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Highlights

- M. yunnanensis enhanced root **growth** of Z. elegans under heavy metal stress.
- BC-MY and CS-MY promoted biomass yields of Z. elegans cultivated in landfill soil.
- **Bacterial inoculated plants exhibited higher Cd and Cu uptake in plants tissues.**
- Micro-XRF confirmed Cd, Cu, and Pb distribution in root and shoot tissues.
- **BC-MY and CS-MY improved the key indices of phytoremediation efficiency in** Z. elegans.

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Synergistic effect of biochar-immobilized plant growth-promoting bacterium on multi-heavy metal phytoremediation by *Zinnia elegans* L. in landfill soil

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Abstract

This research investigated the capacity of *Micrococcus yunnanensis* (MY), a heavy metal-resistant plant growth-promoting bacterium (PGPB), in promoting root growth and development of *Zinnia elegans* L. under heavy metal stress conditions. The study compared the effectiveness of *M. yunnanensis* applied as biochar-immobilized cells (BC-MY) and as cell suspension (CS-MY) in enhancing *Z. elegans* phytoremediation of cadmium (Cd), copper (Cu), and lead (Pb) in municipal solid waste landfill soil. The results demonstrated that *M. yunnanensis* significantly promoted root growth in *Z. elegans* seedlings under heavy metal stress compared to the uninoculated controls. Pot experiments revealed that both BC-MY and CS-MY inoculation enhanced plant growth and biomass when *Z. elegans* was cultivated in multi-heavy metal-contaminated landfill soil. The application of BC-MY or CS-MY increased Cd and Cu concentrations in the root and shoot tissues relative to the uninoculated plants, whereas Pb accumulation was not significantly different ($p < 0.05$). Micro-X-ray fluorescence analysis confirmed the spatial distribution and accumulation of Cd, Cu, and Pb in root and shoot tissues. Furthermore, both BC-MY and CS-MY treatments significantly improved key phytoremediation indices, including root uptake, shoot uptake, phytoextraction efficiency, and uptake efficiency. Notably, bacterial inoculation enhanced the accumulation and translocation factors for Cd and Cu. These findings demonstrate the promising potential of *M. yunnanensis* in both BC-MY and CS-MY forms to improve the phytoremediation performance of *Z. elegans* for Cd and Cu removal from polluted landfill soils.

Keywords: bamboo-derived biochar; common zinnia; immobilized cells; landfill soil; *Micrococcus yunnanensis*; micro-X-ray fluorescence

1. Introduction

The disposal of municipal solid waste (MSW) in developing countries frequently relies on land disposal practices, primarily because of its low cost, ease of operation, and the absence of the need for advanced equipment. Improper management of land disposal, including practices such as open dumping and unsanitary solid waste landfills, result in significant environmental challenges. In addition, the co-disposal of household hazardous waste within MSW landfills contributes to the contamination of leachate and landfill soil with hazardous substances, particularly heavy metals. Heavy metal concentrations in leachate have also increased, mainly because of enhanced dissolution under low-pH conditions created by organic acids (Fang et al., 2016; Haroon et al., 2019). Soils within the MSW landfill and those in the surrounding areas are contaminated with heavy metals at varying levels (Kucherova et al., 2025; Shahamat et al., 2025). Pu et al. (2024) identified elevated heavy metal levels in humus soil at an MSW landfill, with Cu and Cd posing the greatest pollution and ecological risks. Moreover, plants, including those that are edible and cultivated near MSW landfills, can absorb and accumulate heavy metals (Qiao et al., 2023).

Consequently, soil contamination by heavy metals, particularly co-contamination by multi-heavy metals, is a significant issue that requires intervention. Nevertheless, the high operational costs associated with physicochemical methods restrict their widespread adoption for soil decontamination in developing countries (Bakshe and Jugade, 2023). Phytoremediation refers to the utilization of specific plant species for the remediation of contaminated soils through various plant-based mechanisms, such as phytoextraction, phytovolatilization, and phytostabilization (Babu et al., 2021). It is considered highly promising because of its affordability, environmental friendliness, non-invasiveness, and effectiveness in removing heavy metals (Yang et al., 2022). In addition to their ability to minimize soil erosion and manage the spread of heavy metals, plants play a crucial role in boosting soil fertility by emitting a range of organic compounds into soil (Zhang et al., 2024). The selection of suitable plant species is crucial for effective implementation of phytoremediation. To prevent heavy metals from entering the food chain through biomagnification, they should generate a significant amount of biomass, adapt well to their surroundings, and repel herbivores (Jadaun and Pandey, 2024).

Some ornamental plants can thrive and effectively accumulate contaminants, and most ornamental plants are non-edible, resulting in the absence of heavy metals from the food chain (Ziabari et al., 2024). *Zinnia elegans* L. is a well-known annual ornamental flowering plant belonging to the Asteraceae family and is known for its variety of colors. It is characterized by ease of cultivation, rapid growth, high biomass production, and a strong capacity to tolerate and grow under adverse environments and toxic metal stress (Saini et al., 2019; Panda et al., 2020). *Z. elegans* demonstrated tolerance to Pb stress by activating a strong antioxidative defense system, which facilitated its capacity to accumulate Pb (Jahromi et al., 2022). This species demonstrates significant potential for application in the phytoremediation of soils contaminated with heavy metals and presents advantages over other ornamental plant species (Panda et al., 2020). However, the use of *Z. elegans* to remediate soils contaminated with multi-heavy metals has not been investigated extensively.

Phytoremediation is an effective method for the removal of heavy metals from soil; however, it faces several challenges, such as slow plant growth, limited dry matter yield, and lengthy time required to achieve successful remediation. Some microorganisms can potentially improve phytoremediation through various mechanisms (Kour et al., 2021). Plant growth-promoting bacteria (PGPB) are a specific group of beneficial bacteria that positively influence plant growth and offer potential long-term sustainable solutions for enhancing plant biomass. The production of indole-3-acetic acid (IAA), an essential phytohormone categorized as an auxin by PGPB, not only affects the development of lateral roots and the emergence of root hairs but also plays a pivotal role in interactions between plants and microbes, thereby facilitating phytoremediation (Etesami and Glick, 2024). The use of heavy metal-resistant PGPB has become a common strategy to support heavy metal phytoremediation of contaminated soils (Harindintwali et al., 2020). Enhanced plant growth and heavy metal phytoremediation by inoculating heavy metal-resistant PGPB have been reported in several ornamental plants, such as *Celosia cristat*a L. (Yuan et al., 2022), *Helianthus annuus* L. (Kumar et al., 2021), and *Wedelia trilobata* A.St.-Hil. (Lin et al., 2018). Despite the growing interest in phytoremediation, research on the role of PGPB in promoting the multi-heavy metal phytoremediation efficiency of *Z. elegans* remains limited.

Additionally, bacterial inocula for soil amendment, especially in soils contaminated with multi-heavy metals, must be explored. The use of free bacterial cells or cell suspensions in this type of soil is challenging because of the need for fresh preparation and a short shelf life (Ketaubon and Prapagdee, 2023). Owing to its porous nature and extensive surface area, biochar serves as an excellent carrier for PGPB growth and proliferation (Kamyab et al., 2025). To address these challenges, the use of biochar-immobilized bacterial cells can be an effective method to improve landfill soils contaminated with various heavy metals. Nonetheless, there is a lack of research on how biochar-immobilized PGPB affects the phytoremediation of multi-heavy metals in *Z. elegans*. Therefore, this study focused on the application of biochar-immobilized *Micrococcus yunnanensis* (BC-MY), a potent heavy metal-resistant PGPB, to stimulate the growth and efficiency of heavy metal phytoremediation in *Z. elegans* planted in multi-heavy metal-contaminated landfill soil.

2. Materials and Methods

2.1 Bacterial strain and plant seeds

Micrococcus yunnanensis MU1, an IAA-producing PGPB that is resistant to multi-heavy metals, was originally isolated from plant roots and is known for its high IAA production (Ketaubon et al., 2024). The strain was cultivated on Luria-Bertani (LB) agar (Difco, USA) at 28 °C. *Z. elegans* seeds were sourced from a commercial seed supplier. The seed germination rate was 95.8 ± 3.1%.

