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Utilizing N-Fixing Bacteria to Biomanagement of Heavy Metals for Sustainable Husbandry Future Directions: A Review

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THE ISSUE of heavy metals (HMs) contamination has become a matter of significant international concern. As a result, various techniques employed by biological systems to eliminate HMs are being examined. Specific HMs, such as cadmium, zinc, nickel, copper, and cobalt, are necessary for the physiological functions of both symbiotic and non-symbiotic nitrogen-fixing bacteria (NFB) when present in minor amounts. However, elevated levels of HM concentrations can impair the functioning of NFB and their symbiotic relationship. The present investigation focuses on how the NFB, resists the HMs toxicity and increases plant growth to assist in accomplishing crop cultivation in metal-contaminated locations. Numerous mechanisms were reviewed for HMs resistance such as organic ligands and thiols which annoy the metal ions constructing a complex and blocking cell impairment. This review summarizes that soil inoculation with HMs-resistant NFB reflects numerous beneficial effects in agricultural systems, therefore its application is considered a bioremediating tools that have economic and ecological importance. In addition, the current review discussed the role of NFB in reducing the toxic impacts of HMs on plant life.

Keywords: Biomanagement, Heavy metals N-fixing bacteria, sustainable Husbandry.

1. Introduction

The incidence of heavy metals (HMs) in the environment has suited a considerable issue due to the escalating accumulation of these metals to the environment. Due to the growing global human population and the rising need for food, feed, biodiesel, timber, fibers, etc., farmers will be compelled to grow crops in soils that are of low quality, degraded, or moderately polluted (Mahar et al., 2016; Mitra et al. 2022).

HMs have a stubborn and resistant nature, causing them to build up in many living forms and infiltrate the food chain. This poses a significant threat to the environment, as well as to humans and animals (Hassan et al. 2024). In a previous study by Doelman et al. (1994), it was found that some HMs are necessary for growth, but at elevated levels, they can be harmful to humans, animals, and other organisms. The primary reason for this is the formation of complexes between HMs and protein molecules, which leads to their inactivation, including the inactivation of enzymes. Multiple pieces of evidence have been documented as a consequence of heavy metal stress in agricultural soil. Qadir et al. (2015) provided an explanation for the reduction in plant biomass during HMs stress. They found that this reduction was mostly caused by the negative impact of HMs stress on mineral uptake, photosynthesis, chlorophyll synthesis, water balance, and hormone balance. The present review

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aimed to evaluate the role of nitrogen-fixing bacteria to management of HMs in agricultural soil.

2. Sources of heavy metals

HMs released during manufacturing processes can accumulate in the environment and establish a significant hazard to many agro-ecosystems (Abd El-Ghany and Abdel-mongy 2009; Scutarașu and Lucia 2023; Shoayb et al. 2023; Elsadany et al. 2024). As a result of industrial activity and technological advancements, hazardous materials are consistently being discharged into the environment (Hashimet al. 2017). They represent a substantial peril to the environment, public health, and soil health, causing disruption to the structure and functioning of ecosystems for an extended period. HMs can be certainly present in soils (Adewumi and Ogundele 2024) or can be introduced into soils through industrial operations (Fig. 1). Natural sources of HMs in soils include volcanic emissions, the transmission of continental dusts, and the weathering of metal-enriched rocks. These sources contribute significant amounts of HMs to soils over time, as they are exposed to air for extended periods (Wan et al. 2024). Soil can be contaminated by HMs due to various human activities, such as mining and smelting operations, the use of metal-based pesticides and metal-enriched sewage sludge in agriculture (Zheng et al., 2020), the burning of fossil fuels, metallurgical industries, electronics, military training, and weapons (Xiao et al. 2024).

Adewumi and Ogundele (2024) provided data estimated presence of HMs during the period of 2010–2022 in the soils 174 region across the world. The range values (mg/kg) were 0.95–152.00, 2.27–5780.00, 0.05–178.19, 0.03–298.90, 1.10–1631.43, 0.41–2560.00, 2.08–12,986.00, 1.14–3420.00 and 28.10– 88,531.00 for Arsenic, lead, mercury, cadmium, chromium, cobalt, nickel, copper, zinc and iron, respectively

Other sources of HMs contamination can originate from the emission of fly ash from coal-dependent power plants (Deng et al., 2023), as well as the utilization of polyvinyl chloride products, color pigments, different alloys, and rechargeable nickel-cadmium batteries (Blumbergs et al., 2021). The pollution of agricultural soil with HMs is a prominent problem that has experienced a substantial rise in industrial and defense-related locations globally (Rashid et al., 2023; Vardumyan et al., 2024). The rise in usage can be ascribed to the widespread utilization of specific heavy metals, such as copper and zinc, in the manufacturing of chemical fertilizers and pesticides. One additional aspect that contributes to the pollution of agricultural soil with HMs is the utilization of industrial waste fluids for irrigation (Ampofo & Awortwe, 2017).

