

Postprint: Remediation Capability of Alfalfa, Ryegrass, and Pennisetum for Cu-Pb Co-contaminated Soil

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Date: 2018-01-05T00:00:00+00:00

Abstract

With the development of economy and society, soil heavy metal pollution poses a tremendous threat to food security and human health, and phytoremediation currently represents the primary approach for soil heavy metal pollution management. To identify suitable forage grasses for remediating Cu and Pb co-contaminated soil, a pot experiment was conducted to investigate the remediation effectiveness of these three common forage plants on Cu and Pb co-contaminated soil by comparing the adaptability and enrichment characteristics of alfalfa (*Medicago sativa*), ryegrass (*Lolium perenne*), and fountain grass (*Pennisetum alopecuroides*).

The results indicated: (1) The shoot and root biomass of alfalfa were both maximal in the Pb1 treatment group, significantly greater than those in other treatment groups; the shoot biomass of ryegrass was maximal in the Cu1Pb1 treatment group, while its root biomass was maximal in the Pb1 treatment group; the shoot biomass of fountain grass was maximal in the Cu2Pb2 treatment group, while its root biomass was maximal in the Cu2 treatment group.

- (2) Under conditions of single Cu pollution, fountain grass exhibited the highest resistance coefficient; under single Pb pollution, alfalfa showed the highest resistance coefficient; under Cu-Pb co-contamination conditions, fountain grass generally demonstrated a higher resistance coefficient. In the high-concentration Cu treatment group, the shoot biomass, root biomass, and resistance coefficients of the three forage plants all followed the trend: fountain grass > ryegrass > alfalfa, with fountain grass being significantly greater than ryegrass and alfalfa.
- (3) Following cultivation of the three forage plants, the heavy metal Cu and Pb contents in soil were both reduced. At certain concentrations, soil Cu

and Pb heavy metals mutually promoted the absorption of each other in the forage plants.

- (4) Among the three forage grasses, the enrichment coefficient of Cu in the shoots of alfalfa was maximal in the Cu2Pb2 treatment group, reaching 1.61, while the enrichment coefficient of Cu in the roots of ryegrass was maximal in the Cu2Pb2 treatment group, reaching 3.8; the enrichment coefficients of Pb in the shoots and roots of the three forage grasses exceeded 1 only in the roots of ryegrass in the Cu1Pb1 treatment group, reaching 1.46.
- (5) Ryegrass demonstrated strong absorption capacity for Pb, with primary accumulation in the root system; alfalfa exhibited the best overall remediation effect for Cu-Pb co-contamination. The translocation factors for Pb in alfalfa under Cu-Pb co-contaminated soil and in ryegrass under single Pb-contaminated soil exceeded 1, being 2.72 and 2.06 respectively, indicating their potential for Pb enrichment in soil.

Overall, ryegrass exhibits strong tolerance to heavy metal Pb and can be prioritized as a selected material for phytoremediation of single Pb-contaminated soil and vegetation reconstruction in tailings wastelands; alfalfa demonstrates strong tolerance to both heavy metals Cu and Pb, and can be prioritized as a selected material for phytoremediation of single Cu or Cu-Pb co-contaminated soil and vegetation reconstruction in tailings wastelands.

Full Text

Phytoremediation of Single and Combined Pollution of Cu and Pb by *Medicago sativa*, *Lolium perenne*, and *Pennisetum alopecuroides*

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Abstract

With rapid economic and social development, heavy metal contamination in soil has become an increasingly serious problem that threatens food security and human health. To date, phytoremediation remains the primary approach for treating heavy metal pollution. This study investigated the remediation potential of three common forage plants—alfalfa (*Medicago sativa*), ryegrass (*Lolium perenne*), and Chinese pennisetum (*Pennisetum alopecuroides*)—for soils contaminated with copper (Cu) and lead (Pb) through pot experiments comparing their adaptability and enrichment characteristics.

