (Mao et al. 2016). The collected suspension was filtered using a polyethersulfone membrane (0.45 μm), and the supernatant was gathered for measurement of water-soluble metals. The centrifugation and filtering procedures in the two chemical fraction extraction processes were identical.

The residue from the water-soluble fraction was mixed with 25 mL NH_4NO_3 (1 mol/L) and shaken for 30 min at a constant velocity, and the pH of the solution was adjusted to 7.0 using NH_4OH during the shaking process (Mao et al. 2016). Because the metal-polluted soil treated in this study was acidic, only pH-adjusted neutral salt was applied in this experiment.

Supernatants collected after the corresponding extraction procedures were diluted up to 10-fold using deionized water, and metal content in the supernatants were determined using ICP-MS.

Certified soil (GBW07410), plant (GBW10012), and water (GBW(E)080194) standard reference materials determined each 12 samples for quality control and quality assurance. In addition, multi-element calibration standards were analyzed every 25 samples to control the operating state of the equipment. The recovery rates of elements for each standard reference material were within the acceptable levels.

Statistical analysis

The experiment was performed in eight replicates, and results were expressed as average \pm SD. One-way analysis of variance (ANOVA) followed by the Duncan's test for post hoc comparison of averages was used to judge the statistical differences of metal concentrations among different sampling positions and soil layers at a 95% confidence level. The statistical analyses in the present study were performed using SPSS 15.0.

Bioaccumulation factors (BCF) (Bagheri et al. 2019; Stoltz and Greger 2002), transfer factors (TF) (Guterres et al. 2019; Whicker et al. 1999), and extraction factor (EF) (Li et al. 2011) of metals were calculated to estimate the phytoremediation potential of *N. caerulea* as follows:

BCF = metal concentrations in plant tissues/metal concentrations in substrate.

TF = metal concentrations in aerial parts/metal concentrations in roots.

EF = metal concentrations in plant tissues \times biomass of the corresponding tissues.

For the concentration measured method, only the decrease in metal content was used, and the variation in mass of metal in each soil compartment was not calculated, because the standard deviations of metals increased over time and only one soil column was sampled in each compartment.

Results and discussion

Variation in metal distribution in different soil layers

Variations in the soil concentrations of all metals during the current experiment were insignificant ($p > 0.05$). The concentration of Cd in the surface rhizosphere soil (marked as A) increased from 0.83 ± 0.15 to 0.87 ± 0.22 mg kg^{-1} during the first 20-day experimental period and decreased continuously to 0.68 ± 0.31 mg kg^{-1} until 100 DAS of the experiment.

The concentration of Cd in the two different surface bulk soils (marked as B and C) showed a general decreasing trend during the experimental period, although some slight rebounds could be observed. In the deep layers, in both the rhizosphere (marked as A') and the bulk soils (marked as B' and C'), concentration of Cd decreased with increase in duration and treatment. However, the standard deviation of Cd increased over time, especially in the deep layers, indicating that phytoremediation resulted in a higher heterogeneity of the soil. The average decrement of Cd in soil compartments A, A', B, B', C, and C' was 1.5, 1.0, 0.5, 0.5, 0.8, and 0.6 $\mu\text{g kg}^{-1}$ per day, respectively. The difference between the determined Cd content and its statutory limit is the excessive concentration of this metal in the soil. The excessive concentration divided by the average decrement of Cd in soils was the required time to remediate the soil, and thus, it would require 1.0, 1.6, 2.6, 3.1, 2.2, and 2.3 years, respectively, to reach the target of 0.3 mg kg^{-1} in these sampling points based on the reduced average Cd concentration.

The highest Zn concentration in soil compartment A appeared at the second sampling time, 20 DAS, followed by the third, fourth, first, fifth, and last sampling times. Surface rhizosphere soil Zn concentration decreased at 80 DAS

compared with that at previous sampling times and further decreased at 100 DAS. The concentration of Zn in soil compartments B and C presented a general decreasing trend from 182.8 ± 23.5 and 177.5 ± 19.8 mg kg^{-1} at the beginning of the study to 176.2 ± 55.3 and 171.2 ± 50.2 mg kg^{-1} , respectively, at the termination of the experiment. Content of Zn in the deep layers irrespective of bulk or rhizosphere soil presented a decreasing trend as the experiment progressed, and the reduction was higher in the deep rhizosphere soil. The standard deviation of Zn in all sampling points increased with time, and the increment observed in bulk soil was higher than in the rhizosphere. The decrease in the amount of Zn in soil compartments A, A', B, B', C, and C' was 0.140, 0.082, 0.066, 0.049, 0.063, and 0.070 mg kg^{-1} per day, and it required 0.7, 1.5, 1.4, 2.5, 1.2, and 1.6 years to decrease concentrations of Zn to 150 mg kg^{-1} in these sampling points.

