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WHEN ESSENTIAL TURNS EXCESSIVE: THE EMERGING ROLE OF FUNGI FOR BIOREMEDIATION OF ZINC AN ESSENTIAL YET TOXIC SOIL ELEMENT

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Abstract

Zinc (Zn) contamination in soil, resulting from industrial discharges, mining activities, and the overuse of agrochemicals, poses serious environmental and ecological threats. Although Zn is an essential micronutrient, its excessive accumulation in soil can lead to phytotoxicity, reduced crop productivity, disruption of soil microbial communities, and bioaccumulation in the food chain, ultimately affecting human and animal health. Therefore, effective remediation of Zn-contaminated soils is crucial for environmental sustainability, food security, and ecosystem health. In recent years, fungi have emerged as promising bioremediation agents due to their high metal-binding efficiency, adaptability to extreme conditions, and ability to transform toxic metals into less harmful forms. This review explores the multifaceted mechanisms of fungal-mediated Zn remediation, including surface adsorption via functional groups on the fungal cell wall, intracellular sequestration through metal-binding proteins like metallothioneins, and biomineralization through enzymatic activity. Critical factors influencing Zn uptake—such as pH, temperature, biomass dosage, and metal ion concentration are discussed. Recent advances highlight the potential of strains like Aspergillus terreus SJP02 and white-rot fungi (Phanerochaete chrysosporium, Trametes versicolor), achieving Zn biosorption capacities of up to 70 mg/g under optimal conditions. Despite its promise, fungal bioremediation faces challenges related to environmental variability, presence of co-contaminants, and scale-up feasibility. However, ongoing research in fungal genomics, metabolic engineering, and the development of fungal consortia offers new opportunities to enhance the efficiency and field applicability of this eco-friendly, cost-effective remediation strategy. A deeper understanding of fungal biosorption mechanisms will play a pivotal role in advancing sustainable solutions for heavy metal-contaminated environments.

***Keywords**: Heavy Metals; Bioaccumulation; Bioremediation; Metallothioneins; Biosorption*

INTRODUCTION

Zinc (Zn) is an essential trace element vital for the proper functioning of all living organisms. It plays a crucial role in numerous biological processes, including catalytic activity in over 300 enzymes, immune function, DNA and protein synthesis, wound healing, cellular signaling, and cell division (Fouda et al., 2018). Zn is particularly critical during periods of rapid growth such as pregnancy, infancy, childhood, and adolescence, and also contributes to sensory functions like taste perception (Nagraj et al., 2017). In plants, Zn is indispensable for optimal growth, enzyme activity, and metabolic regulation at trace levels. However, when present in elevated concentrations, Zn becomes phytotoxic, inhibiting plant development and disturbing rhizospheric microbial communities. Although plant growth-promoting rhizobacteria (PGPR) have shown potential in mitigating metal-induced toxicity, the persistent accumulation of Zn in soils poses a significant environmental and agricultural concern (Vidyashree et al., 2016). Anthropogenic activities such as mining, industrial discharge, improper waste management, and the excessive use of agrochemicals have drastically elevated Zn levels in the environment, transforming it from a vital nutrient into a toxic contaminant (Li & Christie; Wani et al., 2001). Excess Zn not only impairs soil fertility but also leads to bioaccumulation through the food chain, posing risks to human and animal health. Zn toxicity has been linked to gastrointestinal distress, immune suppression, anemia, and in severe cases, neurological damage (Lesmana et al., 2009; Bhattacharya et al., 2006; Vilar et al., 2007; Marin et al., 2009). Soils contaminated with Heavy metals (HMs) like Zn are often inhospitable to native microbiota, creating selective pressure that favors metal-tolerant microbial strains. These organisms have evolved various resistance mechanisms, including metal exclusion, efflux, intracellular sequestration, and enzymatic detoxification (Ayangbenro & Babalola, 2017; Igiri et al., 2018). Among these, fungi have gained attention due to their remarkable ability to tolerate and immobilize HMs. Fungal-mediated bioremediation—or mycoremediation—involves diverse mechanisms such as metal biosorption through cell wall functional groups (e.g., carboxyl, phosphate, hydroxyl, amino), intracellular accumulation, extracellular chelation via phytochelatins and metallothioneins, and transformation through redox reactions (Cervantes et al., 1994). Recent studies have highlighted the efficacy of specific fungal strains in Zn remediation. For instance, Aspergillus terreus SJP02 demonstrated a high Zn^{2+} sorption capacity of 10.7 ± 0.2 mg/g within 60 minutes, primarily through surface adsorption involving amino, hydroxyl, carbonyl, and phosphate groups. The study also noted a 71.46% Zn^{2+} recovery rate using 0.01 N HNO_3, indicating efficient metal recovery and potential for industrial-scale applications (Shobham et al., 2025). Similarly, white rot fungi such as Phanerochaete chrysosporium, Pleurotus ostreatus, and Trametes versicolor have been extensively explored for heavy metal remediation due to their robust enzymatic systems and ability to withstand adverse environmental conditions (Singh & Singh, 2024).

Fungi offer several advantages in Zn biosorption: high surface area due to filamentous hyphae, resilience to harsh conditions, and the production of extracellular polymeric substances that facilitate metal binding. These complex processes are influenced by environmental variables like pH, temperature, biomass concentration, metal ion concentration, and the presence of competing ions. Advances in molecular biology, genomics, and proteomics have further clarified the genetic and metabolic foundations of Zn resistance and uptake in fungi, opening avenues for strain improvement and industrial application.

Globally, Zn production exceeds 13 million metric tons annually, with China, Peru, and Australia as major contributors. Though essential for human physiology—where total body Zn is about 1.5 g in women and 2.5 g in men (Ryu & Aydemir, 2020) excessive intake can be harmful. Regulatory bodies like the European Food Safety Authority (EFSA) and U.S. Food and Drug Administration (FDA) have set the tolerable upper intake level (UL) at 25–40 mg/day. Chronic intake above this level may lead to nausea, copper deficiency, weakened immunity, and metabolic imbalances (Plum et al., 2010; Spencer et al., 1994; Doherty et al., 2011). Environmental Zn contamination is widespread. The U.S. Environmental Protection Agency (EPA) has identified Zn at 985 of the 1,662 national priority hazardous waste sites (ATSDR, 2005). This metal, along with others such as lead, cadmium, mercury, and arsenic, is classified among the most persistent and toxic pollutants due to its ability to bioaccumulate and resist degradation. Consequently, Zn contamination threatens not only soil quality but also agricultural sustainability and global food security (Agorboude & Navia, 2009; Kumar et al., 2006).

