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Measures to enhance a plant intercropping – crop rotation system for Zn and Cu remediation in sewage river sediments

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ABSTRACT

Heavy metals (HMs) contamination in river sediment has caused potential risks to humans and the environment. In this study, an amending method to enhance phytoremediation of zinc (Zn) and copper (Cu) in sewage river sediment was developed, and its assistant mechanisms were investigated, by comparing the effects of four treatments: plants alone, plants treated with ethylenediaminetetraacetic acid, plants treated with arbuscular mycorrhizae (AM), or plants treated with AM plus indigenous bacteria. The plants were grown in intercropping and crop rotation systems including ryegrass and either maize or alfalfa. The results showed that intercropping and crop rotation effectively reduced Zn and Cu pollution in sediment, particularly when plants were treated with AM. In AM treatment, the Zn remediation effect was better in the maize/ryegrass system, and Zn concentrations in rhizosphere soil decreased the most by 64.37%. Cu was removed better by alfalfa/ryegrass system, with the highest removal rate of 26.93%. AM treatment assisted phytoremediation mainly by increasing the exchangeable and carbonated fractions of HMs and improving the activity of dehydrogenase, polyphenol oxidase and urease in sediment. AM application promoted the uptake of HMs from belowground to aboveground of plants, however, interspecies competition may occur between AM and indigenous bacteria.

Keywords: Phytoremediation; Plant intercropping – crop rotation; Arbuscular mycorrhizae; Ethylenediaminetetraacetic acid; Indigenous bacteria

1. Introduction

Heavy metals (HMs) pollution, particularly in river sediment, has become a global problem [1]. River sediment can act as reservoirs and carriers of pollutants in the aquatic environment [2]. When environmental conditions change, HMs accumulated in sediment can enter the environment as secondary pollution sources to circulate through the soil-water-biological system [3–5], directly or indirectly harming the health of humans and other animals through bioconcentration within the food chain [6,7]. This problem has attracted great interest among the scientific community, particularly in the fields of environmental conservation and protection.

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Remediation methods to remove HMs in contaminating soil include physical, chemical, and/or biological approaches. Chemical and physicochemical methods such as chemical precipitation, ion exchange, electrolysis, reverse osmosis, extraction, and activated carbon adsorption have rapid removal rates [8–10], but destroy the natural structure and physical and chemical properties of soil [11]. By contrast, biological approaches or bioremediation, consisting of remediation using microorganism (bacteria and Fungi) only, phytoremediation (plants) only, or the combination of both the processes [10,12,13] are cost effective, environmentally friendly, and can be applied over large areas of mildly to moderately contaminated soil [14,15]. However, for example, for phytoremediation, the plants used are slow-growing and strongly seasonally restricted. Some plants have good remediation effects on only specific HMs, and little effect on soils polluted with HMs in combination. Therefore, auxiliary measures have been proposed to improve HMs uptake by plants [16].

Methods for enhancing phytoremediation include physical-assisted, chemical-assisted and microorganism-assisted measures [10]. Anning et al. [16] reported the removal efficiency of Hg, As, Pb, Zn and Cu by chemically assisted cattail and vetiver was all above 80%. Rees et al. [17] found that Zn and Cd could be removed by biochar-assisted *Noccaea caerulescens*, and the removal rates were 4% and 40%, respectively. Wei et al. [18] showed that Agrobacterium rhizobia in the roots of wild beans was resistant to Pb, Cu, Cd and Zn, and could promote plant growth. Previous studies have shown that the HMs chelator ethylenediaminetetraacetic acid (EDTA), a commonly used agent for removing HMs in soil, can enhance the phytoremediation of rhizosphere soil HMs and facilitate the transport of HMs from underground to aboveground of plants [19,20]. Therefore, EDTA significantly strengthens plant remediation and has good application potential for the remediation of HMs – contaminated soil. Previous researches have also shown that combined plant – arbuscular mycorrhizae (AM) system has a higher HMs removal rate than plants alone, by enhancing plant growth, root stabilization, HMs extraction, etc [21]. AM is an important functional group of soil microorganisms that can survive in extreme environments with high concentrations of various HMs [22,23], and the extra-rooted mycelium of AM can colonize the soil and transport nutrients to improve plant growth [24]. AM can help to immobilize HMs in plant roots and reduce their transportation to the aboveground, mitigating the adverse effects of HMs on plant growth [25,26]. In addition, inoculation of rhizosphere microorganisms in HMs – contaminated soils has been reported to significantly increase the biological activity of HMs and promote HMs absorption by plants [27,28], depending on a number of factors, such as the characteristics of the fungal symbionts, the availability of HMs and the mycorrhizal growth, etc.

In intercropping systems, different types of plants are sown alternately between seasons. Intercropping plants with different restoration functions can improve the overall restoration effect and reduce remediation time [25,29]. By contrast, rotation is sequential sowing of different crops, which takes advantage of mutually beneficial and competitive relationships among species [30]. Different crop species have different nutrient requirements. Thus, both intercropping and crop rotation can be applied to coordinate the absorption of different HMs by different plants. Some studies have shown that intercropping and/or crop rotation have positive impacts on HMs accumulation in plants, improving phytoremediation efficiency. Yang et al. [31] found that rapeseed – sunflower rotation improved the removal of HMs from soil compared to monoculture. Pavla et al. [32] proved that crop rotation facilitated plant extraction of HMs. Ma [25] showed that corn – ryegrass intercropping or alfalfa – ryegrass intercropping degraded HMs better than monocropping. However, few studies have combined intercropping and rotation to evaluate their effects on the efficiency of HMs uptake.

Zn and Cu are essential trace elements for plants, but the phytotoxicity of excessive both HMs may inhibit plant growth. The interaction of Zn and Cu stress on plants may also affect the transportation of HMs in plants, and further stunt the growth of plants [17,33]. Therefore, in this study, measures to enhance a plant intercropping – crop rotation system were implemented to evaluate their effect on Zn and Cu accumulation in polluted sediment collected from a sewage-contaminated river. In the first round, ryegrass was intercropped with maize, followed by intercropping of ryegrass and alfalfa. We evaluated the effects of four treatments, plants alone (control) and plants treated with EDTA, AM, or AM plus indigenous bacteria, on Zn and Cu soil remediation.

2. Materials and methods

2.1. Experimental materials

2.1.1. Sediment sampling

Sediment samples were collected from the vicinity of two important sewage outlets (*A* and *B*) located in a sewage river in North China. First, sediment samples from outlets *A* and *B* were placed in a cool, ventilated place to dry, and debris such as small and large gravel and animal and plant residues were removed by passing the samples through a 2 mm sieve. Sediments *A* and *B* were then mixed evenly with river sand for a final ratio of 1:1:1, and the samples were sterilized using high-pressure steam at 101 kPa and 121°C for 2 h. The main physicochemical properties and the total amount of major HMs (Cu, Zn, Cd, Ni, Cr and Mn) in the mixed sediment are listed in Table 1. Slurry samples from sewage outlets *A* and *B* were put in a plastic and stored at 4°C, for the cultivation of indigenous bacteria.

2.1.2. Test microbial flora

The original AM fungi were provided by Shandong Agricultural University, China, and the host plant was clover. Since the growth of AM fungi needs air, the river sand is selected as the medium of culture expansion. During this period, nutrient solution was applied, and after four months of reproduction, an inoculum containing spores, mycelia, and infected roots were ready to reserve.