2.2 *In vivo* root elongation assay

Seeds of *Z. elegans* were surface-sterilized by immersion in 70% ethanol for 5 min, followed by treatment with 2% sodium hypochlorite (HClO) for 10 min, and subsequently rinsed twice with sterile deionized water. The experiments were conducted in Petri dishes containing Cd and Cu at concentrations of 10, 25, and 50 mg L^{-1}, and Pb at 25, 50, and 100 mg L^{-1}. Each heavy metal solution was added separately to the Petri dishes. Surface-sterilized seeds were soaked in cell suspensions of *M. yunnanensis* for 1 h and then were placed in sterilized Petri dishes corresponding to each condition. Each heavy-metal concentration was evaluated using two Petri dishes containing 10 seeds per dish, yielding a total of 20 seeds (n = 20). Shoot and root growth were observed and measured using ImageJ software (Duarte et al., 2016), following incubation at 25±2 °C in the dark.

2.3 Landfill soil collection and preparation

The soil surrounding the MSW landfills was collected from the Comprehensive Waste Management Center, Rayong Province, Thailand (47P 742963.85, 1410503.75). Air-dried soil was prepared and sieved using a 2-mm grid sieve chamber. The concentrations of Cd, Cu, and Pb in the landfill soil were measured after acid digestion in a microwave oven (MARS 6, CEM Corporation, USA) and were then assessed using a flame atomic absorption spectrophotometry (FAAS) (240FS AA, Agilent, USA). The physicochemical properties and heavy metal concentrations were previously reported by Ketaubon et al. (2024) as follows: sandy clay loam; pH 6, 8 dS m^{-1} EC, 3.9 cmol kg^{-1} CEC, 4.87% organic matter, 2.82% organic carbon, 0.18% total nitrogen; 15.67 of C/N ratio, 323 mg kg^{-1} available P, 11.2 mg kg^{-1} extractable K, 0.89 mg kg^{-1} Cd, 5.91 mg kg^{-1} Cu, and 55.05 mg kg^{-1} Pb.

2.4 Pot experiments of heavy metal phytoremediation

2.4.1 Preparation of bacterial inoculum and plant seedlings

M. yunnanensis was used to formulate biochar-immobilized *M. yunnanensis* (BC-MY). Freeze-dried cells of *M. yunnanensis* were prepared following the method outlined by Ketaubon and Prapagdee (2023), combined with bamboo derived-biochar produced via slow pyrolysis (500 °C), along with dextrose and tapioca starch to support bacterial growth and act as a binder. To obtain a cell suspension of *M. yunnanensis* (CS-MY), the strain was cultured in LB broth and incubated at 30 °C for a duration of 24 h. The cell pellet was obtained by centrifuging at 8000 rpm for 20 min at 4 °C, followed by two rinses with sterile normal saline. The final pellet was resuspended in sterile normal saline to obtain a cell suspension with an optical density of 0.2 at 600 nm (OD_{600}), corresponding to an approximate viable cell number of 1.5 ×10^7 CFU mL^{-1}.

24.2 Phytoremediation experiments

To assess the effectiveness of BC-MY and CS-MY in enhancing the phytoremediation potential of *Z. elegans*, pot experiments were conducted under greenhouse conditions. The experimental design included four treatments: (1) control, (2) biochar, (3) BC-MY, and (4) CS-MY, arranged in a completely randomized design with three replicates per treatment. Individual plants were cultivated in separate pots containing contaminated landfill soil. The viable bacterial cell counts in the BC-MY formulation were approximately ten times greater than those in CS-MY. To ensure consistent application across treatments, biochar and BC-MY were each added to the landfill soil at a rate of 0.1% (w/w), whereas CS-MY was applied at 1.0% (v/w), followed by thorough mixing prior to planting (Ketaubon et al., 2024). Soil moisture was maintained at 60% of its water-holding capacity through daily irrigation with deionized water (pH 6.7).

2.4.3 Analysis of plant growth and heavy metal concentrations in plants

To evaluate plant growth, the chlorophyll content, root and shoot lengths, and dry biomass weight were measured. Chlorophyll content was recorded weekly using a portable chlorophyll meter (SPAD-502Plus, Konica Minolta, Japan). After a month of growth, the plants were collected and the lengths of their roots and shoots were recorded. Before being weighed, the plant samples were dried in an oven at 60 °C for 72 h and then kept in a desiccator. The dried root and shoot tissues were finely ground, subjected to acid digestion, and analyzed for Cd, Cu, and Pb concentrations using FAAS.

2.4.4 canning of heavy metals distribution in plant tissues using micro-XRF

The elemental composition (% by weight) of Cd, Cu, and Pb and their localization in the roots and shoots of *Z. elegans* were analyzed using a micro-X-ray fluorescence (XRF) microscope (XGT-9000, Horiba Scientific, Japan). Micro-XRF is a technique used to analyze the spatial distribution of elements in different types of samples. Synchrotron-based micro-XRF has been used to investigate the distribution of elements in plant tissues (Fittschen et al., 2017).

2.4.5 Analysis of rhizosphere soil

Soil samples from the rhizosphere were collected at the same time that the plants were harvested. The soil pH was directly determined using a pH meter (FiveGO F2, Mettler Toledo, Switzerland). The activity of soil microbes was evaluated using the fluorescein diacetate (FDA) hydrolysis test (Green et al., 2006). FDA is hydrolyzed by several enzymes including esterases, proteases, and lipases (Navarro et al., 2020). The soil concentrations of Cd, Cu, Pb, and Zn were subjected to acid digestion following the procedures outlined by McGrath and Cunliffe (1985) and were then examined using FAAS.

2.5 Calculation and statistical analysis

The effectiveness of the phytoremediation of heavy metals was evaluated using various quantitative indices, including root uptake, shoot uptake, phytoextraction efficiency (PE), and uptake efficiency (UE), as described by Razmi et al. (2021), along with the accumulation factor (AF), also known as the bioconcentration factor (BCF) and translocation factor (TF), following the methodology outlined by Ali et al. (2013), with some modifications. The calculations for each of these indices are presented in Equations 1–6:

$$\text{Root uptake (RU)}\ (\mu g\ plant-1) = \text{HM conc. in root}\ (\mu g\ g-1) \times \text{Root dry weight}\ (g\ plant-1) \qquad \text{(Eq 1)}$$

$$\text{Shoot uptake (SU)}\ (\mu g\ plant-1) = \text{HM conc. in shoot}\ (\mu g\ g-1) \times \text{Shoot dry weight}\ (g\ plant-1) \qquad \text{(Eq 2)}$$

$$\text{Phytoextraction efficiency (PE)}\ (\mu g\ g-1) = \frac{\text{Shoot uptake}\ (\mu g\ plant-1)}{\text{Root dry weight}\ (g\ plant-1)} \qquad \text{(Eq 3)}$$

$$\text{Uptake efficiency (UE)}\ (\mu g\ g-1) = \frac{\text{(Shoot upatke)+(Root uptake)}\ (\mu g\ plant-1)}{\text{Root dry weight}\ (g\ plant-1)} \qquad \text{(Eq 4)}$$

$$\text{Accumulation factor (AF)} = \frac{\text{HM conc. in the plant tissues}}{\text{HM conc. in the soil}} \qquad \text{(Eq 5)}$$

$$\text{Translocation factor (TF)} = \frac{\text{HM conc. in shoot}}{\text{HM conc. in root}} \quad \text{(Eq 6)}$$

To assess the significance of plant growth and the levels of heavy metals in the plant samples, one-way analysis of variance (ANOVA) was conducted, along with the Duncan multiple range test (DMRT), using a significance threshold of $p < 0.05$.