In this context, microbial biosorption offers a promising, eco-friendly, and cost-effective solution for the remediation of heavy metal-contaminated environments. Fungi, with their diverse metabolic arsenal and structural adaptability, stand out as efficient biosorbents of Zn and other toxic metals. This review provides a comprehensive overview of Zn as a dual-faceted element—essential yet toxic—and explores fungal-based biosorption mechanisms, environmental variables influencing uptake, and the current challenges and future prospects of mycoremediation in managing Zn-contaminated soils.

1.1. Zn in Daily Life: Functional Roles Across Sectors

Zn is widely used in various household and industrial applications due to its excellent corrosion resistance, antimicrobial activity, and integration in numerous daily-use products. One of its most common uses is in galvanization, where a protective Zn coating is applied to iron or steel to prevent rusting—commonly seen in kitchenware, buckets, and water pipes. Zn-carbon and Zn-air batteries are popular in devices like remote controls, clocks, and hearing aids. Recent studies highlight that rechargeable Zn-air batteries are promising candidates for future energy storage due to their high energy density, reversibility, and environmental safety. A novel concept of magnetic Zn-air batteries is under development to meet the needs of next-generation energy solutions (Liu et al., 2020; Zhang et al., 2023). Brass, an alloy of Zn and Cu, is commonly used in faucets, plumbing fittings, musical instruments, and ornamental objects due to its strength and resistance to corrosion. In building construction, Zn-coated (galvanized) metal sheets are used for roofing, gutters, and rainwater harvesting systems. In healthcare and cosmetics, Zn oxide is a vital component found in diaper rash creams, antiseptic powders, and anti-dandruff shampoos (Patra et al., 2024). Additionally, its UV-blocking capability makes it an important ingredient in sunscreens and mineral-based makeup formulations. Recent research also explores nanostructured Zn oxide for use in smart textiles, antimicrobial coatings, and photoprotective agents (Jiang et al., 2022).

ROLE OF ZN IN HUMAN HEALTH AND DISEASE

Zn is included in multivitamins and dietary supplements to support immune function, wound healing, and overall health. The metal is a key ingredient in cold and flu medications due to its role in reducing the severity and duration of symptoms (e.g., Zn lozenges for sore throat). Zn oxide (ZnO) is widely used in ointments, creams, and powders for treating wounds, burns, and rashes (e.g., diaper rash creams and sunscreen). Zn is included in antiseptic creams and oral solutions to help treat infections. It is a cofactor for many enzymes involved in DNA synthesis, cell division, and protein production. It is used in the formulation of enzymatic drugs and pharmaceutical excipients to stabilize active ingredients. Zn supplements are sometimes used in the management of depression, Alzheimer's disease, and schizophrenia, as it is involved in neurotransmission. It plays a crucial role in male fertility by enhancing sperm motility and acrosomal exocytosis through the GPR39 receptor. This suggests its potential in the prevention, diagnosis, and treatment of male infertility (Allouche-Fitoussi D et al 2021, Fallah A et al 2018). Zn is essential for the development and proper functioning of the central nervous system (CNS). It helps regulate neural tube formation and stem cell proliferation during development. Additionally, various Zn-dependent enzymes contribute to CNS function (Gower-Winter SD et. al 2012).

Table 1. Therapeutic and Biomedical Applications of Zn – Mechanistic Insights and Clinical Implications Across Human Health Domains

Application Category	Mechanism of Action	Implications
General Health Support	Supports immune function, wound healing, enzyme activity, and DNA synthesis	Multivitamins, immune-boosting supplements, enzymatic drugs
Cold and Flu Management	Inhibits rhinovirus replication, modulates inflammation	Zn lozenges/syrup reduce cold duration and severity
Dermatological Treatment	Anti-inflammatory, promotes tissue regeneration	Zn oxide in diaper rash creams, sunscreens, ointments for burns and wounds
Antiseptic Applications	Antimicrobial, anti-inflammatory	Zn in oral antiseptics and topical creams
Neurological Health	Cofactor in neurotransmission, affects CNS development	Investigated in treatment of depression, Alzheimer's, schizophrenia
Male Reproductive Health	Enhances sperm motility via GPR39 receptor	Potential biomarker and therapeutic target for male infertility
Upper Respiratory Health	Inhibits virus binding/replication, modulates cytokines	Zn lozenges, nasal sprays
Respiratory Infection	Boosts macrophage and T-cell function	Reduces infection severity and mortality, especially in children
HIV Supportive Care	Restores immune function, counteracts Zn loss from diarrhoea and malabsorption	Supplementation considered, but only at RDA levels as per WHO guidelines
COVID-19 Management	Antiviral, reduces inflammation, preserves epithelial integrity	Studied as adjunct therapy; potential to reduce symptom severity, not a standalone treatment
Pharmaceutical Formulation	Stabilizes active drug compounds, acts as a cofactor	Used in drug delivery systems and excipients

2.1. Common Cold: Researchers suggest that Zn may help reduce the severity and duration of colds by inhibiting rhinovirus binding and replication in the nasal mucosa while also suppressing inflammation. Studies typically use Zn lozenges or syrup, which adhere to the mouth and throat, allowing direct contact with the virus. Zn interferes with the ability of rhinoviruses (the main cause of colds) to bind to receptors in the nasal mucosa, preventing viral entry into cells. It may also inhibit viral replication, reducing the overall viral load. Zn helps regulate immune responses by reducing inflammation, which can lessen symptoms such as congestion and sore throat (**Table 1.**). It may modulate cytokine production, preventing excessive immune reactions that can worsen

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symptoms. While Zn can help reduce the severity and duration of colds, its effectiveness depends on the form, dosage, and timing of administration.

2.2. Pneumonia: Poor Zn status is associated with greater susceptibility to pneumonia, more severe disease, and higher mortality risk in children. Zn enhances the activity of immune cells, such as macrophages and T-cells, which help fight infections (Walker CLF et.al, 2013) Zn plays a significant role in preventing pneumonia by strengthening the immune system and reducing infection risks. While it may aid in recovery, it should not replace antibiotics in treating pneumonia. Instead, it can be used as a complementary supplement, especially in children with Zn deficiency.