The occurrence of HMs in the environment even at low doses is still an environmental proplem due to their toxicity. The minor alterations in their dose over the acceptable level, whether because of anthropogenic or natural factors is a great concern since they cause severe environmental and subsequent health problems. Soil type is expected to effect HMs accumulation in agricultural soils, since diverse soil kinds are consisted of different soil parent matrixes, which can effect the levels of HMs (Nyiramigisha 2021; El-Shabasy et al. 2024; Gupta et al. 2024; Vardumyan et al. 2024). Chopin et al. (2008) state that there are multiple factors that influence the presence and buildup of HMs in agricultural soil (Fig. 2).

3. Nitrogen-fixing bacteria

Nitrogen is a necessary element for all living creatures, both prokaryotic and eukaryotic. Cellular synthesis of proteins, enzymes, and nucleic acids necessitates it. Nitrogen is crucial for soil fertility and plays a vital role in plant development, as well as in the production of food and feed at the agricultural level. Soil fertility and quality rely on soil biology and microbes, which have important functions in soil fertility and primary production by breaking down organic compounds and cycling nutrients (Abdel Ghany et al., 2013; Shen et al., 2023).

Through the process of biological nitrogen fixation (BNF), certain living organisms could transform the inorganic form of atmospheric dinitrogen (N_2) into a biologically usable form of nitrogen, such as ammonia. These organisms, commonly referred to as nitrogen-fixing bacteria (NFB), are responsible for this process. Multiple studies have shown that the BNF is responsible for 65% of the nitrogen now utilized in agriculture. Furthermore, it will remain an ongoing necessity in future sustainable agricultural practices (Matiru and Dakora, 2004; Aasfar et al., 2024).

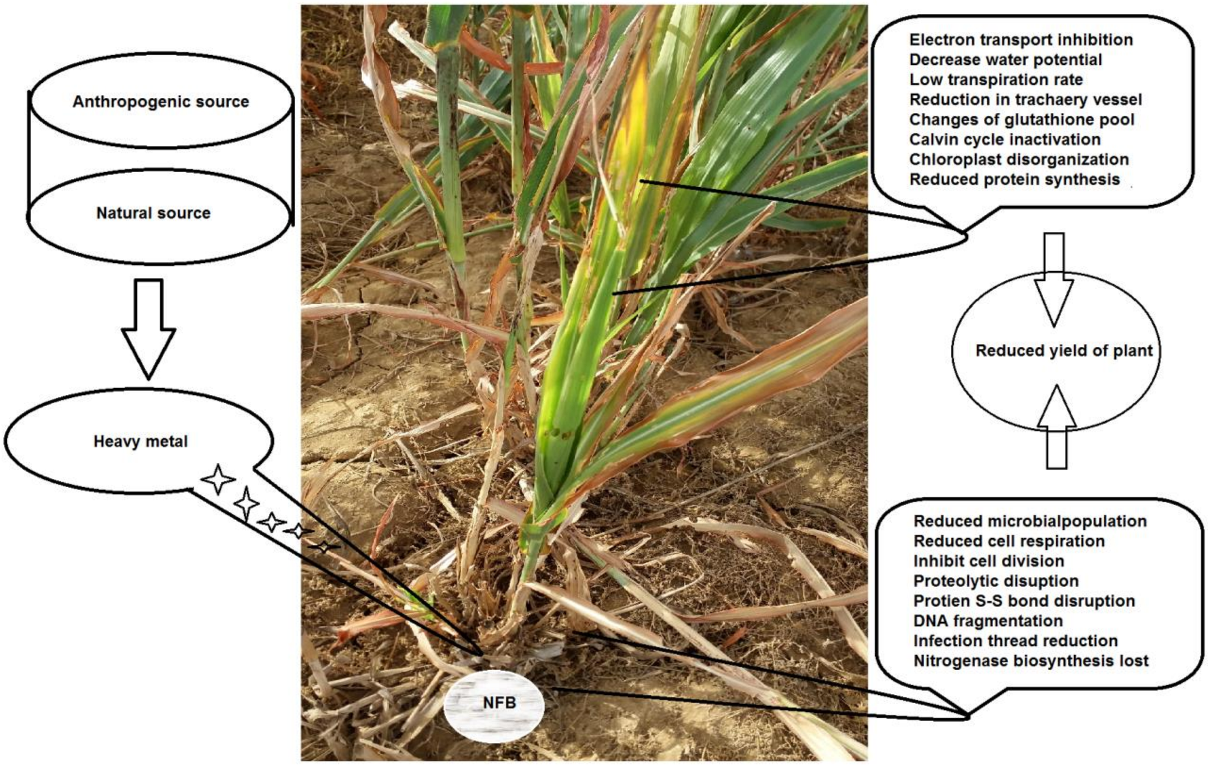

Fig. 1. Heavy metals sources and its impact on plant growth and nitrogen fixing bacteria.

