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The culture inoculation method could influence heavy metals levels in soil, plant uptake, and biomass: a meta-analysis

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ABSTRACT

Heavy metals (HMS) contamination in soil is a major issue that significantly impacts plants and human health. Various approaches have been employed to mitigate the effects of heavy metals, including the application of microorganisms (MO). This study aims to analyze the impact of bioinoculation application on HMS content in plants and soil through a meta-analysis approach. Twenty-nine publications reviewed between 2001 and 2023 reported the effects of microorganism applications on HMS content in plants and soil. A systematic review was applied to select relevant studies, and effect sizes (ES) were calculated using Hedges'*d* to quantify the impact of microbial treatments on heavy metal content. The parameters observed were As, Hg, Cd, Cr, Ni, Co, Pb, Ni, Mn, Zn, and Fe in plants (shoots, roots, fruit, and total plants), soil, and plant biomass. The ES values of Hedges' microorganisms HMS on soil, plants, and plant biomass were -3.257 ($p<0.001$), 1.234 ($p<0.001$), and 2.301 ($p<0.001$), respectively. The results showed that the greatest reduction in HMS content in the soil was the combined application of fungi and bacteria (ES -5.519; $p<0.001$), and the highest metal content absorbed by the soil and plants was Cu (ES -13.642; $p<0.001$) and Pb (2.645; $p<0.001$), respectively. This study showed that *Orychophragmus violaceus* had the highest metal absorption rate (ES 15.528, $p<0.001$) to help clean up heavy metal contamination, especially in agricultural land and industrial areas. This approach can improve soil quality, enhance plant growth, and reduce health risks, which benefits farmers, policymakers, and environmental agencies.

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1. INTRODUCTION

Modern industrialization, mining activities, agricultural practices, and waste management contribute to heavy metals (HMS) pollution (Sarma et al., 2024). Industrial and mining activities are the main sources of heavy metal pollution, such as lead, cadmium, mercury, and arsenic. Waste and emissions from metal plating processes, chemical synthesis, oil refining, and mining operations significantly pollute the air, water, and soil. Studies show that human activities contribute up to 80% of the risk of heavy metal contamination in the environment (Ali et al., 2021; Bargah, 2024; Mishra & De, 2024; Wen et al., 2025). Heavy metal pollution refers to the accumulation of toxic metals such as cadmium (Cd), mercury (Hg), copper (Cu), arsenic (As), lead (Pb), chromium (Cr), nickel (Ni), and zinc (Zn) in the environment, including soil and plants. This contamination can pose serious risks to human health and the environment (Li et al., 2019). Many countries are affected by heavy metal pollution in their soil, water, and ecosystems (Escobar-Mamani et al., 2023; Lubal, 2024; Wang et al., 2022). This is particularly concerning in many countries worldwide, especially in developing nations with numerous industrial hubs. Food crops contaminated with high levels of heavy metals can lead to various health issues, including

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neurological, kidney, and developmental issues (Rai et al., 2019). Moreover, contaminated soil can hinder plant growth, disrupt ecosystems, and contaminate groundwater (Timothy & Williams, 2019).

It is important to note that heavy metal toxicity is dose-dependent, meaning that the risk of harm increases with higher levels of exposure (Diaconu et al., 2020). A UN-ECE Protocol on heavy metals atmospheric emission control was signed in 1998 (Mocanu et al., 2006). However, regulations in many countries may have set different guidelines and limits. Precautions should be taken to reduce heavy metal pollution and deliberately adopt good agricultural practices to contain the risk (Afonne & Ifediba, 2020). Various studies have adopted a remediation approach using chemical, physical, and biological methods to technically address the problem of metal pollution in soil and the environment. However, the biological approach is an attractive method compared to physico-chemical and chemical processes in eliminating the toxicity of heavy metals (Zheng et al., 2024). The use of biological agents in remediating contaminated soil and environments is commonly known as bioremediation techniques. This technique uses microorganisms, such as bacteria and fungi, which play an essential role in breaking down or changing various pollutants, including organic compounds and heavy metals (Olusegun et al., 2024). Bioremediation is a sustainable and cost-effective approach that can be applied to soil and water environments (Zhang et al., 2020). Bioremediation is defined as the use of living organisms, particularly microbes, to degrade, detoxify, or transform environmental contaminants into less harmful forms (Kuppan et al., 2024; Patel et al., 2020). Phytoremediation, on the other hand, refers specifically to the use of green plants and their associated microbes to extract, stabilize, or degrade pollutants from soil and water environments (Iqbal et al., 2019; Pasricha et al., 2021; Yadav et al., 2018). While phytoremediation is considered a subset of bioremediation, the two terms are often used interchangeably in the context of sustainable remediation approaches.

One form of practical and efficient bioremediation technique is to use plant media, commonly called phytoremediation. Phytoremediation, which relies on phytoextraction with biological organisms, is an essential alternative to expensive physical and chemical methods for treating contaminated soil (Davies et al., 2001). Fungi and/or bacteria can efficiently work synergistically with plants in phytoremediation. They can enhance plants' uptake and accumulation of contaminants, leading to improved removal from soil and water. Some report that *Arbuscular mycorrhiza* (AM) fungi can colonize hyperaccumulators and increase the uptake of heavy metals, e.g., Cu, Zn, Pb, and Cd, in hyperaccumulator *Elsholtzia splendens, Canavalia ensiformis* (Andrade et al., 2010; Wang et al., 2007) and As-hyperaccumulator *Zea mays L.* (Bai et al., 2008), which demonstrated the potential use of mycorrhizae hyperaccumulators in phytoextraction. However, some studies use bacteria such as *Rhizobium, Azotobacter, Bacillus subtilis, B. cereus, B. megaterium, Pseudomonas aeruginosa*, and *Pseudomonas koreensis* as biological agents. Plants associated with these bacteria include *Chrysopogon zizanioides, Orychophragmus violaceus*, and *Miscanthus sinensis* (Babu et al., 2015; Liang et al., 2014; Lu et al., 2023). These bacteria can also be symbiotic with plants to increase the process of heavy metal extraction or as a nitrogen fixer needed by plants, thereby increasing the accumulation of heavy metals. Rhizobium symbiosis with Alfalfa legumes has been shown to reduce stress in the presence of Cu (Duan et al., 2019), *Bacillus subtilis* was applied to rice plants (*Oryza Sativa*) and increased the detoxification of arsenic (As) (Ullah et al., 2024), *Bacillus cereus* showed strong plant growth-promoting properties along with high Cd resistance in *Pigeon pea* plants (Gao et al., 2025), *Bacillus megaterium* inoculation has been shown to increase biomass and Cd concentration in roots and shoots of *Arachis hypogaea L*. compared to controls (without inoculation) (Xiong et al., 2024), *Pseudomonas aeruginosa* bacteria have been shown to increase the phytoremediation ability of *Jatropha gossypifolia* in soil contaminated with metals (Al, Pb, Zn, and Cd) by increasing nitrogen fixation and phosphate solubilization (Chi et al., 2023), and inoculation of *Pseudomonas koreensis* in Manzanita plants has been shown to increase the phytoremediation process against heavy metals (As, Cd, Cu, Pb, and Zn) in soil more than threefold (Zhu et al., 2023).