The results showed: (1) Alfalfa achieved maximum aboveground and root biomass in the Pb1 treatment group, significantly greater than other treatments.

Ryegrass showed maximum aboveground biomass in the Cu1Pb1 group and maximum root biomass in the Pb1 group. Chinese pennisetum exhibited maximum aboveground biomass in the Cu2Pb2 group and maximum root biomass in the Cu2 group. (2) Under single Cu pollution, Chinese pennisetum displayed the highest resistance coefficient; under single Pb pollution, alfalfa showed the highest resistance coefficient; and under combined Cu-Pb pollution, Chinese pennisetum generally demonstrated superior resistance. In high-concentration Cu treatments, the ranking for aboveground biomass, root biomass, and resistance coefficient among the three species was: Chinese pennisetum > ryegrass > alfalfa, with Chinese pennisetum significantly outperforming the other two species. (3) Planting all three forage species reduced soil Cu and Pb concentrations. At certain concentrations, Cu and Pb in soil exhibited mutual promotion of absorption in the forage plants. (4) Among the three species, alfalfa showed the highest aboveground enrichment coefficient for Cu (1.61) in the Cu2Pb2 treatment, while ryegrass showed the highest root enrichment coefficient for Cu (3.8) in the same treatment. The Pb enrichment coefficient in both aboveground and root tissues exceeded 1 only in ryegrass roots in the Cu1Pb1 treatment, reaching 1.46. (5) Ryegrass demonstrated strong Pb absorption capacity, primarily accumulating Pb in roots. Alfalfa exhibited the best overall remediation effect for combined Cu-Pb pollution. Both alfalfa and ryegrass showed Pb translocation factors greater than 1 in Cu-Pb composite and Pb single-polluted soils, respectively (2.72 and 2.06), indicating their potential for Pb phytoextraction.

In conclusion, ryegrass shows strong tolerance to Pb and can be prioritized for remediating Pb-contaminated soils and revegetating mine tailings. Alfalfa demonstrates strong tolerance to both Cu and Pb and can be prioritized for remediating soils with single Cu pollution or combined Cu-Pb contamination, as well as for tailings revegetation.

Keywords: *Medicago sativa*; *Lolium perenne*; *Pennisetum alopecuroides*; heavy metal; Cu; Pb; combined pollution; enrichment; translocation

Introduction

Soil constitutes a vital component of the ecological environment and is intimately connected with human production and life activities, making it highly susceptible to various pollutants, particularly heavy metal salts. Heavy metal pollution is characterized by irreversible accumulation, easy enrichment, and resistance to degradation. These metals can enter the human body through the food chain, posing significant threats to human health and causing severe negative impacts on both society and the ecological environment [1-2].

Copper and lead represent major heavy metal pollutants in China's soil-plant ecosystem. Excessive Pb entering the environment participates in the water-soil-biological system cycle, and plant absorption and enrichment of Pb disrupts

mineral nutrient uptake. While Cu is an essential trace element for plant growth and development, excessive Cu exhibits high toxicity, hindering plant growth and disrupting physiological and metabolic processes [3-9]. Consequently, research on the effects of Cu and Pb on soil ecological environments has attracted widespread attention [10-11]. Pollution sources in soil rarely exist in isolation; multiple contaminants often coexist and produce combined effects. Therefore, studying the effects of combined Cu-Pb pollution on plant stress responses helps explore remediation strategies for complex heavy metal contamination [12-13].