2.3. HIV Infection: HIV makes it harder for the body to absorb and use Zn from food. People with HIV also often have diarrhoea, which causes them to lose more Zn, leading to low Zn levels (King J C et.al, 2020). Zn is important for people with HIV (**Table 1.**) as it helps support immunity and reduce infections. Since HIV can lower Zn levels, proper nutrition and, in some cases, supplements may be beneficial, but they should be used carefully to avoid any negative effects. Notably, higher intakes of certain nutrients, such as vitamin A, Zn, and iron, can lead to adverse effects. Hence, WHO advises that individuals with HIV should aim to meet their micronutrient requirements through a balanced diet. Supplementation with vitamins and minerals, including Zn, at daily recommended levels (Recommended Daily Allowance or RDA) is suggested only when dietary intake is insufficient.

2.4. COVID-19: It's best to get Zn from food sources like meat, shellfish, nuts, and legumes, but supplements can be useful if dietary intake is low. Due to Zn's role in immunity, epithelial integrity, and its antiviral and anti-inflammatory effects, some studies have explored its potential to reduce COVID-19 risk and symptom severity, such as diarrhoea and loss of taste and smell. Interest also grew from evidence that Zn lozenges may shorten the common cold's duration. Some studies suggest that Zn supplementation may reduce the severity and duration of viral respiratory infections, including COVID-19.Zn can inhibit viral replication in some corona viruses, though more research is needed specifically for SARS-CoV-2 (Hemilä H. et al, 2017).It may also help reduce inflammation and lung damage in severe COVID-19 cases. Some clinical trials have tested Zn, often in combination with vitamin C or other treatments, for COVID-19 patients. While Zn plays a supportive role in immunity, it is not a cure or guaranteed treatment for COVID-19. However, maintaining adequate Zn levels may help the body fight infections more effectively.

ZN TOXICITY AND HUMAN HEALTH: A DOUBLE-EDGED SWORD

Excessive intake of Zn, particularly through supplements exceeding the Recommended Daily Allowance (RDA), significantly increases the risk of Zn toxicity. One of the leading causes of this condition is over-supplementation, often driven by the misconception that higher intake automatically translates to better health outcomes (Maret W. et al., 2006). Occupational exposure also poses a considerable risk, particularly among individuals working in industries such as welding, Zn mining, and smelting. These workers are frequently exposed to Zn oxide fumes or dust, which, when inhaled, can result in a temporary condition known as metal fume fever—a well-documented illness characterized by flu-like symptoms such as fever, chills, cough, muscle aches, chest tightness, and fatigue (Brenner B.E. et al., 2024; Vogelmeier C. et al., 1987).

Chronic high levels of Zn can interfere with the absorption of copper, leading to secondary copper deficiency. This imbalance may manifest as anemia, compromised immune function, neurological impairments, and reduced bone mineral density, ultimately increasing the risk of fractures (Gibson R.S. et al., 2008). In the gastrointestinal tract, excess Zn can irritate the stomach lining, potentially leading to ulcers, nausea, and other digestive disturbances (**Table 1.**). Prolonged high exposure has also been associated with renal dysfunction, altered nerve conduction, and disruptions in hormonal homeostasis, notably affecting testosterone and insulin levels (Fukunaka A. et al., 2018). Emerging evidence further suggests that sustained Zn toxicity may impair cognitive function, contributing to symptoms such as memory loss, difficulty concentrating, and even depression.

BIOACCUMULATION OF HMS BY MICROBES

HM pollution has become a serious concern across the globe due to their persistent nature, higher toxicity, and recalcitrance. The use of microbes is considered as a promising approach to combat the adverse impacts of HMs. Therefore, technological advancement allowed to use of bioremediation as an imperative approach to remediate polluted soils. Microbes use different mechanisms including bio-sorption, bioaccumulation, bioleaching, bio-transformation and bio-volatilization to mitigate toxic the effects of HMs. These are known to accumulate in plants and they negatively affect the plant's physiological and biochemical processes and consequently cause serious yield losses (Yan et al., 2020). HMs reduce seed germination, photosynthesis, nutrient uptake, etc. as shown in **Figure 1.,** which in turn reduce the overall stand establishment (Hassan et al., 2019).

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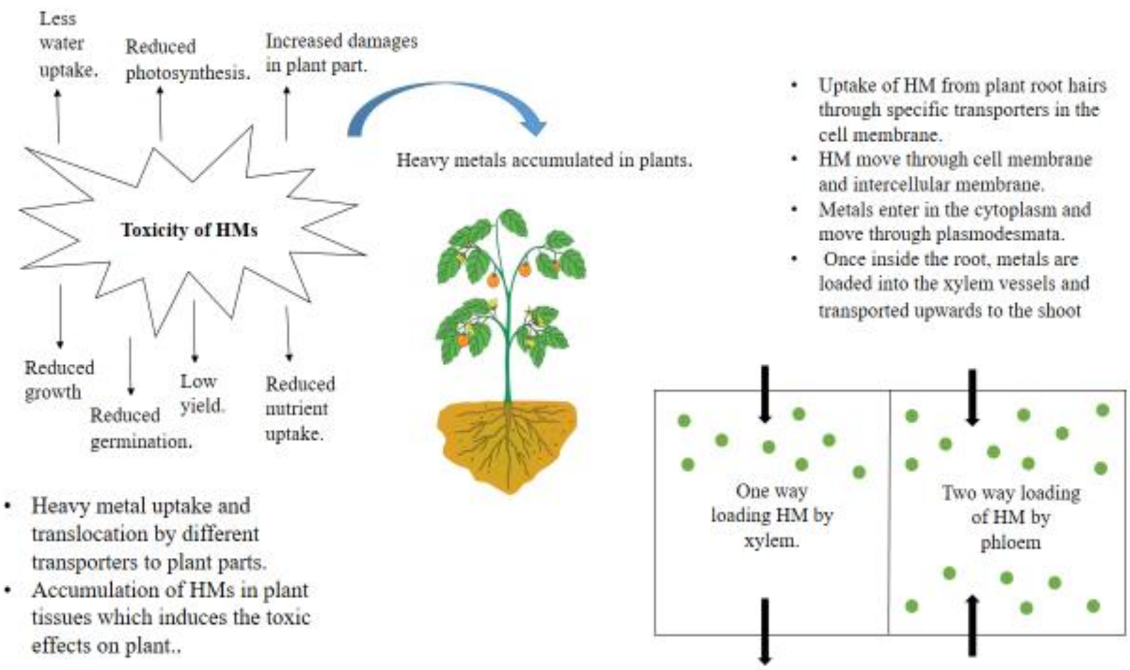

Figure 1. Schematic illustration depicting the translocation of heavy metals (HMs) into plant tissues, leading to toxic effects such as reduced water and nutrient uptake, impaired photosynthesis, decreased germination rates, and the induction of reactive oxygen species (ROS), which collectively result in cellular damage and overall plant decline.