The success of the remediation process depends on various factors such as microbial activity, environmental conditions, contaminant concentrations, and the availability of nutrients and electron acceptors (Chang et al., 2018; Chaturvedi et al., 2018; Chhimwal & Srivastava, 2023; Ullah et al., 2019). Microorganisms can be applied as bioremediation tools in various ways, either individually or in combination with bacteria and/or fungi. Determining the application to be used, both the type of microbe and the method, will undoubtedly impact the efficiency and final result of the remediation process, in the hope that it will not cause additional consequences. This improvement is facilitated through mechanisms such as nutrient cycling, hormone production, and stress tolerance enhancement, which have been widely reported in recent studies (Guzmán-Moreno et al., 2022; Han et al., 2021; Tang et al., 2024). A recent meta-analysis paper examining the efficacy of arbuscular mycorrhizal fungi (AMF) in remediating toxic heavy metals in mine-impacted soils highlights their role alongside various plant species in reducing heavy metal stress and enhancing plant growth (Banerjee et al., 2025). Although using microorganisms for phytoremediation has been widely proven to improve polluted land in field studies, many studies have used different criteria in collecting data under various conditions in the last five years. Therefore, as an extension of the previous work, a comprehensive meta-analysis was conducted to study how microorganisms help in phytoremediation. To understand their effect on cleaning polluted soil, the data were analyzed using meta-analysis, which helps combine results from different studies clearly and reliably (Rosenberg, 2013). It was expected that (i) heavy metal levels in the soil would go down as plants absorb more and grow better because of their interaction with microorganisms, and (ii) this effect would depend on the type of microbes, the plant used, and how the microbes were applied (single or mixed strains).

In our study to investigate the effect of the microorganisms involved in the bioremediation activity of polluted soils, we synthesized the data using meta-analysis, which is useful when integrating a wide range of data and provides a systematic and statistically rigorous way to compare studies with methodological and experimental differences (Rosenberg, 2013). This study aims to identify and quantify the effects of interactions between plants, soil, and microorganisms on the reduction of heavy metal (HM) content in soil through HM accumulation and biomass in plants. In addition, this study aims to examine the role of moderator factors, including types of microorganisms, types of plants, and microbial management methods (single or multistrain applications), in determining the effectiveness of microorganisms on the HM absorption process and plant growth in the context of phytoremediation. We hypothesized that (i) the soil HMs content would decrease as crop HMs uptake and biomass increase caused by the interaction between plants, soil, and microorganisms; (ii) the type of microbes, plant type, and management methods, such as single or multistrain application will influence the effect of microorganisms on HMs uptake and crop growth.

2. MATERIAL AND METHODS

2.1. Data collection

We conducted an exhaustive search for previously published articles in June 2022 using the Scopus database (https://www.scopus.com). Keywords used for literature research are as follows: bacteria, fungi, immobilization, phytoremediation, heavy metals, and soil. The following criteria were applied to select suitable studies. First, articles must have a microorganism application as treatment with non-microorganisms as the control in pairs. Second, the activity of at least one microorganism in reducing the amount of heavy metals in the soil must be measured. Third, experiments were carried out in the field. Fourth, the papers included are between 2000 and 2023. We identified 29 articles from 2000 to 2023 (Fig. 1), in which 165 observations from 13 countries were included (Fig. 2), that met these criteria (Fig. 1 & Table 1). We extracted each study's mean, standard deviation, and number of replications from the target variables. Parameters observed were HMs content (As, Hg, Cd, Cr, Ni, Co, Pb, Mn, Zn, and Fe) in plants (shoots, roots, fruits, and total plant), HMs content in soil, and plant biomass.

