Nitrogen and phosphorus fertilization increases the uptake of soil heavy metal pollutants by plant community

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Research

Keywords: soil heavy metals pollution, phytoremediation, nitrogen and phosphorus fertilizer

Posted Date: April 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-413625/v1>

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Abstract

Background: Soil heavy metal pollution is widespread around the world. Heavy metal pollutants are easily absorbed by plants and enriched in food chain, which may harm human health, cause the loss of plant, animal and microbial diversity. Plants can generally absorb soil heavy metal pollutants. Compared with hyperaccumulation plants, non-hyperaccumulator plant communities have many advantages in the remediation of heavy metals pollution in soil. However, the amount of heavy metals absorbed could be less, and the biomass would be reduced under heavy metal pollution. The application of nitrogen (N) and phosphorus (P) is inexpensive and convenient, which can increase the resistance of plants to adversity and promote the growth of plants of heavy metal polluted soils.

Methods: We designed a comparative greenhouse experiment with heavy metal contaminated soils, and set up four treatments: CK treatment (soil without fertilizer), N treatment (soil with N addition), P treatment (soil with P addition), and N+P treatment (soil with N and P addition).

Results: Our results showed that plant aboveground biomass were 231.17%, 14.84%, 269.86% greater than CK treatment, respectively. N and P fertilizer stimulated plants to allocate more biomass to the aboveground parts. In addition, N treatments significantly reduced the content of Cd in aboveground and belowground biomass of plants ($P < 0.05$); P fertilizer significantly decreased the content of Cu in aboveground biomass ($P < 0.05$). N+P treatments significantly reduced the content of Cd, Cu in aboveground and belowground biomass of plants ($P < 0.05$). Meanwhile, N and N+P significantly increased the accumulation (mg/m^2) of Cd, Cu, and Pb in plant aboveground biomass ($P < 0.05$). N and N+P fertilizer increased aboveground-belowground heavy metals accumulation ratio ($P < 0.05$), promoting plants to uptake more heavy metal pollution out of soil.

Conclusions: N and P fertilizer increased the accumulation of heavy metals in aboveground of the natural plant community and accelerated the absorption of heavy metals by plants, and N fertilizer had a better effect. Our results provide an inexpensive method for remediation of heavy metal pollution in low economic value soils, such as contaminated farmland, abandoned land and mine tailings, etc.

Background

Heavy metal pollution in farmlands is usually caused by the improper disposal of industrial and domestic wastes, and by contaminated sewage irrigation and fertilization, which is widespread around the world [1], and has been frequently reported in European Union, India, the United States, and China [2]. Heavy metal pollutants in soils are easily absorbed by plants, and then enriched in food chain, which could affect animal nerves, liver, bones, etc. It was reported that farmland soil heavy metal pollution also causes some cancers of human beings, like upper gastrointestinal cancers [3], lung, liver, bladder, colon, and skin cancer [4]. Heavy metal pollution also affects the metabolism, heredity and reproduction of life through its toxic effects, leading to the loss of plant, animal and microbial diversity. It was reported that soil heavy metals reduced the microbial diversity by 99.5% [5], resulting in the death of sensitive plants

and affecting plant community composition. Heavy metal pollution also affects the substance cycling, energy conversion and ecosystem function. Human intervention is an important method to reduce the impact of heavy metal pollution.

Human intervention can remove or alleviate the harm of heavy metal pollutants in soil, therefore soil leaching, solidification and stabilization are usually used for the remediation of heavy metal polluted soils, yet these methods are not suitable for agricultural, forestry, abandoned land and other low commercial value soils for their high economic cost[6]. Phytoremediation is a method to remove heavy metal pollutants from soil by harvesting the aboveground biomass which had absorbed soil heavy metals [7–9]. It is an environmentally friendly method for soil heavy metal remediation without damaging the cultivability of soils [10, 11]. Phytoremediation usually use hyperaccumulated plants as this type of plants can enrich high concentrations of heavy metals in their tissue. However, phytoremediation requires a series of steps, such as thoroughly removal of original plants, planting of hyperaccumulated plants and follow-up management, etc. which requires a lot of manpower and economic investment [12]. Meanwhile, a number of studies have reported that hyperaccumulators usually grew slowly and had low biomass production [8, 13]. Plants can generally uptake heavy metal pollutants [14] and non-hyperaccumulator plant communities have the ability to absorb heavy metals in soils [15]. Maric et al. proved that 17 kinds of wild plants can absorb Pb, Cu, and Fe in soil through cultivation experiments [16]. Although some plants are not able to enrich high concentrations of heavy metal pollutants in tissue such as hyperaccumulators, they can absorb more heavy metal pollutants in soil by large planting area, long-term plantation and harvesting [17]. However, there are few studies focusing on how to improve the tolerance and absorption of heavy metals of plant communities.