The bioaccumulation of metals is a complex and dynamic process that varies over time. Its interpretation can be challenging because some metals are essential for an organism's metabolism, and their absorption may be actively regulated (Adams et al., 2011; McCarty et al., 2011). Accumulation of the non-degradable inorganic contaminants such as Cd, Cr, Cu, Ni, and Zn has occurred extensively in soil ecosystems (Adriano 2001; Desaules 2012; Desaules and Studer 1993; Herter and Kuelling 2001) The source of this contamination is primarily anthropogenic, arising from a combination of industrial discharges including mine wastes, dry or wet deposition of coal ashes, urban refuse, agricultural and animal wastes, fertilization with phosphate, compost, or sewage sludge, pesticide application such as fungicides etc. Bioleaching has been proposed for decontamination of solid wastes containing toxic metals such as sludge, soil, sediment, etc. (Bosecker, 2001). Biosorption is a process that uses biological materials to remove HMs from contaminated site. Bioleaching of HMs refers to a process where microorganisms, like bacteria or fungi, are used to extract HMs from solid materials like soil, ore, or industrial waste by converting them into soluble forms through their metabolic activity. Bioleaching processes are based on the ability of microorganisms (such as bacteria, fungi, algae etc.) to transform solid compounds into soluble and extractable elements that can be recovered (Krebs et al., 1997). Biotransformation of HMs refers to the process where living organisms, like bacteria or plants, chemically alter the form of HMs, converting them into less toxic or more readily extractable compounds through metabolic pathways, essentially detoxifying the metal within their system. Microorganisms use enzymes to modify the chemical structure of HMs, often by oxidation or reduction, to make them less toxic, this is a key mechanism used in bioremediation, where microorganisms are employed to clean up heavy metal contaminated sites by converting the toxic metals into less harmful forms. Bio volatilization of HMs refers to the process where microorganisms convert non-volatile HMs into volatile forms, allowing them to be released into the atmosphere as gases. Microorganisms can either directly bind to HMs and transform them into volatile compounds, or indirectly influence the chemical environment to promote volatilization (M. Urík, S. et.al 2007).

Microbial remediation is a crucial strategy with significant potential to enhance crop yield, promote human health, and restore ecological balance (Narayanan and Ma, 2023). Microbial-driven bioaccumulation and bio-magnification are highly effective in eliminating pollutants, ensuring safe and sustainable crop production. Living microorganisms can be used to investigate tolerance to HMs and adsorption specificity to different HMs (Liu et al. 2024). Microbes, such as algae, bacteria, and fungi, are utilized to remediate heavy metal-contaminated soils (Manorma et al., 2023).

2.5. Bacteria: Small size, rapid growth, and ease of cultivation allow them to thrive in a variety of environmental situations. The interaction between microbes and HMs varies based on the metal type, microbial species, and environmental conditions. Factors such as temperature, pH, nutrient availability, and metal ion

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concentration influence the mobility and bioavailability of HMs for microbial transformation. Bacterial cell walls have functional groups such as amino, carboxyl, sulphate, and phosphate groups on which HMs often connects (Yue et al., 2015). The potential of bacteria for HMs uptake can vary from 1-500 mg/g. For example the bacteria such as Pseudomonas aeruginosa strain, Bacillus sp. PZ-1, Arthrobacter viscosus and Bacillus ceres absorbs metals such as Hg, Pb, Cr, Cd respectively from waste water and soil. While Rhodobacter capsulatus also showed a maximum capacity of 164 mg/g to absorb the Zn (II) (Magnin et al., 2014). Extracellular polymeric substances (EPS) help protect microorganisms from the toxic effects of HMs (HMs) by limiting their entry into the cell.

2.6. Algae: Many types of algae have been found to be effective in absorbing HMs from contaminated environments. When algae passively bind metal ions to their cell walls or actively take them up. This makes algae a promising natural solution for cleaning up polluted water and soil. For example Fucus vesiculosus, Cladophora fascicularis, Sargassum sp. marine algae have showed the absorption of Pb (II) and Cu (II) from the aqueous solution and showed a significant potential to detoxify the solution (Barquilha et al., 2017, Demey et al., 2018). On the other hand Chlorella vulgaris can efficiently absorb and store Zn (II)s within its cells and Arthrospira platensis a blue-green alga that can tolerate and accumulate HMs, including Zn. Also Saccharina fusiforme and Saccharina japonica substantially detoxify the Zn (II), Cd (II), and Cu (II) (Poo et al., 2018).

2.7. Fungi: Fungi also have an excellent ability to remediate the HMs polluted soils. Fungi cell walls have chitin and cellulosic structures and also have the polysaccharide, phosphate, and glucuronic acid which are essential for the absorption of the HMs (Purchase et al., 2009). Different functional groups and fungal strains have a significance on the adsorption rate of HMs (Iram et al., 2015). Aspergillus flavus and Aspergillus fumigates has showed potential in the removal of HMs such as Cd, Cr, Cu, Ni, and Zn from the contaminated soils (Shazia et al., 2013). The tolerance limits of the fungal strains to various doses of lead (Pb), cadmium (Cd), zinc (Zn), chromium (Cr), copper (Cu), nickel (Ni), and cobalt (Co) was evaluated. The first report on the study of metal resistance of Aspergillus fischeri and Epicoccum mackenziei. Among the identified fungal species, Aspergillus niger and Fusarium oxysporum were found to be most tolerant with a minimum inhibitory concentration (MIC) of 600 ppm against Cu and Cr respectively. Results indicated removal of considerable amount of HMs by some of the fungi. The highest metal uptake of 8.31 mg/g was found in Fusarium verticillioides for Zn (Amin, 2024). Fungi has a metal-binding features and resistance to metals in unfavourable conditions. Fungal cell wall is the primary interaction site. Fungal tolerance mechanisms involve biotransformation, mobilization and immobilization. Immobilization of metal occurs due to metal sorption with the biomass or exopolymers, intracellular sequestration and precipitation as organic and inorganic compounds (Singh et al., 2018).