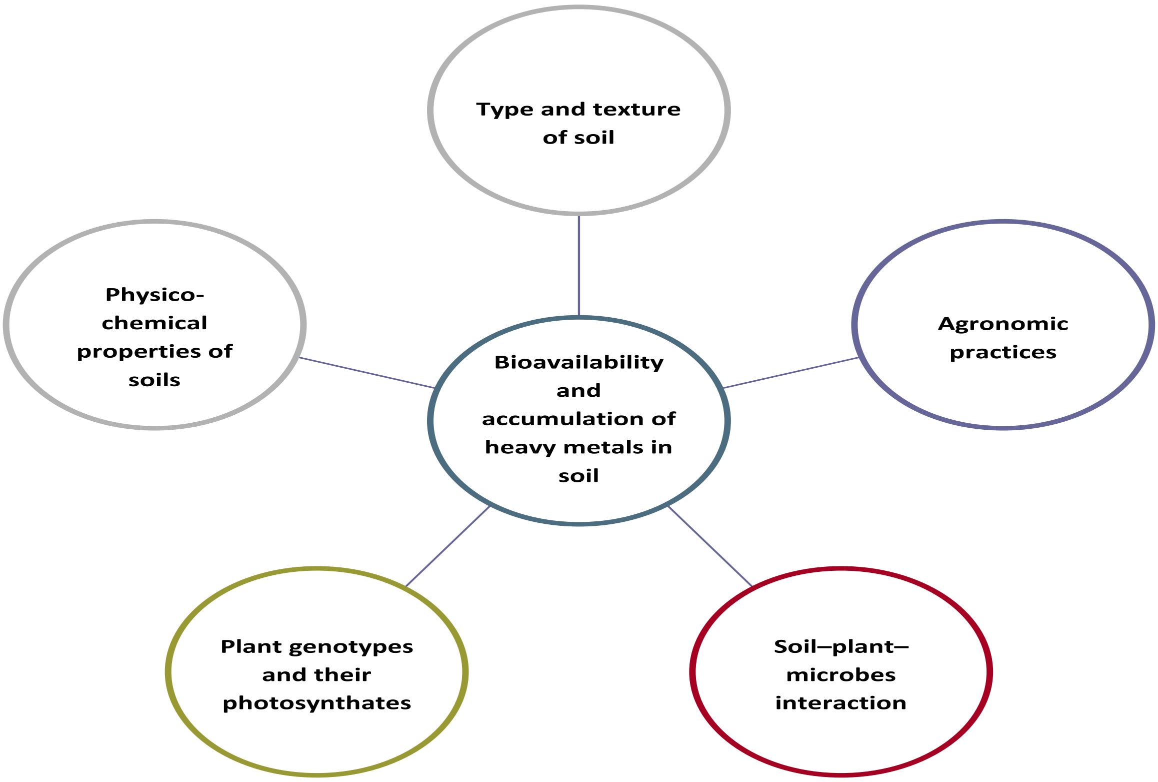

Fig. 2. Factors affecting bioavilability and accumulation of heavy metals in soil.

Based on previous and current research, two nitrogen-fixing groups have been extensively researched. The first group, known as asymbiotic, is exemplified by *Azotobacter* spp. The second group, known as symbiotic bacteria, could form nodules on leguminous plants and is traditionally referred to as Rhizobia. Biofertilizers could increase nitrogen fixation, resulting in a greater crop output (Cañizares-Villanueva et al., 2024).

Several NFB have been discovered, and ongoing research continues to explore new findings in this sector. The NFB associated with nonlegumes, as reported in several studies, encompasses various species including *Derxia, Achromobacter, Arthrobacter, Alcaligenes, Acetobacter, Beijerinckia, Xanthobacter, Mycobacterium, Methylosinus Bacillus, Azomonas, Clostridium, Herbaspirillum, Lignobacter, Klebsiella, Mycobacterium, Enterobacter, Erwinia, Desulfovibrio, Corynebacterium, Campylobacter, Rhodospirillum,* and *Rhodo-pseudomonas* (Wani 1990; Yang et al. 2024). *Azospirillum* and *Azotobacter* species, which are NFB, are mostly found in the rhizosphere of different types of grains. As a result, they have been extensively studied to determine their effectiveness in enhancing agricultural yield in field settings. Various symbiotic bacteria, such as *Rhizobium sp., Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium,* and *Sinorhizobium*, as well as non-symbiotic bacteria like *Azotobacter, Azospirillum, Klebsiella sp.,* and *Bacillus*, are currently being used globally to improve crop productivity (Tan et al., 2024). Symbiotic nitrogen fixers have a crucial role in facilitating the transfer of nitrogen and phosphorous to plants, enabling a sustainable use of nitrogen and phosphorous fertilizers (Tambekar et al., 2009; Awasthi et al., 2011).