Concentration of Pb in soil compartment A increased continually during the experimental period, while in soil compartments B and C, it decreased gradually. The concentration of Pb in the deep rhizosphere soil layer decreased during the first half of the experiment with the lowest value occurring at 40 DAS, followed by an increase until the termination of the experiment. The variation in Pb in the deep bulk soil layer followed a similar pattern, in general, to that of the deep rhizosphere soil. Pb concentration in soil compartments A and C' at the end of the treatment was even higher than the beginning of the treatment, indicating Pb in these points cannot be disposed by phytoremediation, based on the decrease observed in the mean soil Pb concentration. It would require 6.1, 0.5, 5.0, and 1.7 years for *N. caerulescens* to remove excessive Pb in soil compartments A', B, B', and C, respectively.

The concentration of Cu increased continually in the surface and deep rhizosphere soils from 78.2 and 82.3 mg kg^{-1} at the beginning of the experiment to 84.7 and 86.7 mg kg^{-1} , respectively, at 100 DAS. In the surface bulk soil, the concentration of Cu decreased as the experiment progressed, from 86.2 and 81.6 mg kg^{-1} at the beginning of the experiment to 81.2 and 76.3 mg kg^{-1} , respectively, at 100 DAS. However, the trends in variation of Cu concentration in the deep bulk soil were similar to that of Pb, decreasing the most at 20 DAS and then increasing continually until the end of the experiment. It was impossible for *N. caerulescens* to detoxicate Cu in rhizosphere soil based on the decrease in Cu concentration, and it would take 2.0, 1.7, and 31.7 years to remove excessive Cu in the soil compartments B, C, and C' by phytoremediation. The standard deviation of Cu increased from lower than 10 to higher than 30 during the remediation process in different sampling points, indicating plants can destabilize the uniform distribution of Cu in the thoroughly mixed soil.

Plants can absorb nutrients and heavy metals from surrounding soil through soil solution (De Conti et al. 2019), and thus, the concentrations of all metals increased in the

surface rhizosphere soil at 20 DAS. The ability of metals to move in soil is dependent on the characteristics of the soil, i.e., temperature, moisture, pH, EC, and organic matter content and intrinsic properties of metals, such as solubility, atomic weight, and affinity to organic matter (De Medici et al. 2019). Because the bioavailability of Cd to nearly all plant tissues is higher than Zn, Cu, and Pb (Ali et al. 2013; Bi et al. 2011), the increment in the variable coefficient of Cd during the experiment was higher than those of other metals. In addition, *N. caerulescens* is a well-known Cd and Zn hyperaccumulator (Martínez-Alcalá et al. 2013). (Luo et al. 2010) suggested that *N. caerulescens* extended its roots into Cd-rich parts of the soil, indicating the species even required this metal, explaining that the content of Cd and Zn decreased with the growth of the plant species, because these metals were extracted by the species, leading to a smaller pool of water-soluble and exchangeable Cd in the soil. Martínez-Alcalá et al. (2016) reported a similar result that Cd and Zn solubility reduced in the rhizosphere of *N. caerulescens*, relative to the bulk soil. They suggested that the rate of uptake of Cd and Zn by the species was faster than replenishment of the soluble pool. In contrast, content of Pb and Cu increased continually in the surface rhizosphere soil because *N. caerulescens* cannot effectively absorb these from the soil. Plant roots can extract water from the soil, which can increase the transfer of Pb and Cu from bulk toward the roots, and because the plant cannot hyperaccumulate Pb and Cu, the metals may accumulate near the roots. Hu et al. (2019) found that *Populus hopeiensis*, a Cd excluder, significantly increased the accumulation of Cd in the rhizosphere, regardless of the depth of the soil profile, supporting the thesis.

