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

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Abstract

Mines are natural reservoirs of various types of minerals, metals, and metalloids. Several heavy metals (HMs) such as Pb, Cd, Cr, Cu, Ni are major anthropogenic pollutants that cause severe environmental pollution. 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 HMs 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 enhances 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 mining-affected areas. Furthermore, the study highlights the role of various plant species and microbes, particularly AMF, in mitigating HMs stress and its impact on plant growth and nutrition. The meta-analysis also evaluates the efficacy of AMF as a remediation strategy for HMs-impacted mine soils.

Keywords: Mines, Heavy metals, Phytoremediation, Arbuscular mycorrhizal fungi (AMF), Meta-analysis

1 Introduction

Human-driven activities such as agriculture, mining, industrial processes, and the extensive use of fertilizers and pesticides have led to an escalating 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 an unadorned environmental phenomenon worldwide. Metal mining and mineral ore processing have a dual effect on the economy and environment. From one perspective, it provides economic benefits to the country, and simultaneously it causes environmental pollution. Abundant and active mines are the prime source of toxic HMs. During rainy season, due to heavy rainfall and strong winds run-off water washes down the toxic waste material to 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 the living beings as well as the environment (Dhaliwal et al., 2020). Once these toxic HMs are released into the surrounding ecosystem, it could migrate to a distant area, accumulate in various biotic, abiotic components of the system, and adversely affect the food chain, human health, and environment (Peralta-Videa et al, 2009). Lead (Pb), chromium (Cr), mercury (Hg), cadmium (Cd), and arsenic (As) have lethal effects in humans, plants, and animals but depending on the concentration; a few metals such as zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) have another role as an essential micronutrient needed for metabolic activity (Schneegurt et al., 2001; Mohan et al., 2007). Heavy metal contamination significantly alters soil characteristics and the surrounding micro-environment. 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 (qCO_2) (Crowley, 2008; Zhao et al., 2020). The degree of HM pollution has been evaluated by several indices like pollution load index (PLI) and 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 which are usually cost-intensive. Given the limitations of conventional cleanup techniques, bio-logical approaches could be looked into as a potential alternative mitigation option. In some places, bioremediation, 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 despite the fact that 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 up 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 works 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 the high metal contaminated area. Remediation through plant or phytoremediation is one of the most promising eco-friendly management strategies for reducing toxic contaminates (Burges et al., 2018).

In past few decades researchers have worked with various types of plants, their potentiality, and their remediation mechanisms 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 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 in nature (Andra et al., 2009). Also, 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 been conducted in the past focusing 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 HMs 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 HMs 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 Kautilya's Arthshastra, "Mines are Nation's treasury". Mineral resources from mines are abundant in nature. Exploitation of these minerals enhances world's economy and development but at the same time, surface mining, especially open cast mining causes severe environmental problem (i.e loss of surface vegetation, destruction of soil structure etc.). Mines are the source of various metals and minerals like iron and ferro-alloys (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, 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 United States, Russia, Australia, and India etc. (Reichl and Schatz, 2020).

2.1 Coal Mines

Fossil fuel, coal is a predominant element in nature which is mainly composed of carbon with variable amounts of other elements i.e hydrogen, oxygen, nitrogen, and sulphur. China is the largest producer of coal. The open-cast mining generates toxic overburden dumps (OB) and coal dust that contains enormous toxic HMs, is responsible for metal contamination in adjacent agricultural land (Li et al., 2007). In descending order, metals Fe>Mn>Zn>Cu are present in coal mine: the most bioavailable and mobile element is Mn followed by Zn and Cu, and the least mobile metal is Fe. The reason behind Fe less mobility is the residual fraction of Fe, which indicates its strong affinity towards minerals, the solid matrix, and strongly bounded clay minerals (Kartal et al., 2006). The pseudo-total concentration of HMs such as 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. Selectively Pb can accumulate in leaves, stem bark, and root bark whereas Zn and Mn in leaves and Cu in stem wood and root wood. These indicate that accumulation of metals might be tissue specific (Maiti et al., 2016). For reclamation process of OB dumps, the most effective remediation pathway is trees which can accumulate toxic HMs from OB. Trees that are used for reclamations of OB dumps, should be drought resistant, woody, fast-growing, able to grow in metal arid area, and nutrient-deficient area (Pratas et al., 2005). Various woody plant species i.e *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, *Azadirachta indica* A. Juss *etc*. are used for reclamation of coal OB dumps (Maiti et al., 2016). Pb concentration in *A. auriculiformis*, Eucalyptus hybrid trees is significantly higher in root bark as compared to 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 plant which is an important defence mechanism of plant against HM toxicity (Alloway, 2012). In the case of Cu, (BCF > 1; TFleaf, TFstembark and TFstem wood < 1) in both the 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 Predra Verde mine (Brazil) show potential risk to the environment (Perlatti et al., 2021). In Jiuhuashan, Jiangsu Province and eastern China, agricultural soil near abandoned mine 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 produced from La Concordia Mine (Argentina) (Nieva et al., 2018). According to China et al. (2014) environmentally available metal i.e total metal excluding the silicate matrix bound metals, Cu (154 mg kg^{-1}) seems as one of the most abundant heavy metals found in 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 translocation factor for Cu (0.06), Ni (0.36), Co (0.68), 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, Ni in plant is 20-100 mg kg^{-1} and 10-100 mg kg^{-1} respectively (Kabata-Pendias, 2011). Bioavailability of copper also depends on soil pH, soil CEC, and total copper content in soil (Bravin et al., 2009; Brun et al., 2001). Simultaneously copper also plays an essential role in plant growth and development processes such as protein synthesis, CO_2 assimilation, ATP synthesis, maintenance of homeostasis within chloroplast, photosynthesis etc. (Hänsch and Mendel, 2009; Yruela, 2013). On the other hand, high concentration of copper has a toxic effect on seed germination, decreases plant height, causes chlorosis of plant leaves, reduction in plant biomass, and grain yield (Adrees et al., 2015).