Current research on soil heavy metal pollution remediation primarily focuses on phytoremediation, which utilizes plants to absorb, accumulate, and transform heavy metals to achieve remediation goals [14]. This method applies to a wide range of pollutants, requires low investment and cost, does not cause secondary pollution, and can be implemented on a large scale, making it a hot topic in research both domestically and internationally [15-16]. However, most studies on hyperaccumulator screening have focused on crops [17] and vegetables [18], which are typically short, slow-growing, low in biomass, and have long growth cycles without good economic benefits, limiting large-scale application [19]. Forage grasses, in contrast, are not only feed sources but also exhibit strong adaptability, rapid growth, high biomass, and play important roles in ecological restoration and soil and water conservation. To date, dozens of forage species have been used for phytoremediation of heavy metal-contaminated soils, such as alfalfa (*Medicago sativa*) and Italian ryegrass (*Lolium multiflorum*) [20]. Previous studies indicate that alfalfa shows strong Pb enrichment capacity, ryegrass exhibits strong Cu enrichment characteristics and serves as a pioneer plant for Pb-Zn tailing remediation, while research on the combined effects of Cu-Pb pollution on alfalfa, ryegrass, and Chinese pennisetum (*Pennisetum alopecuroides*) remains limited [21-23].

Therefore, this study selected three forage species—alfalfa, ryegrass (*Lolium perenne*), and Chinese pennisetum—as experimental materials to investigate their tolerance, absorption, and translocation capacities for soil heavy metals Cu and Pb, aiming to identify suitable forage species for remediating Cu-Pb contaminated soils and provide a research basis for future applications of forage-based phytoremediation.

1.1.1 Experimental Plants

Seeds of alfalfa, ryegrass, and Chinese pennisetum were purchased from the Jinhua seed market in Zhejiang Province. Plump, uniformly sized seeds were selected as experimental materials and planted in pots for the experiment.

1.1.2 Experimental Soil

Soil was collected from the biological garden base of Zhejiang Normal University. Surface farmland soil (0-20 cm) was air-dried, coarsely screened to remove

large impurities, and passed through a 100-mesh sieve. To minimize root damage during sampling, sand and soil were mixed at a 1:4 ratio as a cultivation substrate.

The background values of the mixed substrate were $35.94 \text{ mg} \cdot \text{kg}^{-1}$ for Cu and $78.22 \text{ mg} \cdot \text{kg}^{-1}$ for Pb, with a pH of approximately 6.8. Analytical grade $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{Pb}(\text{NO}_3)_2$ were used to prepare heavy metal salt solutions at different concentration gradients, which were thoroughly mixed into air-dried soil and allowed to equilibrate for two weeks before use as experimental soil.

1.2 Experimental Design

Based on the Soil Environmental Quality Standard (GB15618-2008), natural background values, and preliminary results on seed germination and seedling responses to single Cu and Pb pollution, nine treatment groups were established (Table 1), with three replicates per group. Each pot contained 1.5 kg of experimental soil spiked with different heavy metal concentrations. Sterilized seeds were sown directly in the contaminated soil. Groundwater from the experimental area was added to maintain soil moisture at 60% of field capacity. Each pot was thinned to six plants (consistent across treatment groups for the same species). Plants and rhizosphere soil samples were harvested after 60 days.

Table 1 Design of heavy metal interaction concentrations and treatment codes

Cu ²⁺ concentration ($\text{mg} \cdot \text{kg}^{-1}$)	Pb ²⁺ concentration ($\text{mg} \cdot \text{kg}^{-1}$)	Treatment code
		Cu1Pb1
		Cu2Pb1
		Cu1Pb2
		Cu2Pb2

1.3 Sample Processing and Heavy Metal Determination

Plant samples were thoroughly rinsed with tap water, washed with $0.1 \text{ mol} \cdot \text{L}^{-1}$ dilute HCl, and finally rinsed 2-3 times with deionized water before surface moisture was removed. Plant samples were separated into aboveground and root portions, oven-dried at 105°C for 30 minutes, then at 70°C to constant weight. Dry weights were recorded, and samples were ground and passed through a 60-mesh nylon sieve. Soil samples were air-dried and passed through a 100-mesh sieve. Plant samples were digested with $\text{HNO}_3\text{-HClO}_4$, and soil samples with $\text{HCl-HNO}_3\text{-HClO}_4$. Copper and Pb concentrations were determined using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

1.4 Data Processing and Analysis

Resistance coefficient = Total biomass in treatment / Total biomass in control
(1)

Bioaccumulation factor (BCF) = C_p / C_s (2)

Where C_p is the heavy metal concentration in plant tissue and C_s is the heavy metal concentration in soil.