For cultivation of indigenous bacteria, 1 mL of slurry sediment sample and 99 mL of beef extract peptone medium were put into a 150 mL flask, sealed with foil, and

Table 1
Physicochemical properties and HMs concentration in the sediment (HMs unit is mg/kg)

Sample	pH	TP (%)	TN (%)	Zn	Cu	Ni	Cd	Cr	Pb
A	7.8	0.12	0.56	3,467.0	128.5	71.4	11.9	279.9	152.5
B	8.2	0.49	0.27	2,908.7	276.4	136.6	16.4	390.8	296.3
A and *B* mixed	8.0–8.1	0.3–0.4	0.45–0.49	2,695–3,303	250–266	119–134	14–15	324–380	242–283

Notes: TP, total phosphorus; TN, total nitrogen.

performed at 30°C. The transfer of the culture solution was made by mixing 10 mL of the culture solution and 90 mL of nutrient solution (beef extract peptone agar medium) into a 150 mL flask. After transferring 2–3 generations, scratched the plate, picked the dominant cluster into the nutrient solution, and continued the enrichment of the cells until the microbial population was sufficient. Finally, 500 mL of the total volume of the enriched culture was obtained for use.

2.2. Test methods

2.2.1. Pot experiment

The mixed sediment samples were placed in a 0.7 m × 0.5 m × 0.4 m polyvinyl chloride (PVC) box that was sterilized with aldehyde. Ventilation was provided via holes in the bottom of the box. Each box was filled with 80 kg sediment. Four treatments were set up: plants alone (P treatment, control), plants treated with EDTA (E treatment), plants treated with AM (A treatment), or plants treated with AM plus indigenous bacteria (AB treatment). Each treatment consisted of three replicates. In A treatment, 200 g of AM fungi inoculum was supplemented. In E treatment, 200 g sterilized AM inoculum was treated to maintain consistent microbial flora, and EDTA was added at a concentration of 3 mmol/kg (dry mud) around the plant roots at 1 week prior to harvest. In AB treatment, 200 g of AM fungi inoculum was added, and 500 mL of indigenous bacterial solution was applied during plant growth. In P treatment, 200 g sterilized AM inoculum was treated as well. At the beginning of the experiment, the AM inoculum was mixed with the sediment, and the moisture of the mixture was adjusted to 30%–60% by adding deionized water. The moisture content was determined by weighing method [34].

The intercropping/crop rotation system consisted of two rounds. The first round was intercropped ryegrass (*Lolium multiflorum* Lam., herbaceous, perennials) and maize (*Zea mays* L. belonging to the grass family Poaceae and tribe Maydeae, Annual cereals), and the second round was intercropped ryegrass and alfalfa (*Medicago sativa* L, herbaceous, perennials). Maize, ryegrass, and alfalfa seeds were soaked in hydrogen peroxide for 10 min for surface disinfection, and the seeds were sown in alternating strips with a row spacing of 15 cm. The seedlings were thinned upon emergence. The first sowing period was from May 21 to August 18, and the second was from August 23 to November 24.

2.2.2. Pretreatment methods

Plant root soil samples were collected regularly and dried in a cool, ventilated place, partly for enzyme activity determination, and partly for the determination of HMs (Zn, Cu) by grinding 180 μm metal sieve with a quartz mortar. The total amount of HMs in each sample was pretreated by hydrochloric acid – nitric acid – perchloric acid – hydrofluoric acid digestion method, and the pretreatment of HMs speciation (exchangeable fraction, carbonated fraction, Fe–Mn oxides fraction, organic bound fraction, residual fraction) was conducted by Tessier's five-step extraction method [35].

Ryegrass and maize in the first round was harvested in the day 88, and ryegrass and alfalfa in the second round was harvested in the day 192. The aboveground part of plants was clipped, and roots were dug out, washed with deionized water three times, then dried the surface of the plants with filter paper, sterilized at 105°C for 30 min, and dried at 80°C to constant weight. The roots, stems, leaves shells and seeds were separated. The pretreatment of in the plants was also done with hydrochloric acid – nitric acid – perchloric acid – hydrofluoric acid digestion method.

2.2.3. Determination of samples

HMs concentration was determined by flame atomic absorption spectrophotometer (AAS, WFX-130, Beijing Rayleigh, Beijing).

Two redox enzymes (dehydrogenase and polyphenol oxidase) and one hydrolase (urease) were measured. For the determination of dehydrogenase activity, 5 g pretreated plant root soil sample was placed in the flasks, and dehydrogenase activity was measured according to the TTC spectrophotometric method [36]. For the determination of polyphenol oxidase activity, 0.1–0.5 g pretreated plant root soil sample was placed in the flasks, and polyphenol oxidase activity was measured using the phenol sodium hypochlorite colorimetric method [36]. For the determination of urease activity, 2–5 g pretreated plant root soil sample was placed in the flasks, and urease activity was measured based on the phenol – sodium hypochlorite colorimetric method [37]. Three repetitions were tested for each sample.

2.2.4. Statistical analysis

One-way ANOVAs and Tukey tests ($p < 0.05$) were performed using the statistical program SPSS 24.0 (PASW Statistics, Chicago, IL) to determine the significant difference between in Zn and Cu removal under the four treatments.

3. Results

3.1. Changes in sediment HMs concentration

According to the Soil Environmental Quality Risk Control Standard for Soil Contamination of Agricultural

Land (GB15618-2018), the critical Zn and Cu soil concentrations for ensuring plant growth are 300 and 100 mg/kg, respectively. The Zn and Cu concentrations in the sediment samples were 9- to 11-fold and 2- to 3-fold higher than the critical limits. Therefore, both Zn and Cu are expected to have serious toxic effects on plants during phytoremediation, which may affect the accumulation and absorption of other HMs in the sediment.

Plants growth of the four treatments is presented in Fig. S1, and Zn and Cu removal rates after remediation are shown in Fig. 1. After the first round of planting, the EDTA treatment attained the highest removal rate of Zn, decreasing in maize and ryegrass rhizosphere soil by 69.00% and 62.30%, respectively. The removal rates were higher in the AM and AM plus indigenous bacteria treatments than in the control, which indicates that both EDTA and AM promoted phytoremediation. However, remediation effects were less significant for Cu than for Zn. Under EDTA treatment, the Cu concentration in maize and ryegrass rhizosphere sediments decreased by 7.90% and 2.20%, respectively, and no significant change was observed after AM treatment. In the control, there was no significant change in Cu concentration in maize rhizosphere soil, whereas a 3.06% decrease was observed in ryegrass rhizosphere soil.

After the second round of planting, the Zn concentrations in alfalfa rhizosphere soil decreased by 10.96%, 8.82%, 10.39%, and 6.33% in the control, EDTA, AM, and AM plus indigenous bacteria treatments, respectively, and those in ryegrass rhizosphere soil 4.85%, 18.22%, 5.81%, and 11.60%, respectively. Therefore, less HMs removal occurred during the second round of planting than during the first round. By contrast, significant remediation effects on Cu were observed in the second round, with Cu concentrations in ryegrass rhizosphere soil decreasing by 24.13%, 26.93%, and 14.91% in the EDTA, AM, and AM plus indigenous bacteria treatments, respectively, which were larger decreases than that in the control (5.07%). In the second round of planting, the removal rate of Cu by AM-treated ryegrass reached 26.93%, the highest rate among both rounds of planting.

In the mid-processing (60 d) and end-of-processing (91 d) periods of cultivation, the temperature of the city dropped sharply, which may have affected plant growth and HMs adsorption in the treatments. Therefore, during the second round of planting with intercropped ryegrass and alfalfa, the Zn and Cu concentrations in rhizosphere sediments were measured during these critical periods, as shown in Fig. 2a and b. In most cases, the maximum HMs removal rate occurred during mid-processing, consistent with a previous report that temperature affects Cu enrichment in ryegrass crops [38]. Another previous study reported that Zn and Cu concentrations in plant leaves increased as temperature increased, demonstrating that temperature influenced phytoremediation [39].

