Original Research

Nitrogen and Phosphorus Fertilization Increased the Remediation Efficiency of Soil Heavy Metal Pollution by *Bidens pilosa*

Junrou Zhou¹, Lei Li¹, Xiaole Zhang², Honglin Li¹, Fuqiong Zhang¹, Chenjiao Wang¹, Yizong Huang¹, Jingxin Huang^{1*}

¹College of Energy and Environmental Sciences, Yunnan Normal University, Kunming, 650500, China ²Public Basic Teaching Department, Yunnan Open University, Kunming, 650504, China

> Received: 12 October 2023 Accepted: 26 January 2024

Abstract

Heavy metal stress can lead to slow growth and dwarfing of hyperaccumulators, limiting their effectiveness in remediating soil heavy metal pollution. Nitrogen (N) and phosphorus (P) fertilization can enhance plant growth, biomass, and stress resistance. We hypothesize that N and P fertilization could improve the soil heavy metal remediation efficiency of Bidens pilosa (B. pilosa). We conducted a greenhouse experiment with four treatments: control (CK), nitrogen fertilization (NF), phosphorus fertilization (PF), and nitrogen and phosphorus fertilization (NPF). Then, we analyzed the growth status, heavy metal content, and accumulation of B. pilosa under various treatments to explore the impact of N and P fertilization on its potential to remediate soil heavy metal pollution. The shoot height, root length, and shoot biomass of B. pilosa significantly improved under NF and NPF treatments (P<0.05). The root tolerance index of B. pilosa in the NF and NPF treatments also increased, exceeding 1. The NF and NPF treatments significantly increased the accumulation of heavy metals Cd, Cu, and Pb in the shoots of B. pilosa (P<0.05). The transfer coefficient of these heavy metals also increased in the NF and NPF treatments. Accordingly, N and NP fertilization can promote the growth of B. pilosa, increase the accumulation of heavy metals Cd, Cu, and Pb in B. pilosa, and improve the remediation efficiency of these heavy metals. In the context of rising global soil heavy metal pollution, our findings indicate that B. pilosa can aid in the remediation of soil heavy metal pollution.

Keywords: Bidens pilosa, nitrogen and phosphorus fertilization, soil heavy metal pollution, remediation

^{*}e-mail: huangjingxin17@163.com

Introduction

Soil heavy metal pollution, characterized by strong toxicity, irreversibility, and persistence, currently poses one of the most serious global environmental problems [1, 2]. Soil pollution, exacerbated by human activities, continues to worsen [3, 4], especially for heavy metals such as Cd, Cu, and Pb [5]. Given the foundational importance of soil in human production and life, contamination poses substantial risks and hazards to both humans and ecosystems [6, 7]. Accordingly, addressing soil heavy metal pollution is an urgent issue. Current remediation methods primarily comprise physical remediation, chemical remediation, and phytoremediation [8]. Despite their effectiveness, physical and chemical approaches carry numerous disadvantages, such as high costs, substantial energy consumption, risk of secondary pollution, and disruption of soil physical and chemical properties [9-11]. In contrast, the study of phytoremediation - an economical and green technology - is gaining wider traction [12]. This method employs hyperaccumulating plants and their respective rhizosphere microbial systems to absorb or stabilize heavy metals in the soil, thereby mitigating soil heavy metal pollution [13-15]. Despite the many advantages of phytoremediation - including environmental sustainability, in-situ remediation, and low cost [16, 17] - hyperaccumulating plants are often stunted, exhibit low biomass, and require prolonged remediation cycles under heavy metal stress, resulting in a slow, inefficient process [14, 18]. As such, enhancing the efficiency of hyperaccumulating plants in remedying soil heavy metal pollution currently galvanizes interest in plant remediation research.

Many studies have aimed to augment the tolerance and remediation capabilities of hyperaccumulators to soil heavy metals through the addition of chelating agents, inoculating agents, fertilization, and other methods [19-22]. Among these, fertilizers are often used to remediate heavy metal-contaminated soil because of their cost-effectiveness and availability. Nitrogen (N) and phosphorus (P) are typically the limiting nutrients in most ecosystems [23]. Applying N and P significantly spurs plant growth and boosts plant biomass [24-26]. Concurrently, adding N or P fertilizers to the soil can diminish the toxicity of heavy metals [27]. Research affirms that applying N fertilizer to heavy metalpolluted soil augments the transpiration rate and net photosynthetic efficiency of plants, elevates antioxidant enzymatic activity, and increases antioxidant matter in plants, thus strengthening cellular resistance to heavy metals and increasing biomass [28-30]. A moderate application of N fertilizer can further activate heavy metal ions in the soil by amending soil pH and conductivity, thereby enhancing plants' remediation efficiency for soil heavy metal pollution. This deems it an efficacious enhancer of plant remediation for soil heavy metal pollution [31, 32]. Conversely, P is another plant-limiting element [33]. P application can invigorate

root formation and growth in early stages, enhance plant stress resistance, and escalate heavy metal accumulation, thereby facilitating the remediation of heavy metalpolluted soil [34, 35]. Studies also discovered that the addition of N and P elements augments the shoot biomass of plant communities, and when both are added, they significantly increase plant shoot biomass [36, 37]. Our previous research also ascertained that N and P fertilizers in soils deficient in N and P can recuperate plant diversity in heavy metal-polluted soil [38], possibly attributed to the fact that N and P fertilizer increased plant tolerance to soil heavy metals [39]. Consequently, the addition of N and P fertilizers can enhance plant growth and alleviate the stress caused by heavy metals on plants. This implies that applying N and P fertilizers can ameliorate the remediation efficiency of soil heavy metal pollution by hyperaccumulators.