2.3 Chromite Mines

Ferrochromium is only natural and economical resources 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 grasp most chromite ores followed by Kazakhstan, India, Albania, and Turkey (ICDA, 2022). The active and abandoned mine wastes are the reservoirs of heavy metals which 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 are present in lesser quantity (Bueno et al., 2009). Chromium (Cr) is generally utilized by different industrial activities such as processing and finishing of leather, production of refractory steel by stainless steel industry, electroplating cleaning agent, muds drilling, production of chromic acid and other chemicals, and use to preserve food for a short time (Shanker et al., 2005). Chromium exhibits different levels of toxicity depending on its chemical form, pH, reacts with other elements and solubility index (Thatoi et al., 2014). Cr (VI) exhibits high toxicity and bioavailability due to it has better solubility than Cr (III) (Abyaneh and Fazaelipoor, 2016). For humans and mammals, lack of Cr (III) in diet can causes metabolic deterioration, cardiac problem, diabetes but 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 plant in solution is as low as 0.5mg kg^{-1} and 5 mg 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 larynx and pharynx, edema, coughing, asthma etc. (Bloise et al., 2008; Pugnaloni et al., 2013). Cr is mostly accumulated in the root than shoot due to its less mobility in root vacuoles but in case of Ni, accumulation is higher in shoot than root due to greater mobility of Ni through xylem tissue (Pulford et al., 2001; Shanker et al., 2005). The Cr and Ni concentration in Roro chromite mine waste soil is 3120 mg kg^{-1} and 1620 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). Similar studies in Almadén mine site in Spain and southern Togo mine sites, Cr and Ni concentration has 86–35 Cr mg kg^{-1} and 21.2–126 Ni mg kg^{-1}) and (182–1029 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 Daduk mine area of Korea, due to dispersion of metals from tailings and watercourses, various types of toxic metals are reported in nearby paddy fields (Lee et al., 2001). Another study in Co Dinh mine of Vietnam, high level of potentially toxic elements is also detected in rice field (5750 Cr mg kg^{-1}, 375 Co mg kg^{-1} and 5590 Ni mg kg^{-1}) (Kien et al., 2010). Based on dynamic translocation factor (TF dyn>1), Cr and Ni accumulation is higher in plant parts of *Oryza sativa* growing in the contaminated agricultural fields which might have potential risk of transfer of toxic HMs to livestock or human (Kien et al., 2010; Kumar and Maiti, 2014).

2.4 Iron Mines

Globally, since 2000, to meet the increasing demand of crude steel the production has expanded drastically. Iron ores are the backbone of world economy (World steel Association 2021). Australia, Brazil, and China are the top 3 countries for production of iron (around 69%) in the world (Holmes et al., 2022). To maintain the growing demand, iron ore industries have continuously amplified the mining activity in iron mines. As a result, huge concentrations of Cd, Mn, As, Ni, Pb, Zn, and Cr has found in the agricultural soil nearby iron mines (Hosseini et al., 2018). Tailing toxic wastes from iron mining are dumped into the tailings pond which is located at the Noamundi–Jodda belt, India. As a result, during monsoon season, the toxic fine particle wash-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 synthesis of chlorophyll, chloroplast structure and its functions but excessive concentration of iron might be entered into the food chain and showed toxic effect in plant, animal and human health (Maiti et al., 2005). According to Dhatrak et al. (2017), the health status of mine workers and nearby population around open cast iron mine showed NIHL (noise-induced hearing loss) and anemia as a major health effect. 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. Thus, these air pollutants would affect the flora and fauna of the local environment (Tripathi et al., 2014; Chaturvedi and Patra, 2016). There are some native plant species such as *Cassia sophera Eupatorium odoratum, Techtona grandis, Alstonia scholaris, Cassia tora* etc. found in Fe-tailings. *Techtona grandis* can accumulates higher concentration of HMs than *Alstonia scholaris* but these HMs have not showed any detrimental effect on native plants rather higher Fe contents 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 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). Amphoteric, silvery -white, hard radioactive element uranium which is occurs naturally in the earth's crust with a mean concentration about 3 mg kg^{-1} (Gupta and Singh, 2003). Hexavalent U is the most soluble form and is present as uranyl cation $(UO_2)^{+2}$ normally in 80-90% of the soil. It prevails in solutions predominantly as stable ion $(UO_2)^{+2}$ and as soluble carbonate complexes i.e. UO_2CO_3, $UO_2(CO_3)_2^{-2}$, $UO_2(CO_3)_3^{-4}$, $(UO_2)_2CO_3(OH)^{-3}$ and $(UO_2)_3(CO_3)_6^{-6}$. In the absence of dissolved inorganic ligands (fluoride, carbonate, sulfate, and phosphate) and the pH range within 4 to 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 which 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 (^{206}Pb) is formed (Sarangi, 2003). The radiation which emits from these naturally occurring isotopes is very low and does not penetrate due its high density which acts as a shield against its own radiation (Wang et al., 2009). According to WHO (2012), the mean concentration of U in ambient air has been reported around 0.02 ng m^{-3} in Tokyo, Japan and 0.076 ng m^{-3} in New York City, USA. Through water or food, uranium enters kidney and the uranyl ion forms bicarbonate and citrate complexes in blood plasma and affects the proximal tubules of kidney, causes tubular degeneration, liver damage, genetic malfunction, cancer, and necrosis (Miller et al., 2004; Sethy et al., 2011). The Environmental Protection Agency (EPA) of United States has categorized U as a carcinogenic element and in drinking water the maximum contaminant level (MCL) of U is 30 ug L^{-1} (EPA, 1999). The proposed interim maximum acceptable level (IMAC) of U in Canada is 20 ug L^{-1} whereas WHO strictly recommended the permissible level to be 2 ug L^{-1} (Shin et al., 2002). In human the ingestion and intake dose are very low (2 $uSv.Y^{-1}$) 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 are conducted for the accumulation of U in native plant species to determine the mechanism of U uptake, absorption by plant and for biological exploration of U from soil (Petrova, 2006). Uranium accumulation varies depending upon plant species as well as genotypes, lines within species, cultivars; moreover, U is accumulated higher in the root portion in comparison to the shoot. Therefore, only a small portion is translocated to the shoot and 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 phyto extraction (84-95% of recovery) of U from hydroponic solution at a concentration below 200 ppm under controlled experimental condition. U is generally restricted in root portion of Vetiver but above 1000 ppm of U concentration, 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 determine the relative comparison between a selected number of peer-reviewed articles and to find out the potential risk of toxic metals on soil health using global datasets. We searched pieces of literature published in the Web of Science database between 2012 to 2022 and select the quality research work according to our objectives. The keywords were 'Mine', 'pollution' 'Copper', 'Cadmium' 'Nickel', 'Chromium', 'Lead', 'World'. From > 2500 published reports, we excluded the 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 Figure S3.