Translocation factor (TF) = C_o / C_u (3)

Where C_o is the heavy metal concentration in aboveground plant tissue and C_u is the concentration in roots.

2.1 Effects of Single and Combined Cu and Pb Treatments on Growth of Three Forage Species

As shown in Figure 1 [Figure 1: see original paper]A, under single Cu pollution, alfalfa aboveground biomass decreased with increasing concentration, while ryegrass and Chinese pennisetum showed an initial decrease followed by an increase. Under high Cu concentration, Chinese pennisetum aboveground biomass was significantly higher than that of alfalfa and ryegrass ($P < 0.05$), indicating stronger tolerance to high Cu contamination. Under single Pb pollution, aboveground biomass of all three species initially increased then decreased with concentration. Alfalfa aboveground biomass was significantly higher than ryegrass ($P < 0.05$) and significantly higher than Chinese pennisetum at low concentrations ($P < 0.05$), demonstrating stronger Pb tolerance. Under combined Cu-Pb pollution, alfalfa showed maximum aboveground biomass in the Cu1Pb2 group, ryegrass in Cu1Pb1, and Chinese pennisetum in Cu2Pb2. Chinese pennisetum aboveground biomass under combined pollution was significantly higher than ryegrass ($P < 0.05$), while alfalfa was significantly higher than ryegrass only in the Cu1Pb2 group ($P < 0.05$) but not significantly different from Chinese pennisetum ($P > 0.05$). Significant differences in aboveground biomass also existed among treatment groups for each species: alfalfa Pb1 treatment was significantly higher than other treatments ($P < 0.05$); ryegrass Cu1Pb1 was significantly higher than Cu2Pb2 ($P < 0.05$); Chinese pennisetum Cu2Pb2 was significantly higher than other treatments ($P < 0.05$), with no significant differences among remaining groups ($P > 0.05$).

As shown in Figure 1B, under single Cu pollution, alfalfa root biomass decreased with increasing concentration, while ryegrass and Chinese pennisetum showed the opposite trend. Under high Cu concentration, Chinese pennisetum root biomass was significantly higher than alfalfa and ryegrass ($P < 0.05$), again indicating stronger Cu tolerance. Under single Pb pollution, root biomass of all three species initially increased then decreased with concentration. Under high Pb concentration, ryegrass and Chinese pennisetum root biomass were significantly higher than alfalfa ($P < 0.05$), indicating weaker Pb tolerance in alfalfa. Under combined Cu-Pb pollution, alfalfa and Chinese pennisetum showed maximum root biomass in Cu1Pb2, while ryegrass peaked in Cu1Pb1. Chinese pennisetum root biomass under combined pollution was significantly higher than alfalfa ($P < 0.05$), while ryegrass root biomass differed significantly from Chinese

pennisetum only in the Cu1Pb1 group ($P>0.05$) and from alfalfa only in the Cu1Pb2 group ($P>0.05$). Significant differences in root biomass among treatment groups were observed: alfalfa Pb1 was significantly higher than other treatments ($P<0.05$); ryegrass Pb treatments were significantly higher than Cu1Pb2, Cu2Pb1, and Cu2Pb2 ($P<0.05$); Chinese pennisetum Cu2 was significantly higher than Pb2, Cu1Pb1, and Cu2Pb2 ($P<0.05$).

Figure 1 Effects of single and combined Cu and Pb pollution on shoot (A) and root (B) biomass of three forage species. Different uppercase letters indicate significant differences among the three species at each treatment level ($P<0.05$). Different lowercase letters indicate significant differences among treatment levels for the same species ($P<0.05$).