Bidens pilosa (B. pilosa) is an annual herbaceous plant found worldwide [40]. Studies have indicated that B. pilosa is a Cd hyperaccumulator plant with specific enrichment capacities for Cu and Pb [41-43]. Compared to other hyperaccumulator plants, B. pilosa showcases stronger tolerance to harsh environments, a faster growth rate, and a higher biomass [44]. These traits make it an ideal hyperaccumulator for remedying soil heavy metal pollution. Chen et al. reported that B. pilosa has a potent absorption and utilization rate of N and P elements in the soil, and certain levels of N and P can enhance root system growth and biomass accumulation [45]. Recent studies have demonstrated that the application of N fertilizer influences the evolution of photosynthetic products, antioxidant enzymes, and lipid peroxidation in B. pilosa, consequently affecting Cd extraction from the soil [46]. As a result, B. pilosa is more sensitive to N and P fertilizers, and a slight increase in N and P elements will enhance its biomass accumulation [47], which may improve its effectiveness in remediating soil heavy metal pollution. However, there is currently limited research on the influence of N, P, and NP fertilizers on the remediation of soil heavy metal pollution by B. pilosa. To address this knowledge gap, we carried out comparative experiments in a greenhouse to study the effects of these fertilizers on the soil heavy metal remediation efficiency of B. pilosa. Our findings serve as a guide for employing N and P fertilizers in the phytoremediation of heavy metal-contaminated soil, as well as offering technical assistance for phytoremediation in mining sites, abandoned lands, and industrial pollution areas.

Material and Methods

Material

Soil samples were collected from abandoned land in the Chenggong District of Kunming City at a depth of 0-10 cm. This soil was classified as acidic red soil. The samples were thoroughly cleared of debris, as well as animal and plant residues, before they were

Table 1. Physicochemical background values of experimental soil.

Properties	Values	
рН	6.4	
Cd(mg·kg ⁻¹)	/	
Cu(mg·kg-1)	/	
Pb(mg·kg ⁻¹)	/	
$NO_3^N(mg\cdot kg^{-1})$	3.75	
$\mathrm{NH_4^+}$ -N (mg·kg ⁻¹)	0.82	
Available P(mg·kg¹)	4.1	

dried, crushed, and sieved through a 5-mesh screen to achieve a uniform mixture for subsequent use. The physicochemical background values of the experimental soil are presented in Table 1.

Experimental Design

To simulate conditions of heavy metal pollution, Cd, Cu, and Pb were incorporated into planting soils to achieve total concentrations of 10, 50, and 20 mg·kg⁻¹, respectively. The soils were then deposited into 20 pots (45 cm × 20 cm× 20 cm), each pot filled with soil weighing 6.31 kg. The greenhouse experiments were divided into four treatments: CK treatment (control only watered), NF treatment (urea added to achieve 30 mgN·kg⁻¹), PF treatment (disodium hydrogen phosphate added to achieve 10 mgP·kg-1), and NPF treatment (both urea and disodium hydrogen phosphate added to achieve 30 mgN·kg⁻¹ and 10 mgP·kg⁻¹, respectively). Each treatment was replicated five times. Seeds of B. pilosa with similar morphologies were used in the experiments, with ten seeds evenly distributed in each pot – a density exceeding the natural population of B. pilosa. All treatments received the same watering and weeding methods. After approximately one year of growth, B. pilosa were harvested and measured. The experiment commenced in September 2021 and concluded in September 2022.

Indicator Measurement

We gently shook off the soil attached to the roots of *B. pilosa* and obtained intact plants at harvest time. Then, we rinsed the residual soil off of the plants using tap water, and measured the shoot height and root length after air drying. The plants were then placed into an envelope and dried in an oven at 70°C for 72 hours. We weighed the shoot and root biomass of *B. pilosa* separately. After drying, we used the *B. pilosa* plants to determine the heavy metal content in their respective shoots and roots. To do this, we cut the shoots and roots into approximately 1 cm pieces, ground them into powder, and passed the powder through

a 100-mesh sieve. Then we weighed 0.3 g of plant powder and digested it using a 10ml HNO_3 -HCl mixture (HNO₃: HCl = 1:3, v/v). After the sample digestion solution was clarified, we diluted it to a constant volume of 50 ml using 5% HNO₃ and determined the heavy metal content of Cu, Cd, and Pb using ICP-MS.

Data Compilation and Analysis

Shoot root ratio (SRR) =
$$L_{shoot} / L_{root}$$
 (1)

SRR represented the ratio of shoot height to root length, where $L_{\scriptsize shoot}$ is the shoot height (cm), and $L_{\scriptsize root}$ is the root length (cm).

Root tolerance index (RTI) =
$$L_{treatment}/L_{CK}$$
 (2)

RTI represented the root tolerance index, which reflected the plant tolerance to heavy metals, where $L_{\textit{treatment}}$ is the root length (cm) of the plant for each treatment and $L_{\textit{CK}}$ is the root length (cm) of the control group.

Accumulation (A) =
$$C_{plant} * M_{plant}$$
 (3)

A represented accumulation, which was one of the most important indicators of phytoremediation of soil heavy metal pollution, and it is closely related to plant growth and biomass accumulation, where C_{plant} is the concentration of heavy metals in plant tissues (mg·kg⁻¹) and M_{nlant} is the biomass of plants (kg).

Transfer Coefficient (TC) =
$$C_{shoots} / C_{roots}$$
 (4)

TC represented the transfer coefficient, which reflected the ability of plants to transport and enrich heavy metals from below to above ground, where C_{shoots} is the concentration of heavy metals in the shoots part of the plant (mg·kg⁻¹) and C_{roots} is the concentration of heavy metals in the roots part of the plant (mg·kg⁻¹).

Microsoft Excel 2021 was used to sort the data and draw the charts, and IBM SPSS Statistics 26 was used to analyze the differences in growth status, biomass, heavy metal content, accumulation, and the transfer coefficient of *B. pilosa* under different treatments.