Nitrogen (N) and phosphorus (P) are limiting nutrient element in most ecosystems. Application of N and P can significantly stimulate plant growth [18, 19], and increase the biomass of plant community [20]. N and P fertilizers are inexpensive and easy to obtain, and are commonly used in agricultural production and vegetation restoration of the disturbed ecosystems [21–23]. Phytoremediation is usually carried out by harvesting the aboveground biomass of plants, and the application of N in the plant community can increase the aboveground biomass of herbs, shrubs, conifers and broad-leaved plants [24–26]. Besides, it is beneficial to plant restoration by harvesting more aboveground biomass. N and P can also increase the adaptability of plants to the adverse and improve the ability of antioxidant system of plant [27]. It was reported that N addition increased the contents of proline and peroxidase in plants, and enhanced the drought resistance and growth performance of plants [28]. Our previous study also found that N and P fertilizer in low level N and P soils could restore plant diversity of heavy metal polluted soils [21], possibly due to that N and P fertilizer increased the tolerance of plants to heavy metal stress [29]. For instant, Zhang et al. [30] found that increasing NO_3^- can improve the tolerance of *Arabidopsis thaliana* to Cd stress. Zhang's study reported that appropriate concentrations of N and P could increase the activity of antioxidant enzymes and reduce the toxicity of heavy metals [31]. Therefore, we speculated that the application of N and P will increase the efficiency of phytoremediation of heavy metal polluted soils.

To test our speculation, we conducted a comparative experiment in greenhouse to study the effect of N and P in phytoremediation of heavy metal pollution soil. Our results may provide an inexpensive approach for remediation of contaminated farmland, abandoned land, tailings and other low commercial value soil heavy metal pollutions.

Methods

Sample

The sampling site of this study is located in Kunming, Yunnan Province, China. This region belongs to a subtropical monsoon climate, and the soil type is acid red soil. The sampling site was abandoned for more than 10 years. The plants in this plot were mainly perennial and annual herbs, including *Bidens bipinnata*, *Euphorbia australis*, *Setaria Setaria*, etc. Our study was begun in November 2018. The plant seed bank of natural plant community was collected by transect method. The specific steps of soil seed bank sampling are as follows: we set up a 500 m sampling line in the sampling site. On this sampling line we further set up 10 sampling plots, and the distance of two adjacent plots were 50 m apart. The size of each sampling plot is 1 × 1 m, and 0-2cm soil in each sample plot was collected.

Greenhouse design

Soils obtained from seed bank collection were used for cultivation experiment. These soils were air dried to 20% water content, and the debris, animal and plant residues were carefully removed. After the soils were crushed and passed through a 5mm sieve, these soils were weighed and put into 20 pots (49 cm×18cm × 14 cm), and each pot was loading 7 kg soils. To simulate the heavy metal pollution, Cu^{2+} , Cd^{2+} and Pb^{2+} were added into the 20 pots with the concentration of 50 mg / kg, 10 mg / kg and 20 mg / kg. In order to verify the role of N and P in the ecological restoration of heavy metal polluted soil, four treatments were set up as follows. Control treatment (CK): soil with heavy metals addition but without N and P fertilizer. Nitrogen fertilizer treatment (NF): soil with heavy metals and fertilized with 30 mg N / kg soil; Phosphorus fertilizer treatment (PF): soil with heavy metals and fertilized with 10 mg P / kg soil; Nitrogen and phosphorus fertilizer treatment (NPF): soil with heavy metals and fertilized with 30 mg N / kg and 10 mg P / kg soil. In our study, each treatment had 5 replicates, except for the treatment applied fertilization in this study, other treatments were identical of all replication. Except for the above treatments, the daily management method of each pot of soil in our study was exactly the same. Our study was ended in October 2019, four treatments totally included 16 types of plant, among which *Bidens bipinnata*, *Euphorbia australis*, *Echinochloa crusgalli* were the dominant species. The plants aboveground and belowground biomass was determined by the following method: the whole plant was harvest of all replications. Soil attached to the root was gently shaken off to avoid loss of biomass. Plant biomass was further cleaned up carefully with tap water in the laboratory. Then it was filled in envelope and dried with a drying oven at 72 °C for 48 h. The dried plant biomass of all replications was separated into aboveground and belowground part, and weighed respectively.