3. Results and Discussion

3.1 Enhanced plant root elongation under various heavy metals

The effectiveness of *M. yunnanensis* under Cd, Cu, and Pb toxicity conditions compared with untreated seedlings is shown in Fig. 1. Root elongation of *Z. elegans* was inhibited under all tested heavy metal concentrations, with Cd exerting the greatest inhibitory effect. As the concentrations of Cd, Cu, and Pb increased, root length gradually decreased. Khanthom et al. (2021) supported these results, indicating that as heavy metal concentrations increased, there was a gradual reduction in the root and shoot lengths of *H. annuus* and *Sorghum bicolor* L. At equivalent concentrations of Cd, Cu, and Pb (25 and 50 mg L^{-1}), the phytotoxic effect on *Z. elegans* seedlings was most pronounced under Cd exposure, followed by Cu and Pb. Cd displays greater phytotoxicity in roots than in leaves, and root elongation is inhibited even at relatively low concentrations (Qin et al., 2020). The accumulation of these heavy metals results in the production of reactive oxygen species, which in turn cause oxidative stress in higher plants (Schützendübel and Polle, 2002). However, plants possess defense mechanisms that deal with heavy metal toxicity. Jahromi et al. (2022) documented that *Z. elegans* produces various antioxidant enzymes, including superoxide dismutase, peroxidase, and catalase, in response to Pb toxicity.

Z. elegans seedlings inoculated with *M. yunnanensis* had significantly longer roots than the untreated control group, indicating that the bacterium had a stimulatory effect on root development. In the presence of 25 mg L^{-1} Cd, Cu, and Pb, inoculation with *M. yunnanensis* enhanced root elongation of *Z. elegans* seedlings by approximately 2.0-, 1.7-, and 1.4-fold, respectively, relative to the uninoculated controls. Our results demonstrate that *M. yunnanensis* enhanced root development in *Z. elegans* seedlings exposed to Cd stress. According to Sangthong et al. (2016), *Micrococcus* sp., an IAA-producing bacterium, facilitates root growth in *Zea mays* L. under Cd exposure. Similarly, our findings revealed that bacterial inoculation may contribute to the increased tolerance of *Z. elegans* to Cd, Cu, and Pb toxicity, while alleviating the detrimental effects of heavy metals. Chen et al. (2024) similarly reported that Cd-resistant bacteria possessing PGP traits facilitated Cd detoxification in *Sorghum bicolor* (L.) Moench by lowering antioxidant enzyme activity and malondialdehyde levels under Cd stress. Evidence from Thooppeng et al. (2023) confirmed that the IAA-producing bacterium, *Streptomyces rapamycinicus* mitigated Cd-induced toxicity in the roots of *Crotalaria juncea* L. Collectively, these findings underscore the pivotal role of *M. yunnanensis* in supporting root elongation and reducing heavy metal toxicity in *Z. elegans*.

3.2 Growth of *Z. elegans* in landfill soil

Prior to transplantation into landfill soil, one-month-old *Z. elegans* seedlings exhibited an average fresh weight of 5.87 ± 0.96 g, with mean shoot and root lengths of 3.9 ± 0.4 cm and 32.6 ± 0.9 cm, respectively. The greenhouse environment was controlled to maintain an average temperature of 28.9 ± 0.8 °C, while the relative humidity was kept at 79.3 ± 5.4%. Fig. 2 shows that *Z. elegans*, when inoculated with BC-MY and CS-MY and grown for a month in landfill soil, exhibited significant increases in both root and shoot lengths, as well as in dry biomass weights, compared to the biochar treatment and uninoculated control groups. The findings indicated that there was no significant difference ($p < 0.05$) in root and shoot lengths and dry biomass yield between plants inoculated with BC-MY and those inoculated with CS-MY. These results confirmed that *M. yunnanensis*, when applied either as biochar-immobilized cells (BC) or as a cell suspension (CS), effectively enhanced the growth of *Z. elegans*. IAA-producing bacteria that promote the growth of both monocot and dicot plants cultivated in heavy metal-contaminated soil have been reported, for example, *Z. mays* (Bruno et al., 2021), *H. annuus* (Kumar et al., 2021), and *Chrysopogon zizanioides* (L.) Roberty (Ketaubon et al., 2024). These findings align with established roles of IAA-producing bacteria in promoting root development, improving rhizosphere microbial interactions, and regulating critical physiological processes (Etesami and Glick, 2024; Wahab et al., 2024).

Furthermore, Zheng et al. (2022) reported that the porous, nutrient-rich structure of biochar provides a favorable habitat for microbial proliferation. Consistently, our results showed that the chlorophyll content of mature *Z. elegans* leaves cultivated in landfill soil increased steadily over time (Fig. 3), ranging from 21.8 ± 1.9 SPAD at planting to 30.8 ± 4.6 SPAD after one month. In mature *Z. elegans* leaves, chlorophyll content did not exhibit any significant variation across the different treatments in landfill soil ($p < 0.05$). These findings indicated that the relatively low levels of Cd, Cu, and Pb in the soil did not have a significant impact on chlorophyll biosynthesis. Our findings align with those of Liu et al. (2021), who observed that *Trifolium repens* L. inoculated with *Pseudomonas putida* and grown in Cd-, Cr- and Pb-contaminated soils exhibited increased biomass and up to 64% greater phytoremediation efficiency without substantial chlorophyll reduction. Similarly, Oubohssaine et al. (2022) reported that *Sulla spinosissima* L. inoculated with *Rhodococcus qingshengii* LMR340 maintained a high biomass and elevated chlorophyll and carotenoid contents under heavy metal stress.

3.3 Changes of soil pH and soil microbial activity *in* landfill soil before and after *Z. elegans* cultivation

One month after transplanting *Z. elegans* into landfill-contaminated soil, the pH values exhibited only slight variation among the treatments, retaining a slightly acidic pH (Table 1). Soil pH is recognized as a key determinant of heavy metal mobility, because alterations in pH can enhance or reduce metal solubility. Consequently, soil pH directly affects the extent to which heavy metals are available for plant uptake (Sintorini et al., 2021). The initial microbial activity of landfill soil without vegetation, as measured by FDA hydrolysis, was 0.74 ± 0.10 µg g^{-1} h^{-1} (Table 1). After one month of *Z. elegans* cultivation, soil microbial activity increased, suggesting that the plant was able to stimulate soil microbial activity. This observation is consistent with the findings of Lange et al. (2015), who reported that increased plant diversity can enhance microbial activity because of greater rhizosphere carbon inputs.

Biochar amendment of *Z. elegans* cultivation led to a 1.2-fold increase in soil microbial activity relative to that of uninoculated control. Similar observations were reported by Navarro et al. (2020), indicating that biochar significantly stimulated soil microbial activity, as reflected by FDA hydrolysis. Notably, Lopes et al. (2021) highlighted a dose-dependent effect, with higher biochar addition leading to progressively greater soil microbial activity. Soil microbial activity was significantly elevated in *Z. elegans* cultivated with CS-MY inoculation, reaching levels 1.7-fold higher than those of uninoculated control and 1.5-fold higher than those of the biochar treatment. This finding aligns with that of Schommer et al. (2023), who demonstrated that biochar-immobilized *Bacillus* species mitigated plant toxicity, thereby promoting both plant growth and soil microbial enzymatic activity.

