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A review and meta-analysis of the efficacy of arbuscular mycorrhizal fungi in remediating toxic metals in mine-affected soils

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Mines are natural reservoirs of various minerals, metals, and metalloids. Several heavy metals (HMs), such as Pb, Cd, Cr, Cu, and Ni, are major anthropogenic pollutants that cause severe environmental pollution. The accumulation of these toxic HMs in soils has raised several concerns for crop growth, food safety, and marketing. Physiological and biochemical processes in plants are severely impacted by HMs, disrupting normal metabolic activities and reducing biomass production. Phytoremediation plays a pivotal role in addressing HM contamination by offering an eco-friendly, economical, and holistic solution. Similarly, arbuscular mycorrhizal fungi (AMF) play a significant role by forming a symbiotic relationship with plant roots. In this association, plants provide root exudates, while AMF enhance plant growth under heavy metal stress by supplying essential nutrients, minerals, and water. These fungi also improve nutrient status, soil quality, and ecosystem stability. The present review and meta-analysis encompass an examination of the global distribution of toxic HMs in miningaffected areas. Furthermore, the study highlights the role of various plant species and microbes, particularly AMF, in mitigating HM stress and its impact on plant growth and nutrition. The meta-analysis also evaluates the efficacy of AMF as a remediation strategy for HM-impacted mine soils.

KEYWORDS

mines, heavy metals, phytoremediation, arbuscular mycorrhizal fungi, meta-analysis

1 Introduction

Human-driven activities such as agriculture, mining, industrial processes, and the extensive use of fertilizers and pesticides have escalated the demand for land resources since the twentieth century (Cheng, 2016). Heavy metal pollution, desertification of land, ecological imbalance of land, soil erosion, land degradation, environmental damage, and decreased soil fertility are all major environmental factors that have severe effects on soil, water, and air (Nosrati and Collins, 2019; Vaverková et al., 2019). Heavy metal (HM) pollution is a global phenomenon. Metal mining and mineral ore processing have a dual effect on the economy and the environment. From one perspective, they provide economic benefits to the country, and simultaneously, they cause environmental pollution. Abundant and active mines are the primary source of toxic HMs. During the rainy season, due to heavy

rainfall and strong winds, runoff water washes the toxic waste material into agricultural fields and surrounding water bodies, simultaneously causing air, water, and soil pollution. HM pollution has an irreversible, long-term residual effect and toxicity that poses an immense threat to living beings as well as the environment (Dhaliwal et al., 2020). Once these toxic HMs are released into the surrounding ecosystem, they could migrate to distant areas, accumulate in various biotic and abiotic components of the system, and adversely affect the food chain, human health, and the environment (Peralta-Videa et al., 2009). Lead (Pb), chromium (Cr), mercury (Hg), cadmium (Cd), and arsenic (As) have lethal effects on humans, plants, and animals. Depending on the concentration, a few metals, such as zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe), have another role as essential micronutrients needed for metabolic activity (Schneegurt et al., 2001; Mohan et al., 2007). Heavy metal contamination significantly alters soil characteristics and the surrounding microenvironment. Microorganisms, serving as dynamic bio-indicators, respond to these changes through variations in microbial biomass, respiration rates, and enzyme activity under HM stress conditions (Hinojosa et al., 2005). Long-term or short-term exposure to various toxic HMs causes significant changes in physiological and ecological parameters, including a reduction in basal respiration, microbial biomass, and an increase in metabolic entropy (qCO₂) (Crowley, 2008; Zhao et al., 2020). The degree of HM pollution has been evaluated by several indices, like a pollution load index (PLI) and a geo-accumulation index (Igeo) (Duncan et al., 2018; Hamad et al., 2019).

Factors such as plant life cycle, plant biomass, bioaccessibility, and bioavailability of HMs in soil can influence the metal removal process (Ali et al., 2013). Various physical and chemical methods are available for decontamination of toxic HMs and are usually costintensive. Given the limitations of conventional cleanup techniques, biological approaches could be considered a potential alternative mitigation option. In some places, bioremediation via phytoremediation of soils contaminated with organic or inorganic pollutants, such as pesticides and hydrocarbons, has become widely accepted. The popularity of bioremediation and phytoremediation for the reclamation of HM-polluted soils is growing even though it has substantial disadvantages due to its economic viability. The term phytoremediation is defined as a green, eco-friendly, low-cost, holistic approach to cleaning toxic contaminants from the environment by a plant-based system (Ali et al., 2013). Numerous phytoremediation projects have been carried out over the past few decades, and as a result, novel phytoremediation techniques, creative concepts, and research have emerged. Several phytoremediation projects have been done in the last few decades, and new phytoremediation strategies, innovative ideas, and research have evolved as a result. More than 500 plant species have been identified as potent HM hyperaccumulators (Ye et al., 2020). A long time span is required for plants to remediate a highly metal-contaminated area. Remediation through plant or phytoremediation is one of the most promising eco-friendly management strategies for reducing toxic contaminants (Burges et al., 2018).

In the past few decades, researchers have worked with various types of plants, their potentiality, and their remediation mechanism strategies for a better understanding of the phytoremediation process. Plants like Cymbopogon citrates (China et al., 2014), Helianthus petiolaris (Saran et al., 2020), Helianthus annuus (Lothe et al., 2016), Bryophyllum laetivirens (Li et al., 2020), Cordyline fruticosa (Herlina et al., 2020), etc., are widely used to remediate heavy metals (Pb, Cd, Cr, Cu, As), and their removal mechanisms have been extensively studied by several researchers in last few years. Vetiver grass (Vetiveria zizanioides) has been widely used for the rehabilitation of mine tailings in several countries like China and Australia. Vetiver is a perennial grass with a huge root system (3-4 m), 1-2 m tall, and non-invasive (Andra et al., 2009). Furthermore, vetiver grass has a strong symbiotic association in the rhizosphere region with a wide range of soil microbes, especially with arbuscular mycorrhizal (AM) fungi, which stipulates phytohormones and essential nutrients for plant development (Bahraminia et al., 2016). The most advantageous properties of mycorrhizal root colonization are an increase in the root surface area to enhance the phytoremediation/phytostabilization potential.