The application of NFB (Kenawy et al., 2024) promoted the production of plant growth hormones, increased disease resistance, improved the plant's ability to withstand environmental stress conditions, enhanced N_2 fixation, and increased nutrient delivery. Soil inoculation with NFB has been shown to have multiple advantageous impacts in agricultural systems (Fig. 3). Several authors have developed a broad concept known as NFB, which encompasses beneficial free-living soil bacteria that enhance plant growth. These bacteria are commonly referred to as plant growth promoting rhizobacteria (PGPR) and they promote plant growth by colonizing the roots (Abdel Ghany et al. 2015a). PGPR, which stands for plant health promoting rhizobacteria, are also referred to as nodule promoting rhizobacteria (Burr and Caesar, 1984 Abdel Ghany et al., 2015b). PGPR were classified into two types, symbiotic and asymbiotic bacteria, based on their association with plants (Khan, 2005). The PGPR has three main functions: it synthesizes specific compounds for plants, enhances the absorption of certain nutrients from the soil, and provides protection against illnesses. Ren et al. (2023) employed plant-associated PGPR to remediate contaminated soils.

4. Heavy metals and its relation to N-fixing bacteria growth and activity

HMs typically hinder the growth of microorganisms by obstructing crucial functional groups, separating necessary metal ions, or modifying the active structures of biological components (Doelman et al., 1994). Nevertheless, at lower concentrations, certain HMs ions, such as cobalt, copper, zinc, and nickel, play a crucial role for microorganisms as they provide essential cofactors for the formation of microbial metallo-proteins and enzymes (Abdel Ghany et al. 2020; Zerrari et al., 2023). The presence of decreased concentrations of HMs resulted in an increase in the activities of specific enzymes, such as proteinases, dehydrogenases, and peptidases. Zinc has a crucial role in the creation of carbohydrates, proteins, auxins, RNA, ribosomes, and phosphate (Abdelhady et al. 2024).

Similarly, copper is essential for other physiological activities such as respiration, photosynthesis, and glucose distribution (Seeda et al., 2023).

The negative consequences of cadmium include disrupted enzyme activity, increased susceptibility of plants to fungal infections, inhibition of DNA-mediated transformation in microorganisms, and reduced symbiotic relationships between plants and microbes (Mohanpuria et al., 2007). Bacterial activity has been demonstrated to be very susceptible to metal contamination, both in laboratory and field settings (Diaz-Ravina and Baath, 1996).

Several reports have shown that the long-term presence of HM pollution in soils has harmful impacts on soil microbial activity, namely on microbial respiration (Doelman and Haanstra, 1984; Dmitri and Maria, 2008). The findings from Bhandal et al. (1990) provide compelling evidence that the relationship between legumes and Rhizobium bacteria is very vulnerable to the harmful effects of HMs.

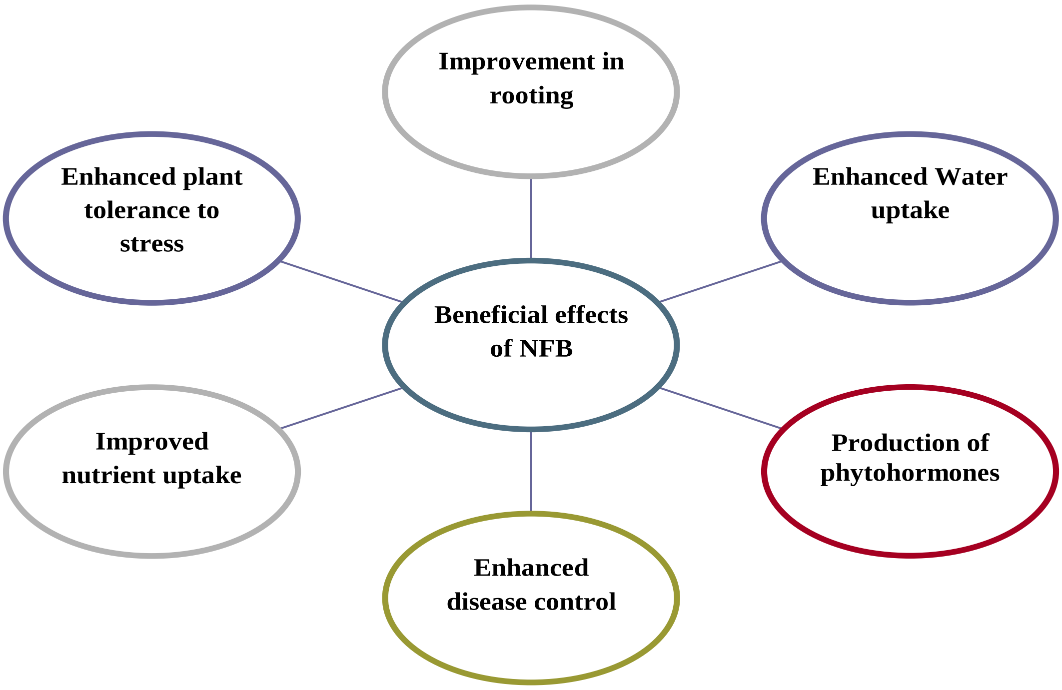

Fig. 3. Helpful effects of nitrogen-fixing bacteria.