Determination of plant heavy metals content

Then aboveground and belowground biomass was cut into pieces to determine the heavy metals content in plants. The methods of measuring plant heavy metals content are as follows: The plant material of a replication was mixed thoroughly, grinded into powder, and sifted through a 100-mesh sieve, then 0.2 g plant powder was digested with 10ml (16mol/L) HNO_3 and (12mol/L) HCl mixed solution using a *Microwave Digestion System* (Touchwin2.0) produced by *Aopule Technology Group (Chengdu) Co Ltd.* After the plant powder has been digested completely, the volume was adjusted to 50ml using 5% HNO_3 , and the content of heavy metals is determined using an *Atomic Absorption Spectrophotometer* (AA-7000) Ltd. Japan. In our study, plant Cd, Cu and Pb contents were determined.

Determination of soil properties

Soils attach to plant root were collected to determine the soil properties. Soil pH was analyzed as follows: First extracting 10 g of soil powder with 10 ml deionized water, and measuring soil pH by a pH meter. Soil NO_3^- and NH_4^+ were analyzed by following methods: First extracting 10 g of soil powder with 100 ml (1mol/L) KCl , and the solution was filtered after shaking 1 hour. Determination of soil NO_3^- and NH_4^+ using *Seal Analytical (AA3)*, Ltd. Germany, which is based on the standard LY/T 1228-2015. Available phosphorus was analyzed by spectrophotometry, extracted with NH_4F solution and determined by molybdenum, antimony and scandium colorimetry (NY/T 1121.7-2014).

Data Analysis

(1) Calculation of plant heavy metal enrichment:

$$H_e = H_{\text{con}} * W_{\text{ab}} \quad (1)$$

H_e : heavy metals enrichment in plant biomass

H_{con} : heavy metal content in aboveground biomass

W_{ab} : weight of aboveground biomass

(2) Calculation of plant aboveground and belowground heavy metal accumulation ratio:

$$R_{\text{ab}} = H_{\text{ea}} / H_{\text{eb}} \quad (2)$$

R_{ab} : Aboveground biomass and belowground biomass heavy metals enrichment ratio

H_{ea} : Heavy metals enrichment in Aboveground biomass

H_{eb} : Heavy metals enrichment in belowground biomass

We used *SPSS Statistics (25.0)* software to analyze the differences of soil properties, plant biomass, plant heavy metal content, quality of plant heavy metals enrichment among different treatments, and conducted one-way ANOVA and Duncan multiple comparisons for comparative analysis. *Microsoft excel 2016* was employed to make charts.

Results

Effects of different treatments on soil properties

Soil properties of different treatments were showed in Table 1. Soil pH, NH_4^+ indicated no significant difference among all treatment ($P > 0.05$). Treatments with N fertilizer had a higher NO_3^- content ($P < 0.05$), and treatments with P fertilizer had a higher available P content ($P < 0.05$).

Table 1 Soil properties of under different treatments.

Treatments	pH	NH_4^+ (mg/kg)	NO_3^- (mg/kg)	Available P (mg/kg)
CK	6.42±0.17 a	0.82±0.13 a	5.05±1.44 b	4.10±0.26 b
N	6.17±0.22 a	0.96±0.10 a	16.41±0.13 a	6.05±0.91 b
P	6.30±0.11 a	0.61±0.07 a	2.20±0.65 b	11.55±2.96 a
N+P	6.17±0.17 a	1.15±0.31 a	14.17±1.91 a	9.88±1.01 a

Effects of different treatments on the aboveground biomass

Plant aboveground biomass of four treatments showed significant differences ($P < 0.05$), N+P treatment had the highest plant aboveground biomass, whereas CK treatment was the lowest. Plant aboveground biomass of N, P, and N+P treatments were 231.17%, 14.84%, 269.86% of CK treatment, respectively. These results indicated that N and P significantly promoted plant growth, and N+P had the highest effect (Fig. 1).