Numerous studies have focused on mining activities and heavy metal contamination, exploring their effects on soil, plants, water resources, ecosystems, and living organisms. Previous studies also examine bioremediation approaches, utilizing plants and microorganisms to mitigate the adverse impacts of HMs effectively. This review aims to provide a comprehensive overview of heavy metal pollution in agricultural soil caused by various mining activities and its associated environmental impacts. Through meta-analysis, the study assesses HM contamination and examines the global distribution of key pollutants, including Cr, Ni, Cd, Pb, and Cu, in mining-affected regions worldwide. This article also sheds light on the role of different plant species and microbes (especially AMF) in mitigating the HM stress condition while supporting plant growth and nutrient uptake. Additionally, through meta-analysis, the study evaluates the efficiency of AMF as a remediation strategy for mine-impacted soils contaminated with HMs such as Cd, Cu, Ni, and Pb.

2 Mines and associated heavy metals

According to the ancient Shamasastry, 1915, "Mines are a Nation's treasury." Mineral resources from mines are abundant in nature. The exploitation of these minerals enhances the world's economy and development, but at the same time, surface mining, especially open-cast mining, causes severe environmental problems (i.e., loss of surface vegetation, destruction of soil structure, etc.). Mines are the source of various metals and minerals like iron and ferroalloys (Fe, Cr, Co, Mn, Mo, Ni, etc.), non-ferrous metals (Al, Sb, As, Bi, Cd, Cu, Pb, Hg, Li, Zn, etc.), precious metals (Au, Pd, Pt, and Ag), industrial minerals (perlite, sulfur, vermiculite, feldspar, graphite, gypsum, kaolin, etc.) and mineral fuels (uranium, petroleum, cooking coal, natural gas, etc.). China is the largest producer of total minerals, followed by the United States, Russia, Australia, and India (Reichl et al., 2020).

2.1 Coal mines

As a fossil fuel, coal is a predominant element in nature. It is mainly composed of carbon with variable amounts of other

elements, including hydrogen, oxygen, nitrogen, and sulfur. China is the largest producer of coal. Open-cast mining generates toxic overburden dumps (OB) and coal dust that contain enormous amounts of toxic HMs and are responsible for metal contamination in adjacent agricultural land (Li et al., 2007). In descending order, metals Fe > Mn > Zn > Cu are present in coal mines: the most bioavailable and mobile element is Mn, followed by Zn and Cu, and the least mobile metal is Fe. The reason behind Fe's lesser mobility is the residual fraction of Fe, which indicates its strong affinity toward minerals, the solid matrix, and strongly bounded clay minerals (Kartal et al., 2006). A pseudo-total concentration of HMs, including Zn (314 mg kg⁻¹), Mn (132 mg kg⁻¹), Pb (82 mg kg⁻¹), Cu (45 mg kg⁻¹), and Co (34 mg kg⁻¹), has been found in reclaimed mine soil (RMS). The bioavailable forms (DTPA-extractable) of Zn, Mn, and Cu are significantly higher in RMS than in control soil. Pb can selectively accumulate in leaves, stem bark, and root bark, whereas Zn and Mn accumulate in leaves, and Cu accumulates in stem wood and root wood. These indicate that the accumulation of metals might be tissue specific (Maiti et al., 2016). The most effective remediation pathway for OB dumps is trees, which can accumulate toxic HMs from OB. Trees that are used for the reclamation of OB dumps should be drought resistant, woody, fast-growing, and able to grow in arid areas and nutrient-deficient areas (Pratas et al., 2005). Various woody plant species, such as A. auriculiformis, M. azedarach, Leucaena leucocephala (Lam.) de Wit, Tectona grandis L. f., Gmelina arborea Roxb., Acacia mangium Wild., Bambusa arundanacea L., Cassia siamea Lam, and Azadirachta indica A. Juss, etc., are used for reclamation of coal OB dumps (Maiti et al., 2016). The Pb concentration in A. auriculiformis, hybrid eucalyptus trees, is significantly higher in root bark than leaf tissue as bark tissue can accumulate lead for longer time while leaves are shed periodically (Sawidis et al., 2011). Through bark exudates, Pb can be removed from plants, which is an important defense mechanism against HM toxicity (Alloway, 2012). In the case of Cu (BCF >1; TFleaf, TFstembark, and TFstem wood <1), two tree species, A. auriculiformis and M. azedarach, might be used for Cu phytostabilization (Sawidis et al., 2011).

2.2 Copper mines

Cu mines are a prime source of potentially toxic HMs (Cu, Zn, As, Cd, and Pb) (Cai et al., 2015). South Africa, Chile, and Peru are the largest producers of copper. Due to the chemical weathering process, waste rocks from the Predra Verde mine (Brazil) show potential risks to the environment (Perlatti et al., 2021). In Jiuhuashan, Jiangsu Province, and in eastern China, agricultural soil near abandoned mines contained high levels of copper (816.8 mg kg⁻¹ and 147 mg kg⁻¹, respectively) contamination (Qin et al., 2012; Wu et al., 2011). Acidic drainage compounds (Cu, Zn, and Fe) have been produced from the La Concordia Mine (Argentina) (Nieva et al., 2018). According to China et al. (2014), of environmentally available metal, that is, total metal excluding the silicate matrix-bound metals, Cu (154 mg kg⁻¹) is one of the most abundant heavy metals found in the Mosabani copper mine (India), followed by Ni (136 mg kg⁻¹) and Pb (9.9 mg kg⁻¹). The underground tissues of this plant have an average concentration of 1959 mg kg⁻¹ Cu, which is much higher than shoot (124 mg kg⁻¹ Cu). Therefore, it indicates that HM mobility is limited inside the plant as the translocation factor for Cu (0.06), Ni (0.36), Co (0.68), and Zn (0.24) is less than 1, while Mn is present in higher concentration in the above-ground tissue (TF > 1; Mn 1.37) (Das and Maiti, 2007). The toxicity level of Cu and Ni in plants is 20-100 mg kg⁻⁻¹ and 10-100 mg kg⁻¹, respectively (Kabata-Pendias, 2011). The bioavailability of copper also depends on soil pH, soil cation exchange capacity (CEC), and total copper content in soil (Bravin et al., 2009; Brun et al., 2001). Copper also plays an essential role in plant growth and development processes such as protein synthesis, CO₂ assimilation, ATP synthesis, maintaining homeostasis within chloroplast, photosynthesis, etc. (Hänsch and Mendel, 2009; Yruela, 2013). A high concentration of copper has a toxic effect on seed germination, decreases plant height, causes chlorosis of plant leaves, and reduces plant biomass and grain yield (Adrees et al., 2015).