3.4 Heavy metal uptake and accumulation by *Z. elegans*

As shown in Table 2, the concentrations of Cd, Cu, and Pb were markedly higher in the roots of *Z. elegans* than in their shoots, by 2.5-, 1.6-, and 3.7-fold, respectively. Root tissues serve as the primary interface for heavy metal exposure, with root exudates contributing to the stimulation of heavy metal uptake by root hairs (Souri et al., 2019). Toxic heavy metals are commonly concentrated and sequestered in plant root tissues, and their accumulation is facilitated by strong interactions between heavy metal ions and root epidermal cell surfaces (Kumar and Prasad, 2018; Qin et al., 2020; Zhang et al, 2023). Elevated Pb concentrations were observed in the root tissues of *Z. elegans*, which can be attributed to the relatively low bioavailable fraction of Pb in the landfill soil compared with its total concentration. The bioavailable (DTPA-extractable) concentrations of Cd, Cu, and Pb were determined as 0.10 ± 0.02, 0.21 ± 0.04, and 1.27 ± 0.02 mg kg^{-1}, respectively (Ketaubon et al., 2024). In the roots, heavy metals accumulated in the order of Pb, Cu, and Cd, whereas in the shoots, the order was Cu, Pb, and Cd. The limited Cd uptake in *Z. elegans* can be explained by the low proportion of bioavailable Cd in landfill soil. Additionally, the landfill soil contained Zn 17.83 ± 1.14 mg kg^{-1}. Antagonism between Cd and Zn during active root absorption leads to decreased Cd uptake mediated by Zn competition (Sahito et al., 2025). Consistent evidence was provided by Madanan et al. (2021), who reported that *Tagetes erecta* L. exhibited higher Zn accumulation than Cd in the root tissues. These findings highlight that heavy metal uptake and accumulation in plants are strongly dependent on plant species, growth stage, concentrations of heavy metals in the soil, and soil properties (Souri et al., 2019; Eid et al., 2020; Dinu et al., 2020).

After one month of growth in landfill soil, inoculation with either BC-MY or CS-MY markedly increased Cd and Cu accumulation in both the root and shoot tissues of *Z. elegans* compared to the uninoculated control, demonstrating enhanced efficiency in facilitating Cd and Cu uptake (Table 2). Accumulation of Cd and Cu in the roots and shoots of *Z. elegans* was not significantly affected by inoculation with BC-MY or CS-MY, except for Cu accumulation in the shoots. Inoculation with BC-MY increased Cd concentrations by 1.4-fold in the roots and 1.6-fold in the shoots compared to the uninoculated plants. Moreover, Cu concentrations increased by 1.4-fold in the roots and 1.7-fold in the shoots of *Z. elegans* inoculated with BC-MY relative to the uninoculated control. Nevertheless, Cu accumulation in the shoots of *Z. elegans* inoculated with BC-MY was 1.2-fold lower compared to those inoculated with CS-MY. Consistent with this finding, our previous research showed that free or suspended Cd-resistant bacterial cells promoted Cd accumulation in *Chlorophytum laxum* R.Br. more efficiently than chitosan-immobilized cells, suggesting that immobilized bacteria may require additional time for adaptation and proliferation in soils polluted with heavy metals (Prapagdee and Wankumpha, 2017).

However, Pb uptake and accumulation in *Z. elegans* was unaffected by BC-MY and CS-MY inoculation. The lack of an effect could be explained by the restricted solubility and bioavailable fraction of Pb in landfill soil. The landfill soil contained bioavailable concentrations of Cd, Cu, and Pb of 0.10 ± 0.02, 0.21 ± 0.04, and 1.27 ± 0.02 mg kg^{-1}, respectively (Ketaubon et al., 2024). The ratios of bioavailable fractions to the corresponding total concentrations (acid-digested) were 11.2, 3.6, and 2.3%, respectively. Notably, Pb displayed very low bioavailability in landfill soil. Generally, plants are able to absorb only the bioavailable fractions of heavy metals, their uptake remains limited. Supporting evidence was provided by Dinu et al. (2020), who observed that *Ocimum basilicum* L. cultivated in mining soil contaminated with multi-heavy metals decreased the solubility of these heavy metals and consequently limited their translocation from soil to plant tissues. In addition, certain bacteria can enhance the solubility of heavy metals through various mechanisms, including the production of siderophores, exopolymers, organic acids, and biosurfactants as well as processes such as biomethylation and metal binding to bacterial cell walls (Ullah et al. 2015; Prapagdee and Wankumpha, 2017).

M. yunnanensis does not produce siderophores, biosurfactants, exopolymers, or organic acids. Its ability to solubilize Cd in Cd-contaminated soil is attributed to electrostatic interactions between the negatively charged bacterial cell wall and positively charged Cd cations, thereby enhancing Cd solubilization (Prapagdee et al., 2013). Ketaubon et al. (2024) further reported that soil inoculation with BC-MY increased Cd, Cu, and Pb accumulation in the root tissues of *C. zizanioides* grown in landfill soil. These findings highlight that enhanced heavy metal accumulation in plant tissues is influenced not only by bacterial mechanisms but also by plant species. *C. zizanioides* possesses a dense and complex root system (Banerjee et al., 2016), which likely contributes to its uptake capacity. Overall, our results underscore that the interactions among heavy metal types, bacterial mechanisms, and plant species play a critical role in determining the success of heavy metal phytoremediation.

Considering the speciation of heavy metals in landfill soil, Pb generally exhibits low bioavailability because it predominantly occurs in the residual fraction, where it is strongly bound to the soil matrix (Osakwe et al., 2012). In contrast, Cd is primarily associated with the exchangeable and carbonate fractions, conferring high bioavailability, whereas Cu is mainly distributed within the organic and Fe–Mn oxide fractions, indicating moderate bioavailability (Osakwe et al., 2012; Kennou et al., 2015). Moreover, given that only three replicates ($n = 3$) were used in our experiments, the statistical power was likely limited, reducing the ability to detect subtle effects of bacterial inoculation on Pb uptake and accumulation. Although the Pb concentrations in the root tissues of *Z. elegans* with and without bacterial inoculation did not differ significantly ($p > 0.05$), the effect size revealed a 1.1-fold increase in Pb accumulation in plants inoculated with BC-MY compared to the uninoculated control. This suggests that

BC-MY inoculation may contribute to enhanced Pb accumulation in root tissues, despite the lack of statistical significance.

Application of a small amount of biochar (0.1% w/w) did not significantly affect the absorption and accumulation of heavy metals in *Z. elegans* compared to the untreated plants. A biochar dose of up to 2.0% (w/w) has been suggested to reduce the phytotoxic effects of pollutants (Liu et al., 2017). These findings indicate that biochar can be effectively utilized as a carrier material for bacterial immobilization. Its porous structure provides a suitable microhabitat by retaining nutrients, water, and bacterial cells, whereas its strong affinity for bacterial adhesion creates a favorable microenvironment for a wide range of soil bacteria (Yang et al., 2018; Kamyab et al., 2025). Therefore, the synergistic association between biochar, heavy metal-resistant PGPB, and plants highlights the role of biochar as an effective microbial carrier and underscores its potential as a green technology for restoring heavy metal-polluted soils (Harindintwali et al., 2020).

3.5 Distribution of heavy metals in plant tissues after plantation in landfill soil

Micro-XRF mapping results and elemental distributions of Cd, Cu, and Pb in the roots and shoots of *Z. elegans* are presented in Table 3. As highlighted in previous studies, XRF is a highly effective technique for confirming the presence of elements and illustrating their spatial distribution within plant tissues (Teferaa et al., 2020; Ramakrishna, 2023), with the benefit of avoiding complex sample preparation procedures. The spatial localization of these heavy metals in the root and shoot tissues of *Z. elegans* was determined by micro-XRF spectroscopy mapping. The elemental signals are visualized as blue (Cd), orange (Cu), and green (Pb) spots on a black background, highlighting specific accumulation zones. Our findings offer definitive evidence for the presence of heavy metals in plant tissues and present semi-quantitative elemental compositions in terms of weight percentage (% wt). The relative abundances of Cd, Cu, and Pb in plant tissues were minor, with silicon (Si), potassium (K), and calcium (Ca) constituting the major elemental composition. Silicon, the second most abundant element in soil, has been reported to alleviate the harmful effects of heavy metal stress on plants (Adrees et al., 2015).

Our findings revealed that the proportions of Cd, Cu, and Pb in the root and shoot tissues of *Z. elegans* across all treatments were generally consistent with directly measured concentrations of the heavy metals (Table 2). In root tissues, Pb represented the highest percentage, followed by Cu and Cd, which corresponded to the order observed in the direct heavy metal concentration analysis. In contrast, the shoot tissues displayed a composition order of Pb > Cu > Cd, which was not entirely aligned with direct measurements. This variation may be attributed to the very low concentrations and uneven distribution of heavy metals within the tissues, emphasizing the need for random sampling of plant tissues in replicate analyses. Our findings indicate that micro-XRF is a reliable method for the qualitative and semi-quantitative assessment of heavy metals, and for mapping their spatial distribution in plant tissues. Similar findings were reported by Hu et al. (2019), who applied micro-XRF to investigate Cd localization in the stems of *Sedum alfredii* Hance, and by Teferaa et al. (2020), who analyzed Cd distribution in *Oryza sativa* L. root hairs. Additionally, Mera et al. (2019) applied synchrotron radiation micro-XRF to investigate the spatial distribution of Pb in roots and leaves of *Brassica napus* L.