3.2. Changes in HMs speciation in sediments

Zn and Cu are harmful HMs involved in the biogeochemical cycle, and their mobility depends strongly on their specific chemical form or different combined forms, rather than the total element concentration. The plant rhizosphere secretes acidic substances that can activate HMs and alter HMs speciation in sediments [40,41]. Therefore, we monitored the speciation of different HMs in sediments in the four treatments.

The speciation of Zn and Cu in the soil in the first round is shown in Fig. 3. HMs speciation in the sediments was most affected by EDTA treatment, particularly in terms of the exchangeable fraction, which was greatly increased. The amount of exchangeable Zn in sediments near the maize and ryegrass rhizosphere was 4.16 mg/kg in the control, compared to 241.83 and 384.45 mg/kg under EDTA treatment, respectively. Similarly, under AM treatment, the exchangeable fractions of Zn and Cu increased compared to the control. These results are consistent with a previous study that reported significantly

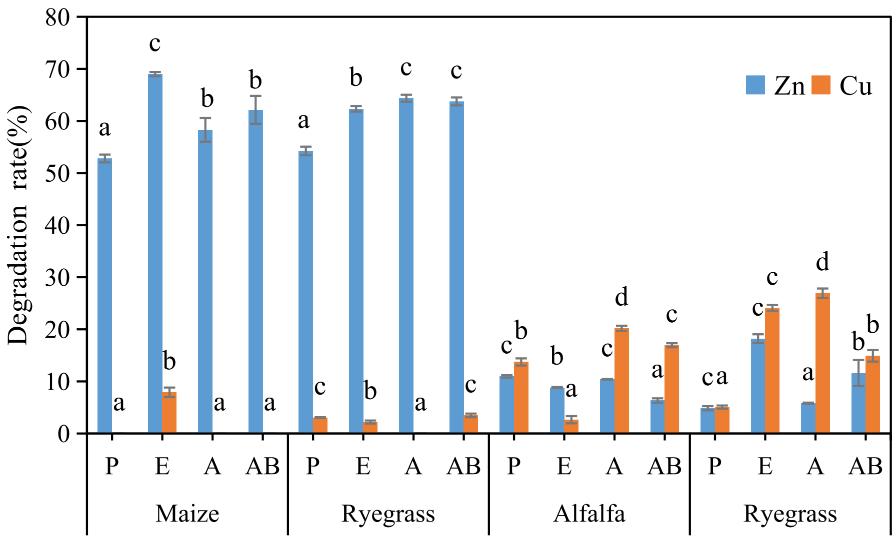


Fig. 1. Zinc (Zn) and copper (Cu) removal rates in sediment samples from China. The four treatments were plants alone (control, P), plants treated with EDTA (E), plants treated with AM (A), or plants treated with AM plus indigenous bacteria (AB). a, b, c, d stand for different significance levels ($P < 0.05$).

higher amounts of exchangeable Cu in maize rhizosphere soil under AM treatment [42]. In addition, AM treatment effectively increased the fraction of carbonated Zn in maize rhizosphere soil by 26.52 mg/kg.

Fe-Mn oxides have a relatively large specific surface and a strong adsorption capacity for Zn, leading to strong ionic bonding that resists release, absorption, or utilization by plants [43]. Similarly, the organic matter-bound Zn fraction is stable and not easily absorbed by plants. Therefore, Zn concentrations in the treatments were significantly higher in the treatments than in the control.

Zn and Cu speciation in the soil after the second round of planting are shown in Fig. 4. Under AM treatment, the exchangeable and carbonated fraction of Cu increased, particularly for carbonated Cu in alfalfa rhizosphere soil, which increased by 7.45 mg/kg, whereas residual Cu decreased significantly. Under EDTA treatment, the exchangeable Cu in ryegrass rhizosphere soil increased, which is consistent with the good Cu removal effect observed in EDTA-treated ryegrass. Similar to the first round, both Fe and Mn oxide-bound and organic matter-bound Zn fractions increased significantly in the treatments compared to the control.

3.3. Changes in sediment enzyme activity

Enzymes are important drivers of ecosystem metabolism in soils, and enzyme decomposition catalyzes the acceleration of all biochemical processes in soil. Most oxidation–reduction reactions in active organisms are catalyzed by dehydrogenase and oxidase. In this study, dehydrogenase, polyphenol oxidase and urease levels were examined in the four treatments at 0, 20, 44, and 88 d in the first planting round, and at 0, 30, 60, and 91 d in the second round.

3.3.1. Changes in dehydrogenase activity in sediment samples

Dehydrogenases are enzymes that catalyze oxidation–reduction reactions, and dehydrogenase activity can be used as a sensitive marker of soil microbial activity. Changes

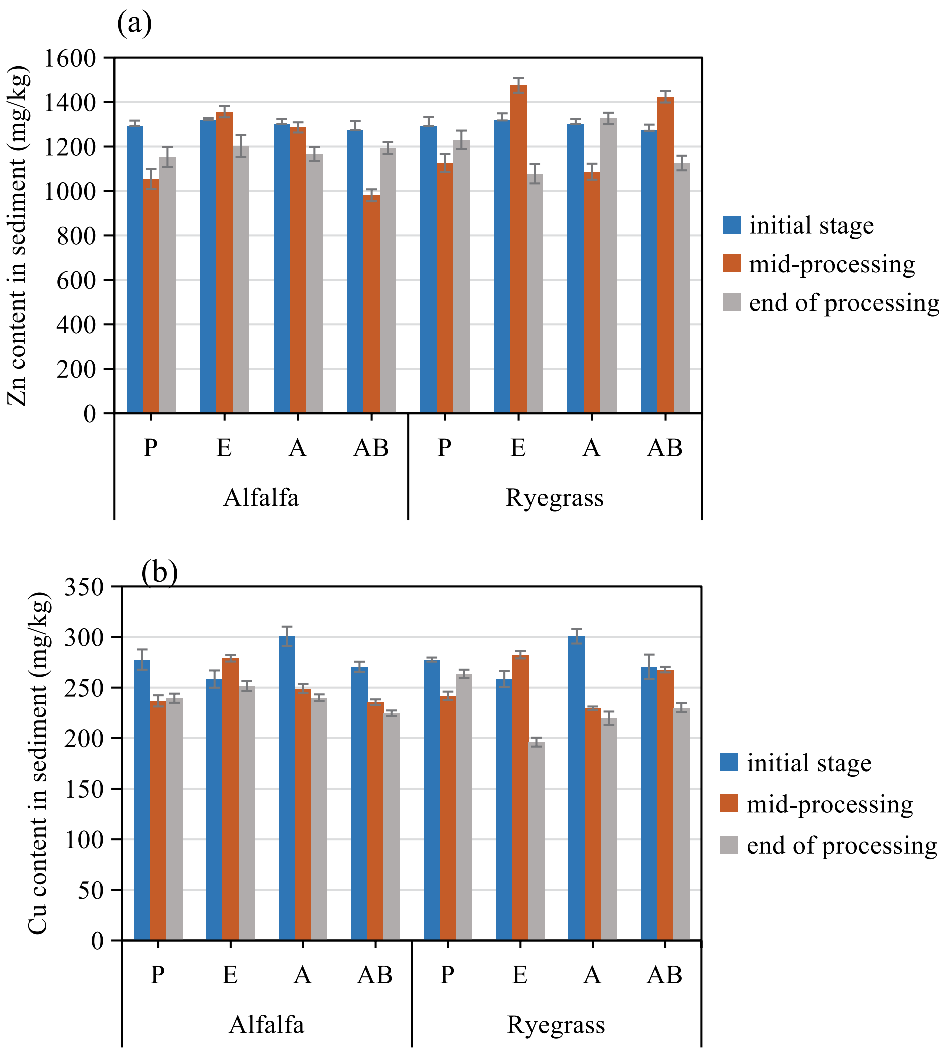


Fig. 2. Zn (a) and Cu (b) concentration in sediments in the second round of the four treatments. The four treatments were plants alone (control, P), plants treated with EDTA (E), plants treated with AM (A), or plants treated with AM plus indigenous bacteria (AB).