From the literature survey, we considered the parameters like standard error, sample size, and the difference between the mean of tested and the mean of control. 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) as suggested by WHO 1996 (Cu: 36 mg kg^{-1}, Ni: 35 mg kg^{-1}, Cr: 100mg kg^{-1}, and Pb: 85 mg kg^{-1}). The ultimate result was expressed on mean difference as a continuous factor for statistical analysis at the 95% confidence level (CI) between the group of individual study and permissible limit of metals (Cr, Ni, Pb, Cd, and Cu) in the mines area. Therefore, from 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, commonly known as the zero-effect line, was present in the middle part of the forest plot depicting that there was no difference between the study group mean and permissible limit. So that mean difference is zero at this point.

From Figure 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 index (I^2) of 98.58% and 96.05% respectively which represented substantial heterogeneity [43,49,63,68, 84-87]. Similarly for Cd, Pb, and Cu (Figure 3, 4, 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 statistical significance at $p < 0.05$. The overall inconsistency index (I^2) of Cd, Pb, and Cu was 47.34%, 99.24% and 98.02% respectively, indicating substantial heterogeneity. The positive value indicated that the total concentration of Cr, Ni, Cd, Pb and Cu in mine areas was higher than the permissible level as recommended by WHO. In a meta-analysis of the summary mean, most metals (Cr, Ni, Pb and Cu) present in mine areas were found to be significant with $p<0.05$ as the confidence intervals did not overlap with the zero-effect line except 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 be able to influence the potential risk of metal toxicity and 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 one of the serious nuisances to the environment as well as for living beings (Sun et al., 2012; Cachada et al., 2018). As we know remediation of heavy metals is very much complicated than any other organic pollutants. Various traditional, mechanical and chemical techniques such as 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, time-intensive, 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 a lot of attention due to its cost-effectiveness, viability, no generation of secondary waste and eco-friendly (Akande et al., 2018; ALAM et al., 2018). It includes plants, various microbes (bacteria, fungi, mycorrhiza etc) which are utilized to decontaminate the hazardous compounds from soil. For soil purification, the applications of plant alone or association with microorganisms that helps to stabilize, mineralize, transfer, remove the 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). By the definition, 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). Table S1 showed different types of mechanisms of phytoremediation and pictorial representation is in Figure S2. Generally, plants can extract essential nutrients (Fe, Zn, Ni, Mn, and Cu) as well as non-essential metals (Cr, Cd, As, Pb and Hg) which 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). There are several studies have been done regarding different mechanisms of phytoremediation strategies, as shown in Table S2. There are many advantages of phytoremediation listed 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, however, one limitation is there, that plant requires time for their growth and development (Morikawa and Erkin, 2003). Several plant species have demonstrated the ability to absorb, bioaccumulate, immobilize, and degrade heavy metals (HMs) from contaminated sites. Techniques like phytoextraction and phytostabilization are commonly used for remediating HMs-polluted sites. Some examples include *Cymbopogon citrates*, *Helianthus petiolaris*, *Vetiveria 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 and enhance soil quality. Successful phytoremediation 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 USA and Iran (Yoon et al., 2008; Zazouli et al., 2014). Phytovolatilization occurs when plants transform the contaminant into volatile compounds and emit 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 formsforms (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 USA, 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 large number of microorganisms in their rhizospheric region. Beneficial microorganisms especially arbuscular mycorrhizal fungi (AMF) have a symbiotic association with plant roots where AMF increases plant nutrient uptake ability, improves biomass accumulation, amplifies photosynthesis capacity, and provide protection against heavy metal toxicity, successively, plant provides exudates of amino acids, carbon, photosynthetic product to the AMF for growth and developments (Mitra et al., 2020).

Around 80% terrestrial plants and 90% agricultural plants have mycorrhizal association in their roots where fungal hyphae enter in the cortical cells of plant roots forming vesicles, hyphae and arbuscles (Smith and Read, 2010). Figure S1 denotes the schematic diagram of heavy metal detoxification mechanism through AMF. AMF helps in immobilization of heavy metals by binding them at cortical region, prevent translocation to the upper ground part (shoot, stem, leaves) and prevent damage of leaves. Plants are categorized based on TF value into hyper accumulator (TF>1) and non- hyper accumulator (TF<1). The translocation factor (TF) is higher in non-mycorrhizal associated plant than in mycorrhizal associated plant (Arshad et al., 2008). Endomycorrhizal fungi AMF, belongs to phylum Glomeromycota which considered as an eco-friendly sustainable strategy to enhance plant growth, increase shoot biomass, improving soil health and water uptake capacity, provide protection to the plant against biotic, abiotic stress and detoxify heavy metal induced stress (Mishra et al., 2019). Glomeromycota are obligated symbiotic organisms, so they require around 20% of carbon from host plant cell for their survival and simultaneously they provide water and nutrients (P, N) through their arbuscles, intracellular and extracellular hyphae to the host plant (Parniske, 2008). AMF combat the heavy metal stress by immobilization, precipitation, chelation and sequestration in rhizosphere, vacuoles and activates the plant anti-oxidant defense system (Mitra et al., 2020). Another way of defense mechanisms of AMF is to secrets a hydrophobic unique glycoprotein called 'Glomalin' which is composed of (39-59%) carbon, (0.03-0.1%) phosphorus, (3-5%) nitrogen, (4-6%) hydrogen, (33-49%) oxygen and trace amount of iron (Schindler et al., 2007; Zhang et al., 2017). This protein is basically 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) both are simply quantified from soil with the help of 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 nutrient, water and the ions which are generally present in immobilize form in soil. AMF also improves the plant ability to acquire nutrient from nutrient depleted zone of rhizosphere (Smith and Read, 2010; Smith and Smith, 2011). As stated by Nakmee et al., (2016), native species of AMF *Glomus aggregatum*, *Acaulospora scrobiculata*, *F. mosseae* provide positive effect on plant nutrient uptake (total N, P, K), 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 1.13-2.76 times higher concentration of Zn than non-inoculated wheat plants (Coccina et al., 2019). Various abiotic stresses like salinity, heavy metals, drought, flooding, extreme temperature etc. AMF community withstand independently against these unfavorable stress condition for its host plant and provide sufficient water in drought stress, supply nutrients (phosphorus) and balance osmotic pressure in flooding stress condition (Zhu et al., 2017; Caradonia et al., 2019). Under drought condition, tomato plant containing AMF (*R. intraradices*) colonization on their roots, provides sufficient water-nutrient to the tomato plant for better growth during water stress situation. Inoculation with *G. etunicatum* enhances total chlorophyll content, root- shoot height- weight, increased N, P, K, Ca, Zn, Cu concentration, flavonoids content, soluble sugar, proline, glycine betaine, polyamine, POD, CAT activity in *Pistaciavera* L under stress condition (Abbaspour et al., 2012). Studies raveled that *G. etunicatum F. mosseae* and *R. irregularis* increased the growth, grain yield of *Triticum aestivum* L. and regulates nutrient uptake capacity and decreases Na+ and Cl- concentration at the time of salinity stress (Daei et al., 2009). According to Hashem et al., (2016) oxidative stress generates high concentration malonaldehyde and hydrogen peroxide on *Solanum lycopersicum* L. AMF strain (*Glomus mosseae*, *Glomus intraradices* and *Glomus etunicatum*) helps to decrease the concentration of these elements and boost up plant 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 plant diseases. However, the presence of AMF significantly reduces pathogen-induced damage and infection by enhancing nutrient availability, stimulating root growth, and improving root morphology. Additionally, 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 on *Cicer arietinum* L plant but treatment with AMF strain (*Glomus hoi*) provide protection against wilt disease and increase the nitrogen and phosphate content in treated plants as compared with non-treated plants