Effect of different treatments on aboveground and belowground biomass ratio

Plant aboveground and belowground biomass ratio (ABR) of four treatments showed significant differences ($P < 0.05$). N treatment had the highest ABR, while CK treatment had the lowest ABR. ABR of N, P, and N+P treatments were 227.20%, 80.59% and 154.04% of CK treatment, respectively. These results suggested that N and P fertilizer stimulated plants to allocate more biomass to aboveground parts under heavy mental pollution (Fig. 2).

Effects of different treatments on aboveground and belowground biomass heavy metals content.

N and N+P treatments significantly decreased the content of Cd in aboveground and belowground biomass of plants ($P < 0.05$), P treatment tended to reduce the content of Cd in aboveground and underground parts of plants, though the effect was no significant ($P > 0.05$). P and N+P treatment significantly lowered Cu content in aboveground and belowground biomass of plant ($P < 0.05$), and N+P treatment significantly decreased Cu content in belowground biomass of plant ($P < 0.05$). N, P and N+P treatments tended to reduce the content of Pb in aboveground and belowground biomass, though it had no significant effect ($P > 0.05$, Fig.3). These results indicated that N and P fertilizer reduced the content of Cd, Cu and Pb pollutants by plant.

Accumulation of heavy metals in aboveground biomass

Cd, Cu, Pb accumulation in aboveground biomass showed significant increases under N and N+P fertilizer addition ($P < 0.01$). Among 4 treatments, CK treatment accumulated the lowest quantity of Cd, Cu, Pb. Two N fertilizer treatments, including N and N+P treatments, accumulated significantly higher quantity of heavy metals ($P < 0.01$). P fertilizer slightly increased plant heavy metals accumulation, yet the effect was no significant. These results confirmed that N and N+P fertilizer increased the accumulation of heavy metals in the aboveground parts of plants.

Plant aboveground and belowground heavy metals accumulation ratio among different treatments.

The accumulation ratio of Cd, Cu, Pb for plant aboveground and belowground part showed significant difference among 4 treatments ($P < 0.01$), whereas N and N+P fertilizer had a similar effect. N, P, and N+P fertilizer increased the proportion of heavy metals in aboveground parts of plant. The results implied that that N, P and N+P fertilizer stimulated plant to accumulate more heavy metal pollutants in plant aboveground parts (Fig. 5).

The influence of N, P treatments and their interaction on the accumulation of heavy metals in plants

N fertilizer significant influenced the aboveground biomass, ABR, heavy metal content and accumulation in aboveground biomass, and aboveground and belowground heavy metals accumulation ratio ($P < 0.01$). P fertilizer significant affected aboveground and belowground biomass ratio, heavy metal content in aboveground biomass. N and P interaction significant impacted aboveground biomass, Cd and Cu content in both aboveground and belowground biomass, Cd, Cu, Pb accumulation in aboveground biomass, and aboveground and belowground heavy metals accumulation ratio ($P < 0.05$). These results suggested that N and P fertilizer increased the uptake of soil heavy metals by plant community, and the uptake effect was best when N, P fertilizers were applied at the same time (Table 2).

Table 2 Summary of results from standard method of moments ANNOVA from aboveground biomass, aboveground to belowground biomass ratio, heavy metal content, aboveground heavy metal enrichment, and aboveground to underground heavy metal enrichment ratio response to experimental treatments.