Results

Effects of Different Treatments on the Growth of *B. pilosa*

We found that the shoot height and root length of *B. pilosa* were significantly higher in the NF and NPF treatments than in CK (P<0.05), with shoot height being higher by 107.6% and 145.9%, respectively (Fig. 1). The PF treatment had no significant effect on shoot height or root length. The SRR in the PF, NF and NPF treatments was significantly higher (P<0.05), especially

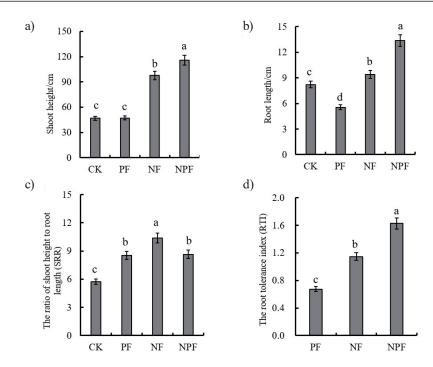


Fig. 1. Effects of different treatments on shoot height a), root length b), SRR c), and RTI d) of *B. pilosa*. The values of different lowercase letters on the bars indicate a statistically significant difference between different treatments, with *P*<0.05.

for the NF treatment where SSR was 81.1% higher. There were significant differences (P<0.05) in the RTI between PF, NF, and NPF treatments. These results indicated that N and NP fertilization increased shoot height, root length, SRR, and RTI, and that NP fertilization had the strongest overall effect.

Effects of Different Treatments on the Biomass of *B. pilosa*

We also found that both biomass and the root-shoot ratio of B. pilosa exhibited significant differences among various treatments (P<0.05) (Fig. 2). In comparison to the CK treatment, the NF and NPF treatments notably augmented the shoot biomass of B. pilosa (P<0.05) – the NPF treatment even boosted the shoot biomass of B. pilosa by a factor of 4.22. Conversely, the root-shoot

ratio from the NF and NPF treatments was significantly lower (*P*<0.05). The PF treatment did not significantly influence either the biomass or the root-shoot ratio. These findings suggested that N and NP fertilization significantly encouraged biomass accumulation – particularly the shoot biomass – whereas P fertilization did not have a substantial impact on the biomass of *B. pilosa*.

Effects of Different Treatments on the Contents of Heavy Metals Cd, Cu, and Pb in Each Part of *B. pilosa*

As demonstrated in Fig. 3, the NPF treatment had significantly lower Cd, Cu, and Pb contents in both the roots and shoots of *B. pilosa* (P<0.05). While the NF treatment also had lower Cd contents in the roots and

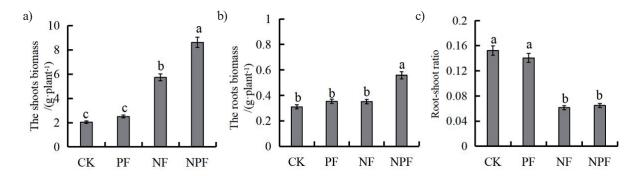


Fig. 2. Effects of different treatments on the shoots a), roots biomass b), and root-shoot ratio c) of *B. Pilosa*. The values of different lowercase letters on the bars indicate a statistically significant difference between different treatments, with P < 0.05.

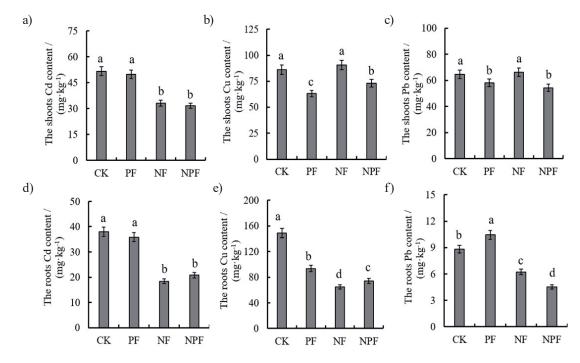


Fig. 3. Effects of different treatments on the contents of Cd, Cu, and Pb in the shoots and roots of *B. pilosa*. The values of different lowercase letters on the bars indicate a statistically significant difference between different treatments, with P < 0.05.

shoots and lower Cu and Pb contents in the roots of *B. pilosa* (*P*<0.05), it did not have a significant effect on the Cu and Pb levels in the plant's shoots. In contrast, the PF treatment had significantly lower Cu content across all components and lower Pb content within the shoots, the PF treatment did not significantly affect Cd content. However, the PF treatment did have notably higher Pb content within the roots of *B. pilosa* (*P*<0.05). These findings suggest that NP fertilization methods considerably decrease the content of Cd, Cu, and Pb in each component of the plant, and P fertilization methods simultaneously increase Pb levels within the root system of *B. pilosa*.

Effects of Different Treatments on the Accumulation Of Cd, Cu and Pb in Each Part of *B. pilosa*

As shown in Table 2, the NPF treatment had significantly higher accumulations of Cd, Cu, and

Pb in the shoots of B. pilosa (P < 0.05) – levels were 2.60, 3.58, and 3.49 times higher, respectively. When compared to the control group (CK), NPF treatment did not significantly affect the accumulation of Cd, Cu, and Pb in B. pilosa roots. The NF treatment had higher accumulations of Cd, Cu, and Pb in the shoots, but lower accumulations of these elements in the roots (P<0.05). The PF treatment did not significantly impact Cd, Cu, or Pb accumulation in the shoots, or Cd accumulation in the roots. When comparing the PF treatment with the CK group, the PF treatment had significantly lower Cu accumulation in the roots, but higher Pb accumulation (P< 0.05). Our results indicated that both N and NP fertilization caused B. pilosa to have significantly higher Cd, Pb, and Cu accumulation in the shoots, with NP fertilization having the strongest effect. P fertilization, in contrast, did not have a significant effect on Cd, Pb, and Cu accumulation in the shoots, but led to higher Pb accumulation in the roots.

Table 2. Effects of different treatments on the accumulation of heavy metals Cd, Cu, and Pb in the shoots and roots parts of B. pilosa.