Various organics including phenolics, alcohols, proteins, citrate, and malate secreted from plant roots can not only promote bioavailability of metals indirectly by increasing activity and diversity of soil microorganisms and enzymes which can be used as catalysts in different chemical reactions (González-Valdez et al. 2018), but can also mobilize soil metals directly by acidizing the soil or providing ligands (Martínez-Alcalá et al. 2016). All metals in the soils used in the present study were activated by *N. caerulescens*, but only specific ones (Cd and Zn) were accumulated, and thus, the ecological risk of non-hyperaccumulated metals increased, exhibited by the decrement in Cu and Pb concentrations in the deep soil layers, irrespective of their concentrations in the bulk and rhizosphere soils being lower than those of Cd and Zn.

According to previous studies, it will take decades even centuries for plant species to remediate superfluous metals in the substrate (Luo et al. 2017; Mao et al. 2016; Niazi et al. 2012). Therefore, the duration of normal greenhouse experiments is insufficient to vary the metal content. In addition, the success of phytoremediation is often estimated on a point-by-point basis rather than a mean of data points from the whole site, and if one sampling point fails to achieve the remediation

objectives, the whole site fails. Thus, a more accurate estimation method should be applied.

Average metal concentration = $\text{Sum}(C_i \times V_i) / \text{Sum}(V_i)$ (C_i is the metal concentration in compartment i , and V_i is the total volume of soil of the corresponding compartment) cannot give a correct estimation of phytoremediation time, because the standard deviations of metals increased as time went by. Therefore, the soil in each pot was blended completely at the end of the treatment after all plant debris has been sieved out, and metal contents in the blended substrate were determined again. It showed that the phytoremediation lead to insignificantly small differences in metal concentrations between the beginning and the end of the treatment. The concentrations of Cd, Pb, Cu, and Zn were 0.84 ± 0.09 , 92.3 ± 6.3 , 82.3 ± 7.9 , and $186.2 \pm 15.3 \text{ mg kg}^{-1}$, respectively, at the termination of the 100-day experiment. Based on the reduction in Cd and Zn concentration in the mixed substrate between the beginning (0.85 ± 0.05 and $187.8 \pm 6.7 \text{ mg kg}^{-1}$) and the end (0.84 ± 0.09 and $186.2 \pm 15.3 \text{ mg kg}^{-1}$) of the treatment, it would take 15.1 and 6.7 years, respectively, to decontaminate Cd and Zn.

Therefore, the remediation time calculated based on the decrease in the mean soil metal concentrations after the 100-day experiment was inappropriate, even when the soil was thoroughly homogenized at the end of the treatment, because the remediation efficiency not only depended on sampling points but was also determined by sampling time. For instance, the results were very different when soil metal concentrations at 20 or 100 DAS were used to estimate the efficiency of remediation.

Change in metal fractions

Owing to the long pollution history, availabilities of metals in soils were lower in this study compared with artificially spiked soil (Römken et al. 2002). Before the treatment, water-soluble fractions of Cd, Zn, Cu, and Zn were less than 5%, 3%, 1%, and 3% of their concentrations, respectively, while exchangeable forms of above elements were no more than 4%, 3%, 2%, and 4%, respectively (Fig. 2). The bioavailability of Pb was lower than that of other metals because of the high charge density of this ion, corresponding well with results of previous studies (Bech et al. 2012; Shi et al. 2019).

At the end of the experiment, the proportion of soluble Cd (sum of water-soluble and exchangeable fractions) in the surface and deep rhizosphere soil decreased from 7.9 and 7.1% to 2.0 and 4.4% after the treatment, and the decrements were lower in the bulk soils. The change in soluble Zn followed a similar pattern as that of Cd (Fig. 2), indicating the plant species can extract Cd and Zn effectively from rhizosphere, and replenishment of the soluble Cd and Zn pools was slower than the extracting by the species during the phytoremediation. In contrast, the proportion of soluble Pb in surface and deep rhizosphere soils increased from 1.6 and 1.8% to 4.4 and

6.7%, respectively, during the phytoremediation process. In the surface and deep bulk soil layers, the metal also increased from 2.2 and 1.4% to 2.5 and 3.4%, respectively. Whether in bulk or rhizosphere soils, the increments in soluble Pb were higher in the deep layers, and a similar trend was observed with Cu (Fig. 2), indicating that *N. caerulea* aggravated the leaching risk of non-hyperaccumulated metals by mobilizing them.

