A meta-analysis on crop growth and heavy metals accumulation with PGPB inoculation in contaminated soils

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Abstract: Plant growth-promoting bacteria (PGPB) have shown great potential in amelioration of heavy metals (HMs) stress on crops and received widespread attention. However, the PGPB remediation effect and its underlying mechanisms remain poorly understood in the soil-plant system. We therefore conducted a meta-analysis with 2037 observations to quantitatively evaluated the effects and determinants of PGPB inoculation on the crop growth rate and HMs accumulation in contaminated soils. Soil organic matter (SOM) and exogenous fertilizers (N, P, K) significantly enhancing crop nutrient uptake and prevent the entry of HMs were important overall drivers of the inoculation efficiency. Inoculation increased shoot and root biomass of all crops (rice, maize, wheat, soybean and sorghum) and the decreased metal accumulation in rice and wheat shoots together with wheat roots. This PGBP effect was significant for biomass production or HM accumulation in four of five bacterial inoculates employed. However, the phylum *Proteobacteria* was the keystone taxa, being the most effective in reducing the heavy metal stress of crops in both the shoot and roots. More antioxidant enzyme activity, photosynthetic pigment and nutrient absorption were induced. Overall, using PGPB inoculation improved the growth performance of all five crops, significantly increased crop biomass in shoots, roots, and grains by 33%, 35%, and 20%, respectively, while concurrently significantly decreasing heavy metal accumulation by 16%, 9%, and 37%. These results are vital to grasping the benefits of PGPB and its future application in enhancing crop resistance to HMs.

Keywords: Plant growth promotion bacteria (PGPB), Heavy metals (HMs), Food crop security, Meta analysis

1 Introduction

Heavy metals (HMs) pollution is considered a main source of soil pollution worldwide, which has a robust negative influence in agricultural land (Oumenskou et al., 2018; Singh et al., 2021; Wang et al., 2022b). Excessive concentration of HMs will poison crops by interfering with their normal physiological and biochemical process to suppress growth and quality, resulting in human health risks through the food chain, such as digestive problems, neurological dysfunction and even cancer (Mungai et al., 2016; Tumanyan et al., 2020; Wang et al., 2022a). In order to ensure food security, strategies increasing crop yields and minimizing the accumulation of HMs during HMs stress spells is urgently needed. Recently, the inoculation of PGPB has been extensively researched and is highly sought after due to its environmentally friendly nature and high efficiency (Carreiras et al., 2023; Etesami et al., 2023).

Plant growth-promoting bacteria (PGPB) are beneficial bacteria that reside in the rhizosphere soil or inside plants, including rhizosphere, endophytic, and other bacteria that are known to promote plant growth and phytoremediation (Kong and Glick, 2017). It has been reported that mycorrhiza-assisted interactions between bacteria and crops can improve their yield and resistance to abiotic and biotic stress (Ma et al., 2022; Ramakrishna et al., 2019； Reddy et al., 2023). PGPB has emerged as a biological inoculant for alleviating crop stress in HM-contaminated soil, due to its ability to mitigate the negative effects of HMs through siderophore, auxin, dissolved phosphate, and nitrogen fixation (Pathania et al., 2020; Tirry et al., 2018). Additionally, it can improve the absorption of nutrients by roots, stimulate the activity of antioxidant enzyme in plants to minimize oxidative damage caused by HMs, and enhance plant tolerance (Chen et al., 2017). Among them, the most dominant group of bacteria, *Proteobacteria*, is considered to play a major role in aiding plant growth and protecting against various biotic and abiotic stresses (Negi et al., 2023). Shabayev and Ostroumov (2023) introduced three *Pseudomonas* strains to soil heavily contaminated with nickel, resulting in improved wheat resistance to high metal concentration through the stimulation of root growth and enhancement of plant mineral nutrition, which was reflected in an increased yield. Studies have suggested that rhizosphere microorganisms typically facilitate positive plant-soil feedback under heavy metal stress (Venturi and Keel, 2016). However, little attention has been given to the varied impacts of different PGPB taxa on crop growth under heavy metal stress. Identifying the distinct advantages of taxa and pinpointing the most beneficial species can serve as a valuable reference for selecting specific microorganisms to achieve effective remediation (Gopalakrishnan et al., 2015; Banerjee et al., 2018).

The primary source of heavy metals in the human food chain is the effective accumulation of these metals in the edible parts of food crops. The transport and transformation of heavy metals in plants are mainly determined by their bioavailability (Etesami et al., 2023). Bacteria can greatly affect the mobilization of metals in soil through the secretion of organic acids, siderophore and metal carriers for adsorption, complexation, redox and other mechanisms (Caracciolo and Terenzi, 2021; Flores et al., 2023). With the potential to regulate HMs accumulation in plants through selective inoculation, PGPB develops multiple strategies (such as accumulation, bioabsorption, and biotransformation) to reduce the transport of toxic metal ions in plant tissues (Pramanik et al., 2021). For example, the inoculation of *Enterobacter aerogenes* MCC 3092, as isolated by Pramanik et al. (2018), into rice rhizosphere significantly reduced the bioavailability of cadmium, decreased the absorption of cadmium in rice tissues, and improved the growth of rice seedlings under cadmium stress. In most studies with PGPB inoculation, a higher metal uptake in roots relative to shoots has been emphasized (Zhuang et al., 2009; Pinto et al., 2010), and it was evident that roots appeared to have a barrier to prevent the transfer of harmful HMs to shoots (El-Meihy et al., 2019). The effects of PGPB inoculation on the accumulation of HMs in crops may be related to soil properties, temperature, humidity and other environmental factors (Tian et al., 2022). Despite numerous studies conducted on the effect size of PGPB inoculation on crops in HMs-contaminated soils, there is still a lack of estimation of the overall effect and the effect size of influencing factors (Lopes et al., 2021).

In order to enhance the efficiency of safe agricultural production, it is imperative to utilize PGPB with a stronger ability. Therefore, in this study, we conducted a global meta-analysis of recent studies published before June 2022 to investigate the response of crop growth to PGPB inoculation in HMs-contaminated soils. We made the following hypothesis: (1) PGPB will have a positive effect on crop growth and effectively alleviated HM stress; (2) The response of crops to PGPB will predominantly be regulated by soil physicochemical properties and temperature; (3) There will be significant differences in crop growth performance among the taxonomic groups at PGPB phylum level. And we evaluated which PGPB bacteria are the most effective species for relieve HMs toxicity, providing a useful source of knowledge for determining the differential benefit of PGPB taxonomic groups.