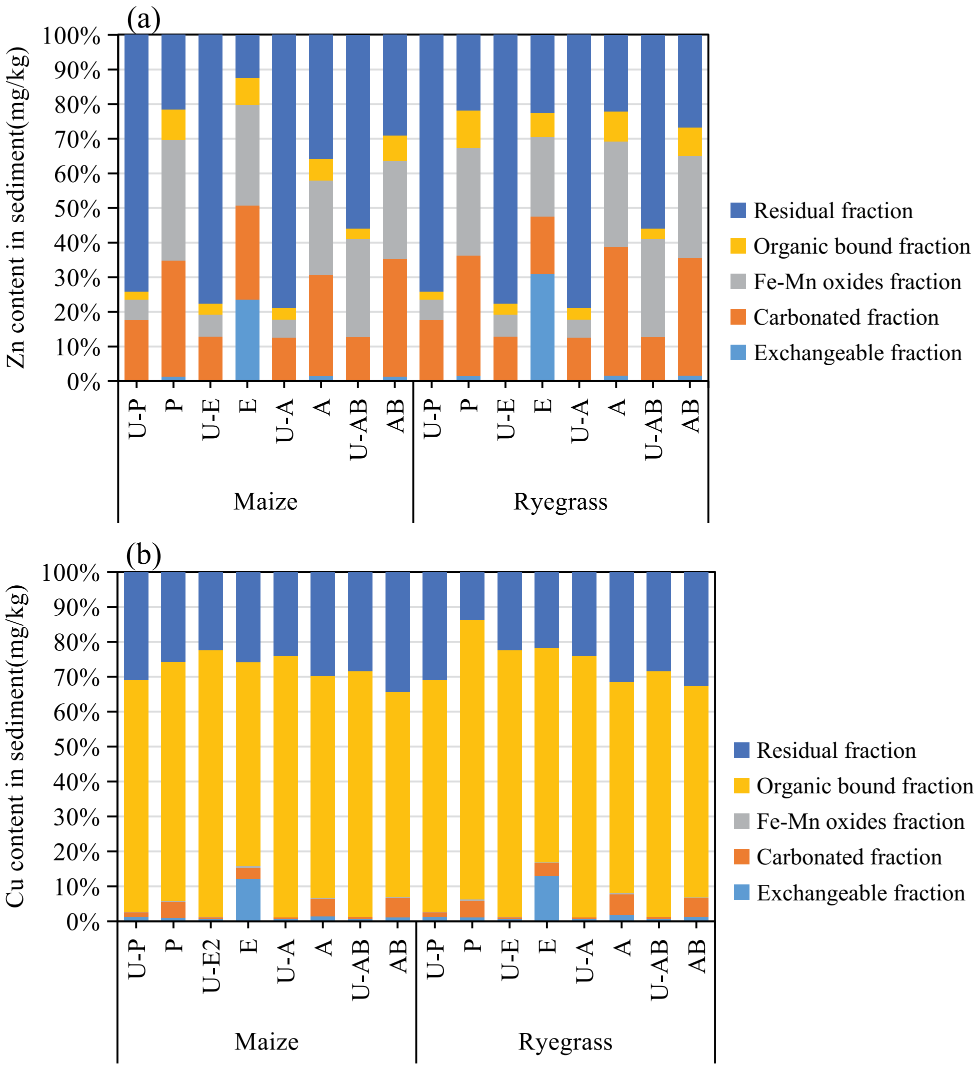
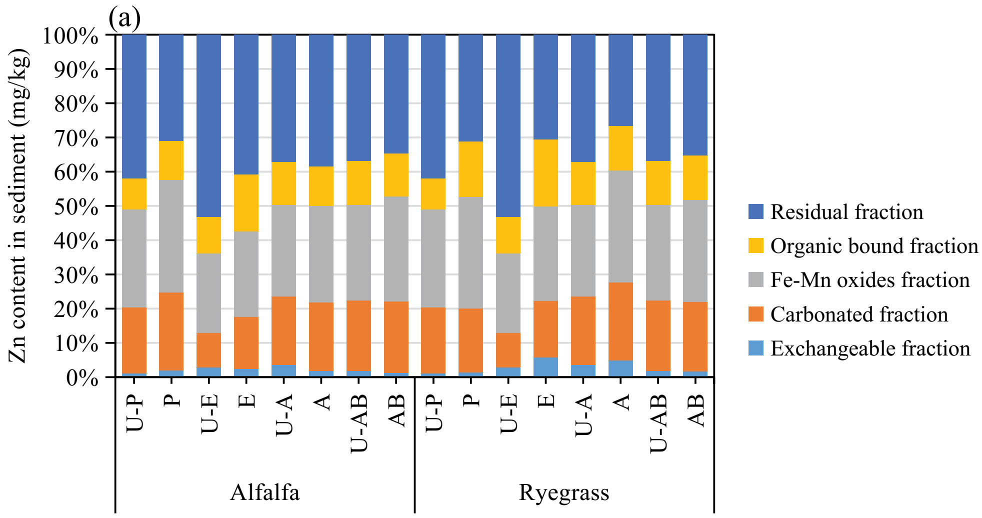


Fig. 3. The speciation of Zn (a) and Cu (b) in the soil in maize/ryegrass system. The four treatments were plants alone (control, P), plants treated with EDTA (E), plants treated with AM (A), or plants treated with AM plus indigenous bacteria (AB). U-P represents the initial value of the speciation of HMs in the sediment in P treatment, U-E represents the initial value of the speciation of HMs in the sediment in E treatment, U-A represents the initial value of the speciation of HMs in the sediment in A treatment, U-AB represents the initial value of the speciation of HMs in the sediment in AB treatment.

of dehydrogenase activity in rhizosphere soil of the four treatments are illustrated in Fig. 5.

In the first round of maize and ryegrass intercropping, dehydrogenase activity in the rhizosphere sediments presented a clear upward trend in the control, EDTA, and AM treatments. Ryegrass showed the best recovery of dehydrogenase activity in rhizosphere soil in the AM treatment, whereas that in the EDTA treatment was low. Dehydrogenase activity in maize rhizosphere soil in the AM plus indigenous bacteria treatment increased rapidly in the first 20 d and then decreased slowly, whereas that in ryegrass rhizosphere soil increased rapidly in the first 44 d, before decreasing slowly. This difference may have been caused by interspecies competition between AM and indigenous bacteria, resulting in a reduction in the number of microorganisms and decreased enzyme activity [44].