Effect	Aboveground		aboveground and		Heavy metals content in aboveground						Heavy metals content in belowground biomass						Heavy metals accumulation in per unit						Aboveground :belowground heavy metals					
	biomass per		belowground		biomass (mg/kg)						(mg/kg)						area of aboveground biomass(mg/m ²)						accumulation ratio					
	unit area(g/m ²)		biomass ratio		Cd		Cu		Pb		Cd		Cu		Pb		Cd		Cu		Pb		Cd		Cu		Pb	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
N(df=4,16)	10792.77	<0.01	34.79	<0.01	3.27	<0.01	1.16	0.71	199.30	0.94	19.54	<0.01	15.63	<0.01	3.03	0.54	2.23	<0.01	53.44	<0.01	0.69	<0.01	36.45	<0.01	31.14	<0.01	3133.91	<0.01
P(df=4,16)	993.73	0.81	47.17	<0.01	9.28	0.39	7.69	0.13	173.66	0.70	20.27	0.68	162.85	<0.01	13.19	0.54	2.79	0.47	7.15	0.95	1.17	0.76	43.44	0.15	26.95	0.13	8190.30	0.98
NxP(df=4,16)	16864.49	<0.01	15.18	0.06	127.23	<0.01	28.56	0.04	30.27	0.12	28.57	<0.01	125.76	<0.01	5.45	0.15	3.98	<0.01	75.08	<0.01	2.48	<0.01	35.97	<0.01	1.67	<0.01	859.10	<0.01

Discussion

Our results indicated that N, P fertilizers increased the biomass of plant communities under heavy metal pollution (Fig. 1). N and P are the limiting nutrients in terrestrial ecosystem, which are supposed to significantly promote plant growth and biomass [32, 33]. For instance, N or P increased plant community biomass at an alpine meadow in the Tibetan Plateau [34] and enhanced the biomass of the hyperaccumulator plant *T.caerulescens* [35, 36] and *Pteris Vittata* [37]. P application stimulated the plant biomass in alpine meadows [34] and typical temperate grasslands [20], and increased biomass and coverage of *Cyanolichen Pseudocyphellaria* [33]. The coupling of N and P nutrients are common in natural ecosystems. In our study, N and P applied at the same time had significant effects on plant growth and biomass (Fig. 1), and their interaction had a significant better effect (Table 2). Previous studies showed similar results, for instant, the combined N and P fertilizer had the greatest effect on the biomass of grass plants [38, 39] and alpine meadow plants [40]. This may be because that N and P promoted photosynthesis and biomass accumulation of plant [28, 41, 42]. Other studies have found that N fertilizer improved the drought tolerance and growth performance of poplars [28], and reduced the toxicity of Cd to plant [28, 42]. N and P addition might enhance plant antioxidant defense ability and the stress to adversity by showing increased level of free proline and carotenoid, which increases the plant's resistance to heavy metal pollutants [43, 44]. Therefore, we speculate that these reasons might jointly promote the plant biomass under heavy metal pollution.

Our study found that the content of heavy metals Cd, Cu, and Pb of plant communities was reduced by adding N and P (Fig. 3). Previous research found N and P had different effects on the absorption of heavy metals by plants. Adding N and P promoted the absorption of Cd by *Brassica napus* [45] or inhibited the content of Cd in corn [46]. Fertilization also significantly reduced the content of Cd and Pb in rice [47], which was consistent with our results. A large number of studies have shown that fertilization reduced the bio-availability of heavy metals in soil, affecting the morphology and complexation of heavy metals, and thus influencing the movement of heavy metals to plant roots and the absorption of heavy metals [48, 49]. In addition, our results suggested that N and P fertilizers are beneficial to increase the

accumulation of heavy metals by plant communities, and the effect was highest as adding N (Fig. 4). Some other studies had similar results, such as N fertilizer increased hyperaccumulators *Pteris Vittata* [37] to accumulate heavy metals As; and increased hyperaccumulators *Sedum alfredii Hance* to accumulate of heavy metals Cd, Pb, and Zn [50]. In our study, N reduced the contents of heavy metals Cd, Cu, and Pb in plants by 31.49%, 8.42%, and 1.28%, respectively, but the biomass increased to 231.17%. The stimulation of plant biomass was greater than the decrease in the content of heavy metals in plants under N and P fertilization (Fig. 1, Fig. 2), resulting in an increase in the accumulation efficiency of heavy metals. Other studies reported that N and P addition changed the morphology of heavy metals in soils, thus affecting the uptake and enrichment of heavy metals by plants [51, 52], which was consistent with our results.