2.3 Chromite mines

Ferrochromium is the only natural and economical resource of chromium produced in chromite mines by carbothermic smelting (Beukes et al., 2010). It is a crystalline alloy generally composed of chromium and iron compounds. Globally, South Africa has the most chromite ores, followed by Kazakhstan, India, Albania, and Turkey (ICDA, 2022). The active and abandoned mine wastes are reservoirs of heavy metals that have lethal effects on water, soil, and living beings (Fernández-Caliani et al., 2009). These mine wastes are generally composed of different types of toxic HMs, mainly chromium (Cr) and Nickel (Ni), along with other metals such as Cu, Cd, Pb, Ni, and Mn present in lesser quantities (Bueno et al., 2009). Chromium (Cr) is generally utilized in industrial activities such as the processing and finishing of leather, the production of refractory steel by the stainless steel industry, electroplating cleaning agents, drilling muds, the production of chromic acid and other chemicals, and food preservation (Shanker et al., 2005). Chromium exhibits different levels of toxicity depending on its chemical form, pH, reaction with other elements, and solubility index (Thatoi et al., 2014). Cr (VI) exhibits high toxicity and bioavailability due to its better solubility than Cr (III) (Abyaneh and Fazaelipoor, 2016). A lack of Cr (III) in human and animal diets can cause metabolic deterioration, cardiac problems, and diabetes, but an excess presence in the body has harmful health effects (WHO, 2000). Hexavalent chromium tends to act as a strong oxidizing agent; therefore, Cr (VI) is 10-100 times more toxic than Cr (III) (Zayed et al., 1998). The toxicity level of hexavalent chromium for plants in solution is as low as 0.5 mg $\rm kg^{-1}$ and 5 mg $\rm kg^{-1}$ for soil (Turner and Rust, 1971). Highly carcinogenic chromium and asbestos exposure may lead to cancer, mesothelioma, pneumoconiosis, skin irritations, and other respiratory problems such as irritation of the larynx and pharynx, edema, coughing, asthma, etc. (Bloise et al., 2008; Pugnaloni et al., 2013). Cr is mostly accumulated in plant roots rather than in the shoot due to its reduced mobility in root vacuoles. However, Ni accumulation is higher in the shoot than in the root due to greater mobility of Ni

through xylem tissue (Pulford et al., 2001; Shanker et al., 2005). The Cr and Ni concentration in the Roro chromite mine waste soil is 3,120 mg kg⁻¹ and 1,620 mg kg⁻¹, respectively, which exceeds the safety level (Cr:75-100 mg kg⁻¹; Ni: 100 mg kg⁻¹) of metals present in soil (IS, 1993). In similar studies in the Almadén mine site in Spain and southern Togo mine sites, Cr and Ni concentrations are 86-35 Cr mg kg⁻¹ and 21.2-126 Ni mg kg⁻¹ and 182-1,029 Cr mg kg⁻¹ and 15–432 Ni mg kg⁻¹, respectively, due to deposition of mine tailings in agricultural soil (Gnandi and Tobschall, 2002; Bueno et al., 2009). In the Daduk mine area of Korea, due to the dispersion of metals from tailings and watercourses, various toxic metals are reported in nearby paddy fields (Lee et al., 2001). In another study in the Co Dinh mine of Vietnam, high levels of potentially toxic elements are also detected in rice fields (5,750 Cr mg kg⁻¹, 375 Co mg kg⁻¹, and 5,590 Ni mg kg⁻¹) (Kien et al., 2010). Based on the dynamic translocation factor (TF dyn>1), Cr and Ni accumulation is higher in plant parts of Oryza sativa growing in contaminated agricultural fields that might have a potential risk of transfer of toxic HMs to livestock or humans (Kien et al., 2010; Kumar and Maiti, 2014).

2.4 Iron mines

Globally, the production of crude steel has expanded drastically since 2000 to meet increasing demand. Iron ores are the backbone of the world economy (World Steel Association, 2021). Australia, Brazil, and China are the top three countries for the production of iron (around 69%) (Holmes et al., 2022). To maintain the growing demand, iron ore industries have continuously increased mining activity. As a result, huge concentrations of Cd, Mn, As, Ni, Pb, Zn, and Cr have been found in the agricultural soil near iron mines (Hosseini et al., 2018). Toxic tailing wastes from iron mining are dumped into a tailings pond located at the Noamundi-Jodda belt, India. As a result, during monsoon season, the toxic fine particles washed off by heavy wind and rain are deposited into nearby water bodies and soil, thereby causing air, water, and soil pollution. Although Fe is essential for the synthesis of chlorophyll, chloroplast structure and its functions, excessive concentrations of iron might enter into the food chain and show toxic effects on plant, animal, and human health (Maiti et al., 2005). According to Dhatrak et al. (2017), the health status of mine workers and a nearby population around an open-cast iron mine showed noise-induced hearing loss (NIHL) and anemia as major health effects. Iron mining plays an important role in economic development and simultaneously causes air pollution by blasting, drilling, unloading, and loading minerals and overburdens by wind at mineral handling plants, workshops, etc. These air pollutants affect the flora and fauna of the local environment (Tripathi et al., 2014; Chaturvedi and Patra, 2016). Some native plant species, such as Cassia sophera Eupatorium odoratum, Techtona grandis, Alstonia scholaris, Cassia tora, etc., can be found in Fe tailings. Techtona grandis can accumulate a higher concentration of HMs than Alstonia scholaris, but these HMs have not shown any detrimental effect on native plants. Rather, higher Fe content promotes lavish growth, as stated by Maiti et al. (2005).