The findings of Bansal and Jitendra (2012) demonstrated that the presence of sewage water contaminated with HMs led to a reduction in the population of symbiotic NFB, resulting in a reduction in nitrogen mineralization. However, there was no significant impact on the growth of sulfur-oxidizing bacteria in the sewage water under the same circumstances.

Nitrifying bacteria are a collection of microorganisms that are very susceptible to changes in soil environmental variables, such as temperature, moisture, pH, and the presence of substrates such NH_4-N, O_2, and CO_2 (Montagnini et al., 1989). Additionally, they are also highly affected by soil contamination (Torstensson, 1997).

A specific assemblage of soil microorganisms, including heterotrophic bacteria and symbiotic nitrogen-fixing bacteria, exhibited vulnerability to prolonged contamination. Oliveira and Pampulha (2006) and Durga Devi et al. (2012) assessed the impact of HMs on soil microbial growth and physiological activities by quantifying dehydrogenase activity. For instance, the nitrogenase enzyme relies on a cofactor that consists of either vanadium and iron, molybdenum and iron, or two iron molecules.

The addition of a low concentration of nickel as a supplement was found to stimulate nitrogen fixation by *Rhizobium leguminosarum* bv. viciae and promote the growth of soybeans. Additionally, it was observed that this treatment increased hydrogenase activity (Lavres et al., 2016). Nevertheless, an overabundance of HMs has a detrimental impact on the symbiotic relationship, leading to a reduction in the quantity of symbiotic nodules, the pace at which nodulation occurs, and the rate of nitrogen fixation (Hao et al., 2014).

Edulamudi et al. (2012) identified certain strains of rhizobia that shown the ability to thrive in the presence of 30 parts per million of mercuric chloride. The population of *Azotobacter* spp. fell in soil contaminated with HMs. In chromium-affected heavy loamy sand, these bacteria entirely disappeared, whereas in light silty loam their count decreased by a factor of 27. According to a study conducted by Wyszkowska et al. in 2007, cadmium was found to be the second most toxic HMs to *Azotobacter* spp., with copper, lead, nickel, and zinc following in toxicity.

Other microbial groups, including copiotrophic, spore-forming copiotrophic, oligotrophic, spore-forming oligotrophic, ammonifying, nitrogen immobilizing, and cellulose-decomposing bacteria, as well as *Arthrobacter* spp., *Pseudomonas* spp., fungi, and actinomyces, exhibited varying levels of susceptibility to these HMs (Wyszkowska et al., 2007). According to study of Dutta et al., (2018) *Klebsiella pneumoniae* was introduced into soil grown with *Vigna mungo* under cadmium stress, so an increase in the amount of chlorophyll was recorded. Additionally, the application of *Azotobacter chrococcum* to soil, which was then subjected to high levels of copper and lead, resulted in an increase in the chlorophyll levels of *Zea mays* plants (Rizvi et al., 2018).

Dabrowska et al. (2017) observed an improvement in the development of *Brassica napus* L. when subjected to stressors caused by cadmium, copper, lead, and zinc. Thus, Kamran et al. (2017) stated that NFB has been extensively employed to enhance the ability of host plants to tolerate and absorb HMs from the soil, while reducing the plant's susceptibility to the stress caused by these HMs. The future strategies for cultivating soil contaminated with HMs or irrigated with HMs polluted water should rely on the use of HMs-tolerant bacterial inoculums. The selection of these inoculums can be based on the isolation site, as indicated by previous research (Klonowska et al., 2012). This study demonstrated that NFB isolated from areas affected by copper, zinc, cadmium, lead, and arsenic mining activities have the ability to promote plant growth and improve soil organic compounds. Some NFB species are tolerant to certain HMs but not to all. This knowledge has been corroborated by a recent study conducted by Shen et al. in 2023. The study found that only twenty strains of rhizobia from 57 isolates presented various levels of tolerance to nickel, copper, manganese and zinc.

The NFB (nonferrous bacteria) found in copper mine tailings, which contain a high concentration of copper and have a significant tolerance to copper, are susceptible to zinc. A noticeable decrease in the ability of free-living nitrogen-fixing bacteria to reduce acetylene has been seen in the presence of HMs, as reported by Owaresat et al. (2023). Paudyal et al. (2007) examined the effects of aluminium, iron, and molybdenum on two strains of Rhizobia that were isolated from the root nodules of two tropical legume species, *Mucuna pruriens* and *Trigonella foenum-graecum*. The study was conducted both in laboratory circumstances (*in vitro*) and in living organisms (*in vivo*). The adverse impacts on rhizobial strains were seen when exposed to varying concentrations of aluminium.

On the other hand, Rhizobia isolates that were boosted with iron showed an increase in the growth, and supported the symbiotic parameters, such as nodulation and biomass production, up to a concentration of 25 mM. However, beyond this concentration, there was a detrimental impact. Molybdenum, when present at a concentration of 75 mM, enhanced bacterial growth. However, at concentrations of up to 20 mM, molybdenum promoted both plant production and nodulation in the legumes being tested.