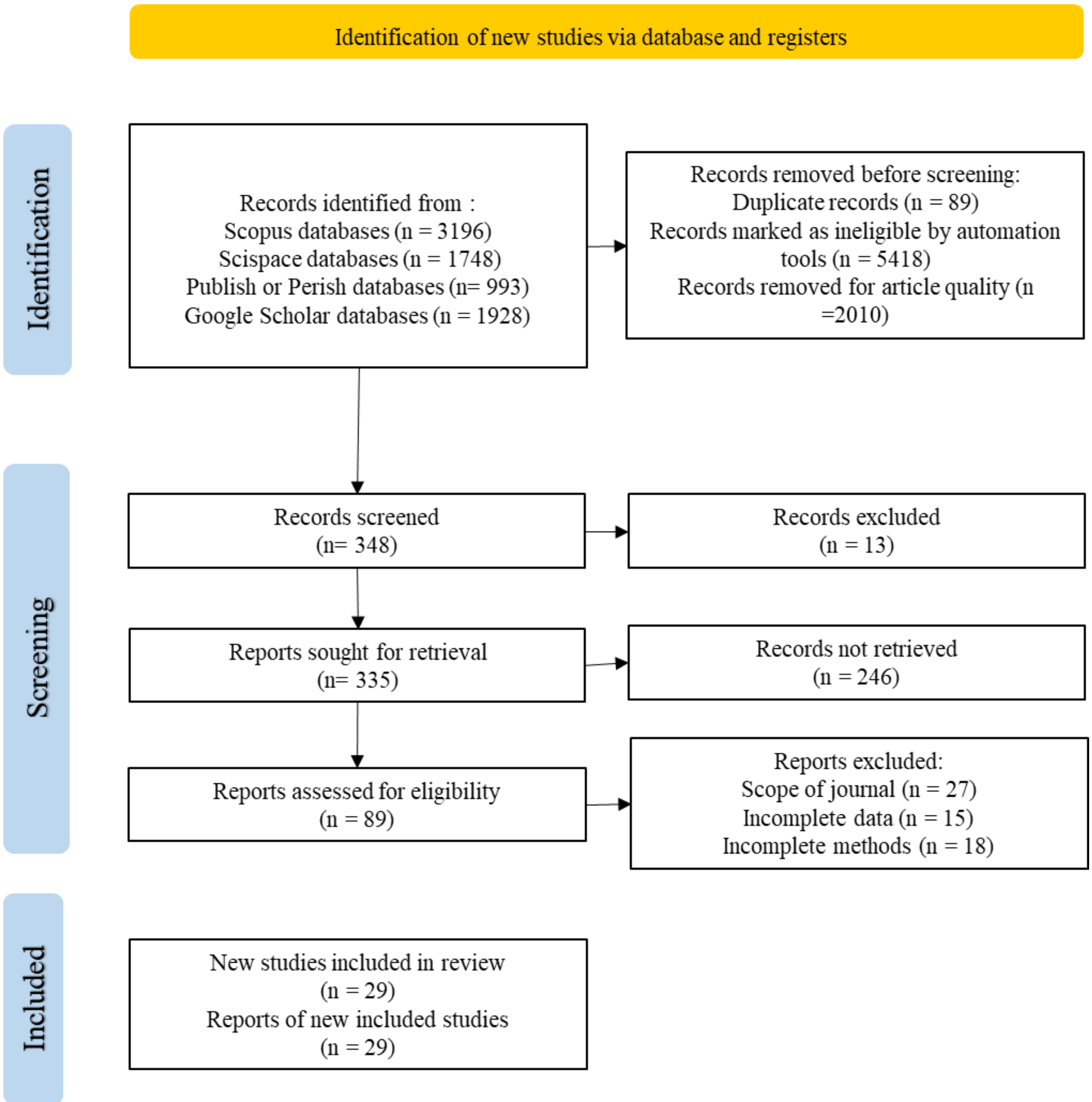


Figure 1. Diagram of literature search based on PRISMA protocols

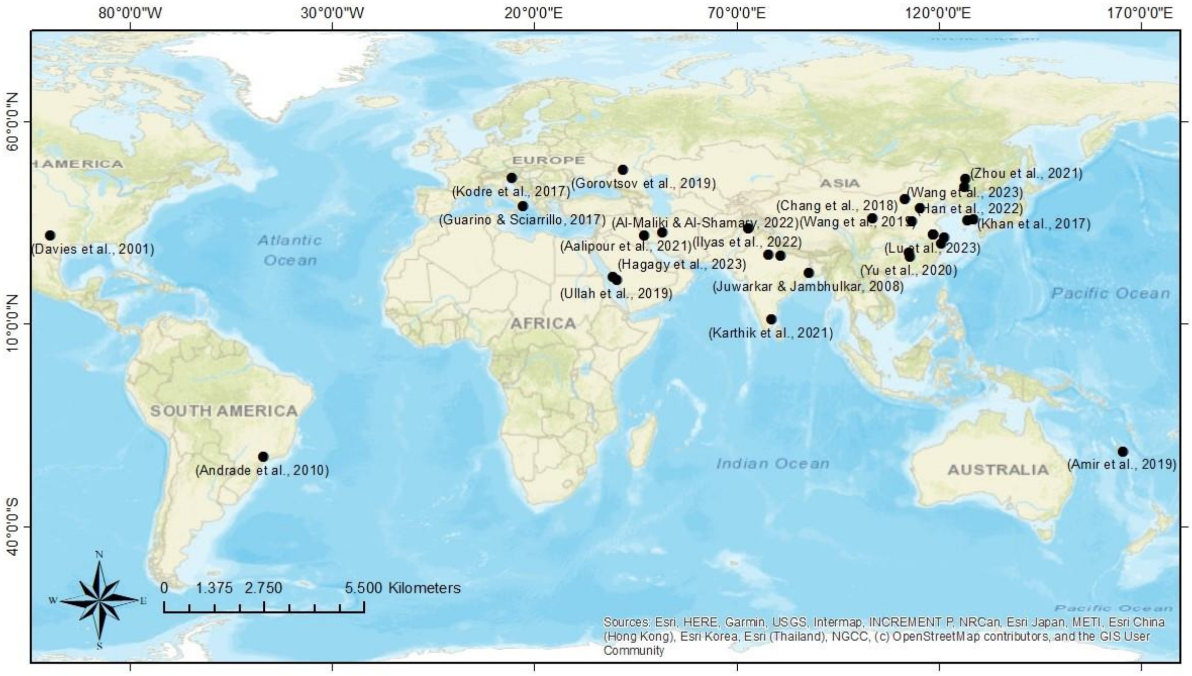


Figure 2. Distribution of paired studies from 29 journal articles selected

2.2. Data categorization and treatment

For each diversity index that showed significant results, mean effect sizes were also analyzed when statistics were grouped into other categories. In the subgroup meta-analysis, microorganisms were categorized based on the microbial groups: bacteria, fungi, fungi+bacteria, and archaea. Microbial applications were grouped into single culture and mixed culture. Plant species were categorized into eighteen types: *Helianthus annuus, Elsholtzia splendens, Canavalia ensiformis L., Orychophragmus violaceus, Miscanthus sinensis, Solanum nigrum, Zea mays L., Solanum melongena, Metrosideros laurifolia, Hordeum vulgare L., Brassica juncea, Pinus massoniana, Arizona cypress, Glycine max L., Brassica rapa, Typha latifolia, Triticum aestivum L.,* and *Triticum spp.* Meanwhile, microbial species/genera were categorized into *Glomus intraradices, Glomus caledonium, Glomus spp., Acaulospora spp., Glomus, Gigaspora, Azotobacter, Rhizobium spp., Pisolithus tinctorius, Entrophospora colombiana, Glomus clarum, Glomus etunicatum, Glomus intraradices, Bacillus licheniformis, Bacillus megaterium, Bacillus polymyxa, Bacillus subtilis, Bacillus thuringiensis, Paenibacillus azotofixans, Claroideoglomus etunicatum; Acaulospora rugosa, Actinobacteria, Firmicutes, Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Gemmatimonadetes spp., Proteobacteria spp., Suillus luteus, Trichoderma harzianum L., Bacillus subtilis L., Trichoderma harzianum L., Comamonas testosterone, Mycoriza, Bacillus spp., Bacillus cereus, Rhizophagus intraradices.*

2.3. Data computation and numerical analyses

For each standard pairwise comparison (control and microorganism treatment), Hedges' (Hedges et al., 1999; Hedges & Olkin, 1985) standard mean differences were calculated between the control and microorganism-treated groups to determine the metric effect size. The mean effect size for each grouping and the 95% bootstrap confidence interval (CI) were calculated using OpenMee software (version build date: 26-07-2016) (Wallace et al., 2017). The mean effect size was considered significantly different from zero if its 95% confidence intervals (CIs) did not include zero (Koricheva et al., 2013). We assessed the potential for publication bias in the entire database using the "trim and fill" method (Jennions et al., 2013). To assess the robustness of the overall observed effects of microbial inoculation on HMS content in soil, HMS in plants, and plant biomass, the fail-safe number (Nfs) was calculated using Rosenberg's weighted method ($\alpha = 0.05$) (Rosenberg, 2005). Nfs Rosenberg shows how much research reporting an effect size of zero needs to be added to the meta-analysis to make the observed effect not significantly different from zero (Rosenberg, 2005). If Nfs > $5 \times n + 10$, the result is considered robust, although there may still be a possibility of publication bias (Jennions et al., 2013).