The interaction between soil microbes and plants plays a pivotal role in mitigating heavy metal (HM) stress through mechanisms such as metal chelation, biotransformation, and phytoremediation strategies including phytoextraction, phytostabilization, and phytovolatilization, as illustrated in **Figure 2.**

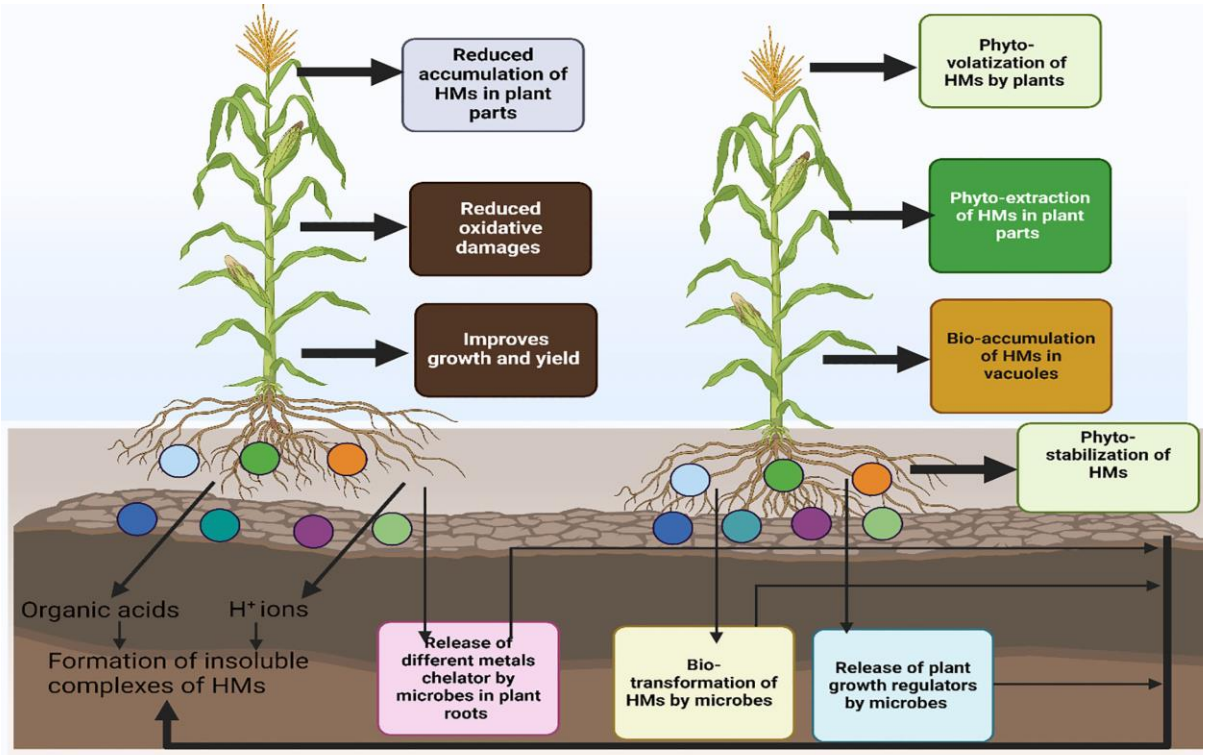

Figure 2. Mechanisms of microbial and plant-mediated mitigation of HM stress in contaminated soils. Microbial activities in the rhizosphere, such as the secretion of organic acids, H^+ ions, metal chelators, plant growth regulators, and biotransformation processes, contribute to HM immobilization and reduced uptake. These microbial interactions support plant health by reducing oxidative damage, enhancing growth, and limiting HM accumulation. Plants respond through various phytoremediation strategies, including phytovolatilization, phytoextraction, phytostabilization, and vacuolar sequestration of HMs.

FUNGAL CELL WALL COMPONENTS AND ITS ROLE IN METAL BINDING

The fungal cell wall is a dynamic and essential structure that provides protection, maintains cell shape and regulates interactions with environment. The cell wall is a complex structure made up of glucan, chitin, chitosan, mannans, and glycosylated proteins as well lipids and pigments. Proteins usually bind with polysaccharides to form glycoproteins, which help strengthen the cell wall. In many yeast and fungi, β-(1,3)-glucan and chitin are essential components of the cell wall, an important structure that surrounds cells and which is responsible for their mechanical protection and necessary for maintaining the cellular shape. In fungi the inner cell wall consists of a core of covalently attached branched β-(1, 3) glucan with 3 to 4% inter chain and chitin (Latgé JP. 2007, Fleet GH. 1991).The outer layers of fungi vary much more than the inner skeletal layer.

2.8. Glucans: The most abundant polysaccharides in nature are made of glucose, that is, they are glucans. Glucans are named according to their type of linkage and the carbons involved in their joining, or receive specific names. Glucans are polysaccharides composed of glucose monomers linked by glycosidic bonds which provides the structural integrity and protection. The most important structural polysaccharide of the fungal cell wall and represents 50–60% of the dry weight of the cell wall. The primary types of glucans found in fungal cell wall are β-Glucans and α-Glucans. β-Glucans forms the fibrillar network. They consist mainly of β-(1, 3)-glucans, with some β-(1, 6)-glucan branches that contribute to the cell wall's flexibility and strength. Whereas the α-Glucans are less abundant and consist of α-(1, 3)-glucans. They play a role in cell wall organization and contribute to fungal virulence in some pathogenic fungi. β-Glucans are significant because human and animal immune system can recognise it and can generate immune response against it. Due to this it can be the target for the antifungal drugs and immune boost therapies. Most polymers of glucan are composed of 1,3 linkage glucose units (65–90%), but there are also glucans with **β** -1,6; **β** -1,4; **α** -1,3 and **α** -1,4 links (**Table 2**.). The enzyme glucan synthases located in the plasma membrane synthesize the **β**-1, 3-D-glucan complex. The α -1, 3- glucan is also a fundamental component of the fungal cell wall and is synthetized by a-glucan synthase. Micro fibrils are formed by the association of several glucan chains through hydrogen bonding. The role of micro fibrillar polysaccharide is mostly structural, they are insoluble in water and almost all solvents, and they are mostly crystalline. The general structures of the different types of fungal beta glucans were described by (Ruiz-Herrera 1992). Seven classes were identified based on their linkages and was found that mostly fungi species had the linear glucan (Narui et al., 1999). Glycogen, that is made of glycosyl units joined by α 1, 4 and α 1, 6 bonds, and glucans in which the glycosyl units are bound solely through α 1, 3 bonds, or α 1, 3 and α 1, 4 bonds; glycogens store and accumulate energy in grain form in cytoplasm (Bacon et al., 1968; Gold et al., 1973, Hochstenbach et al., 1998, Grün et al., 2005).