Treatment	Accumulation of shoots/mg		Accumulation of roots/ug			
	Cd	Cu	Pb	Cd	Cu	Pb
CK	0.11±0.01c	0.18±0.02c	0.13±0.01c	11.73±1.57a	46.15±4.72a	2.74±0.38b
PF	0.13±0.02c	0.16±0.02c	0.15±0.01c	12.67±0.96a	33.04±2.39b	3.69±0.30a
NF	0.19±0.03b	0.52±0.09b	0.38±0.07b	6.46±0.37b	23.40±2.71c	2.14±0.17c
NPF	0.27±0.03a	0.63±0.04a	0.47±0.02a	11.62±1.19a	41.36±3.59a	2.53±0.24b

Data are expressed as mean \pm SD, n = 4. The different lowercase letters after the same column of numbers indicate a statistically significant difference between different treatments, with P<0.05.

		, , , <u>, , , , , , , , , , , , , , , , </u>			
T	Transfer coefficient (TC)				
Treatment	Cd	Cu	Pb		
CK	1.37±0.09b	0.58±0.02d	7.46±0.39c		
PF	1.39±0.07b	0.68±0.05c	5.57±0.44d		
NF	1.81±0.10a	1.44±0.04a 10.74±0.78b			
NPF	1.52±0.11b	0.99±0.06b	12.00±0.66a		

Table 3. Effects of different treatments on the transfer coefficient of Cd, Cu, and Pb of B. pilosa.

Data are expressed as mean \pm SD, n = 4. The different lowercase letters after the same column of numbers indicate a statistically significant difference between different treatments, with P<0.05.

Effects of Different Treatments on the Transfer Coefficient of Cd, Cu and Pb of *B. pilosa*

Table 3 shows that the TC of Cd and Cu in B. pilosa rose under different treatments. In comparison with the control group, NF and NPF treatments had significantly higher TC of Cu and Pb in B. pilosa (P<0.05), with NF treatment yielding the highest TC of 1.44 for Cu, whereas the NPF treatment produced a maximum Pb TC of 12.00. No significant difference was observed in the Cd TC when B. pilosa was treated with PF, although the Cu TC was notably higher. However, the Pb TC was significantly lower in the PF treatment (P<0.05). Our results suggested that N and NP fertilization significantly enhanced the absorption of Cd, Cu, and Pb by B. pilosa and their subsequent transfer to the shoot parts. Of these treatments, N application most robustly facilitated the transfer of Cd and Cu by B. pilosa, while the concurrent application of NP most forcefully promoted the transfer of Pb by B. pilosa.

Discussion

Fertilization Increases Shoot Height and Biomass of *B. pilosa*

Our study found that nitrogen (N) application promoted the growth of B. pilosa, increasing both plant height and shoot biomass (Fig. 1, Fig. 2). Other studies have reported similar findings; for instance, N application increased the heavy-metal cadmium (Cd) resistance of Solanum nigrum L. and promoted biomass accumulation [30]. This effect can be attributed to N being a vital element for plants. Its application, through fertilization, boosts the synthesis of plant stress-resistant substances, enhancing plants' resilience to heavy-metal stress and, hence, fostering growth [19,48]. The coupling of N and phosphorus (P) nutrients is commonplace in natural ecosystems. A significant interaction exists between the soil availability of N and P [49] and adding N and P nutrients results in a synergistic effect on plant growth. The accumulation of plant biomass under the combined application of NP is greater than that under the individual addition of N or

P [50]. Our research results suggest that the combined application of NP significantly influences the growth and biomass of *B. pilosa* (Fig. 1, Fig. 2). For example, the mixed application of N and P has a greater impact on the biomass of grass plants [51,52], alpine meadow plants [53], and angiosperms [54] than the application of N or P fertilizers. This difference might be because N and P enhance plant photosynthesis [55], promoting organic matter accumulation in plant shoot parts [56]. Furthermore, N and P fertilizers can reduce heavy metal toxicity to plants [57] or inhibit oxidative stress caused by heavy metal stress, enhance the enzyme activity of plant antioxidants [30], subsequently increasing plant biomass.

Given that roots are the principal organs that plants utilize to absorb both water and nutrients, their growth and development directly impact plant growth and yield accumulation [58]. The root tolerance index (RTI) is the ratio of root length in each treatment relative to that of the control, and reflects plant tolerance to heavy metals [59,60]. Roots are the first to come into contact with heavy metals during plant growth, which then absorb or reject them. Concurrently, there exist numerous sites on the root cell wall that can exchange, absorb, or fix heavy metal ions - ultimately promoting or inhibiting further transportation of heavy metal ions to the aboveground part [61]. In our study, under NF and NPF treatment, the root length of B. pilosa significantly increased by 14.6% and 62.7%, respectively, and displayed a high RTI (Fig. 1). Consequently, it can be inferred that the addition of N and NP fertilizers expanded the nutrient absorption space of B. pilosa in soil, bolstering its tolerance to heavy metals and thus contributing to the increase in B. pilosa biomass.

Fertilization Decreased Heavy Metal Content but Increased Heavy Metal Accumulation in *B. pilosa*

Our research results demonstrate that the combined application of NP decreases the content of Cd, Cu, and Pb in *B. pilosa*, notably the Cd content. The use of N fertilizer reduced the content of Cd in the shoot portion and Cd, Cu, and Pb in the root portion of *B. pilosa* (Fig. 3). Previous studies have established that N and P differentially impact the absorption of heavy metals

by plants. The addition of N and P either promoted the absorption of Cd by *Brassica napus* [62] or inhibited the content of Cd in corn [63]. Utilizing N fertilizer decreased the Cd and Zn content in *Triticum polonicum L*. [64]. A combination of low N fertilizer and high P fertilizer significantly reduced the Cu content in the shoot portions of castor (*Ricinus communis L*.) [65], aligning with our findings. This reduction may be due to N and P fertilizer reducing the bioavailability of heavy metals in soil by affecting the morphology and complexation of heavy metals, thereby reducing the movement of heavy metals and absorption by plant roots [66, 67].