3. RESULTS

3.1. The overall effect of microorganism inoculation on heavy metal content

The addition of microorganisms as biological agents to the parameters of heavy metal content in soil and plants is described in Figure 3 and Table 2. Our analysis based on Figure 3 and Table 2 revealed that the application of microorganisms to contaminated soil can reduce its heavy metal content with a negative effect size of -3.257 and p<0.001 (n=145; Nfs=9192). The effect of microorganisms in polluted soil on HMS content in plants shows that the effect size is on the right, with a value of 1.234 and p<0.001 (n=211; Nfs=2448). The meta-analysis results also showed that inoculation of microorganisms on polluted soil positively affected plant biomass, as indicated by an effect size of 2.301 and p<0.001 (n=60; Nfs=1792). Based on this, the parameters of HMS content in soil, plants, and plant biomass continued to be tested with a moderator variable.

Table 1: Experiments were included in the meta-analysis of the effect of microorganism inoculation on heavy metal content in polluted soil and plants

Study no.	Reference	Microbes as treatment	Inoculation applications	Plant accumulator	Metals
1	Davies et al. (2001)	Fungi	Single culture	*Helianthus annuus*	Cr
2	Wang et al. (2007)	Fungi	Mix culture	*Elsholtzia splendens*	Cu, Zn, Pb, and Cd
3	Bai et al. (2008)	Fungi	Single and mixed culture	*Zea mays* L.	As
4	Juwarkar and Jambhulkar (2008)	Fungi and Bacteria	Mix culture	*Azadiracta indica, Cassia Saemea, Syzygiumcumini, Artocarpus heterophyllus, Emblica officinalis, Tectona grandis, Delonix regia, Dendrocalamus strictus, Delbergia sissoo, Acacia nilotica, Mangifera indica, Pongamia pinnata*	Cr, Zn, Cu, Fe, Mn, Pb, Ni, Cd
5	Andrade et al. (2010)	Fungi	Single culture	*Canavalia ensiformis* (L.)	Mg, Mn, Zn
6	Liang et al. (2014)	Bacteria	Single culture	*Orychophragmus violaceus*	Cd
7	Babu et al. (2015)	Bacteria	Single culture	*Miscanthus sinensis*	As, Cd, Cu, Pb, Zn
8	Khan et al. (2017)	Fungi	Single culture	*Solanum nigrum*	Cd
9	Guarino and Sciarrillo (2017)	Fungi+Bacteria	Mix Culture	*Acacia saligna, Eucalyptus camaldulensis*	As, Cd, Pb, Zn
10	Kodre et al. (2017)	Fungi	Single and mixed culture	*Zea mays* L.	Hg
11	Chang et al. (2018)	Fungi	Single culture	*Zea mays* L	Cd
12	Chaturvedi et al. (2018)	Fungi	Single culture	*Solanum melongena*	Pb, Cd, As
13	Amir et al. (2019)	Fungi	Mix culture	*Metrosideros laurifolia*	Fe, Mn, Co, Ni, Cr
14	Gorovtsov et al. (2019)	Bacteria	Mix culture	*Hordeum vulgare L.*	Mn, Zn, Cr, Cu, Pb, Ni, Cd
15	Ullah et al. (2019)	Bacteria	Single culture	*Brassica juncea*	Cd
16	Wang et al. (2019)	Bacteria	Mix culture	*Triticum spp.*	Pb
17	Cheng et al. (2020)	Bacteria	Single culture	*Triticum spp.*	Cd, Pb
18	Yu et al. (2020)	Fungi	Single culture	*Pinus massoniana*	As, Cd, Cu, Cr, Mn, Ni, Pb, Zn, Se, Fe
19	Aalipour et al. (2021)	Fungi	Single and mixed culture	*Arizona cypress*	Cd
20	Haider et al. (2021)	Fungi	Single culture	*Glycine max L.*	Cd
21	Karthik et al. (2021)	Bacteria	Single culture	*Lycopersicon esculentum*	Cr
22	Zhou et al. (2021)	Bacteria	Single culture	*Brassica rapa*	Cd
23	Al-Maliki and Al-Shamary (2022)	Fungi, Bacteria, Fungi+Bacteria	Single culture and mixed culture	*Thypa*	Pb
24	Han et al. (2022)	Bacteria	Single culture	*Triticum aestivum* L.	Cd
25	Ilyas et al. (2022)	Bacteria	Single culture	*Triticum aestivum L.*	Cd, Cr
26	Tripathi et al. (2022)	Bacteria	Single culture	*Bacopa monnieri L.*	As
27	Hagagy et al. (2023)	Archaea	Single culture	*Triticum spp.*	Co, Zn, Mn, Fe, Cu
28	Lu et al. (2023)	Bacteria	Single culture	*Chrysopogon zizanioides*	Cr, Cd, Pb, Mn
29	Wang et al. (2023)	Fungi	Single culture	*Glycine max L.; Solanum nigrum*	Cd

Table 2: Summary of the effect size Hedges'd of HMS content and plant biomass.

Response parameter	n	Effect size	Lower bound	Upper bound	Standard Error	P-value
HMS content in the soil	145	-3.257	-3.868	-2.645	0.312	< 0.001
HMS content in plants	211	1.234	0.855	1.614	0.193	< 0.001
Plant biomass	60	2.301	1.57	3.031	0.373	< 0.001

Remarks: *Significant changes occurred when the 95% confidence interval of the effect size did not overlap with zero

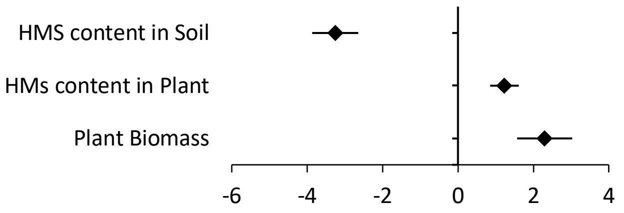


Figure. 3 The effect of microorganism inoculation on heavy metals and plant biomass

3.2. Impacts of moderator variables on HMS content in soil and plant after microbial application

Figure 4 shows that the application of microbes with mixed culture to the HMS content in soil showed the highest effect size (ES) with a value of -3.5 (p<0.001). However, applying a single culture had the highest influence on the HMS content in plants, with an ES value of 1.439 (p<0.001) (Fig. 4b). Application of bacteria and fungi together as treatment can give the highest effect size value in HMS content in soil (ES=-5.519, with p<0.001) (Fig. 4c). Bacterial application had the highest influence on HMS uptake in plants, with an ES value of 4.681 (p<0.001) (Fig. 4d). The HMS content in the soil that decreased the most was the Cu content, with an ES value of -13.642 (p<0.001) (Fig. 2e). The highest amount of HMS uptake in plants is the Pb type, with an ES value of 2,645 (p<0.001) (Fig. 4f). When *Glomus, Gigaspora, Azotobacter, and Rhizobium* are used as treatments along with fungi and bacteria, the HMS level in the soil drops the most, with an ES value of -12.41 (p<0.001) (Fig. 4g). Meanwhile, *Bacillus megantherium* bacteria provided the highest HMS uptake in plants with an ES value of 29,043 (p<0.001).