(Singh et al., 2010). Similarly, *Glomus* sp. synthesis antimicrobial compounds which helps to arrest the mycelia growth of *Fusarium oxysporum* on L. esculentum plants and increase chlorophyll, N, P, K content of the plants. Also in *Capsicum annum*, *Glomus* sp. reduces the activity of pathogen *Pythium aphanidermatum* and provide 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

Research works published between 2005 and 2022 were searched in 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 the assessment of >250 peer-reviewed articles, the articles were excluded based on the following reasons: a) Lacking in analytical techniques (not mentioning the QA/QC), b) remediation through other microbes c) Graphical representation of data. To conclude, a total of 24 studies comprising of 9, 12, 7 and 5 studies for Cd, Pb, Cu and Ni respectively were included in for meta-analysis, which assessed the efficacy of AMF in remediating metal-contaminated mine soils (Table 1). Studies reporting remediation of Cr with AMF was not found during the systemic review. The PRISMA flowchart is displayed in Figure S4.

From RE models at (Figure 6 (a, b,c,d)), the overall mean value 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 index (I^2) of Pb, Cd, Ni, and Cu was 92.94%, 96.39%, 99.30%, and 98.89% respectively, indicating substantial heterogeneity. The positive effect size for all the metals indicated that the AMF has the ability to reduce the metal accumulation capacity in plants. The studies from USA (Punamiya et al., 2010), China (Zhan et al., 2019), Mexico (González-Villalobos et al., 2021), etc. indicate that the Pb accumulation in plants has increased by AMF (*Diversispora spurcum*, *Glomus mosseae*, *Rhizophagus irregularis*) except (Wu et al., 2010; Solís-Domínguez et al., 2011; Bahraminia et al., 2016), the CIs value overlapped the line of zero showing non-significant. (Figure 6a). The 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 has increased in presence of AMF infection of *Glomus deserticola*, *Glomus intraradices* and *R. irregularis*. Other studies from China (Wu et al., 2010; Hu et al., 2013; Liu et al., 2014; He et al., 2020), the Cd accumulation in plant was decreased (*Glomus constrictum*, *Glomus caledonium*, and *Glomus intraradices*) (Figure 6b). For Ni and Cu, the accumulation in decreases in presence of AMF (*Glomus tenue*, *Glomus margarita*) (Orłowska et al., 2011; Lam and Lai, 2018; Manyiwa and Ultra Jr, 2022) and simultaneously increase the accumulation by the help of *Glomus mosseae*, *Glomus etunicatum* (Chen et al. 2005; Lins et al., 2006) (Figure 6c and 6d). Plant roots are symbiotically associated with AMF, which increases plant nutrient uptake ability, increases biomass accumulation, increase photosynthesis capacity, and modulates metal toxicity. Through the formation of extracellular and intracellular hyphae, AMF increases soil surface area for better absorption of soil nutrients (N, and P), toxic metals (Cr, Ni, Cd, Pb, and Cu), improve root growth, root morphology, secrets 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 the 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 HMs 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. There are several areas of research that 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 the use of alternative materials and methods for immobilizing contaminants, such as natural or synthetic zeolites, to overcome the limitations of traditional stabilization/solidification methods.
- Investigate the use of new microorganisms, enzymes or new biotechnology approach 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.
- 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.

Authors contribution

Sonali Banerjee: Review design, Data Analysis, Original draft preparation; Jajati Mandal: Statistical analysis, reviewing; Dibyendu Sarkar and Rupali Datta: Reviewing, Editing; Pradip Bhattacharyya: Supervision, Reviewing, Editing

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Table 1 Summary of research works on arbuscular mycorrhizal fungi in toxic metal remediation

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. (*Gigaspora* sp. and *Glomus tenue*)	650		7020	*Berkheya coddii* Roessle	↓	Orlowska et al., 2011
South Africa	Ni	Pot experiment	Native AMF	650		724	*Berkheya coddii* Roessle	↑	Orłowska et al., 2013
France	Ni	Pot experiment	*Glomus etunicatum* SFONL		60	881	*Cloezia artensis*	↓	Amir et al., 2013
Taiwan	Ni	Pot experiment	AMF	459.5		90.1	*Ipomoea aquatica* Forsk.	↓	Lam and Lia, 2018
South Africa	Ni	Pot experiment	AMF	634.25		66.10	*Colosphospermum mopane*,	↓	Manyiwa and Ultra, 2022
China	Pb	Pot experiment	AMF		600	259.81	*Kummerowia striata*	↑	Chen et al., 2005
China	Pb	Pot experiment	*Glomus mosseae* and *Glomus intraradices*	4418		1.11	*Leucaena leucocephala*	—	Ma et al., 2006
Spain	Pb	Pot experiment	*Glomus deserticola*	595.96		284.1	*Eucalyptus globulus*	↑	Arriagada et al., 2007
USA	Pb	Pot experiment	*Glomus mosseae*		1200	2179	*Chrysopogon zizanioides* (L.)	↑	Punamiya et al., 2010
China	Pb	Field experiment	*Glomus intraradices* and *Glomus mosseae*	209		12.6	*Chrysopogon zizanioides* (L.)	—	Wu et al., 2010