The decontamination effect of *N. caerulea* in the present experiment was consistent with results of Colinet et al. (2019) who reported that about 5 and 2% of the exchangeable Cd and Zn were removed by *N. caerulea* in a 20-week remediation procedure. However, the variations in exchangeable Pb and Cu were not detailed in that study. Li et al. (2014) studied the changes in Cd, Pb, Cu, and Zn in soil particle fractions during repeated phytoextraction using *Sedum plumbizincicola*, another well-known Cd/Zn hyperaccumulator, and revealed that the acid soluble Cd and Zn fractions which were more available to the species than reducible, oxidizable, and residual fractions decreased in the rhizosphere soil with smaller particle size. They also reported that *S. plumbizincicola* decreased labile fractions of Cd and Zn by hyperaccumulating them, but the activity of Cu and Pb in soil was increased by the species after phytoremediation (Li et al. 2018). These findings support the results of the present study, although different plants were used.

Remediation time estimated using metal uptake data

The success of phytoremediation is determined by the dry weight and metal uptake capacity of species. Huge differences in biomass yield of *N. caerulea* were reported among previous studies, even up to 5-fold. McGrath et al. (2006) cultivated *N. caerulea* in the Woburn Market Garden influenced by Cd, Zn, Ni, Cu, and Pb owing to sewage sludge and farmyard manure application, and biomass yield of the species in the field ranged from 0.74 to 3.65 t ha⁻¹. Black et al. (2007) remediated the agricultural land used for livestock feed production with *N. caerulea*, and observed that the biomass yield of the species in such Cd- and Zn-contaminated soils ranged from 1.5 to 4.1 t ha. The highest biomass production (from 2.5 to 5 t ha⁻¹) was reported by Jacobs et al. (2018b), who conducted a field trial in an urban wasteland on the remnants of an old train track polluted by Cd, Zn, and Pb because of the embankment material used and railway activity. The differences were caused by populations, cultivation methods, meteorological conditions, soil characteristics, and experimental duration. Sterckeman et al. (2017) summarized the weight of 60 different *N. caerulea* populations planted in different fields in which the pollution levels varied widely, and the average dry weight of the species in the present study ($39 \pm 9 \text{ mg}$ for roots and $57 \pm 17 \text{ mg}$ for shoots) distributed toward the lower end of their results.