2.2 Effects of Single and Combined Cu and Pb Treatments on Resistance Coefficients

As shown in Figure 2 [Figure 2: see original paper], under single Cu pollution, the resistance coefficients followed the order: Chinese pennisetum > ryegrass > alfalfa. No significant differences in Cu resistance were observed among the three species at low Cu concentrations ($P>0.05$), but at high Cu concentrations, ryegrass and Chinese pennisetum showed significantly higher resistance than alfalfa ($P<0.05$). Under single Pb pollution, alfalfa exhibited a significantly higher resistance coefficient than ryegrass and Chinese pennisetum ($P<0.05$), peaking at low Pb concentration, while no significant differences were observed among the three species at high Pb concentrations ($P<0.05$). Under combined Cu-Pb pollution, high Cu concentration treatments showed the resistance coefficient ranking: Chinese pennisetum > ryegrass > alfalfa, with significant differences among all three species ($P<0.05$).

Significant differences in resistance coefficients were also observed among treatment groups for each species: alfalfa Pb1 was significantly higher than other treatments ($P<0.05$); ryegrass Cu2, single Pb, and Cu1Pb1 treatments were significantly higher than Cu2Pb2 ($P<0.05$); Chinese pennisetum Cu2Pb2 was significantly higher than Pb2 ($P<0.05$), with no significant differences among other treatments ($P>0.05$).

At low Cu concentration, resistance coefficients for alfalfa and Chinese pennisetum followed: Cu1Pb2 > Cu1Pb1 > Cu1, indicating that Pb addition promoted growth in these species with enhancement increasing as Pb concentration rose. For ryegrass, the order was Cu1Pb1 > Cu1 > Cu1Pb2, suggesting that low Pb concentration promoted growth while high Pb inhibited it. At high Cu concentration, alfalfa and ryegrass showed: Cu2 > Cu2Pb1 > Cu2Pb2, indicating that Pb addition inhibited growth with inhibition intensifying as Pb concentration increased. In contrast, Chinese pennisetum showed: Cu2Pb2 > Cu2 > Cu2Pb1, suggesting that low Pb inhibited growth while high Pb promoted it.

At low Pb concentration, alfalfa resistance coefficients followed: $Pb1 > Cu1Pb1 > Cu2Pb1$, indicating that Cu addition inhibited growth with inhibition intensifying as Cu concentration increased. Ryegrass showed: $Cu1Pb1 > Pb1 > Cu2Pb1$, suggesting that low Cu promoted growth while high Cu inhibited it. Chinese pennisetum exhibited: $Pb1 > Cu2Pb1 > Cu1Pb1$, indicating that Cu addition inhibited growth but inhibition weakened as Cu concentration increased. At high Pb concentration, alfalfa and ryegrass showed: $Pb2 > Cu1Pb2 > Cu2Pb2$, indicating that Cu addition inhibited growth with inhibition intensifying as Cu concentration increased. Chinese pennisetum showed: $Pb2 > Cu1Pb2 > Cu2Pb2$, suggesting that Cu addition promoted growth with enhancement increasing as Cu concentration rose.

Figure 2 Resistance coefficients of three forage species under single and combined Cu and Pb pollution. Different uppercase letters indicate significant differences among the three species at each treatment level ($P < 0.05$). Different lowercase letters indicate significant differences among treatment levels for the same species ($P < 0.05$).