2 Material and methods

2.1 Data collection

Peer-reviewed journal articles, which investigating the impacts of PGPB inoculation on crop growth and its related variables in HMs-contaminated soil, were searched using the Web of Science (http://apps.webofknowledge.com/), Google Scholar (https://scholar. google.com) and China National Knowledge Infrastructure (http://www.cnki.net) in June 2022. A number of keywords were used for the search including “PGPB”, “PGPR”, “PGP”, “plant growth promoting bacteria”, “plant growth promoting rhizobacteria”, “metal (cadmium, lead, arsenic, copper, nickel, zinc, and chromium)”, “plant (soybean (*Glycine max* L.), sorghum (*Sorghum bicolor* (L.) Moench) or broom corn or kaoliang or kaffir, rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.) or corn).

In order to enhance the comparability of data, the initial collection of articles must meet the following criteria: (1) Each study must contain both PGPB inoculation treatment in HMs-contaminated soil and control treatment without inoculation. (2) There is at least one target variables with respect to crop growth in a literature. Crop growth indicators include root, shoot and grain biomass of crops, and the concentration of HMs in root, shoot and grain of crops. (3) The mean value, sample size, standard deviation (SD) or standard error (SE) can be extracted from the figures and tables in a paper. SE could be converted to SD by a formula: $SD=SE\times\sqrt{n}$, n is the number of repetition (Chen et al., 2018). Based on the above criteria, 150 published papers were obtained, and the geographical distribution of these researches was shown in Fig 1. For each study, we extracted main information of soil-crop system (crop and soil characteristics, experimental conditions), gathered data from figures with the assistance of WebPlotDigitizer 3.8 (Burda et al., 2017). The data from a study with other extra manipulations (e.g., heat stress, salt stress) would be filtered out (Liu et al., 2023). If a study is measured multiple times during the whole experiment period, the data should be collected from the last sampling period to satisfy the principle of meta-analysis (Hedges et al., 1999). In our research, crop growth characteristics (biomass and heavy metals concentration) were used as response indicators (dependent variables). The related data of soil physical and chemical properties (pH, SOM, available heavy metal content), experimental variables (temperature, humidity, inoculation method, PGPB type, crop type, heavy metal type, fertilization or not) and other plant growth indicators (photosynthetic pigments, antioxidant system, nutrient absorption content) were quantified to analyze the mechanism of crop growth response to PGPB inoculation more comprehensively. Additionally, in order to identify the effect sizes of different bacteria taxa, the phyla and genus to which the bacteria used in the paper belong were also summarized. We finally obtained 990 effect sizes and 1048 effect sizes for the effects of PGPB inoculation on crop biomass and HM concentration of crops respectively.

2.2 Data analysis

Nature log-transformed response ratio was selected to represent the influence of PGPB inoculation on crop growth in heavy metal soil (Hedges et al., 1999), and calculated it by equation (1):

$$y_i=\ln RR=\ln\left(\frac{\overline{X}_t}{\overline{X}_c}\right) \tag{1}$$

where $\overline{X}_t$ and $\overline{X}_c$ are the mean of a variable in PGPB treatment group and control group respectively.

Sampling variance v_i representing the accuracy of a study was calculated by equal (2):

$$v_i=\frac{SD_t^2}{n_t\overline{X}_t^2}+\frac{SD_c^2}{n_c\overline{X}_c^2} \tag{2}$$

where SD_t and SD_c represent the standard deviations of the treatments and control groups, respectively; and n_t and n_c represent the sample sizes of the treatments and control groups, respectively.

Random-effects model was employed in calculating cumulative effect size $\overline{y}$ after weighted average, as shown in formula (3):

$$\overline{y}=\frac{\sum_{i=1}^{k} w_i y_i}{\sum_{i=1}^{k} w_i} \tag{3}$$

$$w_i=\frac{1}{v_i+\tau^2} \tag{4}$$

where the weighting of each response ratio (w) is the reciprocal of sum of v_i and τ^2, τ^2 named between-study variance represents the variation of effect size caused by different studies, which is estimated by restricted maximum likelihood estimator (Veroniki et al., 2016). k is the number of studies.

For a more intuitive result, percent changes of variables influenced by PGPB inoculation were estimated as formula (5):

$$\text{change percent} = [\exp(\bar{y}) - 1] \times 100\% \quad (5)$$

We performed the random-effects model to calculate the weighted effect sizes and 95% confidence intervals (CI) of crop growth responses using the rma function in 'metafor' package (Viechtbauer, 2010). If the 95% CI did not overlap to zero, the effect of PGPB inoculation was considered significant ($p < 0.05$). For each test variable, $y_i = 0$ indicates no effect; $y_i > 0$ represents an increasing effect; $y_i < 0$ represents a decreasing effect (Tian et al, 2021). Between-group Q test was chosen to compare the heterogeneity of the effect sizes of variables among group. A significant between-group Q value (QM, $p < 0.05$) indicated that the effect sizes differed significantly among groups (Liu et al., 2023). Meanwhile, a linear regression model was used to analyze the relationship between other crop growth parameters and biomass and HM concentration in order to facilitate our understanding of the mechanism.

Using Rosenthal's fail-safe numbers method ($p < 0.05$) to assess publication bias (Rosenberg, 2005), and as shown in Table S1 there is no publication bias in most studies. While the fail-safe number is less than 5N + 10, the obtained dataset exhibits a publication bias (N is the number of original observations). If there is publication bias in the data set according to Rosenthal's fail-safe number, trim and fill analysis should be performed to recalculate mean effects and CI (Tian et al, 2021). In this study, meta-analysis was performed *via* metafor package in R-4.2.1, and forest maps and regression maps are also drawn in R-4.2.1.

3 Results

3.1 Overall effects of PGPB inoculation on crop growth with HMs stress

Overall, the operation of PGPB inoculation in HMs-contaminated soil not only increased the biomass of crop tissues, but also reduced the concentration of HMs in crop plant tissues (Fig 2, Fig S1b). Compared with uninoculated control, the shoot and root biomass of crops increased by 33% and 35%, respectively (Fig 2ab). Moreover, all five crops exhibited an extremely positive response to PGPB inoculation, especially for sorghum, which increased the root biomass by 70% (Fig 3b). Differently, HM accumulation in shoots and roots of crops decreased by 16% and 9%, respectively, on the whole (Fig 2cd). Wheat displayed highest efficiency, the accumulation of HMs in shoot decreased by 28%, root decreased by 22% (Fig 3cd). In addition, the analysis indicated that grain biomass of crops increased by 20% and HM accumulation reduced by 37% (Fig S1). A striking result was found to be a significant decrease in corn grain biomass (42%) (Fig S2a), which may be due to the fact that the inoculated PGPB was subjected to nutrient competition and failed to exert growth-promoting ability. In addition, the content of available heavy metals in soil decreased by 11% with the inoculation of PGPB (Fig S3a).