Mertens et al. (2010) proposed that the *Nitrosospira lineage* exhibits a significant level of zinc tolerance. This observation suggests that there may be variations in the formation of a tolerant community across different soils. Conversely, excessive levels of HMs, such as cadmium, copper, zinc, and silver, can also have harmful effects on denitrifying populations (Holtan-Hartwig et al., 2002; Throback et al., 2007). The phenomenon of HMs tolerance in the soil bacterial community is frequently observed in different types of soils (Sazykin et al., 2023). Numerous studies have demonstrated the presence of the soil nitrifying community (Mertens et al., 2010; Corrochano-Monsalve et al. 2024). The correlation between bacterial activity and HMs concentration was shown to be dependent on the specific type of bacteria and other microorganisms present.

Ammonifying bacteria had lower sensitivity to cadmium compared to nitrifying bacteria, as demonstrated by Chenyingxu in (2001). Cadmium was found to be the most potent inhibitor of both ammonification and denitrification, surpassing copper and chromium at the same concentration of 20 mg kg-1 soil. In addition, ecosystems with high levels of HMs pollution exhibit reduced microbial populations compared to uncontaminated habitats (Kandeler et al., 2000). Several HMs, such as copper, nickel, zinc, cadmium, and arsenic, have been found to inhibit the growth, change the appearance, and affect the functions of different types of microorganisms (Abdel-Razek et al., 2008; Abdel-Razek et al., 2009a and b). This includes symbiotic NFB like *R. leguminosarum*, *Mesorhizobium ciceri, Sinorhizobium, Rhizobium sp*., and *Bradyrhizobium sp.* (Bianucci et al., 2011).

The activity of some enzymes from NFB and other PGPR can be influenced by exposure to heavy metals such as nitrous oxide reductase as described by Holtan-Hartwig et al. (2002). This inhibition leads to an inefficient denitrification process, resulting in the release of nitrous or nitric oxides. A study on the toxicity of chromium (VI) on plant growth revealed several notable findings, including an increase in the generation of reactive oxygen species (ROS) and the development of oxidative stress, a decrease in plant biomass, alterations in cellular components, suppression of pigment synthesis, and a reduction in crop yield. The adverse effects were mitigated when the plants established a symbiotic relationship with rhizobia, resulting in the accumulation of HMs in nodules and subsequently reducing their toxicity (Uliana et al., 2018).

Liu et al. (2018) observed the stimulation of plant growth through microbial inoculations in *Z. mays* under cadmium stress in a recent study. *Enterobacter asburiae* KE17 also enhanced the growth and metabolism of soybeans in the presence of high levels of copper and zinc toxicity (Kang et al., 2015). The enhanced plant activity was ascribed to the plant growth promoting characteristics exhibited by the bacteria that were introduced. Jian et al. (2019) found that an excess of HMs negatively affects the nodulation process and reduces the activity of nitrogenase in *Medicago lupulina*. However, they also discovered that co-inoculating the plant with specific bacterial species, such as Sinorhizobium meliloti and Agrobacterium tumefaciens, which possess properties such as nitrogen fixation, resistance to copper and zinc, and production of indole-3-acetic acid (IAA), can mitigate the toxic effects of HMs and promote plant growth. The study conducted by Fatima and Ahmed (2018) found that *Bacillus cereus* mitigated the harmful impacts of chromium and enhanced the growth of *Lens culinaris* in a soil contaminated with chromium. Rhizobium symbiotic systems have a significant impact on enhancing the nitrogen content of soil, resulting in increased biomass and the accumulation of HMs in polluted soil (Hao et al., 2014).

5. Resistance to heavy metals

In order to mitigate the adverse effects of certain HMs on agricultural soil, it is imperative to eliminate excessive concentrations of HMs from the contaminated soil. Various techniques, including pH adjustments, removal, sequestration, and phytoextraction, have been experimented with to extract HMs contaminants from polluted soil (Bano et al., 2018). However, these methods do not limit the investigation of microorganisms that are resistant to high concentrations of HMs while also promoting plant development and increasing yields (Table 1). Many bacteria have developed mechanisms to withstand hazardous HMs (Abd El-Hafez 2014; Shafiq and Rehman 2024). The mechanisms illustrated in Fig. 4 involve the active transportation of the hazardous material (such as HMs) away from the cell organism, the prevention of HMs entry through permeability barriers, the capture of HMs within the cell through protein binding, the capture of HMs outside the cell, the conversion of HMs into a less toxic form through enzymatic detoxification, and the reduction of HMs sensitivity in cellular targets (Bruins et al., 2000). The enhanced survival of NFB under HMs stress circumstances may be attributed to the excessive production of specific organic compounds, such as carbohydrates and proteins (Ravikumar et al., 2002).