3.6 Performance of *Z. elegans* on heavy metal phytoremediation

Table 4 presents the key indices employed to assess the phytoremediation performance in *Z. elegans*: root uptake (RU), shoot uptake (SU), phytoextraction efficiency (PE), uptake efficiency (UE), accumulation factor (AF), and translocation factor (TF). Statistical analysis showed no significant differences ($p < 0.05$) in these indices between the control plants and those that received biochar. The consistently low RU and SU values for Cd, Cu, and Pb in *Z. elegans* were mainly due to low root and shoot dry biomass yields. *Z. elegans* exhibited the highest phytoremediation efficiency indices for Cu, except for AF, which showed values similar to those of Cd. As an essential element, Cu is required for plant physiological processes; however, its absorption and storage are strictly controlled by homeostatic mechanisms to maintain its appropriate concentrations (Liščáková et al., 2022). Moreover, certain soil conditions may alter heavy metal uptake, as exemplified by the enhanced SU of Cd in the calcareous soils of arid and semi-arid regions owing to Cd-Cl complexation (Razmi et al., 2021). The applications of BC-MY and CS-MY significantly improved the phytoremediation efficiency indices of Cd and Cu in *Z. elegans* compared with uninoculated control. In contrast, for Pb, both inoculants increased RU, SU, PE, and UE values. A slight increase in the SU of Pb was observed in the BC-MY and CS-MY treatment groups. This observation aligns with the fact that Pb accumulates in the roots, which is attributed to Pb precipitation on root cell walls (Zhou et al., 2016).

BC-MY inoculation exhibited comparable effects to CS-MY on most indices of Cd, Cu, and Pb phytoremediation efficiency, with no significant differences observed ($p < 0.05$), except for SU and AF of Cu. In *Z. elegans*, BC-MY treatment resulted in 1.2- and 1.5-fold lower SU and AF values of Cu, respectively, compared to CS-MY. Similar enhancements in phytoremediation efficiency through PGPB cell suspension inoculation have been reported for various plants, such as Cd uptake by *Crotalaria juncea* L. (Thooppeng et al., 2022), *Sorghum bicolor* (Chen et al., 2024), Cu accumulation by *Ricinus communis* L. (Li et al., 2025), and *H. annuus* (Kumar et al., 2021). Limited studies have examined the use of heavy metal-resistant PGPB-immobilized cells to enhance heavy metal phytoremediation (Ketaubon et al., 2024). The present findings demonstrate that BC-MY inoculation markedly improved the PE of Cd, Cu, and Pb by 1.3-, 1.3-, and 1.7-fold, respectively, compared with the uninoculated plants. This indicates that *Z. elegans* inoculated with PGPB possesses strong phytoextraction potential for these heavy metals. Additionally, the highest UE value was detected for Cu in CS-MY-inoculated *Z. elegans*, suggesting its pronounced ability to accumulate Cu. The UE values followed the order Cu > Pb > Cd, which was in agreement with the relative bioavailability of these heavy metals in the landfill soil.

To assess phytoremediation mechanisms, AF and TF serve as key indicators for evaluating the potential of plants for heavy metal phytoextraction and phytostabilization (Razmi et al., 2021). In *Z. elegans*, the AF values for Cd and Cu were higher than those for Pb despite the lower bioavailable concentrations of Cd and Cu in the landfill soil. Inoculation with BC-MY and CS-MY enhanced the AF and TF of Cd and Cu, whereas those of Pb remained relatively unchanged across treatments. Notably, AF and TF values greater than 1.0 indicate the capacity of plants to translocate and accumulate heavy metals in their aboveground biomass (Ali et al., 2013). Our findings revealed that *Z. elegans* inoculated with BC-MY and CS-MY exhibited AF values for Cd and Cu greater than 1.0. The TF of Cu also increased, reaching 0.80 ± 0.07 with BC-MY inoculation and 0.98 ± 0.16 with CS-MY inoculation, approaching the threshold indicative of strong translocation ability. According to Jahromi et al. (2022), plants with TF values exceeding 1.0 demonstrate high remediation capacity and are recognized as effective phytoremediators. Nevertheless, AF and TF values for Pb in *Z. elegans*, with or without bacterial inoculation, remained comparatively low, ranging between 0.15–0.18 and 0.25–0.27, respectively.

These results highlight that *Z. elegans* exhibited limited Pb accumulation and translocation capacities. This characteristic is linked to the root-to-shoot restriction mechanism, in which Pb precipitates in the roots by binding to negatively charged groups on the root cell walls (Kumar and Prasad, 2018). Furthermore, root-to-shoot transfer of Pb is influenced by competitive interactions with other heavy metals (Dinu et al., 2020). Notably, *Z. elegans* exhibited low Pb accumulation and translocation capacities. This could be related to the root-to-shoot restriction phenomenon, which explains that Pb precipitates in the plant roots by binding to the negative charges on the cell walls of root tissues (Kumar and Prasad, 2018). In addition, the translocation process from roots to shoots is affected by the competition between heavy metals (Dinu et al., 2020). Based on a bacterial-assisted phytoremediation strategy, our findings suggest that *Z. elegans* inoculated with BC-MY is suitable for Cd and Cu phytoextraction while being more effective for Pb phytostabilization in multi-heavy metal-contaminated landfill sites.

To compare the effects of BC-MY and CS-MY on phytoremediation efficiency, both inoculants exhibited comparable capacities in enhancing several phytoremediation indices (Table 4). Although CS-MY outperformed BC-MY in a few phytoremediation indices, the BC-MY which integrates the benefits of biochar as a carrier material with a highly effective PGPB strain, offers several practical advantages for field application in landfill sites. Biochar can be produced from agricultural residues, resulting in a low-cost and environmentally friendly soil amendment (Patel and Panwar, 2024). The porous structure and large surface area of biochar provides microhabitats that support microbial growth and reproduction and supplies limited nutrients, making it a promising matrix for microbial immobilization (Wu et al., 2022). Immobilization of bacterial cells within biochar can confer protection against adverse environmental conditions, enhance bacterial survival, and promote microbial activity in heavy metal-contaminated soils (Wang et al., 2022; Qi et al., 2023). Moreover, biochar-immobilization is simple to prepare and amenable to scale-up, as it typically relies on adsorption or adherence of cells to the carrier surface (Montreemuk et al., 2024). Importantly, biochar-immobilized bacterial cells exhibit extended shelf life, even under room temperature storage, compared with free-living cells (Ketaubon et al., 2024), thereby improving their practicality for real-world field applications.

4. Conclusion

M. yunnanensis, a heavy metal-resistant PGPB, significantly enhanced root growth in *Z. elegans* under Cd, Cu, and Pb stress, highlighting its protective and growth-promoting functions. Application of *M. yunnanensis*, either as BC-MY or CS-MY formulations, markedly improved plant growth and biomass in *Z. elegans* cultivated in multiple metal-contaminated landfill soil. Increased microbial activity was also observed following *Z. elegans* plantation, particularly in bacterially inoculated treatments. Importantly, inoculation with BC-MY and CS-MY promoted Cd and Cu uptake and accumulation in both the roots and shoots of *Z. elegans*, while favoring Pb retention in the roots, thereby supporting the phytoextraction of Cd and Cu and phytostabilization of Pb. Considering the phytoremediation efficiency, these results indicate that *Z. elegans* combined with bacterial inoculation represents a promising integrated strategy for phytoremediation of landfill soils contaminated with multi-heavy metals. This study provides a foundation for developing sustainable phytoremediation practices using ornamental plants in combination with biochar-immobilized beneficial bacteria.