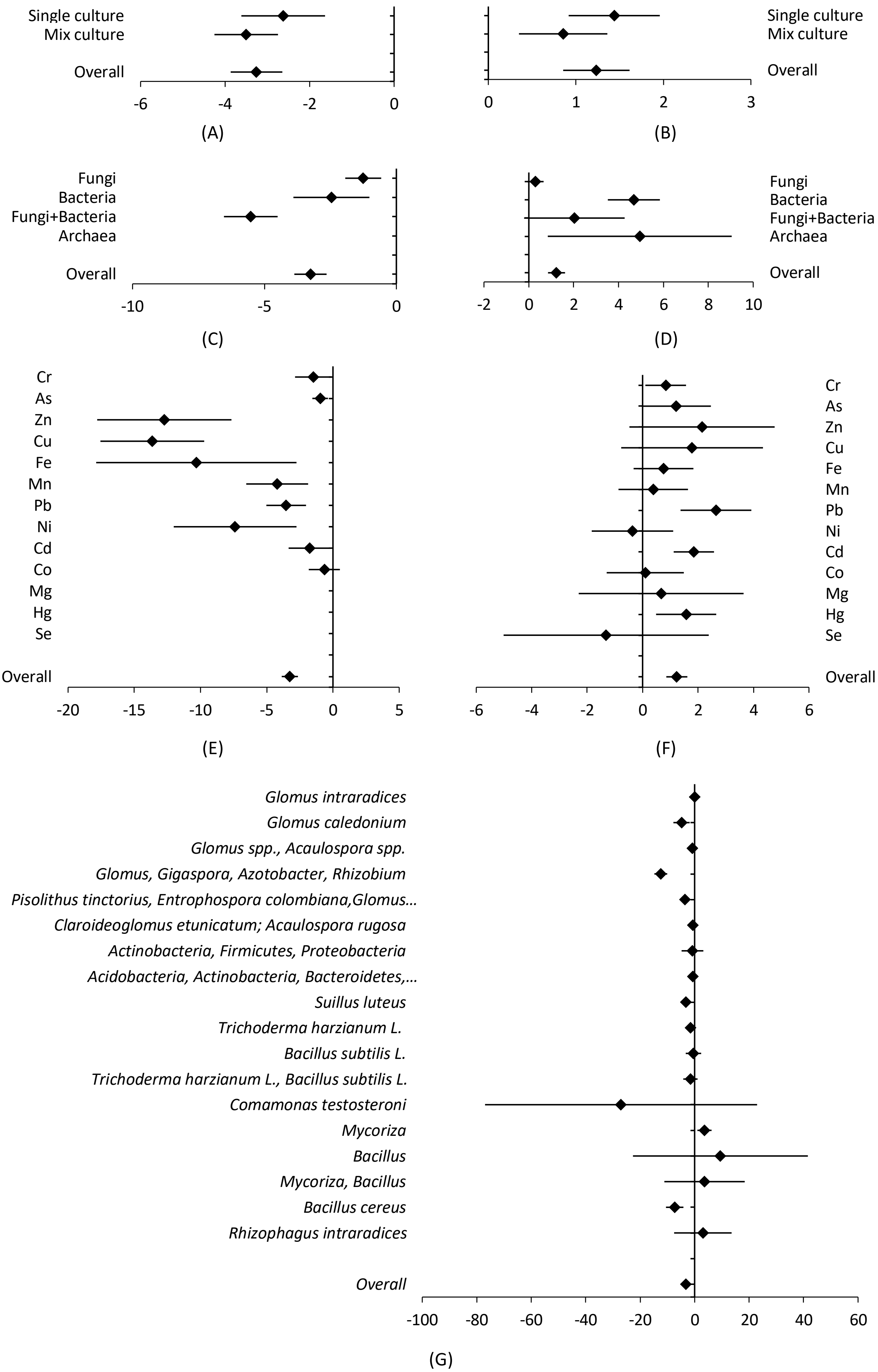
3.3. Impacts of moderating variables on plant biomass after microbial inoculations

Figure 5 shows that the meta-analysis results with Hedges'd show that applying microorganisms to polluted soil can increase plant biomass. The application of microbes with mixed culture had the highest influence on plant biomass with an ES value of 8.206 (p<0.001) (Fig. 5a). The use of mixed cultures of fungi and bacteria simultaneously showed the most significant influence on plant biomass with an ES of 8.774 (p<0.001) (Fig. 5b). The highest HMS uptake was obtained in the shoot part of the plant with an ES value of 1.601 (p<0.001). If HMS uptake in plant parts based on ES values is sorted, then the sequence becomes total plant>shoots>roots>fruit>leaves (Fig. 5c). The use of fungal and bacterial treatments like *Glomus, Gigaspora, Azotobacter*, and *Rhizobium* together led to a rise in plant biomass (ES value = 8.774; P<0.001).

4. DISCUSSION

This meta-analysis study aimed to investigate the impact of microbial inoculation on HMS content in soil, HMS content in plants, and plant biomass. Apart from influencing these three parameters, this meta-analysis study shows that the HMS content in the soil, HMS content in plants, and plant biomass (Fig. 3) also change according to the culture application method, the microbial group used, the type of HMS, and the microbe species/genus used (Fig. 4 & Fig. 5). The application of microorganisms to polluted soil can also increase plant biomass. Cohen's Benchmark provides rough estimates with a mean effect size of d > 0.8, indicating a large effect, 0.2 < d < 0.8 a medium effect, and 0 < d < 0.2 a small effect (Arft et al., 1999; Fedrowitz et al., 2014). The results of the meta-analysis show that the use of microbes has a large effect on the HMS content in soil (negative effect) and plants (positive effect), as well as on plant biomass (positive effect). This big impact can be understood as one illustration of the multi-role function of microbes in soil. These results also show that the presence and number of microbes with special abilities can be increased to solve environmental problems such as pollution. The specific capabilities of microbes in the soil can be enhanced through a combination of inoculation with other functional microbes or with other suitable types of organisms, such as plants, thereby improving the process and quality of soil bioremediation (Chaudhary & Shukla, 2019; Yang et al., 2020).