3.2 Key influencing factors of PGPB effects

The results showed that there was more obvious effect of PGPB under medium temperature (25 °C–30 °C), the biomass of crop shoot and root increased by 26% and 33%, respectively, and the concentration of HMs both significantly decreased by 15% (Fig 4). With regard to the humidity, the crop biomass increase was the largest under the condition of >70% (Fig 4a, b), in the group of 60%–70%, heavy metal concentration in shoot and root of crops were largely attenuated by 25% and 27% respectively (Fig 4c, d). Similarly, different soil pH had different impacts on the biomass and HM concentration of crops with PGPB in contaminated soil ($Q_m = 301$, $p < 0.05$; $Q_m = 170$, $p < 0.05$; $Q_m = 62.3$, $p < 0.05$; $Q_m = 23.3$, $p < 0.05$). For instance, shoot biomass of crops inoculated with PGPB at high pH was 1.9 times higher than that without PGPB inoculation (Fig 4a). However, acidic (pH < 6) and alkaline (pH > 8) conditions were not conducive to the growth and survival of PGPB. Consequently, there was an increased tendency of heavy metal (HM) concentration in the crops after PGPB inoculation (Fig 4c, d), which defeated the purpose of reducing HM stress. In addition, the category of metal is also an important factor that cannot be ignored. In the case of cadmium-contaminated soil, inoculation of plant PGPB has the highest positive effect on crops (Fig S4). As for growth condition, the efficiency of PGPB on hydroponic crops was superior to soil-cultivated (Fig 4). Both single and mixed inoculation exhibited higher biomass yield and lower crop HM concentration. However, it is worth noting that the study of single inoculation has shorter confidence interval, so the results were more reliable and convincing (Fig 4).

Furthermore, the comparative analysis of five influencing factors lent weight to the condition of high SOM content and application of exogenous C, N, P and K fertilizers over others to improve PGPB efficiency and alleviate the HM stress on crops (Fig 5). SOM is signally proportional to crop biomass (slope = 0.41, CI = 0.33–0.48, Fig 5a; slope = 0.26, CI = 0.19–0.33, Fig 5b), especially shoot biomass. Meanwhile, it is inversely correlated with HMs concentration in crops (slope = -0.13, CI = -0.22–-0.044, Fig 5c; slope = -0.083, CI = -0.16–-0.0056, Fig 5d), which also has better effect on the ground part. Notably, the ambient temperature is negatively proportional to the concentration of HMs in crop shoot part (slope = -0.46, CI = -0.83–-0.089, Fig 5c), but ambient humidity is proportional (slope = 0.60, CI = 0.055–1.15, Fig 5c), soil pH was directly proportional to crop root heavy metal concentration (slope = 0.43, CI = 0.10–0.076, Fig 5d).

3.3 Taxonomic effects of PGPB on crop performance under HMs stress

The effects of different PGPB taxa on shoot biomass ($Q_m = 372$, $p < 0.001$), root biomass ($Q_m = 327$, $p < 0.001$), shoot heavy metal concentration ($Q_m = 57$, $p < 0.001$) and root heavy metal concentration ($Q_m = 26$, $p < 0.001$) of crops were significantly different (Fig 6). Among them, *Proteobacteria* as the most widely used taxa is the most beneficial to promote the growth of crops, followed by *Firmicutes* and *Pseudomonas* (Fig 6). *Acinetobacter* and *Serratia* belonging to *Proteobacteria* created a significantly positive contribution to crop growth, not only increasing crop biomass, but also reducing heavy metal concentrations in crops (Fig S5).

3.4 Correlations of PGPB effect sizes on crop performance

We evaluated PGPB capacity by taking into account changes in photosynthetic pigment content, nutrient absorption, phytohormone and oxidative damage indicators of crops (Fig 7). As expected, photosynthetic pigment content of crops increased significantly, with chlorophyll content increased by 29% (yi = 0.25, CI = 0.20–0.30) and carotenoids increased by 16% (yi = 0.15, CI = 0.066–0.22). At the same time, the absorption of N, P, K and S nutrients was also improved, with an increase of 23% (yi = 0.21, CI = 0.15–0.27) and 28 % (yi = 0.24, CI = 0.18–0.30) in the shoot and root parts. The average effect values of H_2O_2 and malondialdehyde (MDA) were -0.32 and -0.41 respectively, which content decreased evidently. By contraries, the antioxidant enzymes (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX)) used as active oxygen scavengers levels all went up. Unexpectedly, the presence of PGPB had no effect on the proline and GSH. The results also demonstrated the mean effect values of crop abscisic acid (ABA) and indoleacetic acid (IAA) were negative and that of succinic acid (SA) was positive.

The relationship between growth performance and physiological indicators were also investigated. Effect values of chlorophyll were directly proportional to shoot biomass ($R^2 = 0.16$, $p < 0.05$) and root biomass ($R^2 = 0.057$, $p < 0.05$), carotenoids had the same response (Fig S6). There was a positive correlation between nutrient uptake and biomass (Fig S6). All the four antioxidant enzymes were in direct proportion to biomass (Fig S6), CAT and SOD showed a significant negative correlation with heavy metal concentrations in crop roots (Fig S7). Shoot heavy metal concentration decreased with the reduced ABA content ($R^2 = 0.42$, $p < 0.05$) (Fig S7). Compared to uninoculated controls, the decline in MDA was accompanied by increase in biomass and by decrease in shoot heavy metal concentration ($R^2 = 0.08$, $p < 0.001$) (Fig S7). We also found H_2O_2 was positively correlated with heavy metal concentration in roots ($R^2 = 0.13$, $p < 0.05$) (Fig S7).

4 Discussion

4.1 PGPB promotes the crops growth under HMs stress

HMs in soil can cause toxic effects on growing crops, leading to crop growth inhibition, crop yield and quality reduction (Wu et.al., 2019; Tang et al., 2023). Fortunately, PGPB inoculation can induce crop metal resistance, cope with heavy metal toxicity by improving plant growth and soil fertility (Yong et al., 2014). Our meta-analysis showed a positive effect on crops with PGPB, inducing a significant increase in shoot and root biomass of 33% and 35% (Fig 1a, b) respectively. Also, grain biomass increased by 20% (Fig S1a), indicating that PGPB had the potential to increase crop yield in HMs-contaminated soils. These results are in conformity to the previous study where there was a great improvement of wheat biomass and grain yield with inoculation of *P. aeruginosa* CPSB1 under the treatment of 2007 mg/kg Cu, 36 mg/kg Cd and 204 mg/kg Cr (Rizvi and Khan, 2017).