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Figure Captions

Fig. 1 Root elongation of *Z. elegans* seedlings treated with *M. yunnanensis* compared with untreated controls under various heavy metal stress conditions. Error bars represent standard deviation across twenty replicates (n = 20). Different letters above each bar indicate statistically significant differences, as determined by DMRT, with a significance level of p < 0.05.

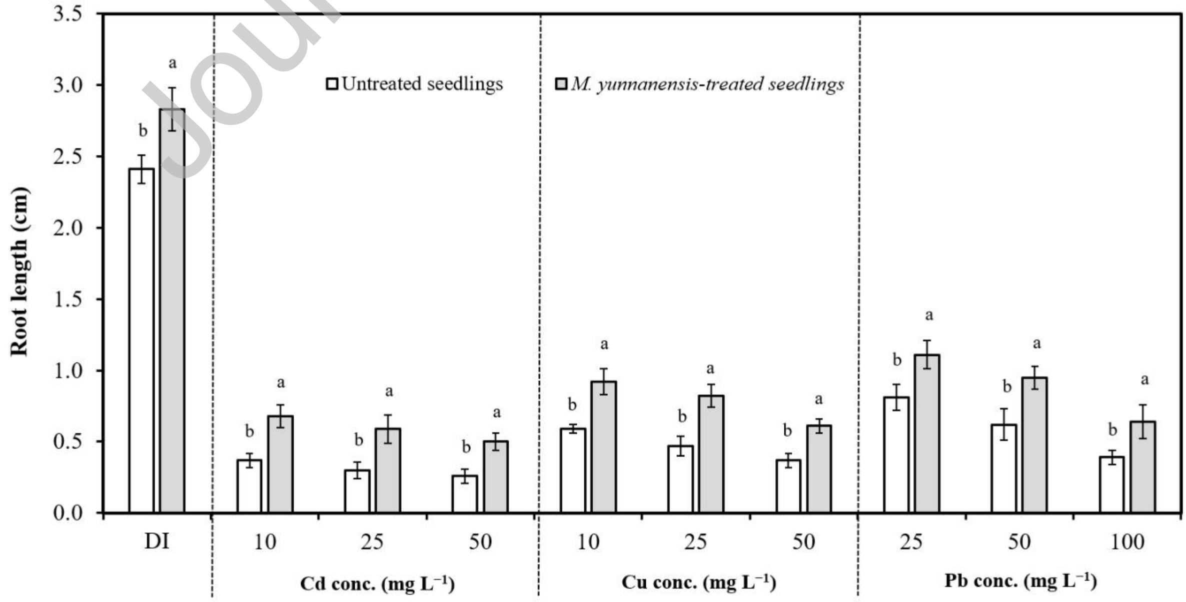


Fig. 2 Growth performance of *Z. elegans* cultivated in landfill soils under different treatments. Error bars represent the standard deviation across three replicates ($n = 3$). Different letters above each bar indicate statistically significant differences among the four treatments, as determined by DMRT, with a significance level of $p < 0.05$.

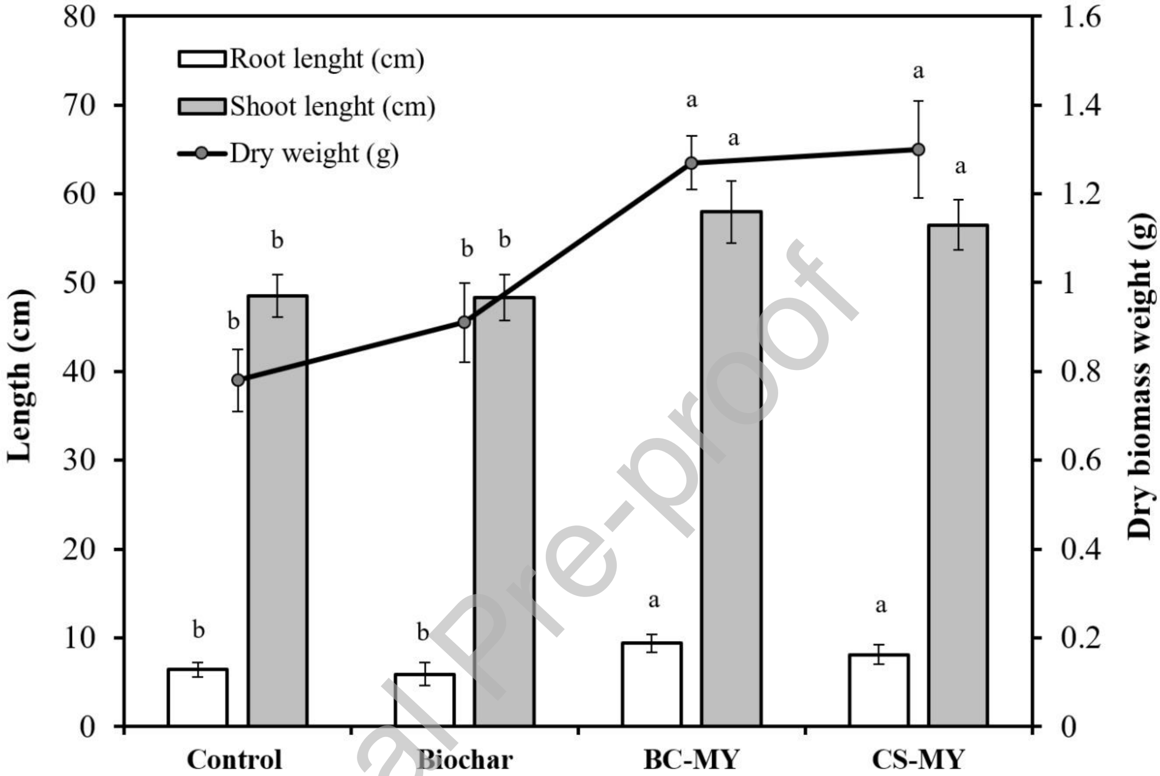


Fig. 3 Chlorophyll contents in *Z. elegans* cultivated in landfill soils under different treatments. The error bars represent the standard deviation across three replicates ($n = 3$).