2.9. Chitin: Chitin is β (1, 4)-linked homopolymer of N-acetyl glucosamine (**Table 2**.), a simple polysaccharide that is embedded deeply in the cell wall and it provides the structural stability (Munro C.A. et al 2001, Latge J.P. et al 2007). Often it allows the fungi to monitor and interact with the external environment. Chitin is an essential component of the cell walls and septa of all pathogenic fungi. Chitin forms the micro fibril 3D network structure which is attached covalently to β (1, 3) – glucan a polysaccharide present in the most of fungal cell walls (Klis F.M. et al 2006). Chitin is synthesised by enzyme chitin synthase (CHS). It is also found that the size of chitin fragments can stimulate the immune cells to generate the immune response (Da Silva C.A. et al 2009). Very large (>100 μm) chitin fragments, from invertebrate sources, are immunologically inert, while intermediate (40–70 μm) and small chitin (<40 μm) are capable of activating macrophages (Da Silva C.A et al 2009). Chitin is a polysaccharide that is found in all known pathogenic fungi hence chitin synthesis is focused for the antifungal therapies (Munro C.A. et al 2001). Chitin plays roles in various ways such as providing the rigidity and strength, helps in the septum formation and cytokinesis, also is involved in the filamentous growth, budding and hyphae extension. It also shields the fungi from osmotic stress, environment and mechanical damage. As chitin has various properties such as biodegradability, biocompatibility and antimicrobial properties it has applications in various fields like in biomedical for wound healing as it promotes the cell adhesion, proliferation and tissue regeneration as it prevent infections in open wounds. Chitin nanoparticles are also used for the controlled drug release. It is also used in the agriculture sector as it enhance the soil structure and nutrient availability.

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2.10. Chitosan: Chitosan is a biopolymer derived from chitin, resulting from the de-acetylation of chitin which distinguishes it from its precursor. Structurally, chitosan is composed of β-(1→4)-linked D-glucosamine units. The presence and proportion of this polymer varies from species to species and also with growth stage. Fungi belonging to the Zygomycetes class, such as species of Aspergillus, Rhizopus, Absidia and Gongronella have significant amount of chitosan in their cell walls during the vegetative growth phases (Nwe, N. et al 2011). Some organisms have high chitosan level than chitin, it also helps in resisting the environmental stresses and external factors (Gow NAR et al 2017). Chitosan has found various applications due to its biodegradable and antimicrobial properties. In agriculture it is used as pesticides and growth enhancer, whereas in food industry it is used as food preservative and food additives to enhance sweetness and shelf life of the product (Abo Elsoud et al 2019). In recent studies it came to know that the fungal chitosan has its application in the cosmetic industry also as it is used in the hair care products to reduce frizz and improve hair (Seino H et al 2020).

2.11. Mannans: This component of cell wall plays a role in disease production and are biomarker in the diagnosis of infection. Mannans are shed into body fluids such as blood and urine during invasive fungal disease. In fact, shed mannans have been recognized as biomarkers for every invasive infection produced by an Ascomycetous fungus. Mannans are mannose polymers located in the outermost part of cell walls; therefore, they may be the first component to interact with the immune system and it can also induce the antifungal protective immunity (Netea, M. G. et al 2006, Robinson, M. J. et al 2009, Saijo, S. et al 2010). Presence of high amount of mannans in the blood stream of candidiasis patient shows us the progression (Chumpitazi, B. F. et al 2014, Mikulska, M. et al 2010).

Table 2. Major fungal cell wall components and their roles in structural integrity, environmental interaction, and metal binding potential.

Component	Chemical Nature	Role in Metal Binding	Other Functional Roles	References
Glucans	Polysaccharides of glucose linked via β-(1,3), β-(1,6), α-(1,3), α-(1,4) glycosidic bonds	Provide binding sites via hydroxyl groups; microfibrillar network supports biosorption	Structural integrity, immune recognition (β-glucans), antifungal drug target	Ruiz-Herrera (1992); Narui et al. (1999); Gold et al. (1973)
Chitin	β-(1,4)-linked N-acetylglucosamine	Forms insoluble, rigid matrices aiding in metal ion entrapment	Provides mechanical strength, immune modulation, target for antifungal therapies	Munro et al. (2001); Klis et al. (2006); Da Silva et al. (2009)
Chitosan	Deacetylated chitin: β-(1→4)-linked D-glucosamine	Positively charged amino groups bind to negatively charged metal ions	Biodegradable, antimicrobial, used in agriculture, medicine, and cosmetics	Nwe et al. (2011); Gow et al. (2017); Abo Elsoud et al. (2019)
Mannans	Polymers of mannose (outer wall)	Potential for metal interaction through hydroxyl groups	Involved in pathogenesis, immune recognition, diagnostic biomarker	Netea et al. (2006); Robinson et al. (2009); Chumpitazi et al. (2014)
Glycoproteins	Proteins bound to polysaccharides (mannans, glucans)	Contribute to metal binding via amino acid residues and glycan chains	Cell wall integrity, signaling, adhesion	Fleet GH (1991); Latgé JP (2007)

MECHANISMS OF FUNGAL-MEDIATED METAL BINDING AND DETOXIFICATION

We can utilizes fungi to degrade or transform harmful substances into less toxic or non-toxic forms. Fungal species such as Pleurotus ostreatus, Rhizopus arrhizus, Phanerochaete chrysosporium, Phanerochaete sordida, Trametes hirsuta, Trametes versicolor etc. Thus, Myco-remediation is the process which use fungi such as filamentous fungi (mould), macro fungi (mushrooms) to eliminate poisons from various environmental components, either alive or