Additionally, our results indicated that applying N fertilizer and NP fertilizer facilitates the increase of heavy metal accumulation in the shoots of B. pilosa, with the combined application of NP producing the best results (Table 2). Other studies have yielded similar results, such as the application of N fertilizer enhancing the accumulation of heavy metals Zn and Cd in hyperaccumulators of Sedum alfredii Hance [68], and boosting the accumulation of heavy metals Pb in Cannabis sativa L. [48]. Research has also discovered that the combined application of NP amplifies the accumulation of heavy metals in the shoot portions of plant communities [39]. In our study, applying NP together reduced the content of heavy metals Cd, Cu, and Pb in the shoot portion of B. pilosa (Fig. 3). Conversely, the accumulation of heavy metals Cd, Cu, and Pb in the shoot portion of B. pilosa increased by 2.0-3.5 times when NP fertilizer was applied (Table 2). This accumulation is attributed to NP fertilizers significantly stimulating the shoot biomass of B. pilosa, which was 4.2 times higher than the control (Fig. 2). The contents of Cd, Cu, and Pb in plants only decreased by 1.1-1.6 times, leading to the accumulation of heavy metals in the shoot portions of B. pilosa treated with NP.

Efficiency of Fertilization on Improving the Remediation Efficiency of *B. pilosa*

The remediation efficiency of plants for heavy metal pollution in soil is primarily mirrored in components such as biomass, heavy metal enrichment, and the transfer coefficient [69-71]. This is because phytoremediation primarily focuses on remediating soil pollution by extracting heavy metals from plant shoot tissues [72]. This study demonstrated that applying nitrogen (N) fertilizer and nitrogen-phosphorus (NP) fertilizer significantly enhanced biomass accumulation in the shoot portion of the B. pilosa plant and increased the enrichment of heavy metals (Cd, Cu, and Pb) in the same part (Fig. 2, Table 2). Fertilizers may indirectly influence the accumulation of heavy metals in plants by affecting soil pH, ion strength, rhizosphere chemistry, and microbial activity, thus altering the uptake of heavy metals by the plants [73, 74].

We also showed that applying N and NP fertilizers combined increased the transfer coefficients of Cd, Cu, and Pb in *B. pilosa*, with transfer coefficients for Cd and Pb all exceeding 1 (Table 3). Similar studies have shown that N or phosphorus (P) fertilizers increase the transfer coefficients of heavy metals in various species, including *B. pilosa* [75], rice [76], and wheat [77]. Thus, applying N and P fertilizer allows for a larger harvest of *B. pilosa* per unit area each growing season, enabling greater Cd, Cu, and Pb transfer to the shoot portion, enriching this part with more heavy metals, and thus enhancing the remediation efficiency for Cd, Cu, and Pb heavy metal pollution in soil by *B. pilosa*.

Overall, we found that applying N fertilizer and NP combined can enhance the efficiency and feasibility of remediating Cd, Cu, and Pb heavy metal pollution in soil using *B. pilosa*. Given the increasing soil pollution of these heavy metals, our research holds guidance value for remediating them with *B. pilosa*, and even for remediating heavy metal pollution in soil with other hyperaccumulators. Further study is needed on the mechanics of the antagonistic and synergistic effects of heavy metals within plants, and the interactions between heavy metals and N and P.

Conclusions

Our findings indicated that nitrogen (N) and nitrogen-phosphorous (NP) fertilization may enhance the growth of B. pilosa shoot height and root length, augment its biomass, and improve the remediation efficiency of B. pilosa concerning heavy metals such as Cd, Cu, and Pb. This improvement could be attributed to the manner in which N and NP fertilization strengthens the B. pilosa root system's tolerance, encourages biomass accumulation in its shoots, and increases the accumulation of heavy metals Cd, Cu, and Pb in soil by the shoots, thus advancing the remediation efficiency of these heavy metals in B. pilosa. Therefore, incorporating N and NP fertilization may significantly contribute to improving the remediation efficiency of soil heavy metal pollution by hyperaccumulators, providing technical guidance for remediating soil heavy metal pollution. Further study is needed on the mechanics of the antagonistic and synergistic effects of heavy metals within plants, and the interactions between heavy metals and N and P.

Acknowledgments

This research was supported by the Applied Basic Research Foundation of Yunnan Province (Grant No. 202301AT070077), the National Natural Science Foundation of China (Grant No. 31800443; 32360317). We appreciate the editors and anonymous reviewers for their constructive comments on our paper.

Conflict of Interest

All the authors declare having no conflict of interest.

References

- 1. ALENGEBAWY A., KHALEK S.T.A., QURESHI S.R., WANG M.Q. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. Toxics, 9 (3), 42, 2021.
- KANG Y., ZHENG S., WAN T., WANG L.J., YANG Q., ZHANG J. Nematode as a biomonitoring model for evaluating ecological risks of heavy metals in sediments from an urban river. Ecological Indicators, 147, 110013, 2023.
- PENG J., ZHANG S., HAN Y., BATE B., KE H., CHEN Y. Soil heavy metal pollution of industrial legacies in China and health risk assessment. Science of the Total Environment. Science of the Total Environment, 816, 151632, 2022.
- CAO J., XIE C., HOU Z. Ecological evaluation of heavy metal pollution in the soil of Pb-Zn mines. Ecotoxicology, 31 (2), 259, 2022.
- ZHOU Z., PENG C., LIU X., JIANG Z., GUO Z., XIAO X. Pollution and Risk Assessments of Heavy Metal (loid) s in the Soil around Lead-Zinc Smelteries via Data Integration Analysis. International Journal of Environmental Research and Public Health, 19, 9698, 2022.
- 6. XIANG M., LI Y., YANG J., LEI K., CAO Y. Heavy metal contamination risk assessment and correlation analysis of heavy metal contents in soil and crops. Environmental Pollution, 278 (2), 116911, 2021.
- PARIDA L., PATEL T. Systemic impact of heavy metals and their role in cancer development: a review. Environmental Monitoring and Assessment, 195, 2023.
- PEIPEI S., DAN X., JINGYUAN Y., YUANCHEN M., SHUJUN D., JING F. Recent advances in soil remediation technology for heavy metal contaminated sites: A critical review. The Science of the total environment, 838, 156417, 2022.
- HAI L., ZIWEI W., CHENJING L., YINGBO D. Technologies for removing heavy metal from contaminated soils on farmland: A review. Chemosphere, 305, 135457, 2022.
- KHALID S., SHAHID M., NIAZI N.K., MURTAZA B., BIBI I., DUMAT C. A comparison of technologies for remediation of heavy metal contaminated soils. Journal of Geochemical Exploration, 182, 247, 2016.
- 11. DHALIWAL S.S., SINGH J., TANEJA P., MANDAL A. Remediation techniques for removal of heavy metals from the soil contaminated through different sources: a review. Mathematical research letters: MRL, 27 (2), 2020.
- 12. YAASHIKAA P.R., KUMAR P.S., JEEVANANTHAM S., SARAVANAN R. A review on bioremediation approach for heavy metal detoxification and accumulation in plants. Environmental Pollution, (May), 301, 2022.
- ALI H., KHAN E., SAJAD M. Phytoremediation of heavy metals-Concepts and applications. Chemosphere, 91 (7), 869, 2013.
- 14. YONG Z., ZHAO S., LIU S., PENG J., ZHANG H., ZHAO Q., ZHENG L., CHEN Y., SHEN Z., XU X., CHEN C. Enhancing the Phytoremediation of Heavy Metals by Combining Hyperaccumulator and Heavy Metal-Resistant