PGPB inhabiting the rhizosphere of crops has a variety of beneficial effects and can promote crop growth directly or indirectly to improve its ability to withstand adverse stress caused by HMs. The results of crop nutrient elements absorption such as N, P and K significantly elevated by inoculation of strains were strong proofs (Fig 7), and directly reflected in the growth of biomass (Fig S6). This is related to the activation of nutrients in the rhizosphere soil by PGPB and the increase of C, N, P bioavailability (Bilal et al., 2018). In addition, through regulating the plant hormones secreted by crops (Fig 7), the growth of crops is positively improved (Figure S6). These results suggest that the mechanisms involved in the benefits under HMs stress could go beyond mere nutrition. Multiple strategies such as nitrogen fixation, phosphorus solubilization, potassium solubilization and production of plant hormones were employed by crops to directly regulate their traits, promote root elongation, cell division and stomatal opening, ulteriorly improve nutrition uptake and promote growth under HMs stress (Sun et al., 2020). Study proved that *Pseudomonas* sp. (strain K32) isolated from rice rhizosphere contaminated by HMs possessed growth-promoting characteristics of IAA production, nitrogen fixation and phosphorus increase under Cd stress, significantly improving the growth of rice seedlings under cadmium stress (Krishnendu et al., 2021). Moreover, research proposed that hormones produced by endophytic bacteria often stimulate growth and metabolic processes, leading to root elongation and effectively promoting nutrient uptake when falling into abiotic stress (Bilal et al., 2018).

The increasing activities of antioxidant enzymes SOD, POD, CAT, and APX, and the reducing contents of MDA and H_2O_2 in crop tissues (Fig 7) were additional proofs promoting the biomass of crops (Figure S6). In response to HMs stress, PGPB protects plants from the harmful effects of ROS by inducing metal resistance in crops, up-regulating the expression of stress-responsive genes, and activating antioxidant defense systems including enzyme components (such as SOD, POD, CAT, APX) and non-enzymatic components (such as ascorbic acid, cysteine, and glutathione) (Gopalakrishnan et al., 2015; Etesami et al., 2018; Pramanik et al., 2021). It has been proved that *Rhizobium* sp. (AS5) inoculated with *Arabidopsis thaliana* induces the expression of GA 30-oxidase gene and up-regulates AUX/IAA1 (transcription suppressor of auxin response gene) gene under nickel stress, therefore its biomass reduction is offset by stimulating antioxidant activity (Sujkowska et al., 2022). Islam et al. (2014) inoculated *Pseudomonas aeruginosa* into Zn-stressed soil and found that its access not only reduced the accumulation of MDA and H_2O_2, but also increased the contents of antioxidant enzymes SOD, POD, CAT and ascorbic acid, which ameliorated the harmful effect of high concentration of Zn on wheat plants. The improvement of antioxidant enzyme activity may be due to the regulation of the abundance of antioxidant enzyme gene /mRNA expression in plants. It was found that under multiple stresses (salt and HMs), bacterial inoculation enhanced the gene /mRNA expression of antioxidant enzyme in plants, resulting in an increase in antioxidant enzyme activity, and compared with uninoculated stressed plants, MDA and H_2O_2 were decreased in inoculated plants (Gururani et al., 2013). What is more, PGPB also enhances the stress resistance of crops by enhancing crop photosynthesis, regulating plant endogenous hormone levels, regulating crop physiology to reduce the toxic damage of HMs. As can be seen from Figure 7, PGPB significantly increased the content of chlorophyll and carotenoids in crops, reflecting the enhancement of plant photosynthesis function in some degree (Cutraro and Goldstein, 2005). They also reduced the production of endogenous abscisic acid (ASA) and auxin, and increased the production of salicylic acid (SA). Consistent with previous results that *Enterobacter cloacae* NP-4 can promote the conspicuous increase of plant growth parameters and self-synthesis of IAA, further, promoting the secretion of root secretions, reversing the inhibition effect of various stresses on growth, and improving light and performance by working with plant endogenous IAA (Singh et al., 2022).

4.2 PGPB reduces the accumulation of HMs in crops

The use of PGPB to prevent heavy metals from entering the food chain through food crops and posing great threat to human health has been extensively studied in recent years (Guo and Chi, 2014; Soto et al., 2019; Zerrouk et al., 2019), we demonstrated the beneficial effects of PGPB with scientific methods, which provided a strong basis for PGPB strategy to reduce the accumulation of HMs in crops. Meta-analysis exhibited that PGPB-mediated the accumulation of HMs in shoot decreased by 16% (Fig 2c) and root by 8.8% (Fig 2d), and the most significant decrease was 37% in edible grains (Fig S1b). The efficacy of PGPB in mitigating the uptake of HMs by crops is largely attributed to their ability to alter the bioavailability of HMs in the soil. By decreasing the bioavailability of metals in the soil, PGPB can reduce the uptake of metals by plant roots, thereby decreasing their accumulation (Burda et al., 2006; Mishra et al., 2017). Previous studies have shown that inoculation with *Streptomyces tendae* F4 can significantly reduce the bioavailability of HMs in soil, and heavy metal accumulation in above-ground sorghum planted in soil also decreased to a certain extent (Phieler et al., 2015). Our results indicated that the availability of HMs in soil was significantly reduced under the induction of PGPB (Fig S3), which provided evidence that PGPB reduced crop accumulation of metals by limiting their mobility. Bacterial cell walls, present in the rhizosphere region, possess a negative charge due to their chemical composition. This allows them to interact with free heavy metal (HM) ions through processes such as electrostatic adsorption, ion exchange, and chelation with negatively charged active groups. Consequently, these bacterial cell walls help reduce the biological availability of metal ions in the soil, preventing plant root cells from absorbing excessive metals and mitigating potential toxicity (Guo et al., 2015). In addition, plant roots interact with rhizosphere microorganisms to produce extracellular polymeric substances (EPS) located on or outside the microbial cell wall during symbiosis and self-growth, which can non-specifically adsorb heavy metal ions, isolate them in soil, reduce their transport and accumulation in crop roots (Lian et al., 2022). Wei et al. (2023) manifested that EPS could regulate the remodeling process of rice root cell wall, affect its substance synthesis efficiency and content, and play a crucial role in limiting the absorption and transport of Cd by rice roots (Wei et al., 2023). Fourier transform infrared spectroscopy was used to analyze the EPS produced by a heavy metal-resistant *Azotobacter* sp., which founding functional groups such as carboxyl (-COOH) and hydroxyl (-OH) groups were mainly involved in the binding of metal ions and inferring nitrogen-fixing bacteria participated in the complexation of metal ions through EPS (Joshi et al., 2009). Complexing with other environment-related metals such as Cd, Cu, Pb, etc., siderophores secreted by microorganism can mediate the HM competitive ion transport channel with similar properties and structures to trace elements to regulate the absorption and transport of HMs (Nazar et al., 2012; Liu et al., 2023). Besides, metallothionein produced by plant microbial symbionts could be combined with metals to form metallothionein crystals or precipitates to reduce metal ion activity, alleviate metal toxicity and inhibit the transfer of HMs from underground to aboveground (Liu et al., 2023). These may be the reason that the accumulation of HMs in the underground, aboveground, and grain tissues decreased successively. In our study, it is worth noting that the accumulation of HMs in the shoot parts of the mixed inoculated crops decreased significantly by 24%, higher than that of the single inoculated crops (Fig 4c). Similarly, co-inoculation of arsenite oxidizing bacteria and cadmium-tolerant bacteria greatly reduced the transfer of arsenic in rice seedlings and reduced the accumulation of arsenic in rice seedlings, which was related to increased sulfide stimulation by co-inoculation (Thongnok et al., 2018). In addition, Chiboub et al. (2020) showed that co-inoculation of *rhizobium* and *pseudomonas* increased the excretion of node genes in the root system of coronium seedlings, which might be related to lateral root yield, root hair density and root hair branching, resulting in relief of HM stress. However, considering the complex symbiotic interaction between rhizosphere microorganisms and crops, more studies are needed to further explain the mechanism of synergistic symbiosis between PGPBs to alleviate the toxicity of HMs on crops (Rojas-Tapias et al., 2014; Chiboub et al., 2020).