2.3 Cu and Pb Accumulation in Three Forage Species Under Single and Combined Treatments

As shown in Figure 3 [Figure 3: see original paper], under single Cu and combined Cu-Pb pollution, Cu accumulation in aboveground and root tissues increased with soil Cu concentration. When soil Cu addition reached $200 \text{ mg} \cdot \text{kg}^{-1}$, Cu concentrations in alfalfa aboveground and root tissues increased by 104.31% and 146.2%, respectively, under combined exposure with $300 \text{ mg} \cdot \text{kg}^{-1}$ Pb, while $800 \text{ mg} \cdot \text{kg}^{-1}$ Pb caused a 13.26% increase in aboveground tissue but a 28.06% decrease in roots. When soil Cu pollution increased to $400 \text{ mg} \cdot \text{kg}^{-1}$, alfalfa aboveground Cu content increased with Pb concentration while root content decreased, indicating that increased soil Pb inhibited Cu absorption in alfalfa roots but promoted aboveground Cu accumulation under high Cu treatment. For ryegrass, increased soil Pb inhibited aboveground Cu absorption at low Cu concentration but promoted it at high Cu concentration. In roots, increased soil Pb promoted Cu absorption at low Cu concentration but inhibited it at high Cu concentration. For Chinese pennisetum, increased soil Pb promoted Cu absorption. In the $Cu2Pb1$ and $Cu2Pb2$ treatments, alfalfa aboveground Cu accumulation was significantly higher than in ryegrass and Chinese pennisetum ($P < 0.05$), while ryegrass root Cu accumulation was significantly higher than in alfalfa and Chinese pennisetum ($P < 0.05$).

Under single Pb and combined Cu-Pb pollution, Pb accumulation in aboveground and root tissues increased with pollution level. At $300 \text{ mg} \cdot \text{kg}^{-1}$ Pb, compared to single Pb treatment, low Cu concentration ($200 \text{ mg} \cdot \text{kg}^{-1}$) reduced alfalfa aboveground Pb content while high Cu increased it, indicating that Cu addition inhibited Pb absorption with inhibition weakening as Cu concentration

increased, eventually promoting Pb absorption at sufficient Cu levels. Alfalfa aboveground Pb accumulation was significantly higher than ryegrass and Chinese pennisetum under combined pollution ($P < 0.05$). Root Pb accumulation differed significantly among the three species under single Pb and combined pollution ($P < 0.05$), with no significant difference between alfalfa and ryegrass in the Cu2Pb1 treatment ($P > 0.05$).

Overall, the three forage species showed substantial differences in heavy metal accumulation between aboveground and root tissues, with generally higher concentrations in roots, likely related to plant heavy metal tolerance mechanisms. Roots typically release various organic compounds (including monosaccharides and amino acids) that help immobilize toxic heavy metals [27]. The complex effects of combined Cu-Pb pollution indicated that, at certain concentrations, Cu and Pb mutually promoted each other's absorption in forage plants.

Figure 3 Cu and Pb contents in three forage species at each treatment level. Different lowercase letters indicate significant differences among the three species at each treatment level ($P < 0.05$).

2.4 Bioaccumulation and Translocation Factors for Cu and Pb in Three Forage Species

As shown in Figure 4 [Figure 4: see original paper], the three forage species generally exhibited higher enrichment capacity for Cu than Pb under both single and combined pollution, indicating stronger Cu enrichment ability. The aboveground BCF for Cu peaked at 1.61 in the Cu2Pb2 treatment, while root BCF values were generally greater than 1, except for 0.85 in the Cu1Pb2 treatment. Aboveground and root BCF values for Pb were generally less than 1, exceeding 1 only in ryegrass roots in the Cu1Pb1 treatment (1.46). Significant differences in BCF values for both Cu and Pb were generally observed among the three species ($P < 0.05$).

Figure 4 Variation of BCF in forage species. Different lowercase letters indicate significant differences among the three species at each treatment level ($P < 0.05$).