USA	Pb	Pot experiment	*Glomus deserticola*	4620		3.89	*Prosopis juliflora*	–	Solís-Domíngue z et al., 2011
China	Pb	Pot experiment	AMF	3683	1500	2655	*Viola baoshanensis*	↑	Zhong et al., 2012
Iran	Pb	Pot experiment	*Glomus versiforme*		800	119.80	*Chrysopogon zizanioides*	–	Bahramini a et al., 2016
Brazil	Pb	Pot experiment	*Acaulospora scrobiculata*	125		103	*Chrysopogon zizanioides* (L.)	↑	Meyer et al., 2017
China	Pb	Pot experiment	*Gaeumannomyces cylindrosporus*		1000	252.25	*Zea mays* L	↑	Yihui et al., 2017
China	Pb	Pot experiment	*Diversispora spurcum*	1426.7		732.9	*Cynodon dactylon* (L.) Pers.	↑	Zhan et al., 2019
Mexico	Pb	Pot experiment	*Rhizophagus irregularis*	640		237.97	*Parkinsonia aculeata* L	↑	González-Villalobo et al., 2021
Spain	Cd	Pot experiment	*Glomus deserticola*	21.48		7.4	*Eucalyptus globulus*	↑	Arriagada et al., 2007
Brazil	Cd	Pot experiment	*Glomus intraradices*		0.02	885	*Helianthus annuus* L.	↑	de Andrade et al., 2008
China	Cd	Field experiment	Glomus intraradices and *Glomus mosseae*	2.25		939	*Chrysopogon zizanioides* (L.)	↓	Wu et al., 2010
China	Cd	Pot experiment	AMF	113	200	6952	*Viola baoshanensis*	↑	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.	↑	Hassan et al., 2013
China	Cd	Pot experiment	*Glomus constrictum*		112	8.27	*Zea mays* L.	↓	Liu et al., 2014
China	Cd	Pot experiment	*Diversispora spurcum*	16.9		14.5	*Cynodon dactylon* (L.) Pers.	↑	Zhan et al., 2019
China	Cd	Field experiment	AMF	19.02		4.8	*Zea mays* L.	↓	He et al., 2020
Brazil	Cu	Pot experiment	*Glomus etunicatum*			125.71	Leucaena leucocephala	↑	Lins et al., 2006
China	Cu	Pot experiment	*Glomus mosseae*	232		1267.34	*Lolium perenne*	↑	Chen et al., 2005
South Africa	Cu	Pot experiment	Native AMF sp. (*Gigaspora* sp. and *Glomus tenue*)	55		108(29)	*Berkheya coddii* Roessle	↑	Orlowska et al., 2011
USA	Cu	Pot experiment	Native AMF	653		21.5	*Prosopis juliflora*	↑	Solís-Domíngue z et al., 2011
South Africa	Cu	Pot experiment	Native AMF	55		26	*Berkheya coddii* Roessle	↑	Orłowska et al., 2013
Brazil	Cu	Pot experiment	*Glomus margarita*	17.7		102	*Chrysopogon zizanioides* (L.)	↓	Meyer et al., 2017
South Africa	Cu	Pot experiment	AMF	768.13		250.11	*Colosphospermum mopane*,	↓	Manyiwa and Ultra, 2022

↑ Metal uptake increased in shoot/root of AMF infected plant as compared with control

↓ Metal uptake decreased in shoot/root of AMF infected plant as compared with control

– Non significant

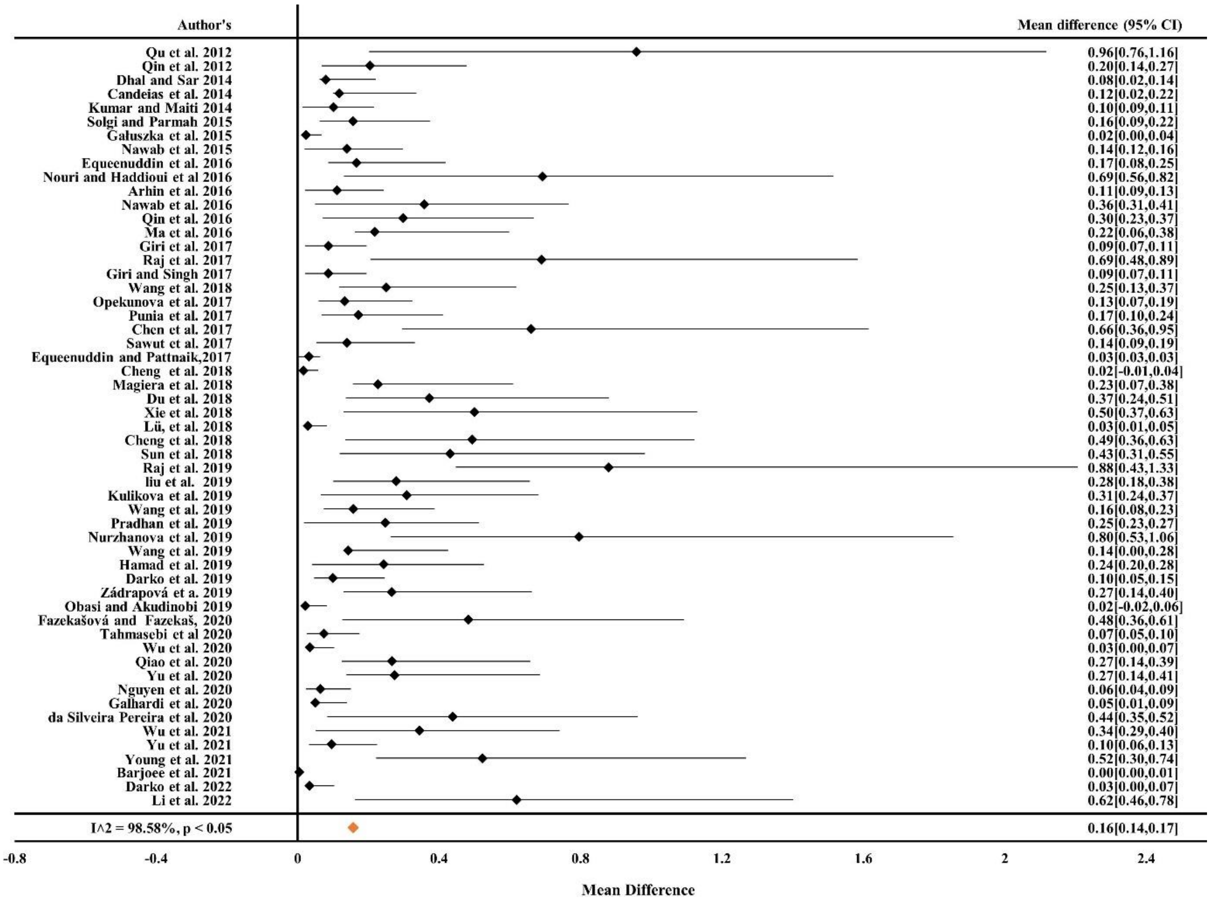

Figure 1 Forest plot indicating the mean difference of individual observation regarding the Cr contamination in mine areas.