2.5 Uranium mines

Worldwide, Kazakhstan is the largest producer of uranium, followed by Australia, Namibia, Uzbekistan, and Canada. Jaduguda, India's first, oldest, and most productive underground uranium (U) mine, consists of uraninite and other associated accessory minerals such as copper, nickel, arsenic, cobalt molybdenum, and magnetite, etc. (Sethy et al., 2014). Uranium occurs naturally in the earth's crust with a mean concentration of approximately 3 mg kg⁻¹ (Gupta and Singh, 2003). Hexavalent U is the most soluble form and is present as the uranyl cation $(UO_2)^{+2}$ in 80%-90% of the soil. It prevails in solutions predominantly as a stable ion $(UO_2)^{+2}$ and as soluble carbonate complexes, that is, UO_2CO_3 , $UO_2(CO_3)_2^{-2}$, $UO_2(CO_3)_3^{-4}$, $(UO_2)_2CO_3(OH)^{-3}$ and (UO₂)₃(CO₃)₆⁻⁶. In the absence of dissolved inorganic ligands (fluoride, carbonate, sulfate, and phosphate) and a pH range within 4–7.5, the hydrolysis ion UO_2OH^+ in water and soil forms complexes with these inorganic ligands. As a result, these complexes increase the total solubility of U (Shahandeh and Hossner, 2002). Uranium is a radioactive element that undergoes a continuous decaying process, emits alpha (α), beta (β), and gamma (γ) rays, and produces various isotopes. This transformation stops when the stable product lead (206Pb) is formed (Sarangi, 2003). The radiation that is emitted from these naturally occurring isotopes is very low and does not penetrate due to its high density, which acts as a shield against its own radiation (Wang et al., 2009). According to WHO (2012), a mean concentration of U in ambient air has been reported to be approximately 0.02 ng m⁻³ in Tokyo, Japan, and 0.076 ng m⁻³ in New York City, United States of America. Uranium enters the kidney through water or food, and the uranyl ion forms bicarbonate and citrate complexes in blood plasma and affects the proximal tubules of the kidney, causing tubular degeneration, liver damage, genetic malfunction, cancer, and necrosis (Miller et al., 2004; Sethy et al., 2011). The Environmental Protection Agency (EPA) of the United States has categorized U as a carcinogenic element, and in drinking water, the maximum contaminant level (MCL) of U is $30 \ \mu g \ L^{-1}$ (EPA, 1999). The proposed interim maximum acceptable level (IMAC) of U in Canada is 20 µg L^{-1,} whereas WHO strictly recommended the permissible level to be $2 \ \mu g \ L^{-1}$ (Shin et al., 2002). In humans, the ingestion and intake dose is very low (2 μ Sv.Y⁻¹), which is far below the WHO permissible level (100 uSv.Y⁻¹). The mean metal pollution index (MPI) value indicates the overall pollution level in ground and surface water to be below the maximum threshold value of 100 (Mohan et al., 1996). Several experiments have been conducted for the accumulation of U in native plant species to determine the mechanism of U uptake absorption by plants and for biological exploration of U from soil (Petrova, 2006). Uranium accumulation varies depending upon plant species as well as genotypes, lines within species, and cultivars; moreover, U is accumulated higher in the root portion than in the shoot. Therefore, only a small portion is translocated to the shoot. Less than 1 mg kg⁻¹ of U is found to be toxic in the soil (Sheppard et al., 1992; Stojanović et al., 2010). According to (Pentyala and Eapen, 2020), Vetiveria zizanioides L. Nash showed good ability for phytoextraction (84%-95% of recovery) of U from hydroponic solution at a concentration below 200 ppm under controlled experimental conditions. U is generally restricted

in the root portion of vetiver, but at concentrations above 1,000 ppm, it is translocated from the root to the shoot.

3 Quantitative evaluation of various toxic metals in mining areas through meta-analysis

The main aim of the meta-analysis was to compare a selected number of peer-reviewed articles and to determine the potential risk of toxic metals on soil health using global datasets. We searched literature published in the Web of Science database between 2012 and 2022 and selected research according to our objectives. The keywords were "Mine," "pollution," "Copper," "Cadmium," "Nickel," "Chromium," "Lead," and "World." From >2,500 published reports, we excluded studies based on data originality. We screened the remaining articles from different origins depending on the title and abstract. From the vast range of published articles, the research papers were selected based on the manuscripts reporting metal toxicity due to mining activity across different types of mines, and proper analytical methods were followed. Finally, based on the inclusion criteria, 55, 41, 98, 52, and 51 research papers were considered for Cr, Ni, Pb, Cd, and Cu, respectively. The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) flowchart is depicted in Supplementary Figure S3.

From the literature survey, we considered parameters like standard error, sample size, and the difference between the tested and control means. The effect size or outcomes were calculated by the mean difference between the maximum concentration of metals (Cr, Ni, Pb, Cd, and Cu) and the permissible limit of metals in mine areas. The maximum permissible limit of metals in soil (Cr, Ni, Pb, and Cu) was that suggested by World Health Organization, 1996 (Cu: 36 mg kg⁻¹, Ni: 35 mg kg⁻¹, Cr: 100 mg kg⁻¹, and Pb: 85 mg kg⁻¹). The result was expressed on mean difference as a continuous factor for statistical analysis at the 95% confidence level (CI) between the group of individual studies and the permissible limit of metals (Cr, Ni, Pb, Cd, and Cu) in the mine areas. Then, from the random effect model (RE), forest plots were designed to summarize all the study information of individual research work, and this plot simultaneously provides a visual representation of heterogeneities. The vertical line in the middle of the forest plot, commonly known as the zero-effect line, shows that there was no difference between the study group mean and the permissible limit. This mean difference is zero at this point.