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dead (P. Jeyakumar et al 2023). Hence we can use Myco remediation as an instrument for pollutant degradation, transformation, or immobilization (F. Bosco et al 2019). Fungi such as Aspergillus niger and Trichoderma harzianum are effective in the bioremediation of HMs due to their ability to secrete organic acids that chelate metals and facilitate their removal. Aspergillus niger produces citric acid, which can dissolve metals like cadmium, lead and Zn transforming them into less harmful forms that can either be immobilized or precipitated from the environment. Studies have shown that under laboratory conditions (S.K. Khan et al 2017, R.C. Wang et al 2017, L. Yang et al 2019). The ability of fungi to bind HMs on their cell walls through functional groups like carboxyl, amino, and hydroxyl groups also enhances their capacity for heavy metal detoxification (H.E. Smith et al 2018). Mycoremediation process reduces the toxicity of the HMs and prevent it from further spreading into the environment. Fungi have a unique advantage because they form vast mycelia networks that help transport nutrients and enzymes across large areas. These networks enable fungi to penetrate deep into the soil, reaching pollutants that bacteria cannot access. Additionally, mycelia networks support co-metabolism, meaning that breaking down one pollutant can aid in the degradation of another. Fungi have more advantages as compared to bacteria for the bioremediation process of pollutants. Such advantages make fungi effective in treating a wide range of contaminants in diverse environmental conditions. Fungi are highly adaptable as they can survive under the extreme conditions such as pH, temperature and moisture condition, which may be a limitation for bacteria. Fungi like Aspergillus niger and Penicillium chrysogenum can grow in highly acidic environments with pH as low as 2, and in alkaline environments with pH up to 11 (S.K. Khan et al. 2017). Symbiotic relationship of fungi with plants helps in the bioremediation process. For example, the mycorrhizal fungi associated with the plant roots which helps the plant to uptake the nutrients and the translocation of the nutrients and minerals along with the degraded pollutants, contaminants and HMs. This interaction not only promotes plant growth in polluted soils but also improves the efficiency of bioremediation by allowing fungi to extend their pollutant-degrading abilities through the plant's root system (J.L. Burton et al. 2019). Organisms such as bacteria, fungi, lichens, actinomycetes and algae can be selected for the bio remediation process; but we choose fungi as they are very opportunistic and are highly adaptable, also they respond very quickly to the external environment stress, environmental disasters, and extreme climatic circumstances (U. Picciotti et al. 2023). Fungi are very good at interacting with metal ions. They can either push metal ions out of their cells or absorb and store them inside. They also have the ability to change toxic metal ions into harmless forms. Scientists have developed different ways to help fungi either trap, release, change, neutralize, or tolerate heavy metal ions (K.S. Anjitha et al. 2021). Fungal bioremediation involves several key reaction processes such as precipitation reaction in which fungi can convert the soluble metals/ metal ions into insoluble form, fungi secretes the organic acids which reacts with metal ions to form metal oxalates or phosphates, to precipitate it out from the solution. Aspergillus niger has been found to facilitate the precipitation of HM such as lead oxalate and Zn oxalate effectively removing it from contaminated site. More examples for the Zn metal precipitation is Penicillium sp. (Penicillium oxalicum), Fusarium sp. and Trichoderma sp. these fungal species secrets the organic acids which leads to the formation of insoluble Zn oxalates which further separates from the solution (J.W. Niehau et al 2018, Price et al 2001, Purchase D et al.2009). Fungi have cell walls rich in functional groups such as carboxyl, hydroxyl, and amine groups, which bind with metal ions through ionic exchange and chelation. For example, Aspergillus niger and Penicillium chrysogenum are known for their high biosorption capacity for metals like cadmium, lead, mercury and Zn (L. Yang et al. 2019). Metals are adsorbed on the fungal surface and cell walls which reduces the mobility and bio availability in the environment. Bioaccumulation, in contrast, refers to the absorption and retention of metals within fungal cells, where they can be isolated and stored in vacuoles or attached to intracellular proteins. Trichoderma harzianum can bio accumulate significant amounts of Cu and Zn, effectively removing these metals from contaminated soil (H.E. Smith, et al. 2018). Phosphate-solubilizing fungi, such as Aspergillus niger, secrete organic acids like citric acid and oxalic acid, which play a crucial role in chelation and precipitation of HMs like Pb, Cd, and Al and Zn. Citric acid can form stable complexes with metals such as Pb, Cd, and Al, reducing their bioavailability and toxicity.

METAL RESISTANCE GENE

Metal resistance genes (MRGs) are the genetic elements found in bacteria, fungi, and plants that help organisms tolerate and detoxify HMs. These genes encode proteins involved in metal transport, sequestration, efflux, and enzymatic detoxification. MRGs are the foundation for microbial responses to the threats of toxic metals. Metals play critical roles in fungal homeostasis as they are required for various biochemical processes, as enzymatic cofactors. Function of MRGs is to remove the toxic metal ions from the cytoplasm with help of Efflux pumps (eg: CzcABC for Cd/ Zn/Co resistance in bacteria). Examples of the common MRGs in microorganisms are (Cadmium-Zn-Cobalt) which encodes efflux proteins in the pseudomonas species; Ars (Arsenic Resistance) which is found in the E.coli and encodes the ArsB (efflux pump) and ArsC (arsenate reductase). Efflux Pumps in Suillus bovinus have identified enhanced Zn efflux as a potential tolerance mechanism (Kumar et al 2021). The mt1 metallothionein gene in Fusarium oxysporum is activated in response to Zn exposure and plays a key role in Zn resistance. The fungus increases the activity of efflux pumps to expel excess Zn ions, maintaining intracellular metal homeostasis. Metals, which are recognized for their importance in fungi, are Cu, Fe, Zn, and Mn. Zn is an essential metal for the survival of the fungi and is needed for various functions such as structuring of the DNA, nucleic acids, physical growth and most importantly in the protein folding (Brown K.H. et al 2001, Feldmann H. et al 2012). Role of Zn in the DNA binding is that its Zn cluster protein ($Zn (II)_2 Cys_6$), found only in Ascomycetes binds with DNA which is important for the transcriptional activation and regulation of the gene products (Zhang C. et al 2019). Metallothionein (MTs) are low-molecular-weight, cysteine-rich proteins that binds with metal ions, also they play very crucial role in metal homeostasis and detoxification by regulating essential metals like Zn and Cu while also protecting against toxic HMs such as Cd, mercury (Hg), and Pb by the oxidative stress protection in which the MTs scavenge reactive oxygen species (ROS), protecting cells from the oxidative damage. Plants growing in the metal contaminated soil express the MTs to tolerate the excess metals, preventing toxicity in their tissues. Genetically engineered plants or microbes with high MT production can be used for bioremediation, aiding in the phytoremediation and microbial assisted soil detoxification (Sreelatha et al 2025, Parida et al 2024). High concentrations of metal bound by MTs may affect the nutrient cycling and plant growth leading to the lower agricultural productivity. MTs play a vital role in heavy metal detoxification in soil but it can also cause risks if metal accumulation and microbial imbalance are not managed properly. MTs are used in genetic engineering to develop plants and microbes for heavy metal removal from the contaminated sites. MTs can also be used as the potential biomarker for cancers, neurodegenerative diseases and diabetes. Examples of the fungi with metallothionein are Neurrospora crarrssa has Cu binding MT which also helps in maintaining the Zn (II) balance, Saccharomyces cerevisiae has MT gene CUP1 which primarily binds Cu but it can also bind to Zn, Aspergillus nidulans has MT gene which plays role in Zn storage and detoxification. These MT help fungi adapt to fluctuating Zn levels, ensuring proper enzyme function and metal detoxification.