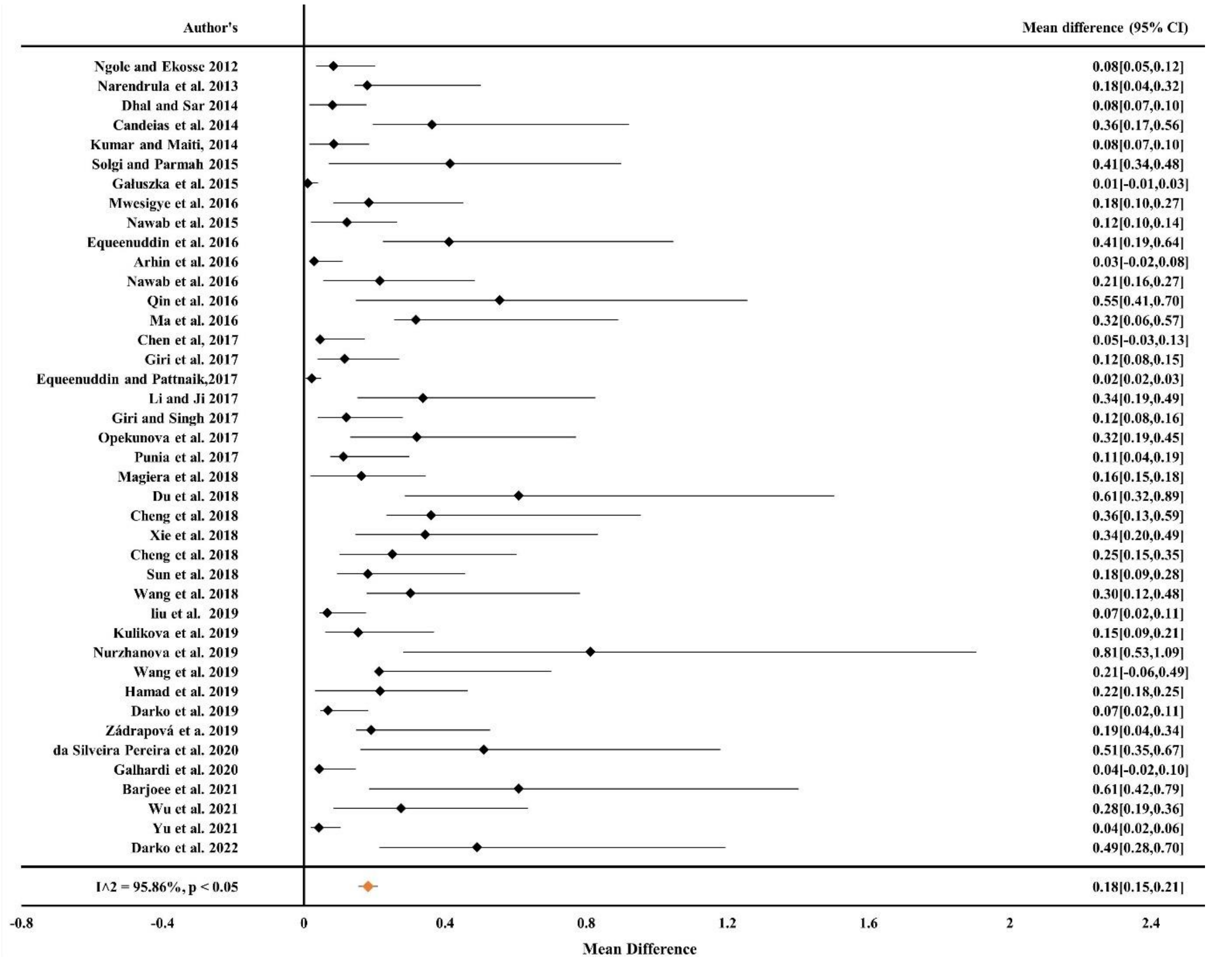

Figure 2 Forest plot indicating the mean difference of individual observation regarding the Ni contamination in mine areas.