Microorganisms have been widely used as biological agents to help reduce soil contamination by HMS. The addition of microorganisms can undoubtedly improve the physicochemical properties of soil and support plant growth, thus significantly affecting phytoremediation methods (Iqbal et al., 2019; Nedjimi, 2021; Sarma et al., 2024). Therefore, these factors directly influence our methodological approach, especially in terms of the selection of the type of microorganisms, the application dose, and the soil and plant parameters observed. Microorganisms provide nutrients and growth hormones to phytoremediator plants through their ability to cycle soil nutrients, secrete growth hormones, produce organic acids, buffer soil pH, or act as decomposers in the soil (Guzmán-Moreno et al., 2022; Han et al., 2021; Tang et al., 2024). Some microbes are essential as mediators of plant-soil relationships. The functions of microbes in the soil are critical for influencing the soil's biological properties, ultimately changing the soil's properties as a whole. Our results show that a combination of microbes plays a significant role in HMS reduction in soil (Fig. 4a). We assumed that HMS accumulation in plants depends not only on HMS levels in the soil but also on microbial interactions within the rhizosphere. The application of microorganisms with mixed cultures is thought to improve soil health biologically, especially in the rhizosphere, by becoming a pioneer in soil microbial diversity so that essential soil functions can be supported. Mixed cultures are considered to be able to provide essential elements in the soil that can be used for the growth of other organisms. However, it is likely that microbial strains isolated from contaminated soil are more tolerant of metals and have developed resistance (Ahemad, 2019). Microbes with a high level of environmental tolerance are better able to play their role in the nutrient cycle in the soil. Mechanisms that can be carried out by microbes, such as the secretion of organic acids, growth regulators, chelating agents, and soil enzymes, are thought to support plant growth so that heavy metal uptake is more effective (Manoj et al., 2020).


Single culture
Mix culture
Overall
-6 -4 -2 0
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Single culture
Mix culture
Overall
0 1 2 3
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Fungi
Bacteria
Fungi+Bacteria
Archaea
Overall
-10 -5 0
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Fungi+Bacteria
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Overall
-2 0 2 4 6 8 10
(D)
Cr
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-6 -4 -2 0 2 4 6
(F)
Glomus intraradices
Glomus caledonium
Glomus spp., Acaulospora spp.
Glomus, Gigaspora, Azotobacter, Rhizobium
Pisolithus tinctorius, Entrophospora colombiana,Glomus…
Claroideoglomus etunicatum; Acaulospora rugosa
Actinobacteria, Firmicutes, Proteobacteria
Acidobacteria, Actinobacteria, Bacteroidetes,…
Suillus luteus
Trichoderma harzianum L.
Bacillus subtilis L.
Trichoderma harzianum L., Bacillus subtilis L.
Comamonas testosteroni
Mycoriza
Bacillus
Mycoriza, Bacillus
Bacillus cereus
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Overall
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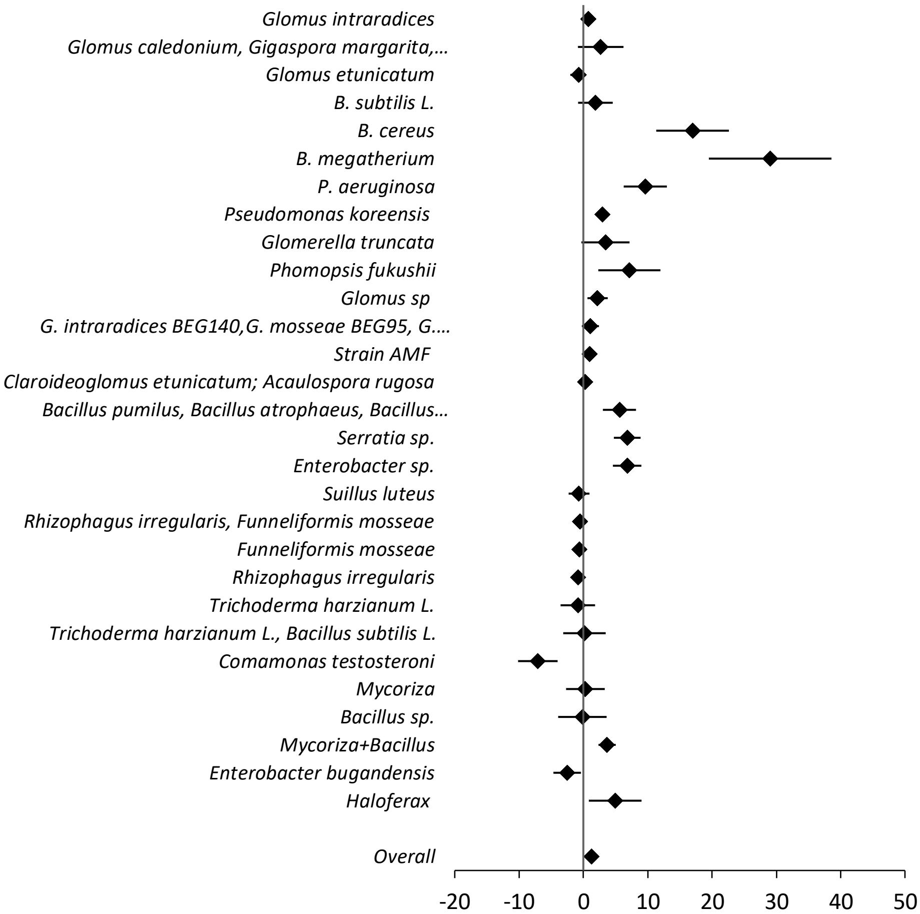

(H)

Figure. 4 Effect size Hedges'd for the HMS content under different applications in soil (A) and plant (B); microbes group in soil (C) and plant (D), HMS type in soil (E) and plant (F), microbes species/genus in soil (G) and plant (H). The effect size was significant if the 95% bootstrap confidence interval did not include zero

On the contrary, only certain microbes can associate with certain plants. In this meta-analysis study, application with a single culture had the highest influence on HMS uptake in plants (Fig. 4b). This is thought to be because the role of microorganisms in plants is more specific when compared to the position of microorganisms in soil (Reynolds et al., 2003). This particular role is the association of microbes with plants, making HMS absorption more effective. For example, arbuscules play an important role in mineral ion exchange in root cortical cells, increasing plant tolerance responses, chelation of organic material around roots, limiting uptake, and direct biosorption by hyphae (Xu et al., 2024).

Mycorrhiza is known as a type of microbe that can colonize the roots of its host plant. Root colonization by mycorrhiza can increase nutrient and/or HMS uptake from the soil to the plant. Our study found that among the types of HMS, the HMS that was most reduced in soil due to the application of microbes as a bioremediation agent was Cu (Fig. 4e). Cu is a micronutrient in the soil that is quite important for plants. Copper (Cu) plays an important role in plant physiology. Cu is a cofactor for many enzymes involved in photosynthesis, respiration, and antioxidant defense systems (Chen et al., 2022). However, it is important to remember that although copper is necessary for plant growth, excessive Cu content can be toxic to plants. Excessive Cu accumulation can disrupt cellular processes and cause damage. Factors such as soil pH, organic matter content, and the presence of competing ions can influence the availability of copper in the soil (Hou et al., 2019). Soil pH plays an important role in copper availability. Cu tends to be more available to plants in slightly acidic to neutral soil (pH 6.0-7.0). The presence of organic material in soil will make Cu more available. This is due to the role of organic material, which is able to release Cu bonds with other compounds and absorb and exchange metal ions such as Cu. The decrease in Cu levels in the soil indicates that phytoremediation is going well.