In the second planting round, the dehydrogenase activity of ryegrass and alfalfa rhizosphere soils decreased in all treatments. After day 30, dehydrogenase activity was higher in the control and AM plus indigenous bacteria treatment, which indicates that HMs removal rates were higher in these treatments than in the EDTA and AM treatments in the initial stage of intercropping. The dehydrogenase activity of the alfalfa rhizosphere soil under AM plus indigenous bacteria treatment was particularly high, which was consistent with the 23.00% Zn removal rate in this period (Fig. 1). However, dehydrogenase activity sharply decreased during the planting process, which further suggested species competition in the soil microbial environment under the AM plus indigenous bacteria treatment. Dehydrogenase activity in the ryegrass rhizosphere soil under AM treatment increased in the first 60 d and then



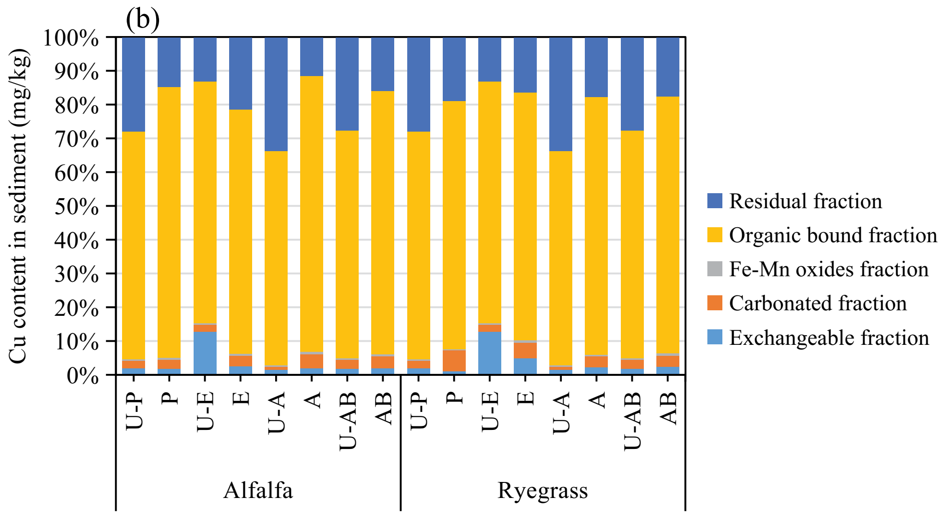


Fig. 4. The speciation of Zn (a) and Cu (b) in the soil in alfalfa/ryegrass system. The four treatments were plants alone (control, P), plants treated with EDTA (E), plants treated with AM (A), or plants treated with AM plus indigenous bacteria (AB). U-P represents the initial value of the speciation of HMs in the sediment in P treatment, U-E represents the initial value of the speciation of HMs in the sediment in E treatment, U-A represents the initial value of the speciation of HMs in the sediment in A treatment, U-AB represents the initial value of the speciation of HMs in the sediment in AB treatment.

began to decrease, and an important reason for this decline in dehydrogenase activity was the rapid decrease in local temperature. Low temperatures inhibited plant and microbial activity, resulting in decreased soil enzyme activity.

3.3.2. Changes in polyphenol oxidase activity in sediment

The dynamic changes of polyphenol oxidase activity in the rhizosphere soil of intercropping-rotary crops under different treatments with plant growth are present in Fig. 6.

In the first planting round, polyphenol oxidase activity showed an upward trend in rhizosphere soil in all four treatments during the first 44 d, and then slightly decreased. This indicates that plant growth increased gradually during the initial planting stage, after which vigorous microbial metabolism promoted HMs absorption and accumulation by plants. However, the resulting increase in plant HMs concentration led to toxic effects that inhibited plant growth, slightly reducing enzyme activity. In both maize and ryegrass rhizosphere soils, polyphenol oxidase activity was higher or increased more rapidly under AM treatment, indicating that AM effectively enhanced plant rhizosphere microbial activity. Under the AM plus indigenous bacteria treatment, the recovery of polyphenol oxidase activity in rhizosphere soil was slowest, but always showed an upward trend. This result may have been due to interspecies competition, such that the decay of large numbers of microorganisms released polyphenol oxidase into the environment, continuing decomposition [42].

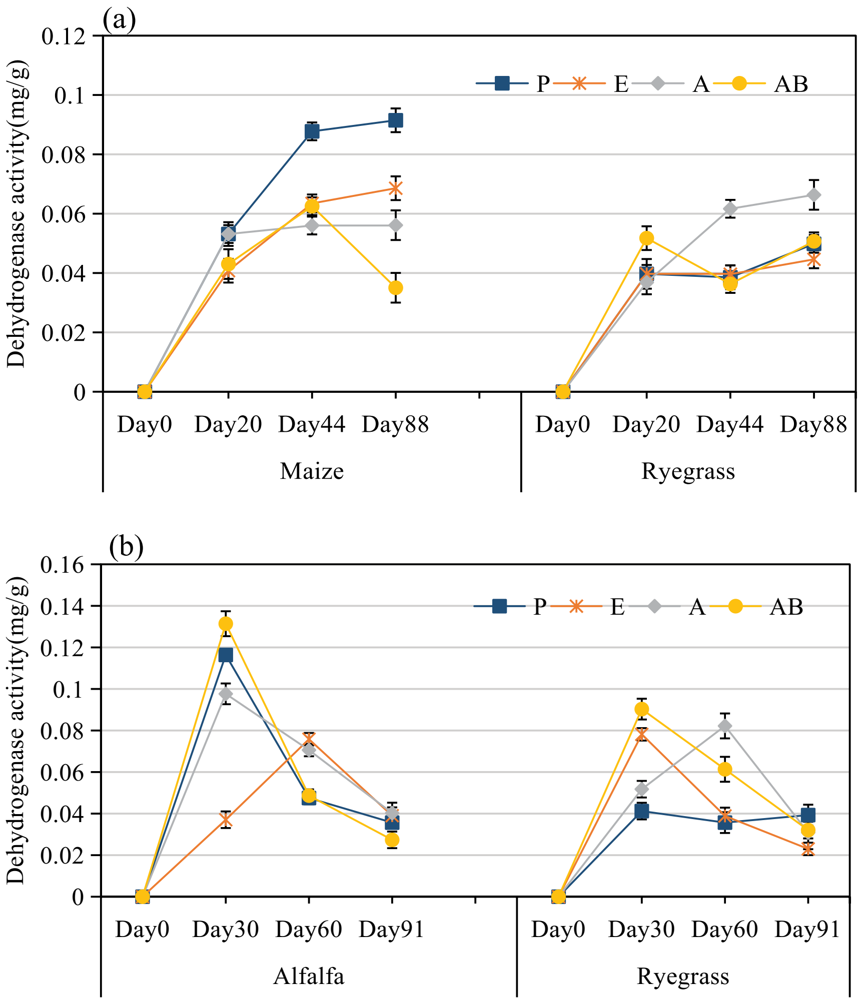


Fig. 5. Changes of dehydrogenase activity in rhizosphere soil of the four treatments in the first (a) and second (b) rounds of plants. The four treatments were plants alone (control, P), plants treated with EDTA (E), plants treated with AM (A), or plants treated with AM plus indigenous bacteria (AB).

In the second planting round, polyphenol oxidase activity in ryegrass and alfalfa rhizosphere sediments generally increased first and then decreased in the control, AM, and AM plus indigenous bacteria treatments. Enzyme activity was higher in ryegrass rhizosphere soil under AM treatment and in alfalfa rhizosphere sediment under AM plus indigenous bacteria treatment, which was consistent with the removal of Zn and Cu from ryegrass rhizosphere soil and of Zn from alfalfa rhizosphere soil.

3.3.3. Changes in urease activity in sediment

Urease is one of the major hydrolases in the soil, and the activity of urease is closely related to the concentration of soil organic matter and microbial activity. Fig. 7 illustrates the dynamic changes of urease activity in the rhizosphere sediments of intercropping – crop rotation under the four treatments.