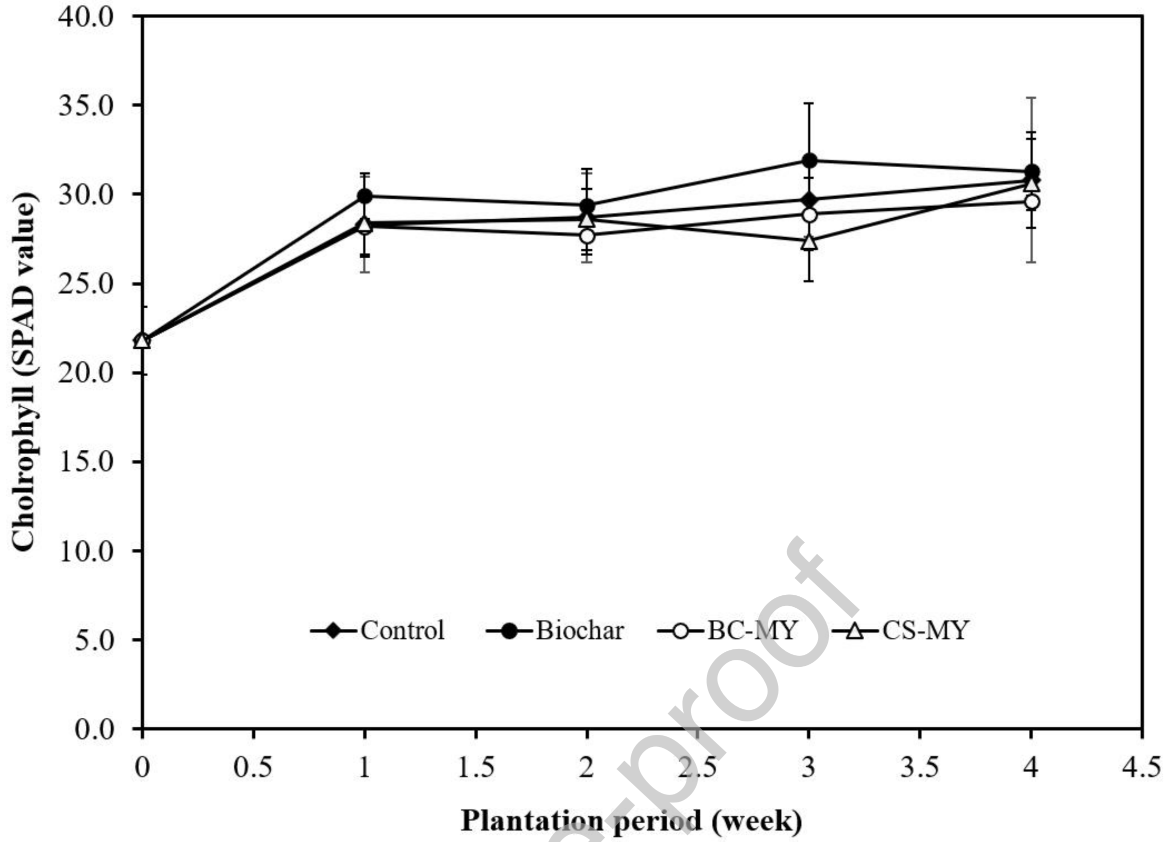


Table 1 Soil pH and soil microbial activity before and after *Z. elegans* plantation in landfill soil

Treatment	Soil pH	Soil microbial activity (FDA hydrolysis) (μg g^{-1} h^{-1})
Before	6.52 ± 0.06 ^{ns}	0.74 ± 0.10 ^{de}
After		
Control	6.66 ± 0.16 ^{ns}	0.88 ± 0.06 ^{d}
Biochar	6.55 ± 0.10 ^{ns}	1.02 ± 0.02 ^{c}

BC-MY	6.64 ± 0.16 ^{ns}	1.50 ± 0.03 ^{a}
CS-MY	6.44 ± 0.08 ^{ns}	1.23 ± 0.07 ^{b}

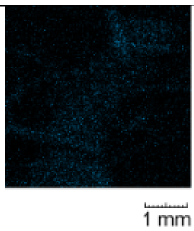
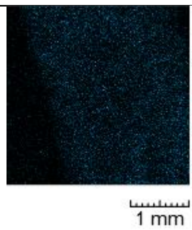
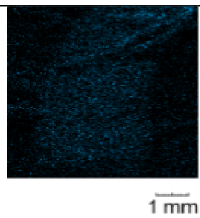
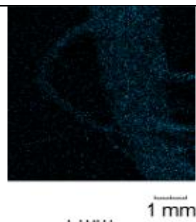
Different superscript letters indicate statistically significant differences among the four treatments (p < 0.05) according to DMRT. The "ns" indicates non-significant differences at the p < 0.05 level.

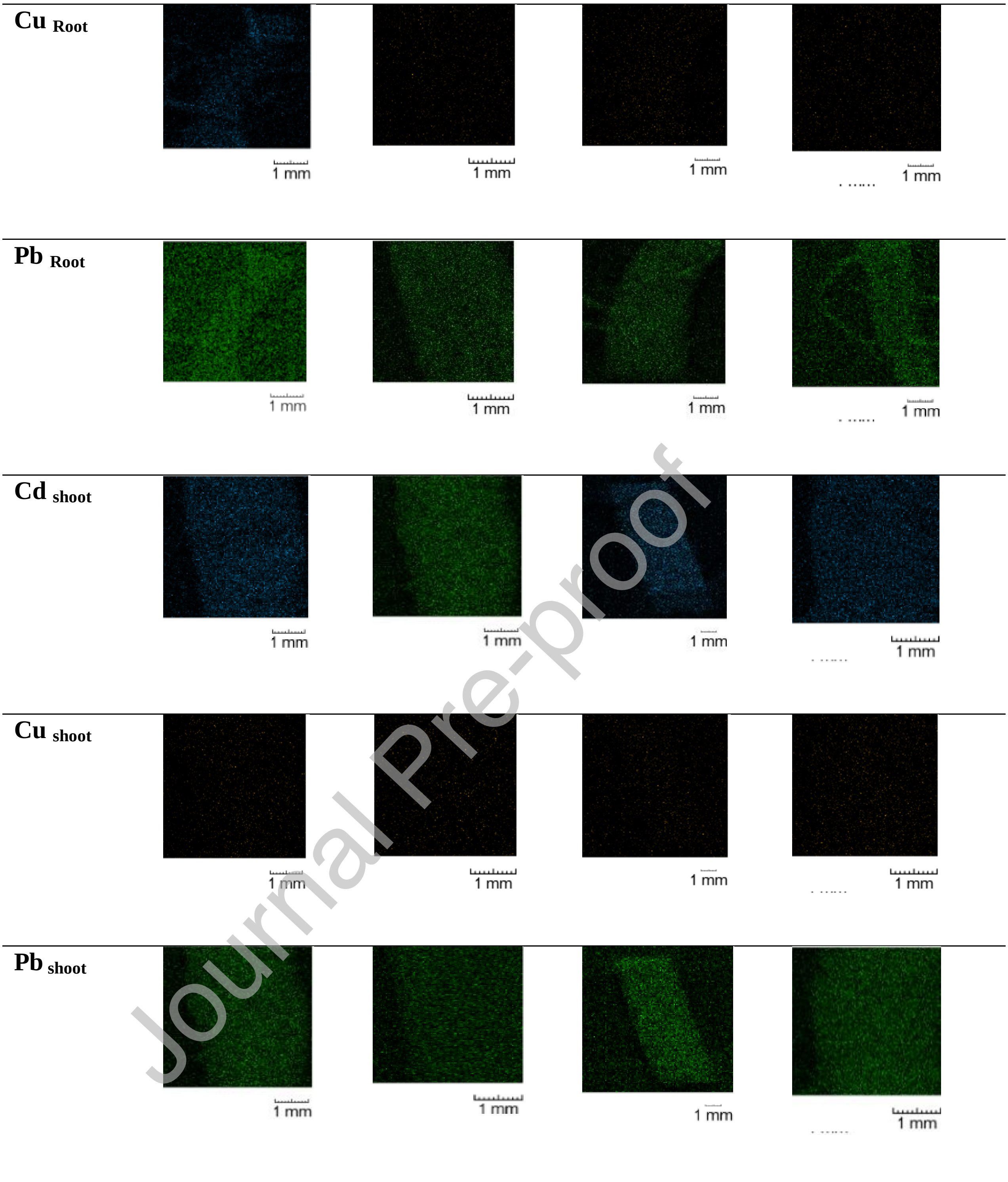
Table 2 Concentrations of heavy metals accumulated in plant tissues in the landfill soil after *Z. elegans* plantation

Treatment	**Heavy metals in root (mg kg^{-1})**			**Heavy metals in shoot (mg kg^{-1})**		
	Cd	**Cu**	**Pb**	**Cd**	**Cu**	**Pb**
Control	1.47±0.19 ^{b}	12.50±0.10 ^{c}	15.15±0.21 ^{ns}	0.60±0.05 ^{c}	7.67±0.52 ^{c}	4.10±0.27 ^{ns}
Biochar	1.37±0.04 ^{b}	11.56±0.21 ^{c}	15.90±1.56 ^{ns}	0.66±0.18 ^{c}	6.63±0.49 ^{c}	3.90±0.14 ^{ns}
BC-MY	1.99±0.33 ^{a}	17.22±1.66 ^{a}	16.67±0.92 ^{ns}	0.98±0.07 ^{ab}	12.70±0.42 ^{b}	4.15±0.21^{ns}
CS-MY	2.10±0.13 ^{a}	15.81±0.68 ^{ab}	15.54±0.87 ^{ns}	1.21±0.16 ^{b}	15.39±0.83 ^{a}	4.27±0.33 ^{ns}

Different superscript letters within each heavy metal category denote statistically significant differences among the four treatments ($p < 0.05$) as determined by DMRT. The "ns" indicates non-significant differences at the $p < 0.05$ level.