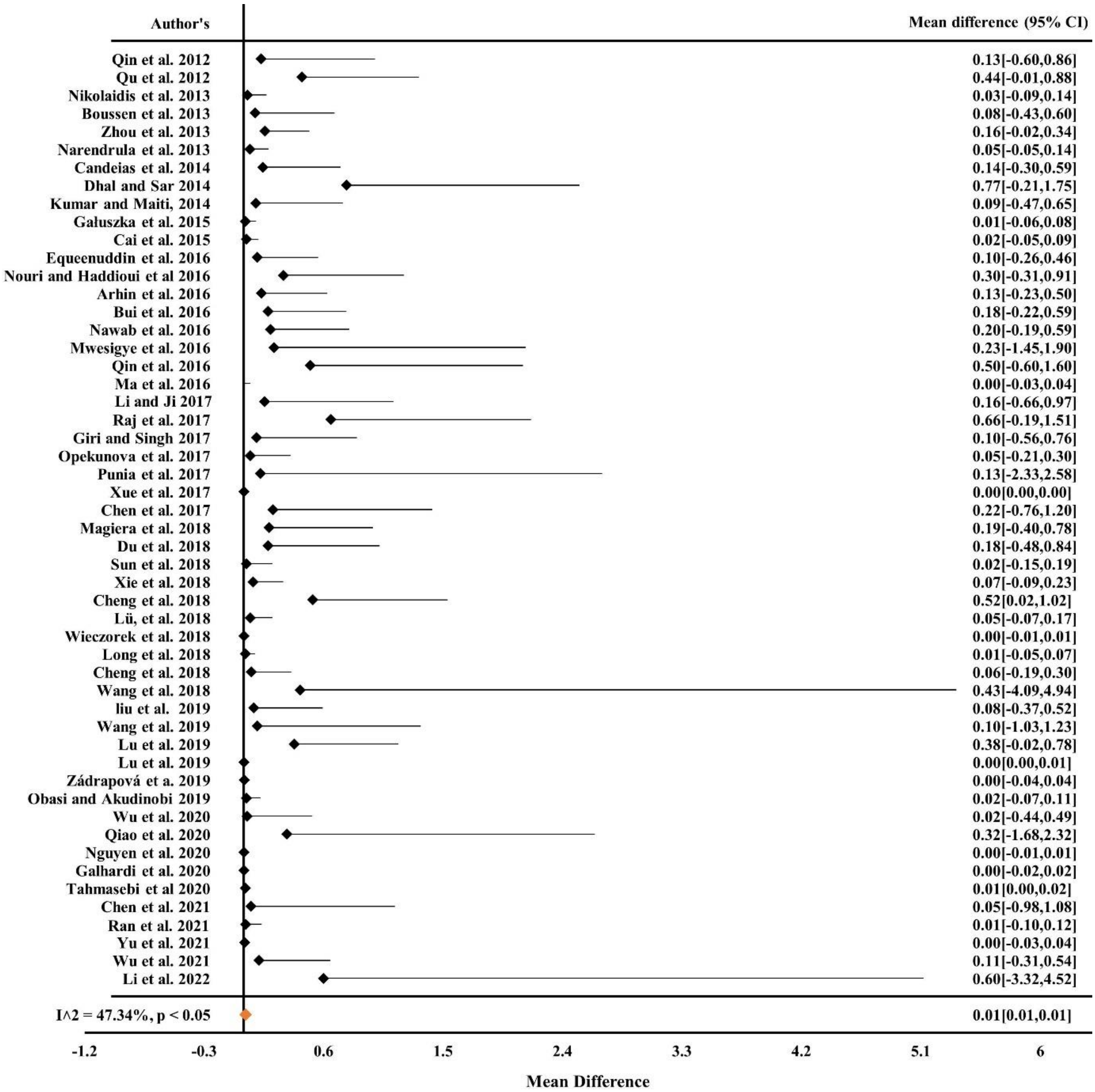

Figure 3 Forest plot indicating the mean difference of individual observation regarding the Cd contamination in mine areas.

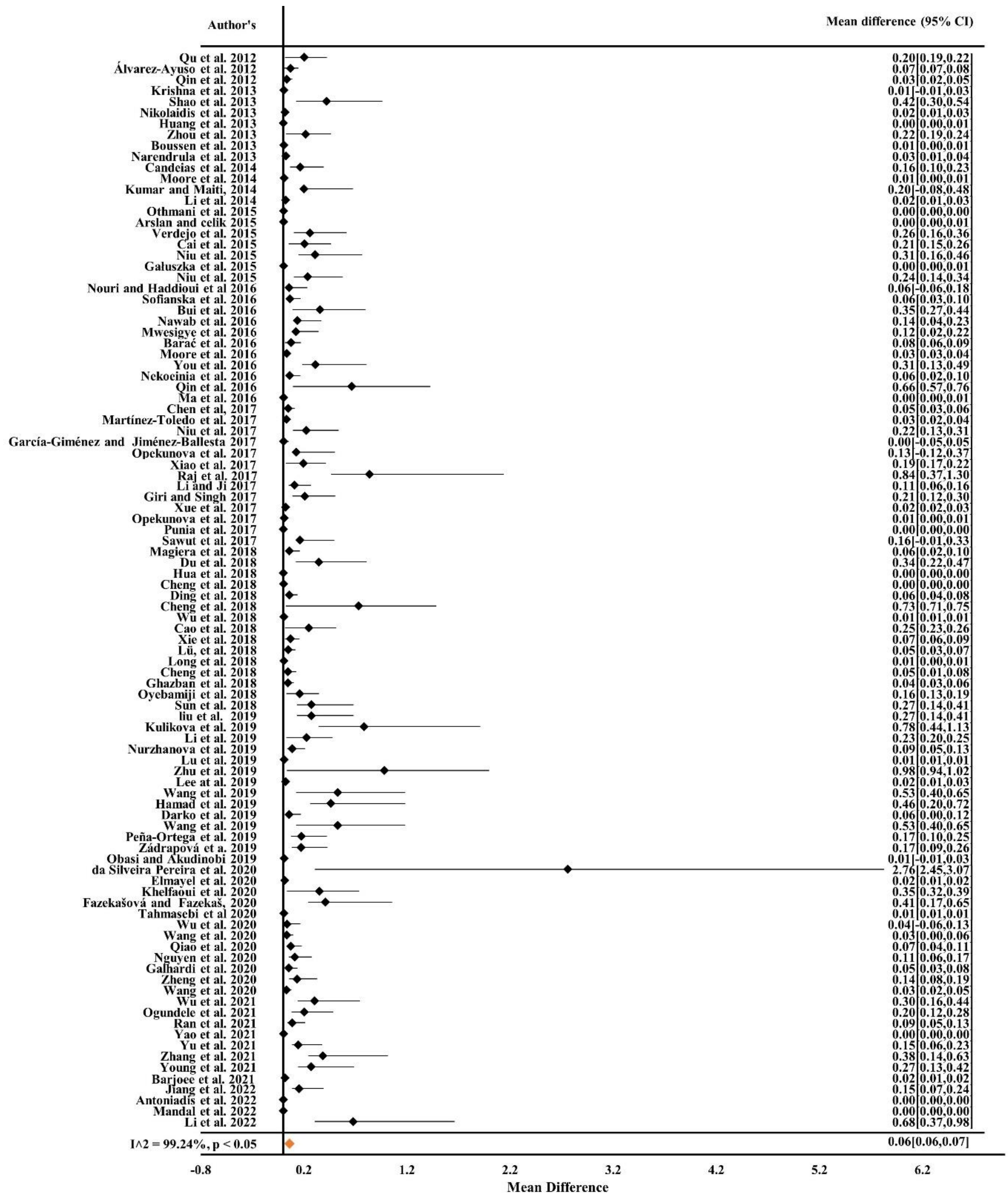

Figure 4 Forest plot indicating the mean difference of individual observation regarding the Pb contamination in mine areas.