In the first round, the urease concentration in the rhizosphere soil of ryegrass and maize in AM treatment was always higher than that of the control. In the early intercropping of maize and ryegrass, the urease activity in the rhizosphere soil showed an increasing trend under the EDTA, AM and AM plus indigenous bacteria treatments. After 44 d of planting, the urease activity in the rhizosphere soil of maize tended to be gentle, while that in the ryegrass rhizosphere soil still showed an upward trend. This indicates that the rhizosphere of plants had an activating effect on microorganisms, and the urease activity was significantly affected by the speciations of soil HMs [45]. The reduction of HMs in the ryegrass rhizosphere soils was generally greater than that of maize, and the exchangeable and carbonated fraction of HMs in the rhizosphere sediments were also greater than those of maize. This shows that the urease activity can reflect the absorption of HMs by plants to some extent.

In ryegrass rhizosphere soil of the second round, the change of the urease was consistent with dehydrogenase in the control and AM plus indigenous bacteria treatment, whereas the urease activity recovery showed an upward trend in AM treatment, indicating that AM fungi played a

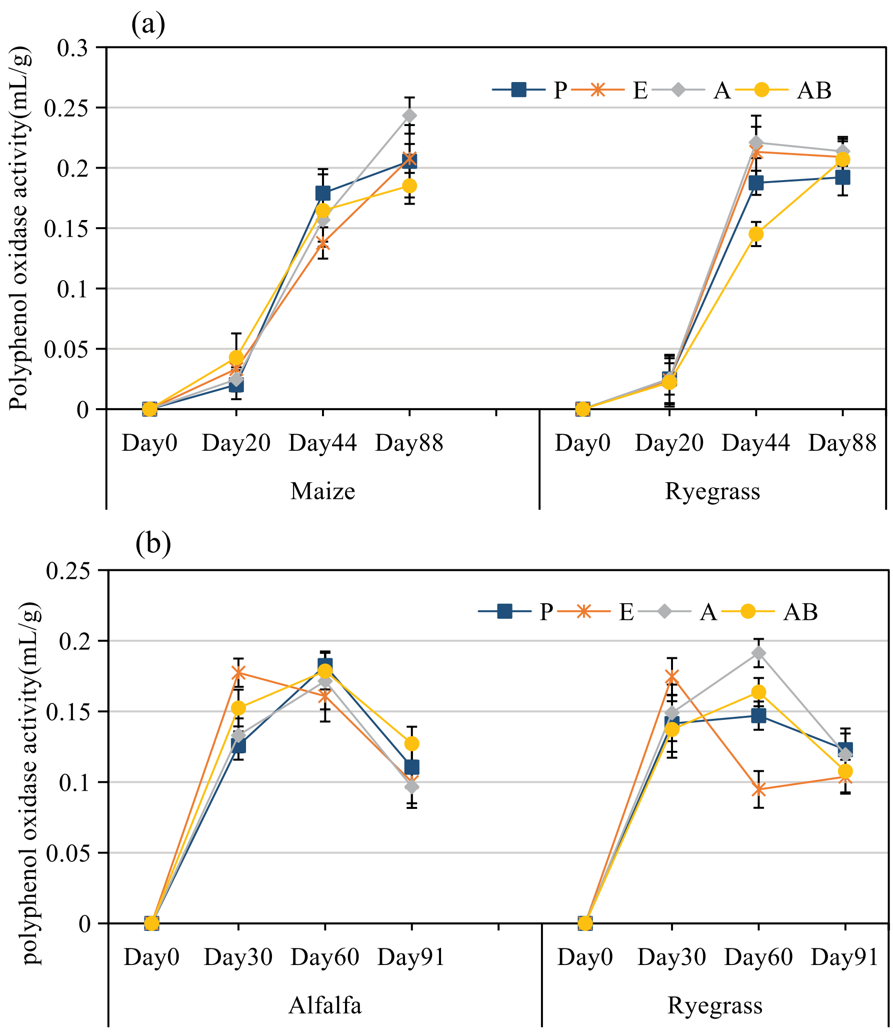


Fig. 6. Changes of polyphenol oxidase activity in rhizosphere soil of the four treatments in the first (a) and second (b) rounds of plants. The four treatments were plants alone (control, P), plants treated with EDTA (E), plants treated with AM (A), or plants treated with AM plus indigenous bacteria (AB).

certain role in enhancing phytoremediation. In alfalfa rhizosphere sediment, under the AM treatment, the curve declined first and then increased. This may be due to the inhibition of urease by high concentrations of HMs and activation of urease by low concentrations. Moreover, the urease activity in the second round was significantly lower than that in the first round. Temperature may be one of the main reasons for inhibiting urease activity as well.

3.4. HMs absorption and accumulation by plants

The accumulation of Zn and Cu in different parts of different plants grown under intercropping and rotation are shown in Fig. 8. In the first round of planting, ryegrass roots showed the highest Zn accumulation, particularly in the EDTA treatment, where Zn accumulation was high in maize roots. EDTA effectively activated HMs and transformed them to exchangeable fractions. Under AM treatment, Zn accumulation in plants was also high, and Zn concentrations in both maize and ryegrass tended to migrate to aboveground parts, consistent with a previous study [46].

In the second round of planting, ryegrass roots showed the highest Cu accumulation, and AM treatment enhanced phytoremediation. Cu was an essential element of active organisms, and showed greater accumulation in belowground plant parts, demonstrating a poor ability to migrate to aboveground parts in ryegrass with larger biomass. AM treatment increased Cu concentration in ryegrass roots and increased Cu removal to the aboveground plant parts. For example, Cu showed less accumulation in alfalfa roots, but better removal to aboveground plant parts.

4. Discussion

We investigated the effects of different treatment techniques on Zn and Cu concentrations in river sediment using