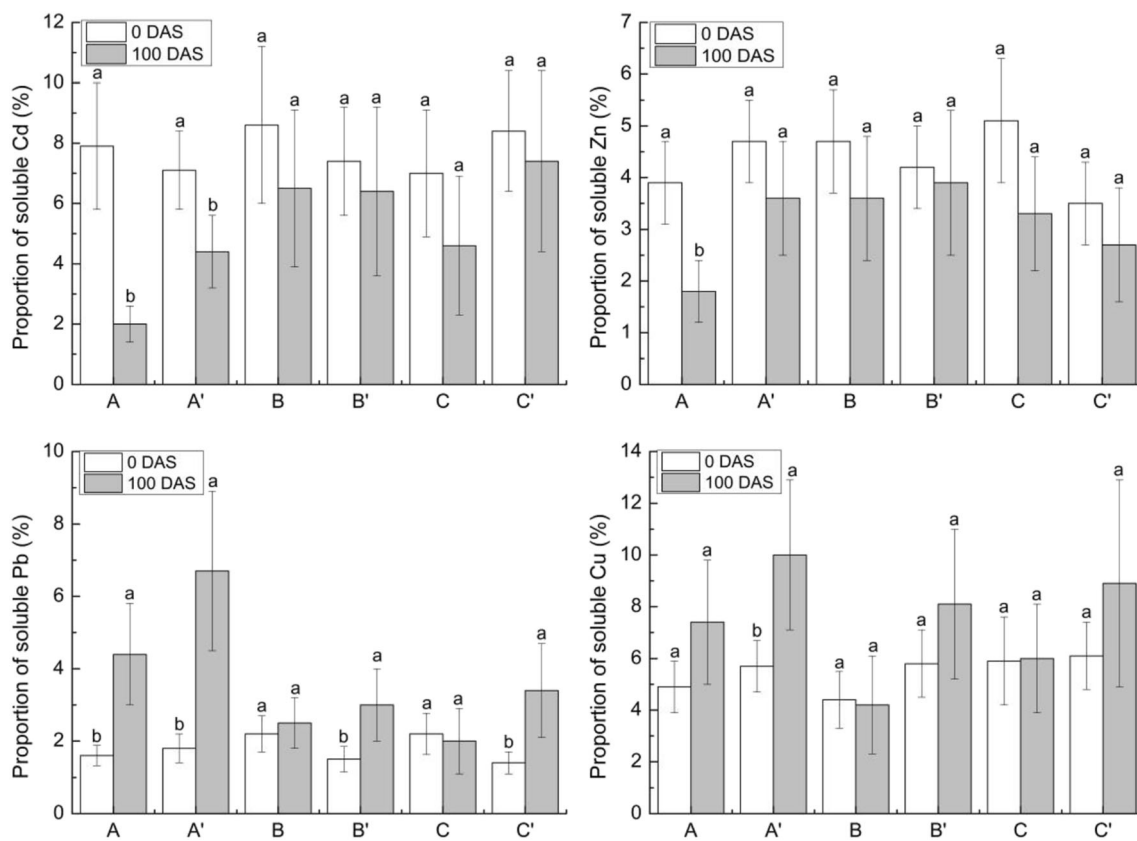


Fig. 2 Soluble fractions of metals in different sampling points at the beginning and the end of the experiment. Different letters indicate significant differences in metal fractions ($P < 0.05$) determined by one-way ANOVA followed by Fisher's LSD test among different sampling points

Comparing with our previous study in which soils were also collected from Guiyu (Luo et al. 2019b), dry weight of *N. caerulescens* in this experiment was lower. Except for Pb concentration in the soil being higher in the previous study, other parameters, i.e., plant population, experimental duration, nutrient contents, and pollution level, were nearly the same.

The concentrations of Cd (13.2 and 87.1 mg kg⁻¹ for below ground and aerial parts, respectively) and Zn (3012.9 and 7386.7 mg kg⁻¹ for below ground and aerial parts, respectively) in *N. caerulescens* did not achieve the criteria of a hyperaccumulator for Cd (> 100 mg kg⁻¹) or Zn (> 10,000 mg kg⁻¹) (Luo et al. 2019b). In addition, plant shoot contents of Cd, Zn, Pb, and Cu in the present study (Fig. 3) were lower compared with previous treatments in which the species was planted in artificially polluted substrate (Bayçu et al. 2017), field-accepted mine sludge (Martínez-Alcalá et al. 2016), or urban soil influenced by embankment material and traffic activity (Jacobs et al. 2018a). Sterckeman et al. (2017) sampled 60 populations of *N. caerulescens* from the western distribution zone of the species, and subdivided them into non-metallicolous, calamine, and serpentine edaphic groups. They found that the average Cd concentration in plant shoots varied greatly according to the edaphic group, and the calamine edaphic group had significantly higher Cd content

than the other two groups. The concentrations of Cd in plant shoots in the present study were at the high end of the range of the Cd levels of the serpentine edaphic group.

Because the pollution history of the used soil in this study was long, *N. caerulescens* could not extract organic-bound and residual Cd and Zn from the soil effectively. The root

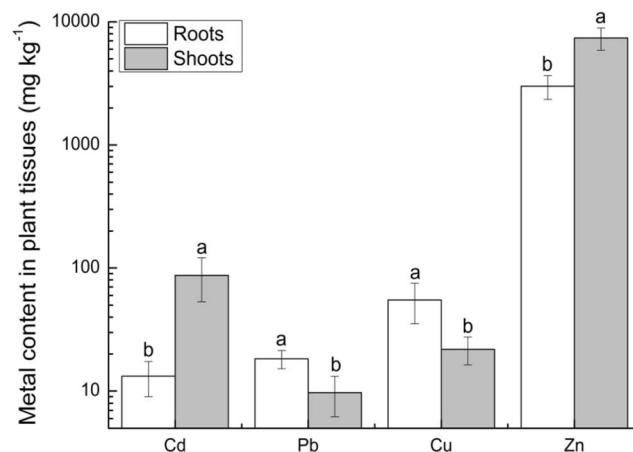


Fig. 3 Concentrations of Cd, Pb, Cu, and Zn in plant roots and shoots. Different letters indicate significant differences in metal concentrations of roots and shoots ($P < 0.05$) determined by one-way ANOVA followed by Fisher's LSD test

Table 1 Bioaccumulation factors (BCF), transfer factors (TF), and extraction factor (EF) for Cd, Zn, Pb, and Cu and remediation time for *Noccaea caerulea* to remove excessive metals

	Roots BCF	Shoots BCF	TF	EF (mg plant ⁻¹)	Remediation time (years)
Cd	15.5	102.5	6.6	0.0055	82.5
Zn	16.1	39.3	2.5	0.5385	9.6
Cu	0.2	0.1	0.5	0.0013	1297.8
Cu	0.7	0.3	0.4	0.0034	1309