The results of meta-analysis studies show that the highest metal uptake in plants is obtained by Pb metal (Fig. 4f). Uptake of lead (Pb) by plants can occur when lead in the soil dissolves in the form of Pb^{2+} ions and is then absorbed by plant roots (Ur Rahman et al., 2024). However, plants usually do not absorb lead in significant amounts, except in certain conditions where the lead content in the soil is very high or certain plant species have a better ability to absorb lead. Lead concentration in soil is the main factor influencing plant uptake. If the lead content in the soil is very high, there is greater potential for plants to absorb lead. Some plant species have a better ability to absorb lead than others. Plants called "lead accumulators" have more efficient root systems and uptake mechanisms for lead (Moogouei & Chen, 2020).

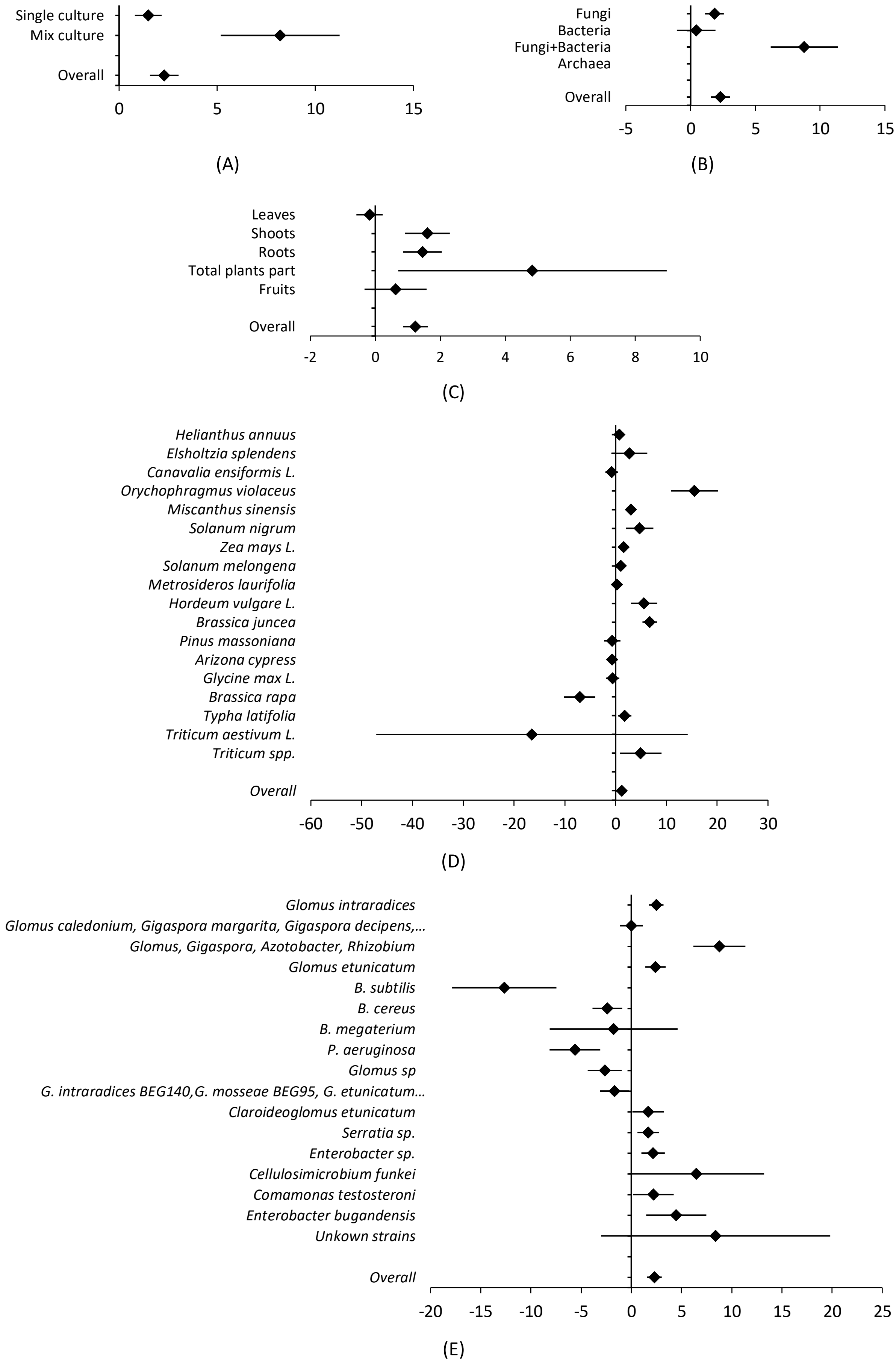

Figure 5 Effect size Hedges'd for the plant biomass under different applications (A), microbes group (B), part of plants (C), plants species (D), and microbes species/genus (E). The effect size was significant if the 95% bootstrap confidence interval did not include zero

This plant is often used in phytoremediation practices to clean lead-contaminated soil. Soil conditions such as pH, moisture, and texture can influence lead uptake by plants. Soil conditions that support microbial activity that reduces lead to the form of Pb^{2+} ions can increase uptake by plants (He et al., 2024).

Other studies have shown that several types of bacteria have been successfully applied to absorb heavy metals in soil with several types of plants. Among others, Azospirillum brasilense can reduce the content of Cd, Ni, Pb, and Zn in pa choi plants (Wu et al., 2025), *B. cepacian* can reduce the toxicity levels of Cd and Pb in tomato plants (Janaki et al., 2024), and *Stutzerimonas stutzeri* and *Pseudomonas Sundara* can increase the absorption of heavy metals Cd, Cr, and Pb in sunflower plants (Waseem et al., 2024). These findings reveal that soil conditions with certain types of plants provide an environment that supports certain bacteria in the absorption of certain metals.

Treatment *of Glomus, Gigaspora, Azotobacter, and Rhizobium* together as a mixed culture was able to achieve the highest HMS in the soil (Fig. 4g). It is known that mycorrhizal fungi (*Glomus, Gigaspora*) are effective in colonizing plant roots, forming an external protective layer that enhances nutrient absorption and protects against pathogens and soil stress. Meanwhile, *Azotobacter* is a free-living bacterium in the rhizosphere, capable of fixing Nitrogen (N) and secreting growth hormones. *Rhizobium* is able to create nodules with its host plant and helps fix Nitrogen better. These results indicate that the association between microbes and suitable host plants is able to increase HMS uptake in plant tissues. However, mixed culture showed a strong influence on HMS uptake. Microorganisms, such as plant growth-promoting rhizobacteria (PGPR) and *Arbuscular Mycorrhizal* fungi (AMF), enhance nutrient uptake and promote plant growth, which is vital for phytoremediation success (Iqbal et al., 2019). Unfortunately, most hyperaccumulating plants frequently accumulate certain metals only, which shows limited applicability to locations with many mixed contaminants (Pasricha et al., 2021). Meta-analysis studies show that the treatment of *Bacillus megatherium* bacteria shows an increase in HMS in plant tissue. *Bacillus megatherium* is one of the many bacteria with potential bioremediation applications, and its effectiveness depends on the specific context and pollutants involved (Guzmán-Moreno et al., 2022). The organic acids produced by these bacteria are able to break the bonds of HMS with soil colloids. Plants easily absorb the breakdown products of HMS.