The accumulation of HMs in crops has a great relationship with soil properties and external environmental conditions, among which PGPB inoculation efficiency is mainly determined by SOM and whether the soil is fertilized or not (Fig 5). Under different soil pH, planting temperature and humidity conditions, the accumulation of HMs in crops varies greatly (Fig 4). It is worth noting that the content of SOM is inversely proportional to the accumulation effect of HMs, and the application of fertilizer in soil can reduce the accumulation of HMs in crops (Fig 5), which is consistent with the research results that N, P and co-existing ions in soil can reduce the accumulation of Cd and Cu in rice grains (Liu et al., 2023). This can be attributed to the higher content of SOM and the application of exogenous fertilizers to increase soil nutrients and fertility, which is conducive to increasing microbial activity and improving microbial working efficiency (Deng et al., 2015; Wu et al., 2021). Besides, SOM contains carboxyl and phenolic functional groups, which can chelate polyvalent metal ions and have strong adsorption capacity for metal oxides. Therefore, a high level of SOM can reduce the mobility of HMs and prevent their absorption by crops at the source (Islam et al., 2022b; Jindo et al., 2023). Available phosphorus also has the capacity to precipitate metal ions and reduce the availability of metals (Bolan et al., 2014), and research shows that application of N (such as urea) reduced the solubility and exchange components of Pb and Cd by changing soil pH (Bolan et al., 2003). Surprisingly, although most studies considered soil pH to be a key factor affecting the availability and mobilization of HMs, our study displayed that there was no special correlation between the effect of HM accumulation and pH. When soil pH was in the neutral range (6–8), the inhibitory effect of PGPB was the most obvious, and the accumulation of heavy metals had a negative effect value (Fig 4). There may be two reasons for this situation: From the perspective of HMs, it is possible that pH value does not directly affect the availability of HMs, but affects their interaction and adsorption with other compounds by influencing the redox potential of soil (Chauhan et al., 2018); From the microbial point of view, beneficial soil microorganisms prefer a pH value between 6 and 7, and acidic and alkaline soil organic biomass and nutrient content are relatively low, reducing microbial activity and unable to exert their growth-promoting properties (Rahman et al., 2021). Therefore, we should pay more attention to the vital role of nutrient content in PGPB inoculation with HM-contaminated soil.

4.3 Mitigation efficiency of Proteobacteria under HMs stress

According to our study, not all the effect sizes of PGPB species on host performance differ from zero (Fig 6). Most PGPB can increase crop biomass, but only *Proteobacteria* has a good inhibition effect on heavy metal accumulation (Fig 6). These results indicate that most PGPB are efficient at providing one benefit, but *Proteobacteria* can provide both benefits. Wu et al. isolated 64 strains of bacteria from the rhizosphere of *Sedum alfredii* in the lead-zinc mining area. The majority of these isolates possessed at least one PGP trait, but all the strains harbored four PGP traits belonged to *Proteobacteria*, and they indicated that *Proteobacteria* was the most dominant group in the inner sphere of plants (Wu et al., 2020). Ulteriorly, they also suggested that *Proteobacteria* might be a keystone taxa in HMs-contaminated soil to trigger the defense mechanism of crops and increase the resistance of crop. Therefore, we boldly speculate that the inoculation of *Proteobacteria* leads to rapid turnover in both structure and function of bacterial communities in contaminated soil, which is more favorable to crop growth (Banerjee et al., 2018).

Studies show that *Proteobacteria* as the most abundant phylum in natural soil, can be highly adaptable to HM pollution conditions and effectively utilize plant-derived carbon (Abdullahi et al., 2020; El Aafi et al., 2015; Hussain et al., 2021). They play a synergistic role with other phyla of bacteria and fungi in dominating the decomposition of soil organic matter, providing plant-available nutrients for growth (Xiao et al., 2022). This may be related to the aspects of their life strategies that might be described as more copiotrophic, which accelerates the degradation of SOM by degrading volatile and refractory compounds such as lignin (Liu et al., 2021). *Proteobacteria* possess several genes encoding heavy metal detoxification enzymes and proteins involved in heavy metal sensing and transcription to improve their competitiveness (Kou et al., 2023). They are preferred to selected in a HMs-contamination soil to colonize (Chen et al., 2018), and as time goes by, the abundance of *Proteobacteria* with the property of high degree adaptability to HMs pollution conditions was proportional to the concentration of HMs (Chauhan et al., 2018). This resistance mechanism of heavy metal is performed by *Proteobacteria* by prohibiting the propagation of pathogens, helping plants absorb nutrients, and secreting foreign hormones or compounds (Chen et al., 2018; Wang et al., 2021). Most of the microorganisms that help fix HMs by producing hydrogen sulfide belong to

Proteobacteria (Carpio et al., 2018). Meanwhile, it reported that functional groups of hydroxyl (-OH), amino (N-H), carboxyl (COO-) and phosphate groups, appeared on the cell surface during bacterial culture, may be used for the binding of metal ions (Wang et al., 2020). Because of these characteristics, *Proteobacteria* has become a more effective control group to reduce the availability of HMs in soil, the accumulation of HMs in plants and promote plant growth. Our results also illustrated that *Acinetobacter* and *Serratia* belonging to *Proteobacteria* were two genera with better stress resistance, significantly reducing the accumulation of HMs (Fig S5). This is consistent with the results of Turpeinen et al. (2004), which reported that *Acinetobacter*, *Enterobacter*, *Edwardsiella*, *Pseudomonas*, *Salmonella* and *Serretia* as dominant genera from arsenic, chromium and copper-contaminated soils using culture-dependent and independent methods. Studies have shown that *Serratia* were capable of using their flagella to form strong C-O-Pb bonds, allowing them to establish self-protection barriers and ultimately resist metal stress (Chen et al., 2019).