From Figures 1, 2 it can be observed from the RE model that the overall mean value for Cr was 0.16 (CIs: 0.14–0.17) and for Ni, it was 0.19 (CIs: 0.16–0.22), statistically significant with p < 0.05 and inconsistency indexes (I²) of 98.58% and 96.05%, respectively, which represented substantial heterogeneity [43,49,63,68, 84–87]. Similarly, for Cd, Pb, and Cu (Figures 3–5) from the RE model, the overall mean values were 0.01 (CIs: 0.01–0.01), 0.06 (CIs: 0.06–0.07), and 0.08 (CIs: 0.07–0.09), respectively, which are statistical significance at p < 0.05. The overall inconsistency indexes (I²) of Cd, Pb, and Cu were 47.34%, 99.24%, and 98.02%, respectively, indicating substantial heterogeneity. The positive value indicated that the total concentrations of Cr, Ni, Cd, Pb, and Cu in mine areas were higher than the permissible level recommended by

WHO. In a meta-analysis of the summary means, most metals (Cr, Ni, Pb, and Cu) present in mine areas were found to be significant at the p < 0.05 level, as the confidence intervals did not overlap with the zero-effect line except for Cd. Various factors like runoff water and aerial deposition from mines lead to the contamination of nearby agricultural lands, water bodies, etc. As a result, the presence of higher concentrations of metals in different mine areas may influence the potential risk of metal toxicity and its relative risk to the ecosystem (Qu et al., 2012; Chen et al., 2017; Liu et al., 2019; Sun et al., 2018).

4 Remediation strategies

Worldwide, the immense development in industrial sectors, especially mining, metal, energy supply, agriculture, chemical production, and transport, causes significant pollution of the ecosystem. Globally, heavy metal contamination is a problem for the environment as well as for living beings (Sun et al., 2012; Cachada et al., 2018). As we know, remediation of heavy metals is more complicated than remediation of other organic pollutants. Various traditional, mechanical, and chemical techniques, including electrochemical treatments, thermal methods, incineration, excavation, vitrification, chemical oxidation, and solvent extraction, are widely utilized to remove or destroy these toxic HMs in soil. However, these methods are often costly, timeintensive, and labor-demanding. Moreover, they can lead to soil degradation and generate secondary waste materials, posing additional environmental management challenges (Khan et al., 2018). Bio-remediation techniques have gained attention due to their cost-effectiveness, viability, no generation of secondary waste, and eco-friendly (Akande et al., 2018; ALAM et al., 2018). These techniques include plants and various microbes (bacteria, fungi, mycorrhiza, etc.) that are utilized to decontaminate the hazardous compounds from soil. For soil purification, applications of plants alone or in association with microorganisms help to stabilize, mineralize, transfer, and remove toxic metals (Wang et al., 2018).

4.1 Phytoremediation

The term "phytoremediation" is derived from Greek ("phyton") and Latin ("remedium"), which means "plant" and "to correct," respectively (Cunningham et al., 1996). Phytoremediation is a bioremediation process in which plants (alone or in association with microbes) are used as purifying agents to remove, stabilize, or destroy the toxic metals from air, water, and soil in an eco-friendly manner (Wani et al., 2012). Supplementary Table S1 shows different mechanisms of phytoremediation, and Supplementary Figure S2 shows images of such mechanisms. Generally, plants can extract essential nutrients (Fe, Zn, Ni, Mn, and Cu) as well as non-essential metals (Cr, Cd, As, Pb, and Hg) that are not required in their physiological process and can store an enormous amount of the toxic metals (hyper-accumulator) in their parts from contaminated soil and water (Tangahu et al., 2011). Several studies have been done regarding different mechanisms of phytoremediation strategies, as shown in Supplementary Table S2. The advantages of phytoremediation are as follows: 1) inexpensive technology (60%-80% lesser than traditional process); 2) minimize soil



deterioration; 3) solar-driven remediation process; 4) no generation of secondary hazardous compounds; 5) suitable and broad-spectrum treatment; 6) sustainable and environment-friendly technique. One limitation is that plants require time for their growth and development (Morikawa and Erkin, 2003). Techniques like phytoextraction and phytostabilization are commonly used for remediating HM-polluted sites. Several plant species have demonstrated the ability to absorb, bioaccumulate, immobilize, and degrade heavy metals (HMs) from contaminated sites. Some examples include C. citrates, H. petiolaris, V. zizanioides L, Pennisetum purpureum cv. Mott, Conocarpus lancifolius, and Cordyline fruticose, etc. (Andra et al., 2009; China et al., 2014; Herlina et al., 2020; Rasheed et al., 2020; Kowitwiwat and Sampanpanish, 2020; Saran et al., 2020). These species are key in cleaning contaminated soils either by extracting HMs into their tissues or stabilizing them in the soil. Vegetation helps limit pollutant transport, reduce wind dispersion, and prevent water erosion (Perronnet et al., 2000). Unlike conventional methods that disturb soil physical properties, phyto-strategies maintain enhance soil quality. Successful phytoremediation and implementation requires considerations of biomass production, heavy metal concentration in plant material, and the time needed for soil remediation (Robinson et al., 1998). Phytodegradation involves the uptake of toxic compounds by plants, where plant enzymes break down these substances into less harmful forms (Sun et al., 2012; Hamdi et al., 2012). Plant species like Arabidopsis

thaliana and Azolla filiculoides are used for phytodegradation of pollutants such as 2,4-DNT and bisphenol A in the United States and Iran (Yoon et al., 2008; Zazouli et al., 2014). Phytovolatilization occurs when plants transform the contaminant into volatile compounds and emit them into the atmosphere through transpiration or radial diffusion from their leaves, stems, and roots (Limmer and Burken, 2016; Peter et al., 2017). Rhizodegradation is the breakdown of contaminants facilitated by rhizospheric microorganisms, where root-released enzymes and exudates help decompose pollutants into non-toxic forms (Jia et al., 2016; Gkorezis et al., 2016). Plants like Pteris vittata (Sakakibara et al., 2010) and Salicornia bigelovii (Shrestha et al., 2006) are involved in phytovolatilization of arsenic and selenium in Japan and the United States, respectively. The efficacy of phytoremediation depends on selecting appropriate plant species and various environmental factors. Overall, phytoremediation is a complex process involving multiple plant mechanisms. Understanding these processes can enhance plant adaptation to metal stress and improve efficiency, providing sustainable solutions for heavy metal contamination and ecosystem restoration.