- Plant Growth-Promoting Bacteria. Frontiers in Plant Science, 13, 912350, 2022.
- PATRA D.K., PRADHAN C., PATRA H.K. Toxic metal decontamination by phytoremediation approach: Concept, challenges, opportunities and future perspectives. Environmental Technology & Innovation. 18, 100672, 2020.
- AWA S.H., HADIBARATA T. Removal of Heavy Metals in Contaminated Soil by Phytoremediation Mechanism: a Review. Water, Air, & Soil Pollution, 231 (2), 2020.
- WAN X., LEI M., CHEN T. Cost-benefit calculation of phytoremediation technology for heavy-metalcontaminated soil. Science of the Total Environment, 563-564, 796, 2016.
- ASAD S., FAROOQ M., AFZAL A., WEST H. Integrated phytobial heavy metal remediation strategies for a sustainable clean environment - A review. Chemosphere, 217, 925, 2019.
- ZHU Y., WANG Y., ZHENG H., XIANG X., WANG H., XIE M., LIU H., FANG Z., LIU L., DU S. N fertilizers promote abscisic acid-catabolizing bacteria to enhance heavy metal phytoremediation from metalliferous soils. The Science of the total environment, 894, 164964, 2023.
- YANG Q., YANG C., YU H., ZHAO Z., BAI Z. The addition of degradable chelating agents enhances maize phytoremediation efficiency in Cd-contaminated soils. Chemosphere, 269, 129373, 2020.
- 21. KHAN A., ULLAH I., KHAN A., PARK G., WAQAS M., HONG S., JUNG B., KWAK Y., LEE I., SHIN J. Improvement in phytoremediation potential of *Solanum nigrum* under cadmium contamination through endophytic-assisted Serratia sp. RSC-14 inoculation. Environmental science and pollution research international, 22 (18), 14032, 2015.
- 22. HE T., XU Z., WANG J., WANG F., ZHOU X., LI W., LI Q. Improving cadmium accumulation by *Solanum nigrum L*. via regulating rhizobacterial community and metabolic function with phosphate-solubilizing bacteria colonization. Chemosphere, 287 (Pt 2), 132209, 2021.
- 23. XIAO J., DONG S., SHEN H., LI S., ZHI Y., MU Z., DING C. Phosphorus addition promotes Nitrogen retention in alpine grassland plants while increasing N deposition. Catena: An Interdisciplinary Journal of Soil Science Hydrology-Geomorphology Focusing on Geoecology and Landscape Evolution, 2022.
- 24. MA Q., SUN L., TIAN H., RENGEL Z., SHEN J. Deep banding of phosphorus and nitrogen enhances Rosa multiflora growth and nutrient accumulation by improving root spatial distribution. Scientia Horticulturae, 277, 109800, 2021.
- KOŁODZIEJEK J. Growth performance and emergence of invasive alien Rumex confertus in different soil types. Scientific Reports, 9, 19678, 2019.
- 26. FANG X., YANG Y., ZHAO Z., ZHOU Y., LIAO Y., GUAN Z., CHEN S., FANG W., CHEN F., ZHAO S. Optimum Nitrogen, Phosphorous, and Potassium Fertilizer Application Increased Chrysanthemum Growth and Quality by Reinforcing the Soil Microbial Community and Nutrient Cycling Function. Plants, 12 (23), 2023.
- SHETTY K.G., HETRICK B.A.D., SCHWAB A.P. Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. Environmental Pollution, 88 (3), 307, 1995.
- 28. YANG Y., XIONG J., TAO L., CAO Z., TANG W., ZHANG J., YU X., FU G., ZHANG X., LU Y. Regulatory mechanisms of nitrogen (N) on cadmium (Cd) uptake and

Nitrogen and Phosphorus Fertilization...