In a prior study, Diaz-Ravina et al. (1994) found that the level of tolerance to different HMs is influenced by the concentration of HMs in the soil. The study also showed that soil contaminated with cadmium, nickel, lead, zinc, or copper not only induced tolerance to the specific metal but also to other trace metals. The soil nitrifying community in soils contaminated with lead also exhibited co-tolerance to zinc, and vice versa (Rusk et al., 2004). The ability to tolerate cobalt and other HMs may be attributed to intracellular protein binding, non-specific efflux systems, or the presence of plasmid or genomic gene clusters that encode various mechanisms for metal tolerance. Dmitri and Maria (2008) propose that the denitrifying microbial group responds to increasing levels of lead by selectively favoring metal-tolerant variants of nitrite reductases. The effects of NFB on some metals have been identified. For instance, the presence of high levels of carbohydrates within cells and the presence of large cell inclusions enhance the resistance of NFB to cadmium, copper, nickel, and zinc. Additionally, the formation of thiols has been found to counteract the rapid oxidation caused by HMs (Zhang et al., 2022). Thiols can react with metal ions and have been demonstrated to be efficacious in mitigating the toxicity of cadmium, gold, mercury, and lead (Zhang et al., 2022). A previous study demonstrated that bacteria associated with plants can enhance the chemical conversion, chelation, or the process of heavy-metal precipitation and sorption (Gadd, 2008). The study conducted by Etesami (2018) demonstrated that certain endophytic bacteria have the ability to mitigate HMs toxicity. This was evident through the promotion of plant growth and increased chlorophyll content in various crop plants that were inoculated with siderophore-producing bacteria (Etesami 2018; Sepulvedacaamano et al., 2018). Bacteria have unique genes on plasmids or chromosomes that provide resistance to the toxicity of HMs (Shafiq and Rehman 2024).) at the genomic level. Park and Ely (2008) documented the up-regulation of 27 genes in Nitrosomonas europaea in response to zinc, including genes associated with mercury tolerance and inorganic ion transport. The predominant bacterial species found in the rhizosphere were acidobacteria, actinobacteria, proteobacteria, and bacteroidetes. Proteobacteria are potentially the most resistant bacteria to HMs, commonly found in areas contaminated by HMs (Sheik et al., 2012; Hamamura et al., 2013; Zhouzhou et al., 2019). Deicke et al. (2019) established that *Frankia* spp. are abundant NFB in soil, forming symbiotic relationships with a diverse array of hosts. Frankia releases organic compounds that have a wide range of attraction to both necessary and harmful HMs. Prior research has indicated that the addition of *Azotobacter chroococcum* to *Z. mays* plants reduces the accumulation of copper and lead in the plant's organs. The presence of various metabolites, such as protons and exudates, leads to the immobilization of lead by acting as metal chelators (Rizvi et al., 2018). According to Dong et al. (2016), it was observed that the metal tolerant strain *Bacillus megaterium* decreased the movement of nikel. The presence of *Exiguobacterium*, a bacteria that can tolerate arsenic, on the surfaces of *Vigna radiata* plant roots resulted in a decrease in the movement of arsenic within the plants (Pandey and Bhatt 2016). Plant metabolites indirectly resist herbicide resistance by facilitating the development of inoculation microorganisms. In their study, Juan et al. (2019) found that three strains of *Pseudomonas* were able to resist high levels of HMs. This led to a significant increase in root enzyme activity in *Nicotiana tobacum* K326 and a decrease in the total lead content by 3.28% to 6.38% in the soil polluted with lead during tobacco planting. *Pseudomonas aeruginosa* is used as a powerful bioinoculant due to its ability to exhibit PGPR activity, produce biofilm and biosurfactants, and play a crucial part in the phytoextraction process of HMs (Chellaiah 2018). Moreover, several bacteria are able to remove HMs with promising levels as showed in **Table 2.**

Table 1. Activity of bacterial inoculants in heavy metals contaminated soil.

Contaminated soil with	Bacterial inoculants	Activity	Reference
Copper and lead	Bradyrhizobium sp.	Increased the accumulation of metals in root	Dary *et al.* (2010)
Cadmium	*Sinorhizobium meliloti*	Increased cadmium phytoextraction	Ghnaya *et al.* (2015)
Zinc	*Sinorhizobium meliloti*	Increased zinc accumulation in root	Zribi *et al.* (2015)
Copper	*Sinorhizobium medicae*	Increased metal accumulation in root	Delgadillo *et al.* (2015)
	Sinorhizobium meliloti	Increased plant growth and copper tolerance	Kong *et al.*(2015)
		Increased tolerance of seedlings	Chen *et al.* (2018)
Zinc and cadmium	*Mesorhizobium loti*	Increased growth of the plant	Fan *et al.* (2018)
Arsenic	*Bradyrhizobium sp.*	Reduce translocation factor	Bianucci *et al.* (2018)
Lead	*Pseudomonas* strains (K03, Y04, and N05)	Promoted root growth and increased the root enzyme activity	Juan *et al.* (2019)
Copper, zinc	*Frankia* spp.	Detoxification of potentially toxic metals	Deicke *et al.* (2019)