Our results demonstrated that N and P fertilization increased ABR (Fig. 2) and enhanced the proportion of heavy metal enrichment in aboveground parts of plants (Fig. 5). In our study, N, P and N + P fertilizer stimulated the aboveground biomass by 231.17%, 14.84% and 269.86%. The ABR of the plants increased after fertilization, which was similar to the Thornley model [53]. Other results from a meta-analysis also showed that N or P fertilizer increased the aboveground biomass ratio of many species [54], including maize [55], *Sporobolus alterniflorus* [26], alpine meadow plants on the Tibetan Plateau, etc [34]. This may be due to that the application of N and P fertilizer can enhance the photosynthesis of plants and promote plant to accumulate organic matter in aboveground part [28], thus increasing plant height, leaf length and stem length [25]. In our study, N + P fertilizer resulted in the highest Cd accumulation in aboveground biomass by 143%. N fertilizer had the highest Cu and Pb accumulation in aboveground biomass, which were 244.02% and 236.26% increases, respectively (Fig. 4). It might be fertilizer indirectly impact the accumulation of heavy metals in plant by influencing soil pH, ionic strength, rhizosphere chemistry, microbial activity, which affected the absorption of heavy metals by plants [51, 56]. Heavy metals plant remediation is usually carried out by harvesting the aboveground parts of plants and subsequent reprocessing. The increase of heavy metal enrichment in aboveground parts of plants may improve the efficiency of phytoremediation. Our results suggested that the application of N or P fertilizer could increase the efficiency of plant remediation of heavy metals in soil.

Soil heavy metal pollution is widespread and increasingly serious around the world [57]. Heavy metal pollution in low economic value soil such as farmland and abandoned land accounts for a large proportion of polluted soils, which leads to the quality reduction of a variety of agricultural products, harms human health and causes the loss of biodiversity [3, 4, 21]. Compared with high-cost methods such as leaching and other engineering remediation, the remediation of low economic value soils is usually carried out through phytoremediation. However, super-enriched phytoremediation requires a series of steps, such as thorough removal of original plants, cultivation and management, and therefore needs a large amount of manpower and economic investment. Our results showed that N and P fertilizer increased the accumulation of heavy metals in the aboveground parts of the natural plant community and accelerated the remediation of heavy metals in soil by plants, thus establishing a new approach for remediation of heavy metals in low economic value soils such as farmland and abandoned land. At the same time, fertilizer can not only improve the structure of organic matter and nutrient elements in

grassland soil, balance the nutrient elements lost due to mowing and play an important role of nutrient replenishment, but also use natural plant community to avoid further cultivation and management process requirement by the use of super-enriched plants [56, 58]. In the context of global soil heavy metal pollution, our results may have a certain practical value. Moreover, the mechanism of antagonistic and synergistic effects of heavy metals in plant, heavy metals and N, P elements still need to be further explored.

Conclusions

N and P fertilizer increased the accumulation of Cd, Cu, and Pb in aboveground of the natural plant community and accelerated the absorption of Cd, Cu, and Pb by plants, and N fertilizer had a better effect. Our results provide an inexpensive method for remediation of heavy metal pollution in low economic value soils, such as contaminated farmland, abandoned land and mine tailings, etc.

Declarations

Authors' contributions

XZ, LQ, and JH designed the study. GT analyzed the data and drafted the manuscript. XD and CW contributed to the data analysis and interpretation of data. LL and JG conducted measurements. ML and JH made key revisions and edits. All authors contributed in drafting and editing the manuscript and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (grant number: 31800443), the Yunnan Provincial Department of Education Fund (grant number: 2018JS144), the Scientific Research Foundation for introduced doctor of Yunnan normal university (grant number: 2017ZB021), the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (grant number: 2019QZKK0308), the Thousand Young Talents Program of Yunnan Province, and Double Top University Plan Fund of Yunnan University (grant number: C176220100044).

Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Plant aboveground biomass per unit area under different treatments.

Figure 2

Plant aboveground and belowground biomass ratio of different treatments.

Figure 3

Plant aboveground (A, C, E) and belowground (B, D, F) biomass heavy metals content.

Figure 4

Heavy metals accumulation in aboveground biomass.

Figure 5

Aboveground and belowground heavy metals accumulation ratio.