- accumulation in plants: A review. Science of the Total Environment, 708 (C), 135186, 2020.
- LI Y., LIU K., ZHU J., JIANG Y., YU F. Manganese accumulation and plant physiology behavior of Camellia oleifera in response to different levels of nitrogen fertilization. Ecotoxicology and Environmental Safety, 184, 109603, 2019.
- 30. MAQBOOL A., ALI S., RIZWAN M., ARIF M.S., YASMEEN T., RIAZ M., HUSSAIN A., NOREEN S., ABDEL-DAIM M.M., ALKAHTANI S. N-Fertilizer (Urea) Enhances the Phytoextraction of Cadmium through Solanum nigrum L. International Journal of Environmental Research and Public Health, 17 (11), 2020.
- 31. FAHAD S., BASHIR S., SALEEM M.H., SIDDIQUI M.H., REHMAN M., PENG D., SHAH R.A., DENG G., ALAMRI S., KHAN S.M. Effects of rice straw biochar and nitrogen fertilizer on ramie (*Boehmeria nivea L.*) morpho-physiological traits, copper uptake and post-harvest soil characteristics, grown in an aged-copper contaminated soil. Journal of Plant Nutrition, 45 (1), 11, 2021.
- 32. KIMANI S.M., BIMANTARA P.O., KAUTSAR V., TAWARAYA K., CHENG W. Poultry litter biochar application in combination with chemical fertilizer and Azolla green manure improves rice grain yield and nitrogen use efficiency in paddy soil. Biochar, 3 (4), 1, 2021.
- MUINDI E.M. Understanding Soil Phosphorus. International Journal of Plant & Soil Science, 31 (2), 1, 2019.
- 34. SU R., ZHANG Z., CHANG C., PENG Q., CHENG X., PANG J., HE H., LAMBERS H. Interactive effects of phosphorus fertilization and salinity on plant growth, phosphorus and sodium status, and tartrate exudation by roots of two alfalfa cultivars. Annals of Botany, 129 (1), 53, 2021.
- 35. GUPTA D.K., CHATTERJEE S., DATTA S., VEER V., WALTHER C. Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. Chemosphere, 108, 134, 2014.
- LI W., GAN X., JIANG Y., CAO F., LU X., CEULEMANS T., ZHAO C. Nitrogen effects on grassland biomass production and biodiversity are stronger than those of phosphorus. Environmental Pollution, 309, 119720, 2022.
- 37. VÁZQUEZ E., BORER E., BUGALHO M., CALDEIRA M., MCCULLEY R., RISCH A., SEABLOOM E., WHEELER G., SPOHN M. The synergistic response of primary production in grasslands to combined nitrogen and phosphorus addition is caused by increased nutrient uptake and retention. Plant and Soil, 490 (1), 371, 2023.
- 38. HUANG J., WANG C., QI L., ZHANG X., LU M. Phosphorus is more effective than nitrogen in restoring plant communities of heavy metals polluted soils. Environmental Pollution, **266** (Pt 3), 115259, **2020**.
- 39. TANG G., ZHANG X., QI L., LI L., GUO J., ZHONG H., LIU J., HUANG J. Nitrogen and Phosphorus Fertilizer Increases the Uptake of Soil Heavy Metal Pollutants by Plant Community. Bulletin of Environmental Contamination and Toxicology, 109 (6), 1059, 2022.
- 40. ZHANG R., CHEN D., LIU H., GUO C., TANG L., WANG H., CHEN Y., LUO K. Effect of temperature and water potential on the germination of seeds from three different populations of Bidens pilosa as a potential Cd hyperaccumulator. BMC Plant Biology, 22 (1), 1, 2022.
- 41. DOU X., DAI H., SKUZA L., WEI S. *Bidens pilosa L.* hyperaccumulating Cd with different species in soil and the

- role of EDTA on the hyperaccumulation. Environmental Science and Pollution Research, **26** (25), 25668, **2019**.
- 42. SALAZAR M.J., WANNAZ E.D., BLANCO A., PAZCEL E.M.M., PIGNATA M.L. Pb tolerance and accumulation capabilities of *Bidens pilosa L.* growing in polluted soils depend on the history of exposure. Chemosphere, **269** (1), 128732, **2020**.
- 43. PERLATTI F., FERREIRA T., ROMERO R., COSTA M., OTERO X. Copper accumulation and changes in soil physical-chemical properties promoted by native plants in an abandoned mine site in northeastern Brazil: Implications for restoration of mine sites. Ecological Engineering, 82, 103, 2015.
- 44. DAI H., WEI S., POGRZEBA M., KRZYŻAK J., RUSINOWSKI S., ZHANG Q. The cadmium accumulation differences of two *Bidens pilosa L.* ecotypes from clean farmlands and the changes of some physiology and biochemistry indices. Ecotoxicology and Environmental Safety, 209, 111847, 2021.
- WEI C., TANG S., PAN Y., LI X. Plastic responses of invasive Bidens frondosa to water and nitrogen addition. Nordic Journal of Botany, 35 (2), 232, 2017.
- DAI H., WEI S., SKUZA L. Effects of different soil pH and nitrogen fertilizers on *Bidens pilosa L*. Cd accumulation. Environmental Science and Pollution Research, 27 (3), 2020.
- 47. ZHANG X., GU P., LIU X., HUANG X., JI J. Effect of crop straw biochars on the remediation of Cd-contaminated farmland soil by hyperaccumulator *Bidens pilosa L*. Ecotoxicology and Environmental Safety, **219** (6), 112332, **2021**.
- 48. DENG G., YANG M., SALEEM M.H., REHMAN M., KHAN S.M. Nitrogen fertilizer ameliorate the remedial capacity of industrial hemp (*Cannabis sativa L.*) grown in lead contaminated soil. Journal of Plant Nutrition, 44 (12), 2021.
- 49. VITOUSEK P.M., PORDER S., HOULTON B.Z., CHADWICK O.A. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications, 20 (1), 5, 2010.
- SCHLEUSS P.M., WIDDIG M., HEINTZ-BUSCHART A., KIRKMAN K., SPOHN M. Interactions of nitrogen and phosphorus cycling promote P acquisition and explain synergistic plant-growth responses. Ecology, 101 (5), 2020.
- 51. HENRY H.A.L., CHIARIELLO N.R., VITOUSEK P.M., MOONEY H.A., FIELD C.B. Interactive Effects of Fire, Elevated Carbon Dioxide, Nitrogen Deposition, and precipitation on a California Annual Grassland. Ecosystems, 9 (7), 1066, 2006.
- ZAVALETA E., SHAW M., CHARIELLO N., THOMAS B., CLELAND E., FIELD C., MOONEY H. Grassland and responses to three years of elevated temperature, CO₂, precipitation, and N deposition. Ecological Monographs, 73 (4), 2003.
- 53. NIU K., LUO Y., CHOLER P., DU G. The role of biomass allocation strategy in diversity loss due to fertilization. Basic & Applied Ecology, 9 (5), 485, 2008.
- 54. GUIGNARD M.S., NICHOLS R.A., KNELL R.J., MACDONALD A., LEITCH A.R. Genome size and ploidy influence angiosperm species' biomass under nitrogen and phosphorus limitation. New Phytologist, 210 (4), 1195, 2016
- 55. VLADIMIR K., MARCEL B. Symbiotic phosphate transport in arbuscular mycorrhizas. Trends in plant science, 10 (1), 22, 2005.