4.2 Mycorrhizal remediation

Naturally, plants interact constantly with many microorganisms in their rhizospheric region. Beneficial microorganisms, especially



arbuscular mycorrhizal fungi (AMF), have a symbiotic association with plant roots where AMF increase plant nutrient uptake ability, improve biomass accumulation, amplify photosynthesis capacity, and provide protection against heavy metal toxicity. Successively, the plant provides exudates of amino acids, carbon, and photosynthetic products to the AMF for growth and development (Mitra et al., 2020).

Around 80% of terrestrial plants and 90% of agricultural plants have mycorrhizal associations in their roots, where fungal hyphae enter the cortical cells of plant roots, forming vesicles, hyphae, and arbuscles (Smith and Read, 2010). Supplementary Figure S1 denotes the schematic diagram of the heavy metal detoxification mechanism through AMF. AMF help immobilize heavy metals by binding them at the cortical region, preventing translocation to the upper ground part (shoot, stem, leaves), and preventing damage to leaves. Plants are categorized based on TF value into hyper-accumulators (TF > 1) and non-hyperaccumulators (TF < 1). The translocation factor (TF) is higher in non-mycorrhizal-associated plants than in mycorrhizalassociated plants (Arshad et al., 2008). Endomycorrhizal fungi AMF belong to the phylum Glomeromycota. They are considered an ecofriendly, sustainable strategy to enhance plant growth, increase shoot biomass, improve soil health and water uptake capacity, provide protection to the plant against biotic and abiotic stress,

and detoxify heavy metal-induced stress (Mishra et al., 2019). Glomeromycota are obligate symbiotic organisms, so they require around 20% of carbon from host plant cells for their survival. Simultaneously, they provide water and nutrients (P, N) through their arbuscles and intracellular and extracellular hyphae to the host plant (Parniske, 2008). AMF combat heavy metal stress by immobilization, precipitation, chelation, and sequestration in the rhizosphere and vacuoles and activate the plant anti-oxidant defense system (Mitra et al., 2020). Another AMF defense mechanism is to secrete a hydrophobic unique glycoprotein called glomalin, which is composed of carbon (39%-59%), phosphorus (0.03%-0.1%), nitrogen (3%-5%), hydrogen (4%-6%), oxygen (33%-49%), and a trace amount of iron (Schindler et al., 2007; Zhang et al., 2017). This protein is basically an N-linked glycoprotein produced from the spores and hyphae of AMF, which helps in soil aggregation, cellular function, toxic heavy metal stress, carbon storage, etc. (Emran et al., 2012; Wu et al., 2015). Easily extractable glomalin-related soil protein (EE-GRSP) and total glomalin-related soil protein (T-GRSP) are both readily quantified from the soil with the help of a citrate buffer (Wright and Upadhyaya, 1996).

For plant growth and nutrition, AMF increase the surface area with the help of extracellular and intracellular hyphae for better absorption of nutrients, water, and the ions that are generally



present in an immobilized form in soil. AMF also improve the plant's ability to acquire nutrients from the depleted zone of the rhizosphere (Smith and Read, 2010; Smith and Smith, 2011). As stated by Nakmee et al. (2016), native species of AMF Glomus aggregatum, Acaulospora scrobiculata, and F. mosseae provide positive effect on plant nutrient uptake (total N, P, K) and enhance plant biomass, leaf number, and plant height of sorghum. It was observed that those wheat plants inoculated with AMF culture (F. mosseae and R. intraradices) contain a 1.13-2.76 times higher concentration of Zn than non-inoculated wheat plants (Coccina et al., 2019). Various abiotic stresses include salinity, heavy metals, drought, flooding, extreme temperature, etc. AMF communities independently withstand these unfavorable stress conditions for their host plants, provide sufficient water in drought stress, supply nutrients (phosphorus), and balance osmotic pressure in flooding stress conditions (Zhu et al., 2017; Caradonia et al., 2019). Under drought conditions, tomato plants containing AMF (R. intraradices) colonized on their roots provide sufficient water-based nutrients to the tomato plant for better growth during water-stress situations. Inoculation with G. etunicatum enhances total chlorophyll content, root-shoot height-weight, increased N, P, K, Ca, Zn, Cu concentration, flavonoid content, soluble sugar, proline, glycine betaine, polyamine, POD, and CAT activity in Pistaciavera L under stress conditions (Abbaspour et al., 2012). Studies revealed that G. etunicatum F. mosseae and R. irregularis increased the growth and grain yield of Triticum aestivum L., regulate nutrient uptake capacity, and decreased Na+ and Clconcentration at times of salinity stress (Daei et al., 2009). According to Hashem et al. (2016), oxidative stress generates a high concentration of malonaldehyde and hydrogen peroxide in Solanum lycopersicum L. AMF strains (Glomus mosseae, Glomus intraradices, and Glomus etunicatum) help to decrease the



concentration of these elements and boost the plant's defense system against Cd stress. AMF also provide protection against biotic stress. Various pathogens, such as root-rot fungi, pathogenic bacteria, nematodes, and other harmful microorganisms, can cause various diseases. However, the presence of AMF significantly reduce pathogen-induced damage and infection by enhancing nutrient availability, stimulating root growth, and improving root morphology. AMF secrete beneficial enzymes in the rhizosphere, strengthening plant defenses and enabling plants to better withstand biotic stress (Vos et al., 2012; Spagnoletti et al., 2020). Fusarium wilt causes damage to *Cicer arietinum* L, but treatment with an AMF strain (*Glomus hoi*) provides protection against wilt disease and increases the nitrogen and phosphate content in treated plants as compared with non-treated plants (Singh et al., 2010). Similarly, *Glomus* sp. synthesizes antimicrobial compounds that help to arrest the mycelia growth of *Fusarium oxysporum* on *L. esculentum* plants and increase the chlorophyll, N, P, and K content of the plants. Furthermore, in *Capsicum annum, Glomus* sp. reduces the activity of the pathogen *Pythium aphanidermatum* and provides better yield of the plant (Kumari and Prabina, 2019; Kumari and Srimeena, 2019).