Table 3 Micro-XRF mapping and relative concentrations (%wt) of heavy metals distribution in the root and shoot tissues of *Z. elegans* after planting in landfill soil

Treatment	**Relative concentrations (%wt) of heavy metals**			
	Control	**Biochar**	**BC-MY**	**CS-MY**
Cd $_{\textbf{Root}}$	0.0266	0.0253	0.0690	0.0816
Cu $_{\textbf{Root}}$	0.0877	0.0467	0.1075	0.2390
Pb $_{\textbf{Root}}$	0.2818	0.3376	0.3411	0.3403
Cd $_{\textbf{shoot}}$	0.0622	0.0817	0.1454	0.1572
Cu $_{\textbf{shoot}}$	0.1064	0.0862	0.3791	0.2131
Pb $_{\textbf{shoot}}$	0.3397	0.2812	0.3623	0.3715
	Micro-XRF mappping of Cd (blue), Cu (orange), and Pb (green)			
Cd $_{\textbf{Root}}$				



Note: The regions of interests (ROIs) were defined manually based on each part of plant tissues to extract element specific fluorescence intensities. Element-specific fluorescence intensities were extracted from each ROI, with quantification based on the integrated peak areas of characteristic Kα and Lα emission lines. Spectral deconvolution and quantification were performed using XGT Analyzer software (Horiba Scientific).

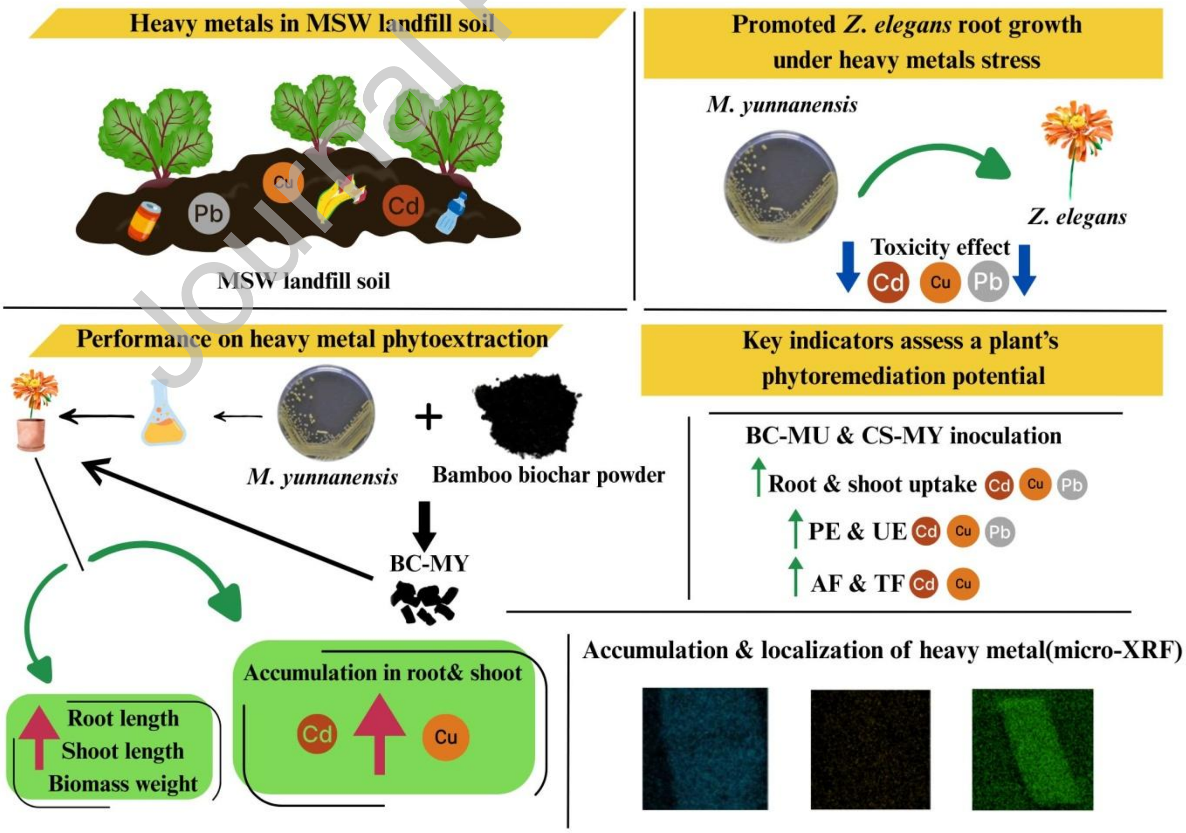
Table 4 Indicies of heavy metal phytoremediation efficiency of *Z. elegans* planted in landfill soil

Efficiency	Control	Biochar (T2)	BC-MY	CS-MY
Root uptake (RU) (µg plant^{-1})				
Cd	0.05±0.03 ^{b}	0.07±0.02 ^{b}	0.25±0.04 ^{a}	0.24±0.06 ^{a}
Cu	0.50±0.18 ^{b}	0.54±0.13 ^{b}	1.92±0.48 ^{a}	2.10±0.33 ^{a}
Pb	0.53±0.11 ^{b}	0.68±0.15 ^{b}	1.75±0.16 ^{a}	1.71±0.07 ^{a}
Shoot uptake (SU) (µg plant^{-1})				
Cd	0.44±0.11 ^{b}	0.48±0.10 ^{b}	1.19±0.10 ^{a}	1.38±0.15 ^{a}
Cu	5.71±0.38 ^{c}	6.17±0.46 ^{c}	15.48±1.09 ^{b}	18.31±0.99 ^{a}
Pb	3.05±0.22 ^{b}	3.28±0.50 ^{b}	5.05±0.21 ^{a}	5.00±0.56 ^{a}
Phytoextraction efficiency (PE) (µg g^{-1})				
Cd	11.97±0.64 ^{b}	9.72±0.69 ^{b}	14.92±1.02 ^{a}	16.45±0.92 ^{a}
Cu	149.56±12.43 ^{b}	147.25±10.97 ^{b}	193.47±19.41 ^{a}	206.19±13.52 ^{a}
Pb	49.67±6.53 ^{bc}	61.68±5.44 ^{b}	86.67±3.92 ^{a}	90.01±4.06 ^{a}
Uptake efficiency (UE) (µg g^{-1})				
Cd	13.67±1.15 ^{b}	11.77±1.58 ^{b}	18.14±1.70 ^{a}	19.04±0.08 ^{a}
Cu	162.06±11.49 ^{b}	159.91±19.28 ^{b}	212.14±23.18 ^{a}	229.09±15.22 ^{a}
Pb	64.67±6.75 ^{c}	76.37±1.28 ^{c}	103.34±11.75 ^{ab}	118.49±13.21 ^{a}
Accumulation factor (AF)				
Cd	0.75±0.04 ^{c}	0.67±0.11 ^{c}	1.11±0.04 ^{ab}	1.54±0.26 ^{a}

Cu	0.73±0.05 [c]	0.76±0.08 [c]	1.31±0.02 [b]	1.90±0.08 [a]
Pb	0.16±0.03 [ns]	0.15±0.01 [ns]	0.16±0.04 [ns]	0.18±0.04 [ns]
Translocation factor (TF)				
Cd	0.35±0.09 [b]	0.38±0.04 [b]	0.52±0.05 [a]	0.58±0.07 [a]
Cu	0.61±0.04 [b]	0.57±0.04 [b]	0.80±0.07 [a]	0.98±0.16 [a]
Pb	0.27±0.09 [ns]	0.25±0.03 [ns]	0.25±0.01 [ns]	0.27±0.07 [ns]

Different superscript letters indicate significant differences among the four treatments ($p < 0.05$) according to DMRT. The "ns" indicates non-significant differences at the $p < 0.05$ level.

Graphical Abstract



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Declaration of competing interest

The authors declare no conflicts of interest regarding the publication of this paper. This article does not contain any studies with human participants or animals performed by any of the authors.