4.4 Limitations of out study

Our study highlights the overall effect of PGPB on crop growth in heavy metal soils and the role of PGPB in inhibiting heavy metals in agriculture. Certainly, the deficiencies and uncertainties remain in this study as follows: First, more than 90% of the studies were conducted based on potting experiments to inhibit and control heavy metals through PGPB. The stability of heavy metals in some simulated HM-contaminated soils differs significantly from that in *situ*, which may not accurately reflect the actual effect of PGPB inoculation. To rigorously investigate the effects of PGPB inoculation on crop growth in HM-contaminated soils, prioritizing field experiments is essential. Second, the symbiotic patterns and interactions of microorganisms regulate the structure of microbial communities and ultimately affect their functions in the ecosystem (Kong et. al., 2019). However, the absence of information on changes in crop rhizosphere microbial communities has limited our understanding of the mechanisms behind PGPB inoculation in inducing heavy metal resistance through microbial characteristics (such as microbial functional groups, genes, and enzyme activities). Therefore, there is a need to strengthen research on the response of crop rhizosphere microbial communities to PGPB inoculation. Third, soil nutrient conditions may mask the effects of bacterial colonization on rhizosphere effects (Gan et al., 2021). However, fully paired data including soil properties, microbial biomass, and enzyme activities are limited, which hinders our ability to compare the relative importance of these factors. To address this gap, future studies should focus more on the potential interaction between soil nutrient availability and crop root bacterial colonization on rhizosphere effects.

5 Conclusions

PGPB not only promoted crop growth, but also effectively alleviated the accumulation of HMs. PGPB inoculation inhibits the migration and transport of HMs through multiple mechanisms, including the regulation of physiological metabolic activities and the induction of HM resistance in crops. The PGPB effect was positively correlated with SOM and fertilizer content. *Proteobacteria* exhibited the strongest adaptability and highest efficiency among all microbial taxa. This study provides a basis for alleviating HM stress in crops by rhizosphere PGPB inoculation, and points towards the direction for the application of PGPB as a biological fertilizer to reduce crop HM content in agricultural safety production.

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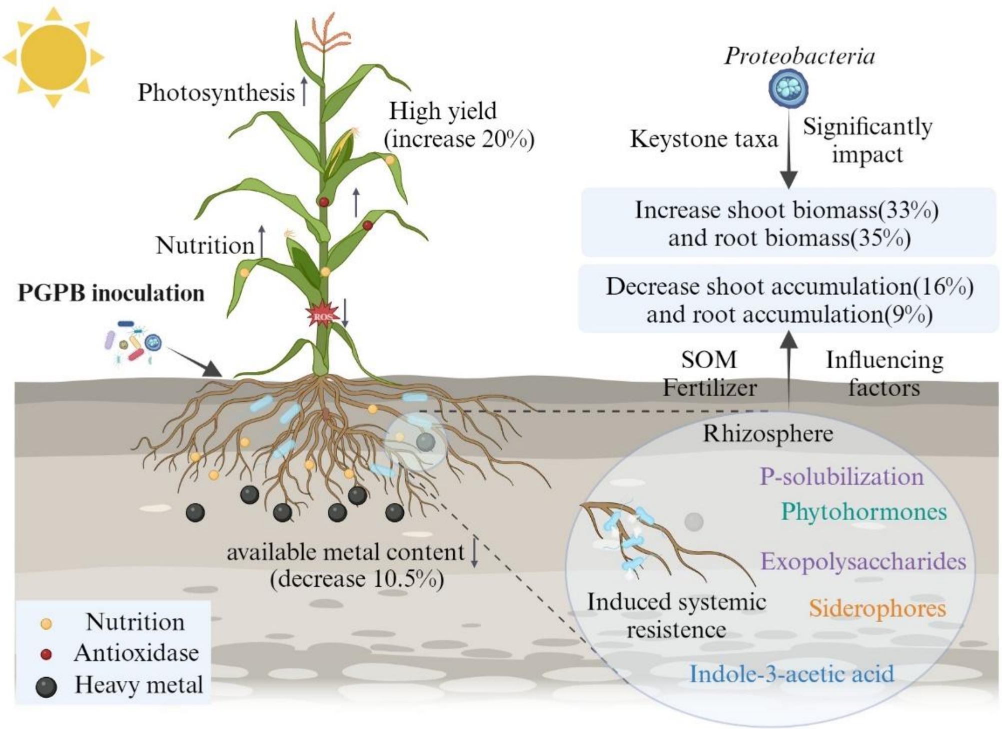
Figure captions