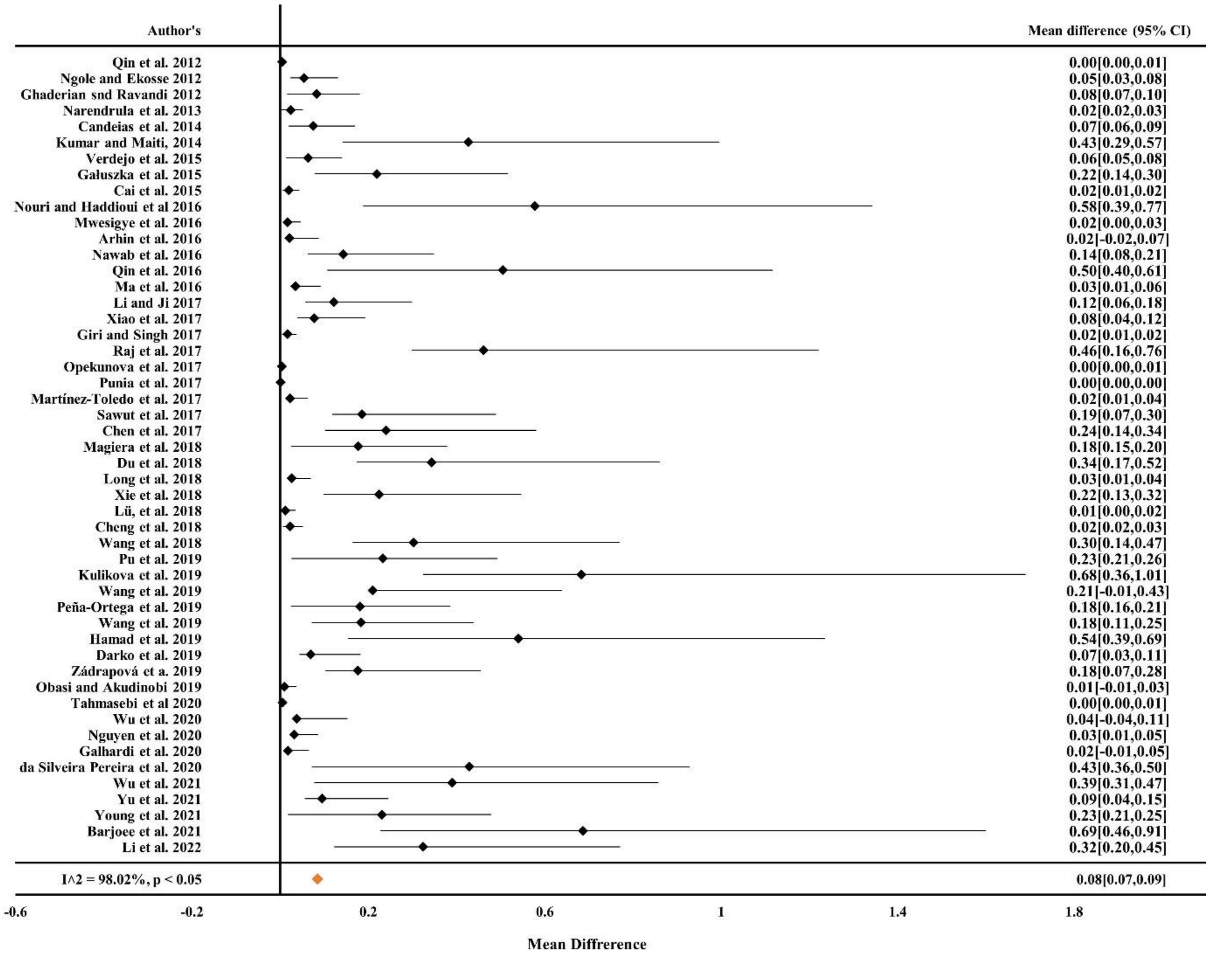

Figure 5 Forest plot indicating the mean difference of individual observation regarding the Cu contamination in mine areas.

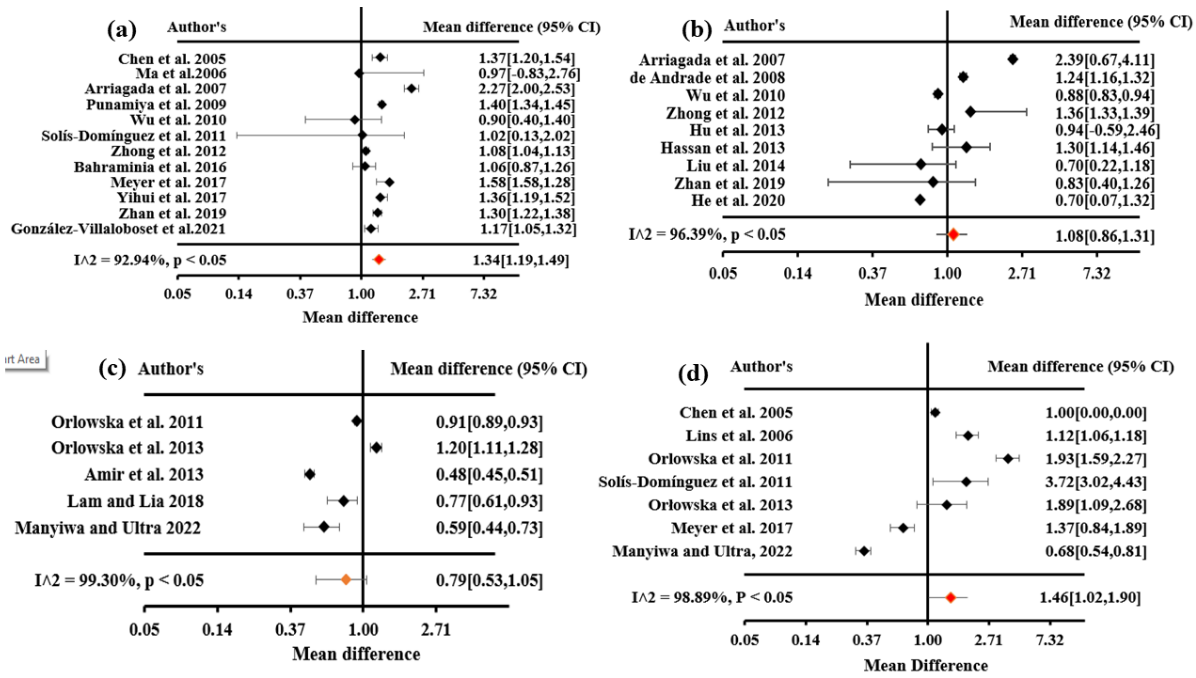

Figure 6 Forest plot indicates the mean difference of individual observation regarding the metal accumulation level in plants in the presence of mycorrhizal infection in different countries. [(a) – Pb; (b)- Cd; (c)- Ni and (d) - Cu]