5 Evaluation of AMF as a tool to remediate metals through meta-analysis

Studies published between 2005 and 2022 were searched in the Web of Science database and selected based on their reporting quality. The keywords were "Arbuscular mycorrhizal fungi," "Mine," "Soil," "World," "Cadmium," "Nickel," "Lead," "Copper," and "Chromium." After assessing more than 250 peer-reviewed articles, articles were excluded based on the following reasons: a) Lack of analytical techniques (not mentioning the QA/QC), b) remediation through other microbes, and c) graphical representation of data. A total of 24 studies comprising nine, twelve, seven, and five studies for Cd, Pb, Cu, and Ni, respectively, were included in the meta-analysis, which assessed the efficacy of AMF in remediating metal-contaminated mine soils (Table 1). Studies reporting remediation of Cr with AMF were not found during the systemic review. The PRISMA flowchart is displayed in Supplementary Figure S4.

From the RE models shown in Figures 6A–D, the overall mean values for Pb, Cd, Ni, and Cu were 1.34 (CIs: 1.19–1.49), 1.08 (CIs: 0.86–1.31), 0.79 (CIs: 0.53–1.05), and 1.46 (CIs: 1.02–1.90), respectively. The data showed statistical significance at p < 0.05. The inconsistency indexes (I²) of Pb, Cd, Ni, and Cu were 92.94%,

96.39%, 99.30%, and 98.89%, respectively, indicating substantial heterogeneity. The positive effect sizes for all the metals indicated that the AMF can reduce the metal accumulation capacity in plants. Studies from United States (Punamiya et al., 2010), China (Zhan et al., 2019), Mexico (González-Villalobos et al., 2021), etc., indicate that the Pb accumulation in plants was increased by AMF (Diversispora spurcum, G. mosseae, Rhizophagus irregularis) except for Wu et al. (2010), Solís-Domínguez et al. (2011), and Bahraminia et al. (2016), for which the CI values overlapped the zero-effect line and were determined to be non-significant (Figure 6A). Case studies from Spain (Arriagada et al., 2007), Brazil (de Andrade et al., 2008), China (Zhong et al., 2012), and Canada (Hassan et al., 2013) showed that the accumulation of Cd in plant systems increased in the presence of AMF inoculation of Glomus deserticola, G. intraradices, and R. irregularis. In other studies from China (Wu et al., 2010; Hu et al., 2013; Liu et al., 2014; He et al., 2020), the Cd accumulation in the plant decreased (Glomus constrictum, Glomus caledonium, and G. intraradices) (Figure 6B). For Ni and Cu, the accumulation decreases in the presence of AMF (Glomus tenue, Glomus margarita) (Orłowska et al., 2011; Lam and Lai, 2018; Manyiwa and Ultra Jr, 2022), and accumulation increases with the help of G. mosseae, G. etunicatum (Chen et al., 2005; Lins et al., 2006) (Figures 6C,D). Plant roots are symbiotically associated with AMF, which increase plant nutrient uptake ability, increases biomass

Country	Metal	Type of experiment	Name of AMF	Inherent total metal concentration in soil (mg kg ⁻¹)	Experimental dose (mg kg ⁻¹)	Plant metal content (mg kg ⁻¹)	Test crop	Effect of AMF inoculation	Reference
South Africa	Ni	Pot experiment	Native AMF sp. (<i>Gigaspora</i> sp. and <i>Glomus tenue</i>)	650		7,020	Berkheya coddii Roessle	ł	Orlowska et al., 2011
South Africa	Ni	Pot experiment	Native AMF	650		724	Berkheya coddii Roessle	Ť	Orłowska et al. (2013)
France	Ni	Pot experiment	Glomus etunicatum SFONL		60	881	Cloezia artensis	t	Amir et al. (2013)
Taiwan	Ni	Pot experiment	AMF	459.5		90.1	<i>Ipomoea aquatica</i> Forsk.	t	Lam and Lai (2018)
South Africa	Ni	Pot experiment	AMF	634.25		66.10	Colosphospermum mopane	ł	Manyiwa and Ultra Jr (2022)
China	Pb	Pot experiment	AMF		600	259.81	Kummerowia striata	1	Chen et al. (2005)
China	Pb	Pot experiment	Glomus mosseae and Glomus intraradices	4,418		1.11	Leucaena leucocephala	-	Ma et al. (2006)
Spain	Pb	Pot experiment	Glomus deserticola	595.96		284.1	Eucalyptus globulus	1	Arriagada et al. (2007)
United States	Pb	Pot experiment	Glomus mosseae		1,200	2,179	Chrysopogon zizanioides (L.)	1	Punamiya et al. (2010)
China	Pb	Field experiment	<i>Glomus intraradices</i> and <i>Glomus mosseae</i>	209		12.6	Chrysopogon zizanioides (L.)	-	Wu et al. (2010)
United States	Pb	Pot experiment	Glomus deserticola	4,620		3.89	Prosopis juliflora	-	Solís-Domínguez et al. (2011)
China	Pb	Pot experiment	AMF	3,683	1,500	2,655	Viola baoshanensis	Ť	Zhong et al. (2012)
Iran	РЬ	Pot experiment	Glomus versiforme		800	119.80	Chrysopogon zizanioides	-	Bahraminia et al. (2016)
Brazil	Pb	Pot experiment	Acaulospora scrobiculata	125		103	Chrysopogon zizanioides (L.)	t	Meyer et al. (2017)
China	Pb	Pot experiment	Gaeumannomyces cylindrosporus		1,000	252.25	Zea mays L	t	Yihui et al., 2017
China	Pb	Pot experiment	Diversispora spurcum	1426.7		732.9	Cynodon dactylon (L.) Pers.	t	Zhan et al. (2019)

(Continued on following page)