The abilities of soil microbes are very diverse, including increasing the availability of nutrients in the soil. The availability of nutrients in the soil is important in supporting plant growth. The results of this meta-analysis study show that the application of functional microbes in soil, apart from reducing HMS content in soil and increasing HMS uptake in plants, can also increase plant biomass. The microbial application method with mixed culture had the greatest influence on increasing plant biomass (Fig. 5a). This is thought to be due to the functional role of each microbe applied to support plant growth. Plants need complete essential nutrients to grow and develop well. Macro and micronutrients must be sufficiently available in the soil. The combination of fungi and bacteria together as a mixed culture treatment provides the highest influence in increasing plant biomass (Fig. 5b). In previous reports, it was shown that the combination of mycorrhizae and plant growth-promoting rhizobacteria (PGPR) can significantly enhance plant growth even under stress conditions. This microbial mixture helps regulate nutrient and hormone balance, produces plant growth regulators, solubilizes nutrients, and induces resistance to pathogens (Nadeem et al., 2014). Other reports showing similar effects include the combination of *Pseudomonas mendocina* and arbuscular mycorrhizal (AM) fungi in lettuce (*Lactuca sativa* L. cv. Tafalla) (Kohler et al., 2008), *P. fluorescence* and *G. mosseae* in legumes (*Phaseolus vulgaris*) (Younesi & Moradi, 2014), and *Planomicrobium chinense* strain P1 and *Bacillus cereus* strain P2 in plants under drought stress (Chieb & Gachomo, 2023). Increased plant growth is a key indicator for assessing the success of phytoremediation efforts. Plants that grow well have good roots, which, apart from being able to absorb nutrients, can also absorb more HMS. Even though all parts of the plant are capable of storing HMS, meta-analysis studies show that shoots are the most abundant part of the plant (Fig. 5c). The order of plant parts in storing HMS from largest to smallest is shoots>root>fruits>leaves. This can be attributed to their respective physiological roles and storage capacities. Each part makes a different contribution to the overall nutrient storage and health of the plant, which is essential for survival and reproduction (Harrison & Morris, 2018).

Meta-analysis studies show that *Orychophragmus violaceus* is the plant that absorbs the most HMS. *Orychophragmus violaceus* is a species of *Brassicaceae* that is widely cultivated in China, especially in winter cover crops in northern China, due to its low temperature tolerance and low water requirements (Huang et al., 2023). Having the ability to survive in soil with minimal water availability, it is thought that the strength of this plant lies in its roots (Dutta & Sarma, 2022; Mair et al., 2022). Plants have a wide distribution of roots, and many can absorb nutrients better. A number of plant species in the Brassica genus are suitable for use in phytoremediation because they can withstand the harmful effects of heavy metals (Kaur et al., 2017). Brassica species possess the capacity to engage in the phytoremediation of heavy metals (HMs) through various physiological processes, including phytovolatilization (Kumari et al., 2020), phytostabilization (Yadav et al., 2018), and phytoextraction (Sarwar et al., 2017). We assume *Orychophragmus violaceus* roots adapt to the pressure of high HMS concentrations by transporting more HMS from roots to shoots so that the concentration of HMS in the rhizosphere is reduced. These results may be in line with the research of Xing et al. (2022), which reported that the *Orychophragmus violaceus* plant has a high level of adaptation. When plant roots are able to grow well in soil contaminated with HMS, the exposure of HMS to microbes in the soil is reduced. This produces a good environment for microbes to carry out their functions so that plants are able to absorb essential elements resulting from the work of microbes. Plants have developed tolerance mechanisms for excess metal concentration, including metal efflux, reduced metal uptake by root immobilization or mycorrhizal action, intracellular chelation by metal complexes with phytochelatin (PC), metallothionein, or other organic molecules such as amino acids or organic acids, and compartmentalization within the vacuole (Thakur et al., 2022).

The results of the meta-analysis showed that the joint application *of Glomus, Gigaspora, Azotobacter*, and *Rhizobium* together as a mixed culture showed the highest impact on plant biomass. The biological interactions between plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi are believed to cause this cumulative effect on all components of the rhizosphere. These interactions are also influenced by environmental factors such as soil type, nutrient availability, humidity, and temperature (Shah et al., 2021). Glomus and Gigaspora fungi are known mycorrhizal fungi that can colonize plant roots, and their presence in the soil has been widely reported to improve nutrient uptake, plant growth, and crop yields (Fall et al., 2022). Meanwhile, *Azotobacter* and *Rhizobium* microbes are functional microbes that are known to increase N nutrient uptake in plants. Soils contaminated with HMS usually have low nitrogen content in addition to low organic C content. Inoculated plants with nitrogen-fixing microorganisms will be better because the Nitrogen needs for plants are met. This nitrogen fixer also releases supporting growth-promoting substances and plant growth (Jaiswal et al., 2021). The presence of PGPR and mycorrhiza in the rhizosphere is beneficial not only for plant growth but also for each other. On the other hand, bacteria stimulate hyphae growth by increasing cell permeability, thereby facilitating root penetration by fungi, while mycorrhiza increases the activity of nitrogen-fixing and phosphorus-solubilizing bacteria (Linderman, 1992). Even though the data collected in this meta-analysis study is robust, there is still the possibility of bias that may occur due to unpublished or unreported data. Therefore, it is important to look at each piece of data in detail and thoroughly examine it in terms of the data recording process and research methods used.

5. CONCLUSION

The results showed that microbial inoculation can reduce HMS content in soil, increase HMS in plants, and increase plant biomass. Mixed culture is most effective in reducing HMS in the soil, while single culture is successful in increasing HMS uptake in plants. The mixed culture treatment obtained the highest plant biomass. The combination of bacterial fungi (*Glomus, Gigaspora, Azotobacter,* and *Rhizobium*) together as a mixed culture was able to reduce the HMS content in the soil. In contrast, the pure culture of *B. megatherium* was able to increase the highest HMS uptake significantly. The order of plant parts in storing HMS from largest to smallest is shoots>root>fruits>leaves. *Orychophragmus violaceus* is the plant that absorbs the most HMS. However, there are still research gaps related to the effectiveness of microbes against specific types of heavy metals, the physiological and molecular mechanisms underlying the remediation process, long-term stability in field conditions, and the risk of bioaccumulation in the food chain that need further research.

To reduce heavy metal pollution, sustainable and environmentally friendly practices are essential. This may include adopting cleaner production methods in industry, reducing inputs containing heavy metals in agriculture, proper waste management, soil testing, and remediation, and implementing regulations to limit emissions and exposure to heavy metals.

Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear to influence the work reported in this paper.

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