Graphical abstract

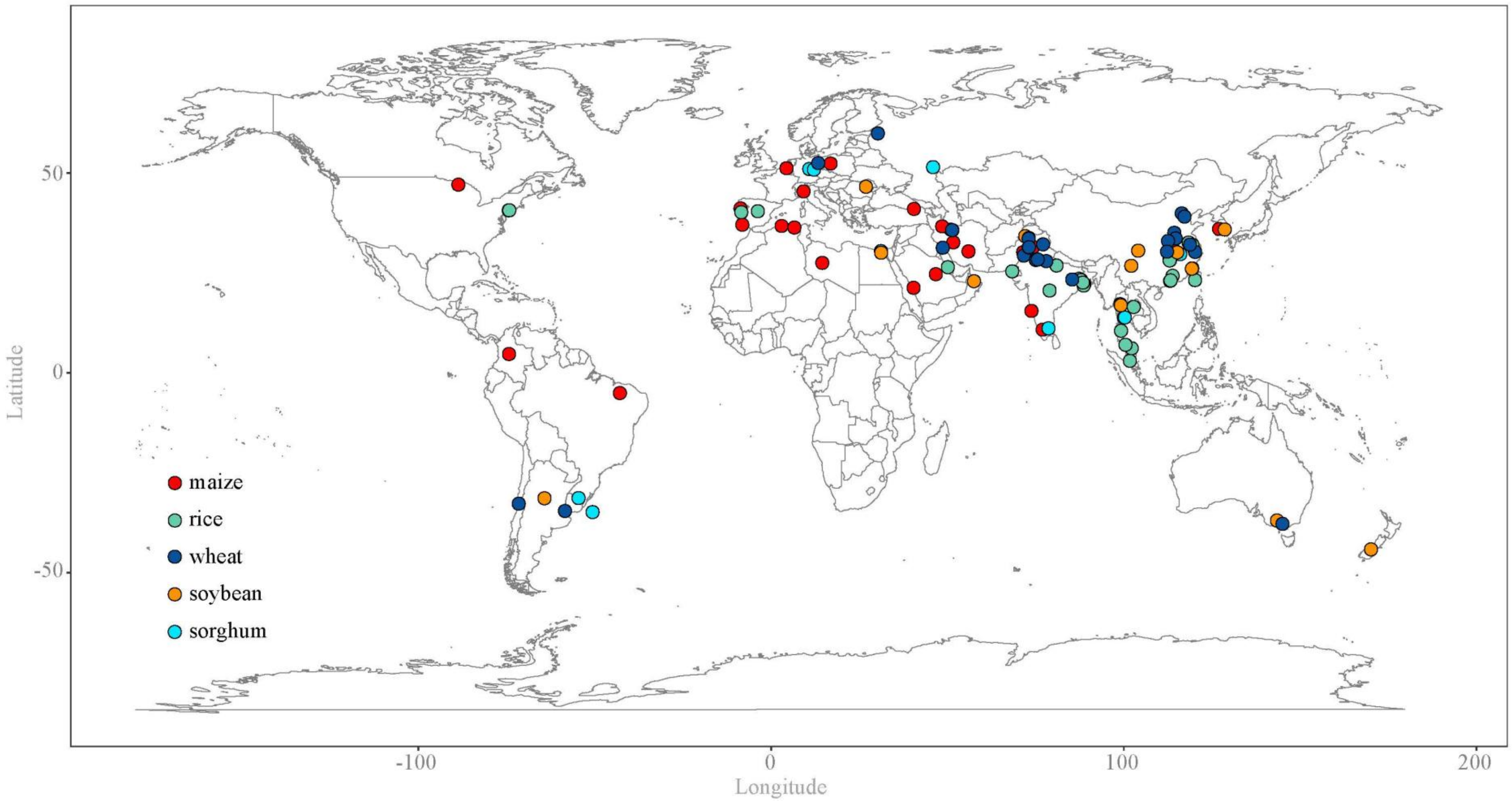


Fig. 1. Distribution of experimental sites

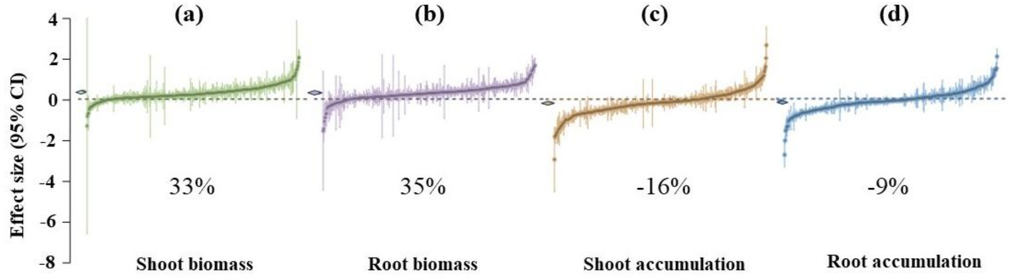


Fig. 2. Forest plot for the effect size estimates of the effect of PGPB on crop biomass and metal accumulation. (a)Shoot biomass, (b)Root biomass, (c)Shoot accumulation, (d)Root accumulation. Purple dots with green lines represent 95% confidence intervals (CI). If the 95% CI does not overlap 0, it denotes a significant effect by PGPB. Overall weighted mean effect size estimate is indicated by purple solid diamond. 33%, 35%, -16% and -9% represent percentage changes in the crop shoot biomass, root biomass, shoot accumulation and root accumulation respectively.

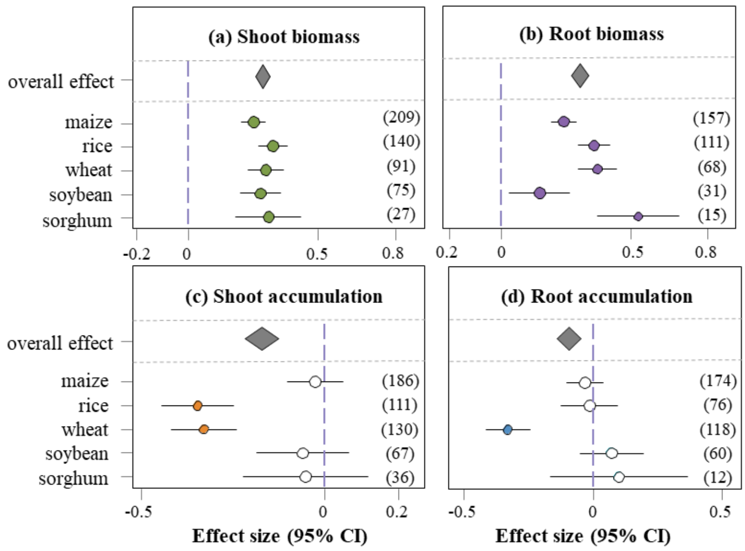


Fig. 3. Changes in the effect of PGPB on crop biomass and metal accumulation in response to various crop type. (a)Shoot biomass, (b)Root biomass, (c)Shoot accumulation, (d)Root accumulation. Horizontal error bars indicate 95% confidence intervals (CI). If the 95% CI does not overlap 0, it denotes a significant effect by PGPB. The sample size for an effect is indicated in parentheses on the right. Overall weighted mean effect size estimate is indicated by purple solid diamond.