Country	Metal	Type of experiment	Name of AMF	Inherent total metal concentration in soil (mg kg ⁻¹)	Experimental dose (mg kg ⁻¹)	Plant metal content (mg kg ⁻¹)	Test crop	Effect of AMF inoculation	Reference
Mexico	Pb	Pot experiment	Rhizophagus irregularis	640		237.97	Parkinsonia aculeata L	Ť	González-Villalobo et al. (2021)
Spain	Cd	Pot experiment	Glomus deserticola	21.48		7.4	Eucalyptus globulus	Ť	Arriagada et al. (2007)
Brazil	Cd	Pot experiment	Glomus intraradices		0.02	885	Helianthus annuus L.	Ť	de Andrade et al. (2008)
China	Cd	Field experiment	Glomus intraradices and Glomus mosseae	2.25		939	Chrysopogon zizanioides (L.)	ł	Wu et al. (2010)
China	Cd	Pot experiment	AMF	113	200	6,952	Viola baoshanensis	1	Zhong et al. (2012)
China	Cd	Pot experiment	Glomus caledonium 90036	1.54		1.44	Sedum alfredii Hance	¥	Hu et al. (2013)
Canada	Cd	Pot experiment	Rhizophagus irregularis	0.75	40	256.44	Helianthus annuus L.	Ť	Hassan et al. (2013)
China	Cd	Pot experiment	Glomus constrictum		112	8.27	Zea mays L.	ŧ	Liu et al. (2014)
China	Cd	Pot experiment	Diversispora spurcum	16.9		14.5	Cynodon dactylon (L.) Pers.	Ť	Zhan et al. (2019)
China	Cd	Field experiment	AMF	19.02		4.8	Zea mays L.	ł	He et al. (2020)
Brazil	Cu	Pot experiment	Glomus etunicatum			125.71	Leucaena leucocephala	Ť	Lins et al. (2006)
China	Cu	Pot experiment	Glomus mosseae	232		1267.34	Lolium perenne	1	Chen et al. (2005)
South Africa	Cu	Pot experiment	Native AMF sp. (<i>Gigaspora</i> sp. and <i>Glomus tenue</i>)	55		108 (29)	Berkheya coddii Roessle	t	Orlowska et al., 2011
United States	Cu	Pot experiment	Native AMF	653		21.5	Prosopis juliflora	Ť	Solís-Domínguez et al. (2011)
South Africa	Cu	Pot experiment	Native AMF	55		26	Berkheya coddii Roessle	Ť	Orłowska et al. (2013)
Brazil	Cu	Pot experiment	Glomus margarita	17.7		102	Chrysopogon zizanioides (L.)	¥	Meyer et al. (2017)

(Continued on following page)

Manyiwa and Ultra Jr Reference (2022) noculation Colosphospermum торапе 250.11 Experimental dose (mg kg^{_} [ABLE 1 (Continued) Summary of studies on arbuscular mycorrhizal fungi in toxic metal remediation concentration in soi 768.13 Inherent total Łg_ fetal uptake increased in the shoot/root of the AMF-inoculated plant compared with the control. Metal uptake decreased in the shoot/root of the AMF-inoculated plant compared with the control Name of AMF AMF experiment experiment Type of Pot Metal G South Africa Country

accumulation, increases photosynthesis capacity, and modulates metal toxicity. Through the formation of extracellular and intracellular hyphae, AMF increase soil surface area for better absorption of soil nutrients (N and P) and toxic metals (Cr, Ni, Cd, Pb, and Cu), improves root growth and root morphology, and secretes various proteins like glomalin (Amir et al., 2013; Lam and Lai, 2018; Zhan et al., 2019; Manyiwa and Ultra Jr, 2022). In this paradigm, the presence or absence of AMF in plant systems could impact the accumulation capacity of toxic metals, thus increasing or decreasing ecosystem risk.

6 Conclusion

Global heavy metal pollution is of great concern to environmentalists. Numerous research papers have explored the toxic effects of HMs on plants, animals, humans, and other living organisms. These studies highlight the detrimental impact of HM contamination on the ecosystem, emphasizing the need for effective remediation strategies. Plants play an efficient role in the remediation of poisonous HMs. However, the effectiveness of phytoremediation is often limited by slow plant growth and lower efficiency in removing HMs. To address these challenges, the use of plant-associated microbes, especially arbuscular mycorrhizal fungi (AMF), can significantly enhance the removal efficiency of HMs from contaminated soils. These microbes can also improve plant health, nutrient uptake, and stress tolerance, thereby boosting the overall phytoremediation process. The success of this bioremediation technology depends on the proper selection and screening of plant species and AMF cultures to optimize their effectiveness in mitigating HMs from contaminated environments. Future research should focus on optimizing AMF-based remediation strategies, particularly in metal-polluted soils, to enhance ecological sustainability and agricultural productivity. Several areas of research could potentially improve the remediation of metal-contaminated soils in the future. Some of these include:

- Developing more efficient and cost-effective methods for removing or treating metal contaminants.
- Improving our understanding of the behavior and mobility of metal contaminants in the environment, which could lead to more targeted and effective remediation methods.
- Developing new technologies for detecting and measuring metal contaminants in soil, which could enable more accurate assessments of contamination levels and the effectiveness of remediation efforts.
- Investigating alternative materials and methods for immobilizing contaminants, such as natural or synthetic zeolites, to overcome the limitations of traditional stabilization/solidification methods.
- Investigating the use of new microorganisms, enzymes, or new biotechnology approaches for bioremediation and making it more efficient.
- Investigating the use of hybrid approaches, such as combining phytoremediation with bioremediation or chemical treatment, to increase the efficiency of remediation.
- Investigating the use of machine learning and AI tools to optimize the effectiveness of remediation methods and better predict the behavior of contaminants in different soil types.

Non-significant



FIGURE 6

The forest plot indicates the mean difference of individual observations regarding the metal accumulation levels in plants in the presence of mycorrhizal infection in different countries. (A) Pb, (B) Cd, (C) Ni, and (D) Cu.

• Conducting more long-term studies to assess the effectiveness of different remediation methods and to identify any potential negative effects on the environment or human health.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

SB: Data curation, Formal analysis, Methodology, Software, and Writing–original draft. JM: Software and Writing–review and editing. DS: Conceptualization, Resources, and Writing–review and editing. RD: Conceptualization, Resources, and Writing–review and editing. PB: Conceptualization, Supervision, and Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2024.1532169/ full#supplementary-material

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