BCF = metal concentrations in plant tissues/metal concentrations in substrate; TF = metal concentrations in aerial parts/metal concentrations in roots; EF = metal concentrations in plant tissues × biomass of the corresponding tissues, and the quotient of the excessive amount of metal and its metal mass extraction divided by 2 (two plants per pot) was the required harvest cropping for metal decontamination

BCF, shoot BCF, and TF for Zn and Cd were higher than 1 (Table 1), indicating *N. caerulea* accumulated more Cd and Zn in roots compared with the surrounding soil, and transferred them into shoots. However, all these parameters for Pb and Cu were less than 1, proving the species was not a hyperaccumulator for these metals.

Remediation time for the sampled field was also estimated using the data of metal uptake by the species obtained after the experiment. Metal concentrations in plant tissues multiplied with biomass yield were defined as total metal mass extraction per plant. The product of the mass of soil (6 kg) and the difference between the determined metal content and its statutory limit is the excessive mass of this metal. The quotient of the superfluous amount of metal and its metal mass extraction divided by 2 (two plants per pot) was the required harvest cropping for metal decontamination. Considering initial soil metal concentrations, experimental duration, and metal mass extraction of the species, it would require 82.5, 1297.8, 1309.0, and 9.6 years for the plant species to remove superfluous Cd, Pb, Cu, and Zn, respectively. This result differed significantly with the remediation time estimated by the decrease in the mean total soil metal concentrations. The estimation result of remediation efficiency based on change in post-experiment Cd and Zn concentrations were 81 and 77 times higher than the uptake of these metals by *N. caerulea*, whereas in the deep rhizosphere soil, they were 54 and 45 times higher.

The soil in each pot was mixed thoroughly at the end of the experiment, and metal concentrations in the mixed substrate were analyzed again. It would take 15.1 and 6.7 years, respectively, to remove excessive Cd and Zn in the soil, which was closer to the results obtained from the metal uptake data. The chemical balances of all metals were not achieved even though soils were mixed thoroughly again at the termination of the experiment. This may be partly caused by sampling errors because it was impossible to collect soils from exactly the same position, especially in the rhizosphere soil.

The results from this experiment exhibited that phytoremediation of these multiple metals-influenced soil using *N. caerulea* will take a long time and the leaching risk of non-hyperaccumulated metals (Pb and Cu) by the

species was aggravated. The results also proved that evaluation of the metal phytoremediation efficiency based on the changes in post-experiment soil metal concentrations was not an accurate option, if the soil can be mixed thoroughly at the end of the phytoremediation. It is impossible to blend the metal-polluted soil at the end of the treatment to assess the remediation efficiency in real site; therefore, phytoremediation time based on plant metal uptake data is a better scheme.

Conclusion

The results from the pot experiment showed that *N. caerulea* can decontaminate soil Cd and Zn as demonstrated by the decreased concentrations and soluble fractions of these metals in both the bulk and rhizosphere soils. However, the species cannot extract soil Pb and Cu effectively, because their concentrations did not vary significantly during the experiment. Water-soluble and exchangeable forms of Pb and Cu increased at the end of the treatment, especially in the deep soil layers, indicating that *N. caerulea* exacerbated the leaching risk of these metals. Phytoremediation time based on plant metal uptake data showed that it required 82.5, 1297.8, 1309.0, and 9.6 years for the plant species to remove superfluous Cd, Pb, Cu, and Zn, respectively, which was significantly more than the results calculated on the basis of the measured changes in the post-experiment soil metal concentrations. These results suggested that plant metal uptake data can estimate remediation efficiency more accurately than changes in soil metal, because the results of the latter not only depended on sampling points but were also determined by sampling time.

Authors' contributions Conceptualization: Wenxiang He, Jie Luo; data curation: Aogui Long, Jie Luo; methodology: Chunming Zhang; funding acquisition: Wenxiang He, Chunming Zhang, Jie Luo; writing—original draft: Wenxiang He, Aogui Long, Jie Luo; writing—review and editing: Min Cao. All authors read and approved the final manuscript.

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Data availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Disclaimer The manuscript entitled, “*Mass balance of metals during the phytoremediation process using *Noccaea caerulea*: a pot study*” is prepared in accordance with the Guide for Authors available on the journal’s website and it has not been published elsewhere in part or in its entirety. All authors attest to the validity of its contents, and agree to its submission in Environmental Science and Pollution Research.

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

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