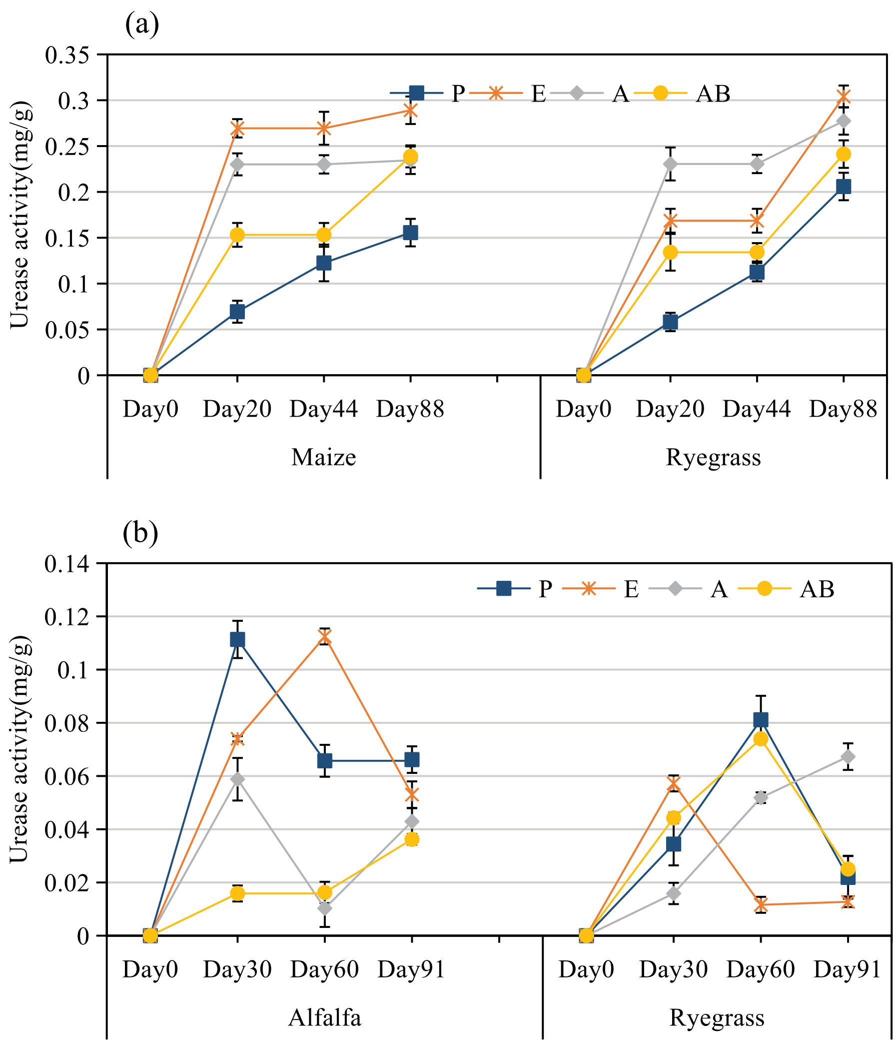


Fig. 7. Changes of urease activity in rhizosphere soil of the four treatments in the first (a) and second (b) rounds of plants. The four treatments were plants alone (control, P), plants treated with EDTA (E), plants treated with AM (A), or plants treated with AM plus indigenous bacteria (AB).

pot experiments and an intercropping and crop rotation system under control, EDTA, AM and AM plus indigenous bacteria treatments.

Plant intercropping and crop rotation formed a complementary system for Zn and Cu remediation. Previous studies have shown that many types of crop plants, for example, corn or ryegrass, present inhibited growth under single-plant continuous cropping for HMs phytoremediation [47,48]. Intercropping effectively uses sunlight, soil space, and nutrients, promoting plant growth and HMs extraction from soil [25], which may explain why intercropped corn, ryegrass and alfalfa showed normal growth in the Zn- and Cu-contaminated sediments of the present study. Zn removal in river sediments by different treatments improved after the first round of planting, whereas Cu concentrations did not change significantly. However, in the second planting round, Cu was effectively removed from soil, perhaps due to HMs activation by roots [30]. After the first round of planting, plants helped to activate Cu in the sediment, improving Cu accumulation by alfalfa and ryegrass in the second round.

4.1. EDTA effects on phytoremediation

In the EDTA treatment, the exchangeable fractions of Zn and Cu in sediments increased greatly, and plant absorption and accumulation of Zn and Cu also increased. Most of the exchangeable HMs in sediments is bioavailable, therefore, plants absorbed more HMs. Exchangeable HMs fractions are unstable in sediment, and EDTA improved the mobility of HMs. However, these HMs fractions are also highly toxic, which makes them harmful to plants, and EDTA is difficult to degrade, which causes secondary pollution [49]. Because EDTA effectively activates HMs to their exchangeable states, plant roots accumulated more Zn and Cu.

In the EDTA treatment, dehydrogenase activity was relatively low in rhizosphere soil, and polyphenol oxidase

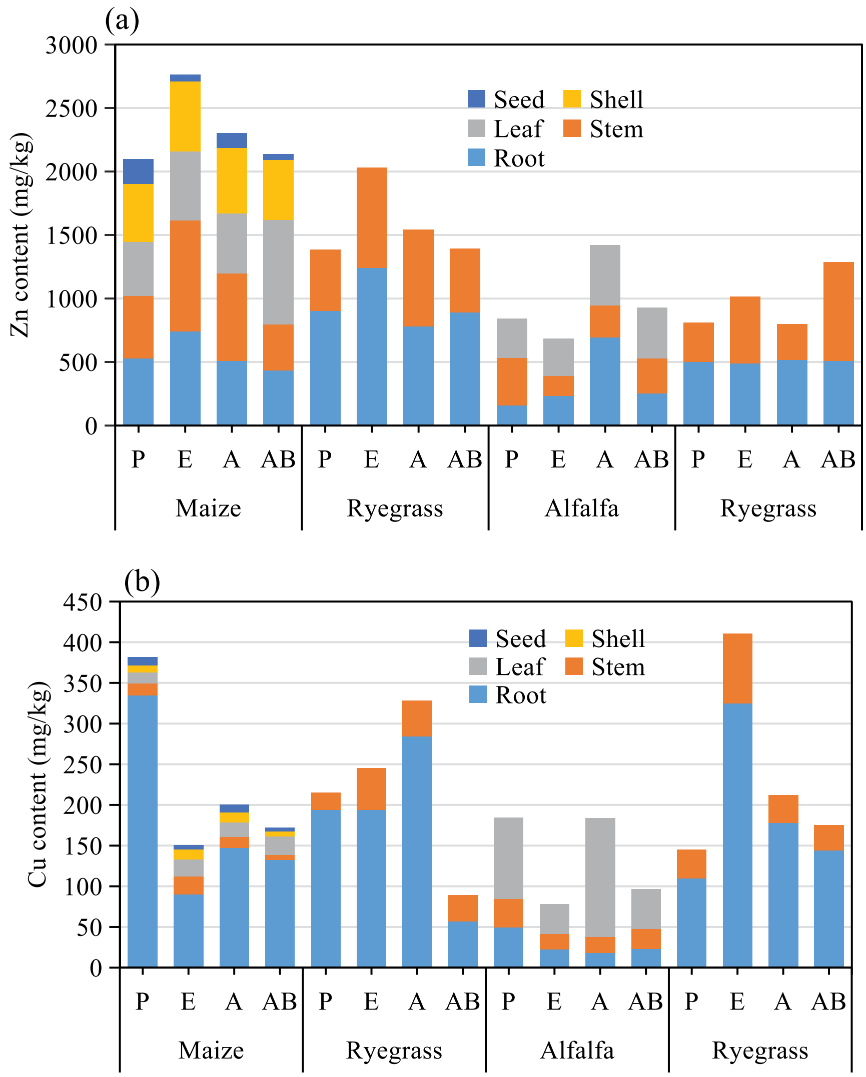


Fig. 8. The accumulation of Zn (a) and Cu (b) in different parts of different plants at the end of planting under intercropping and rotation. The four treatments were plants alone (control, P), plants treated with EDTA (E), plants treated with AM (A), or plants treated with AM plus indigenous bacteria (AB).

decreased rapidly. Dehydrogenase activity is negatively correlated with compound dose and HMs concentration [50,51]. EDTA could inhibit soil microorganism and enzyme activity, thereby reducing the amount of microorganisms in soil [52]. Notably, dehydrogenase activity decreased in alfalfa and ryegrass rhizosphere soils, and polyphenol oxidase and urease activity was significantly lower in the second round of planting, possibly because lower temperature inhibited the metabolism of microorganisms in the sediments, constraining plant growth and limiting Zn absorption by plants [53,54].

4.2. AM effects on phytoremediation

Compared to the EDTA treatment, the AM treatment increased the exchangeable Zn and Cu fractions and effectively increased the fraction of carbonated HMs in sediments, thereby increasing the absorption and accumulation of Zn and Cu by plants. HMs accumulation in plants is related to the change in the total amount or morphology of HMs in rhizosphere soil [45]. AM may change the pH of rhizome sediments by increasing the secretion of acidic substances from plant roots, such that the HMs fractions change to their bioavailable states [31,55]. Carbonated HMs absorbed by plants is less toxic, which is an important reason for the better growth of plants under AM treatment. Zn and Cu accumulation was also higher in plants in the AM treatment. Accumulated Zn and Cu in plants tended to migrate to aboveground plant parts from roots. Mycorrhiza may improve the sediment environment near plant roots through AM secretions, increasing sediment fertility levels and promoting Zn and Cu migration to aboveground plant parts [56]. However, other studies have shown that AM reduces HMs concentrations in host plants, whereas

HMs accumulation by aboveground and belowground plant parts increase significantly [57], mainly because AM significantly increases plant biomass and dilutes HMs concentrations in plants [58].