56. SONG J., WANG Y., PAN Y., PANG J., ZHANG X., FAN J., ZHANG Y. The influence of nitrogen availability on anatomical and physiological responses of Populus alba × P. glandulosa to drought stress. BMC Plant Biology, 19 (1), 2019.

- ZHANG F., WAN X., ZHONG Y. Nitrogen as an important detoxification factor to cadmium stress in poplar plants. Journal of Plant Interactions, 9 (1), 249, 2013.
- QIN H., HUANG R. Auxin Controlled by Ethylene Steers Root Development. International Journal of Molecular Sciences, 19 (11), 3656, 2018.
- HUSSAIN S., AKRAM M., ABBAS G., MURTAZA B., NIAZI N.K. Arsenic tolerance and phytoremediation potential of *Conocarpus erectus L.* and Populus deltoides L. International Journal of Phytoremediation, 19 (1), 2017.
- 60. NAJEEB S., ALI J., MAHENDER A., PANG Y.L., LI Z. Identification of main-effect quantitative trait loci (QTLs) for low-temperature stress tolerance germination- and early seedling vigor-related traits in rice (*Oryza sativa L.*). Molecular Breeding, 40 (1), 2020.
- KHAN I., AWAN S.A., RIZWAN M., ALI S., HUANG L. Effects of silicon on heavy metal uptake at the soil-plant interphase: A review. Ecotoxicology and Environmental Safety, 222, 112510, 2021.
- 62. ERIKSSON J.E. Effects of nitrogen-containing fertilizers on solubility and plant uptake of cadmium. Water Air & Soil Pollution, 49 (3), 355, 1990.
- 63. BOGDANOVIC D., UBAVIC M., CUVARDIC M. Effect of phosphorus fertilization on Zn and Cd contents in soil and corn plants. Nutrient Cycling in Agroecosystems, 54 (1), 49, 1999.
- 64. WANG X., CHENG Y., SHUAI W., ZENG J., ZHOU Y. Nitrate N influences the accumulations and subcellular distributions of Cd and Zn to mediate the Cd/Zn interactions in dwarf Polish Wheat (*Triticum polonicum L.*) seedlings. Soil Science and Plant Nutrition, 65 (2), 1, 2018.
- 65. YAO S., GUO G., ZHOU X, REN C., HUANG G., HONG-QING H. Effect of nitrogen and phosphorus fertilizer application on uptake and accumulation of Cu by castor (*Ricinus communis L.*) in mine soil. Journal of Plant Nutrition and Fertilizers, **24** (04), 1068, **2018**.
- 66. DHERI G., BRAR M., MALHI S. Influence of phosphorus application on growth and cadmium uptake of spinach in two cadmium-contaminated soils. Journal of Plant Nutrition & Soil Science, 170, 495, 2007.
- 67. WÅNGSTRAND H., ERIKSSON J., ÖBORN I. Cadmium concentration in winter wheat as affected by

- nitrogen fertilization. European Journal of Agronomy, **26** (3), 209, **2007**.
- 68. LIN Z., DOU C., LI Y., WANG H., NIAZI N., ZHANG S., LIU D., ZHAO K., FU W., LI Y., YE Z. Nitrogen fertilizer enhances zinc and cadmium uptake by hyperaccumulator *Sedum alfredii Hance*. Journal of Soils and Sediments, **20** (1), 320, **2020**, .
- 69. DINU C., VASILE G.G., BULEANDRA M., POPA D.E., UNGUREANU E.M. Translocation and accumulation of heavy metals in *Ocimum basilicum L.* plants grown in a mining-contaminated soil. Journal of Soils and Sediments, **20** (51), **2020**.
- KUMAR A., TRIPTI., RAJ D., MAITI S., MALEVA M., BORISOVA G. Soil Pollution and Plant Efficiency Indices for Phytoremediation of Heavy Metal(loid)s: Two-Decade Study (2002-2021). Metals, 12 (8), 1330, 2022.
- SOUBASAKOU G., CAVOURA O., DAMIKOUKA I. Phytoremediation of Cadmium-Contaminated Soils: A Review of New Cadmium Hyperaccumulators and Factors Affecting their Efficiency. Bulletin of Environmental Contamination and Toxicology, 109 (5), 783, 2022.
- QUARSHIE D.G., XIAO X., ZHANG L. Enhanced Phytoremediation of Soil Heavy Metal Pollution and Commercial Utilization of Harvested Plant Biomass: a Review. Water, Air, & Soil Pollution, 232 (11), 1, 2021.
- 73. AERTS R., CHAPIN F. The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. Advances in Ecological Research, 30, 1, 2000.
- 74. GRANT C.A., SHEPPARD S.C. Fertilizer Impacts on Cadmium Availability in Agricultural Soils and Crops. Human and Ecological Risk Assessment: An International Journal, 14 (4), 210, 2008.
- XIONG M., GUO J., ZHANG F., HAN F., LIU J., YANG Y., ZHING H., HUANG J. Effects of Different Nitrogen Fertilizers on Remediation of Soil Cadmium Pollution by Bidens pilosa[J]. Acta Agriculturae Universitatis Jiangxiensis, 44 (04), 1592, 2022.
- 76. SONG T., DAS D., HU Q., FENG Y., ZHANG J. Alternate wetting and drying irrigation and phosphorus rates affect grain yield and quality and heavy metal accumulation in rice. Science of The Total Environment, 752, 141862, 2020.
- 77. SABIHA-JAVIED, SIDDQUE N., WAHEED S., ZAMAN Q.U., ASLAM A., TUFAIL M., NASIR R. Uptake of heavy metal in wheat from application of different phosphorus fertilizers. Journal of Food Composition and Analysis, 115, 104958, 2023.