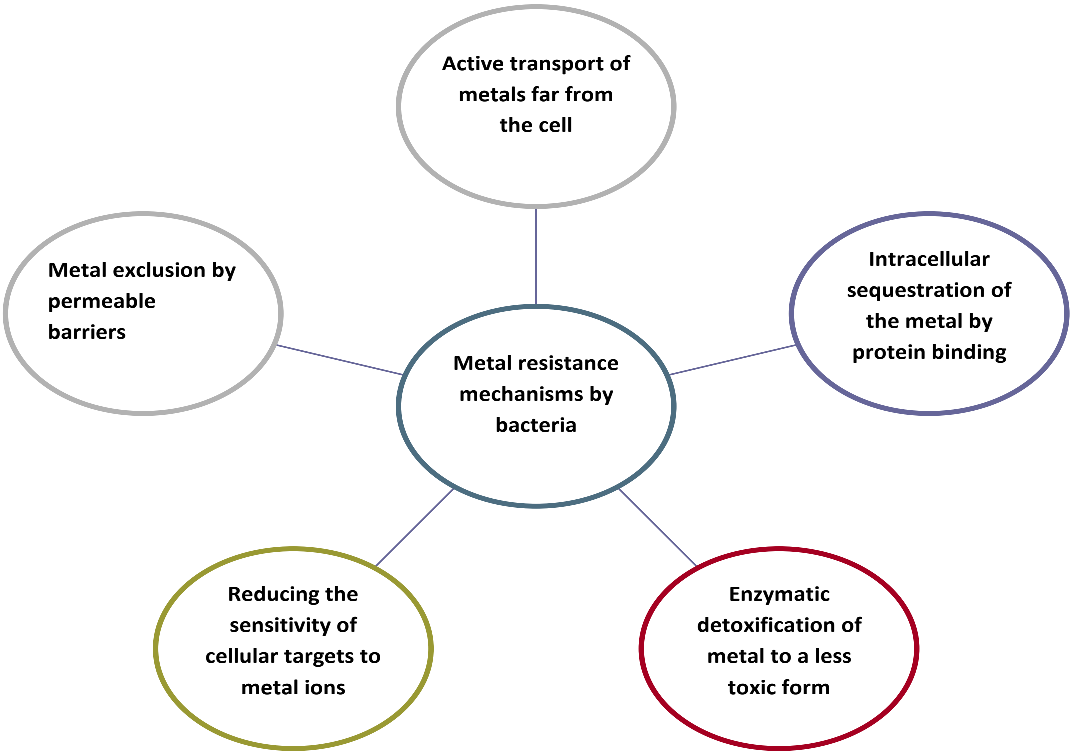

Fig. 4. Resistance mechanisms to heavy metals.

Table 2. Removal % of some heavy metals by some bacteria.

Heavy metal	Used bacteria	Removal Efficiency, %	Reference
Mercury	*Pseudomonas aeruginosa*	60	Imron et al. (2021)
Copper	*Bacillus subtilis*	37.8	Melo et al. (2022)
Copper	*Pseudomonas putida*	25.4	Melo et al. (2022)
Zinc, Lead	*Sporosarcina pastaurii*	96	Jalilvand et al. (2019)
Cadmium, Zinc, Lead	*Variovorax boronicumulans*	84	Jalilvand et al. (2019)
Chromium	Staphylococcus capitis	89	Zahoor and Rehman (2009)
Lead	*Bacillus megaterium*	73	Njoku et al. (2020)
Lead	*Bacillus xiamenenensis*	97	Mohapatra et al. (2019)
Zinc	*Bacillus marisflavi*	70	Kayalvizhi and Kathiresan (2019)
Copper	*Bacillus marisflavi*	62.5	Kayalvizhi and Kathiresan (2019)
Manganese	*Bacillus marisflavi*	44	Kayalvizhi and Kathiresan (2019)

6. Conclusions

Industrial waste that contains high concentrations of HMs causes substantial pollution to the environment, water bodies, and agricultural areas. Soil pollution negatively impacts plant growth. Although plants have the ability to develop, they also have the capacity to absorb and store HMs in their tissues, which can have harmful effects on human health. There has been a substantial rise in the amount of research investigating the application of NFB for phytoremediation in addressing environmental crises. The NFBs are employed as potent bioinoculants and have a vital role in the phytoextraction process of HMs. This brief review specifically examines the utilization of HMs tolerant NFB and their suitability for phytoremediation as symbionts in legumes. Concurrently introducing NFB to host plants mitigated the detrimental impacts of heavy metal toxicity and promoted plant growth. Genomic analyses of HMs-resistant rhizobia have shown that a small subset of genes are crucial for tolerance, while other genes are responsible for the relationship between metal balance and nitrogen fixation efficiency.

List of abbreviations:

HMs: Havey metals
BNF: Biological Nitrogen Fixation
IAA: Indole-3-acetic acid
NFB: Nitrogen-fixing bacteria
PGPR: Growth-promoting rhizobacteria
ROS: Reactive oxygen species

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

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