Enzyme activity increased significantly in the AM treatment. Dehydrogenase activity recovered best in ryegrass rhizosphere soil, indicating that ryegrass and AM formed a symbiotic system that promoted the degradation of composite pollutants by plants, or mycorrhizal plant protection, which blocked the toxic effects of metal ions on plants. Polyphenol oxidase activity was higher or increased more rapidly in the AM treatment, indicating that AM effectively enhanced microbial activity in the rhizosphere of plants. urease activity was higher in root sediments in the AM treatment than in the control, consistent with the results of a previous study [59], which found that AM inoculation in atrazine-contaminated soil increased urease activity. After inoculation with AM, large numbers of microorganisms near the rhizosphere accumulated more metal ions, sharing the stress applied to plants and enhancing plant growth [45]. However, the activities of dehydrogenase, polyphenol oxidase, and urease in alfalfa and ryegrass rhizosphere soil were significantly lower in the second round than in the first round, perhaps mainly due to low temperature. Dehydrogenase activity first increased and then decreased in the AM plus indigenous bacteria treatment, and polyphenol oxidase activity recovered the most slowly, suggesting interspecies competition between AM and indigenous bacteria [27,28].

4.3. Comparisons and suggestions on HMs removal

Continually removal of Cu and Zn was found by the intercropping – crop rotation system, which is different from the previous study. The removal efficiency of Cu and Zn by chemically assisted cattail and vetiver could reach 80%, but there was a risk of secondary contamination of the soil by chemical agents [16]. Zn and Cd were reported to be removed by biochar-assisted *Noccaea caerulescens*, but the initial concentrations of Zn and Cd were much higher than that in this study, and Cu removal was not reported [17]. The highest removal rates of Cu and Zn in the sewage sludge by Goosegrass Herb and Canna were 54% and 79%, respectively, but the plants were much more popular in southern China [60]. We found that AM fungi significantly enhanced intercropping – crop rotation and reduced Zn and Cu pollution effectively in river sediments. The highest removal rates of Zn and Cu in rhizosphere soil were 64.37% and 26.93%, respectively. Zn was removed well by the intercropping of maize and ryegrass, and Cu was removed better by the next intercropping of alfalfa and ryegrass. Intercropping – crop rotation might avoid the interaction of Cu and Zn stress on plants to affect the absorption of HMs in plants to some extent, especially for the AM treatment.

Low temperature was found to inhibit plant and AM activity, resulting in decreased soil enzyme activity, detrimental to the extraction and transformation of HMs by plants. The presence of some indigenous dominant bacteria might create interspecific competition, which could affect the auxiliary phytoremediation effect of AM fungi. Therefore, it was suggested that the environment conditions of the areas for remediation should be settled before the large-scale implementation of this technology, such as the suitable moisture of the soil, the moderate temperature, environment strain preferred, etc.

5. Conclusion

The intercropping – crop rotation system using ryegrass and maize or alfalfa worked best as a remediation method for sewage river sediment under treatment with AM. In the first round of maize and ryegrass intercropping, the application of EDTA or AM effectively improved Zn removal from sediments, whereas the effect of Cu removal was not significant. The second round of ryegrass and alfalfa intercropping perfectly matched the first round, which effectively improved sediment Cu remediation. AM treatment also enhanced the removal of Zn and Cu in the second round. AM assisted phytoremediation by increasing the exchangeable and carbonated fractions of HMs in the soil, and by effectively increasing dehydrogenase, polyphenol oxidase, and urease activity. Applying AM treatment promoted the removal of Zn from belowground to aboveground plant parts in maize, ryegrass and alfalfa, and enhanced Cu migration from belowground to aboveground plant parts in ryegrass and alfalfa in the second round of planting.

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Author contributions

Zhang JH (M.S.) designed the study, supervised the experiment, interpreted the results and drafted the manuscript. Zhang J (M.S.) investigated the research status, did the single factor and orthogonal experiments and conducted statistical analysis. Zhang GK (M.S.) made the contrast experiment, plotted the figures and verified the results. Wang LX (M.S.) and Chen MJ (M.S.) were responsible for the collection of samples for the entire experiment. Rao Z (Professor) and Li YN (Associate Professor) revised this paper and agreed to the final version to be published.

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Supplementary information

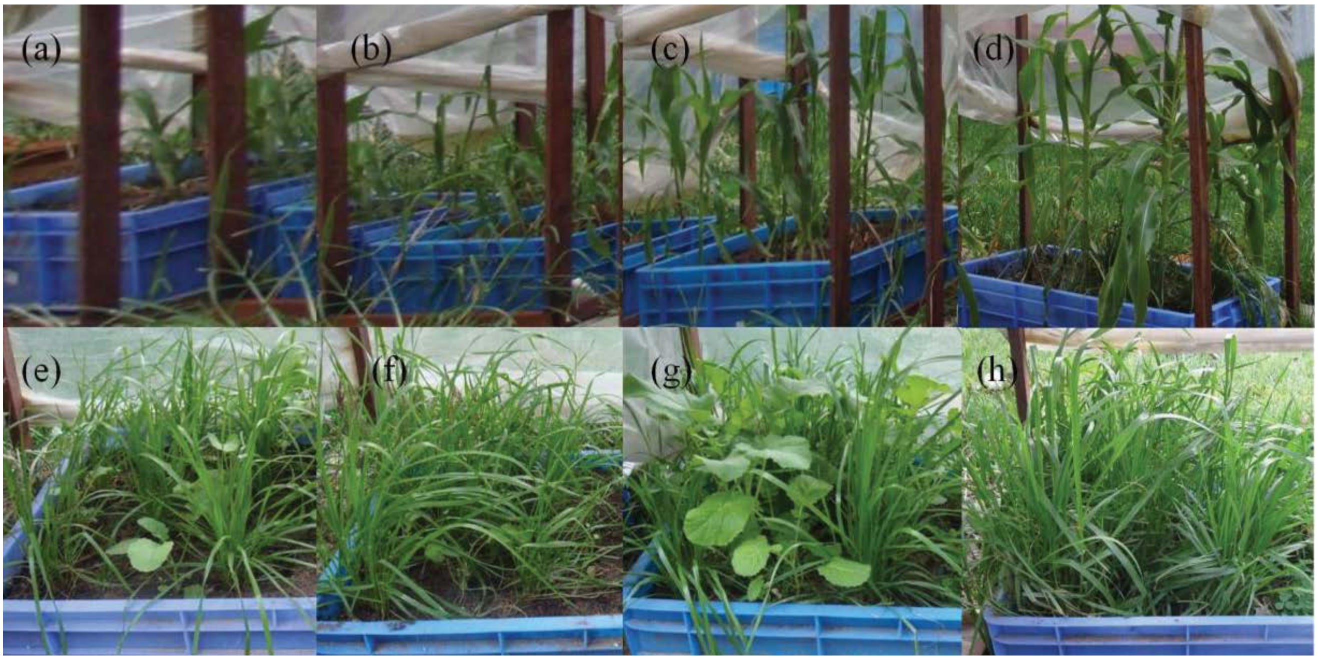

Fig. S1. Plants growth of the four treatment in maize/ryegrass system (a–d) and alfalfa/ryegrass system (e–h), respectively. (a, e) were for the treatment of plant only (control). (b, f) were for the treatment of plants treated with EDTA. (c, g) were for the treatment of plants treated with AM. (d, h) were for the treatment of plants treated with AM plus indigenous bacteria.