As shown in Figure 5 [Figure 5: see original paper], translocation factors for Cu were below 1 for all three species under single and combined pollution. Alfalfa showed the highest Pb translocation factor (2.72) in the Cu2Pb2 treatment, while ryegrass exhibited a Pb translocation factor greater than 1 (2.06) under low Pb concentration, indicating that these two species could readily translocate Pb from roots to shoots, reflecting their potential for Pb phytoextraction. Chinese pennisetum showed translocation factors below 1 for all heavy metals, possibly due to exclusion mechanisms that reduce toxicity. Significant differences in Cu translocation factors were observed among species in all treatments except the control ($P < 0.05$), while Pb translocation factors differed significantly among species in all treatments except the control and Cu1Pb1 ($P < 0.05$). Alfalfa

translocation factors for Cu and Pb were significantly higher than ryegrass and Chinese pennisetum in the Cu1Pb1, Cu2Pb1, and Cu2Pb2 treatments ($P < 0.05$). Alfalfa and ryegrass demonstrated stronger Cu and Pb translocation capacity than Chinese pennisetum.

Figure 5 Variation of TF in forage species. Different lowercase letters indicate significant differences among the three species at each treatment level ($P < 0.05$).

Discussion

Research indicates that plant heavy metal absorption exhibits a concentration effect: as environmental heavy metal ion concentrations increase, plant enrichment and toxicity also increase [24-25]. Plants accumulate heavy metals from soil through physiological and biochemical processes, with substantial variation among species in tolerance, absorption, and translocation capacity [26]. In this study of three forage species for Cu-Pb contaminated soil remediation, heavy metals primarily accumulated in roots, consistent with most plant heavy metal accumulation patterns. This likely reflects plant tolerance mechanisms, as roots typically release various organic compounds that immobilize toxic heavy metals [27]. Since most absorbed heavy metals accumulated in roots, toxicity to above-ground organs was reduced. Additionally, combined Cu-Pb treatment promoted Cu and Pb absorption to some extent, indicating synergistic accumulation of the two heavy metals under combined pollution. This finding aligns with Li et al. [36] who reported similar results for Cu and Pb accumulation in maize seedlings under single and combined treatments. Heavy metal interactions are complex and influenced by multiple factors, requiring further mechanistic investigation.

The bioaccumulation factor reflects plant heavy metal enrichment characteristics and evaluates the capacity to absorb and transfer metals to plant tissues. Results showed that Cu, having relatively high biological activity and high tolerance in the three forage species under combined pollution, was more readily enriched than Pb. Lead has low mobility in soil due to chemical adsorption and stable complex formation, which restricts migration and creates an exclusion effect. Lin et al. [37] reported similar conclusions for pakchoi under combined Cu-Pb pollution. The translocation factor reflects the ability to transfer heavy metals from roots to shoots, with higher values indicating stronger translocation capacity. Alfalfa demonstrated good remediation potential for single Cu and combined Cu-Pb pollution. Lead translocation factors were generally below 1 in all three species except in the Pb1 and Cu2Pb2 treatments, consistent with Ye [21] who reported root accumulation exceeding shoot accumulation. Compared with ryegrass and Chinese pennisetum, alfalfa showed enrichment potential for Pb in combined Cu-Pb contaminated soil. Plant enrichment and translocation capacity for Cu and Pb determine remediation effectiveness for corresponding pollution types.

Conclusion

Based on resistance coefficients, Chinese pennisetum exhibited the highest resistance under high Cu concentration in both single Cu and combined Cu-Pb pollution, making it most suitable for remediating high Cu contamination. Alfalfa showed the highest resistance coefficient for Pb, making it optimal for single Pb pollution remediation.

Under combined Cu-Pb pollution, alfalfa demonstrated higher aboveground enrichment capacity for both Cu and Pb compared to ryegrass and Chinese pennisetum, while ryegrass showed higher root enrichment capacity for both metals than the other two species.

Although ryegrass did not show exceptional enrichment capacity, its easy cultivation and positive effects on Cu, Pb, and combined contaminated soils make it suitable for remediating single Pb pollution. Alfalfa exhibited the strongest overall enrichment capacity for combined Cu-Pb pollution and can be considered for remediating single Cu or combined Cu-Pb contaminated soils. Chinese pennisetum showed relatively poor tolerance to Cu and Pb and is not recommended for combined Cu-Pb pollution remediation.

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