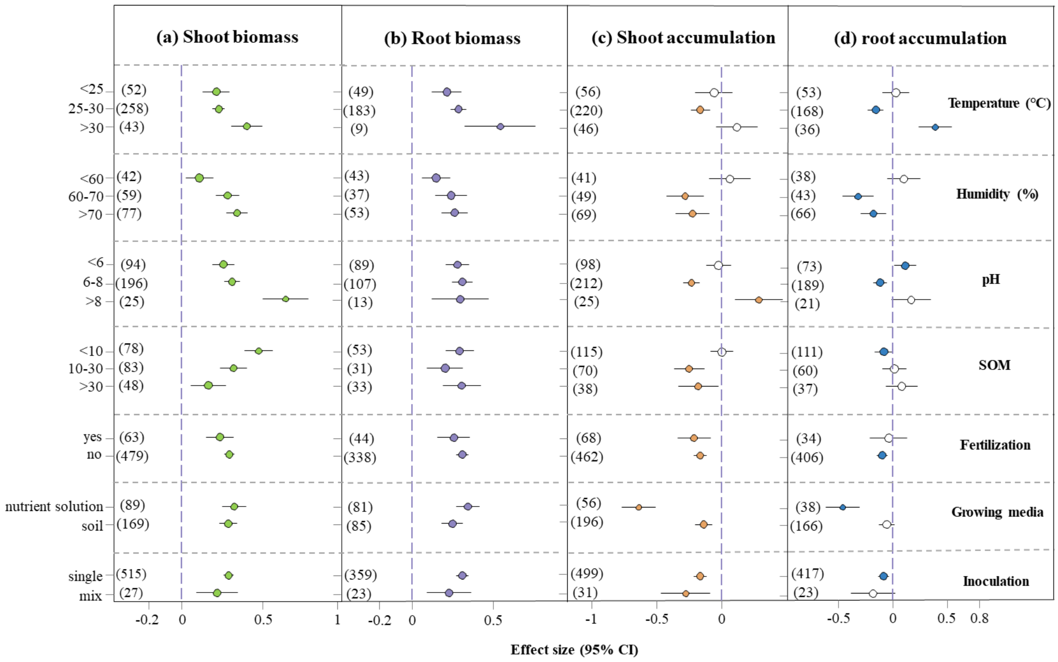


Fig. 4. Changes in the effect of PGPB on crop biomass and metal accumulation in response to various growth environment conditions. The temperature, humidity, soil pH, SOM (g/kg), fertilization, growing media and inoculation were taken into consideration. (a)Shoot biomass, (b)Root biomass, (c)Shoot accumulation, (d)Root accumulation. Horizontal error bars indicate 95% confidence intervals (CI). If the 95% CI does not overlap 0, it denotes a significant effect by PGPB.

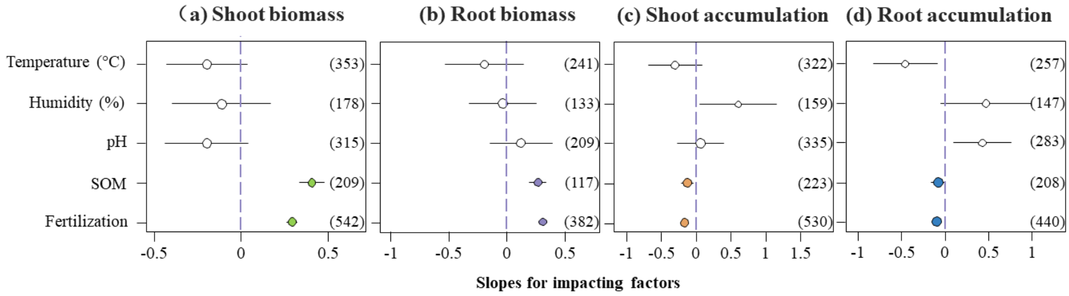


Fig. 5. Factors impacting the biomass and metal accumulation of crops following PGPB inoculation. Circles indicate the slopes of relative changes in crop Shoot biomass (a), Root biomass (b) and Shoot metal accumulation (c), Root metal accumulation (d) with temperature, humidity, soil pH, SOM, fertilization, as well as their 95% confidence intervals (CI). The numbers in the parentheses mean the sample size of examined functions. If the 95% CI does not overlap 0, it denotes a significant effect by influential factors (Crimson circle).

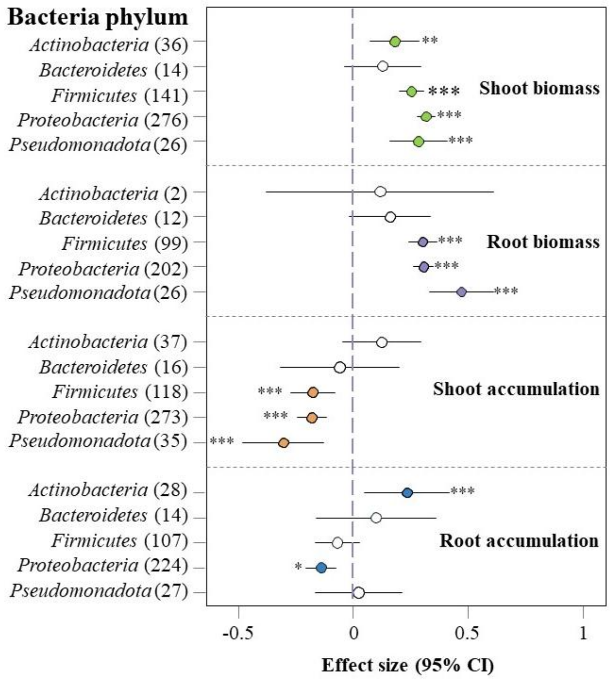


Fig. 6. Changes in crop biomass and metal accumulation in response to various PGPB phylum level. Horizontal error bars indicate 95% confidence intervals. If the 95% CI does not overlap 0, it denotes a significant effect by PGPB. The sample size for an effect is indicated next to each phylum. Asterisks indicate significant effects (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.).

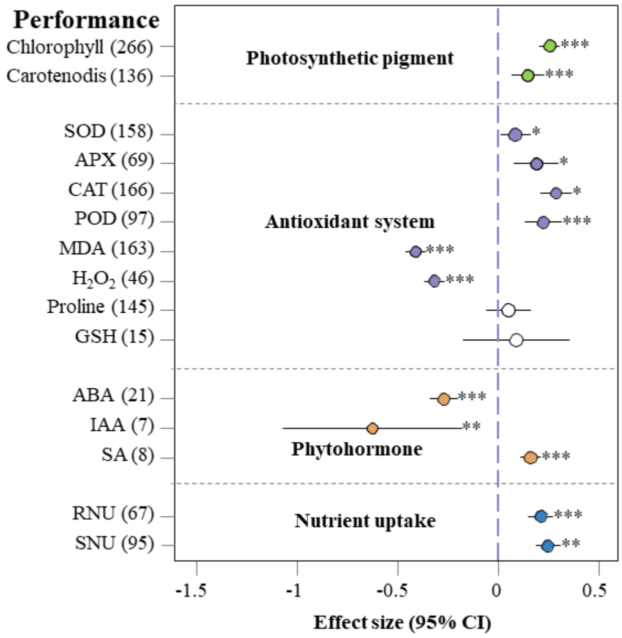


Fig. 7. Effect size of PGPB on crop physiology under heavy metal stress. Horizontal error bars indicate 95% confidence intervals (CI). If the 95% CI does not overlap 0, it denotes a significant effect by PGPB. SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidases; MDA, malondialdehyde; H_2O_2, hydrogen peroxide; GSH, glutathione; ABA, abscisic acid; IAA, indole-3-acetic acid; SA, succinic acid; SNU, shoot nutrient uptake; RNU, root nutrient uptake. Asterisks indicate significant effects (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.)