IMMOBILIZATION METHOD FOR FUNGAL BIOMASS IN HM REMOVAL

Immobilization method is more preferred because it provides the structural stability to fungal biomass, also this method allows to reuse the immobilized fungal biomass in multiple cycles of metal adsorption without loss of its efficacy. Also free fungal biomass can degrade easily whereas the immobilized fungal biomass remain intact for long time, these immobilized fungi has long shelf life than free biomass. The immobilized fungi has one more benefit of having increased surface area and optimized functional group exposure hence it has higher biosorption efficiency. The immobilization matrix prevents biomass aggregation, ensuring the better metal interaction. Free fungal biomass is more fragile and may break whereas the immobilized biomass has mechanical strength, which makes it suitable for large-scale applications. The immobilized biomass is easier to handle and this helps fungi to tolerate harsh environment conditions. Some immobilization techniques are entrapment in alginate, silica gel or polymer matrices which are cost effective and allow prolonged use, also this method is an eco-friendly alternative to chemical and physical methods that generates toxic sludge (B. Hemambika et al 2011).

Now, in all of the above methods we can use both live and dead fungal biomass to remove HMs but to maintain a viable live fungal biomass during metal adsorption is difficult, because it requires a continuous supply of nutrients and avoidance of metal toxicity to the microorganism hence dead fungal biomass is more preferred instead of live. Dead fungal biomass depends on surface adsorption in which the functional groups of the cell wall such as carboxyl, hydroxyl and amino groups bind with the metal ions (C.L. Brierley et al 1990, Sulaymon et al 2013). Live fungal biomass can uptake HMs through both surface adsorption and intracellular accumulation; however, the viability of these cells can be affected by metal toxicity, potentially limiting their long-term application (Malik et al 2004, Chen et al 2003). The filamentous zygomycete fungi Rhizopus sp. absorb / adsorb the metal ion was investigated in which it was found that the immobilized fungi reduced the heavy metal concentration (Gomes et al 2014). Recent advancements have highlighted the development of novel bio-composite materials using fungal biomass and natural polymers, such as alginate-clay or chitosan blends, which have significantly enhanced the biosorption capacities and mechanical stability of immobilized fungal systems.

For instance, a 2023 study by Li et al. demonstrated that Aspergillus niger immobilized in an alginate-bentonite composite matrix achieved over 90% removal efficiency for lead and cadmium ions from aqueous solutions, maintaining its biosorption potential across multiple cycles without significant structural degradation. This supports the growing preference for immobilized, non-viable fungal biomass as an eco-friendly, reusable, and efficient solution for heavy metal bioremediation under harsh environmental conditions. In conclusion, immobilized fungal biomass offers a superior and sustainable approach for HM remediation due to its enhanced mechanical stability, reusability, increased biosorption efficiency, and adaptability to harsh environmental conditions, making it a practical and eco-friendly alternative to conventional methods.

FUTURE PROSPECTS

Zn deficiency in agriculture soil is a challenge globally. Micronutrients are required in small quantity and are necessary for plant growth and development; such as Fe, Mn, Zn, Cu, Mo, B, Cl, Ni, P, K, N etc. Among the micronutrients, Zn is the most lacking in agricultural soils (Alloway B.J.et al 2009). Zn deficiency leads to poor plant growth, reduced chlorophyll production, and poor root development. Crops such as rice, wheat, maize, and citrus are highly susceptible to Zn deficiency, which ultimately affects yield and nutritional quality. Several factors contribute to Zn deficiency, including high soil pH, excessive phosphorus application, and low microbial activity that limits Zn bioavailability. Here, fertilizers plays the important role so the use of fertilizer is being practiced to produce enough food for increasing population. However, particularly the nitrogen (N) and phosphate (P) fertilizers are being used (Duan Y et al 2014, Qaswar M, et al. 2020). Hence, bio fortification can also be done by using Zn fertilizers such as; Zn sulphate, Zn sulphate monohydrate, Zn chelate, Zn oxide, Zn carbonate and Zn chloride (Kihara J., et al. 2020). Over the past two decades, nanotechnology has been investigated as a means to improve nutrient use efficiency and enable the targeted delivery of nutrients to plants, nano-scale fertilizers (1–100 nm) possess a higher surface area-to-volume ratio and can be engineered for surface modifications, allowing for controlled release based on plant needs or environmental conditions (Raliya R et al 2017, White JC et al 2018). Nano fertilizers can be used because of its advantages such as enhanced nutrients uptake due to their small size and increased reactivity, reduced nutrient loss, low environmental pollution etc. Due to their small size, nano-fertilizers penetrate plant cells more efficiently, enhancing nutrient uptake and promoting better growth. Some nano-fertilizers interact with soil microbes, improving nutrient cycling and microbial activity. On comparison of the nano fertilizer with the conventional fertilizer; the nutrient efficiency of Nano fertilizer is high as compared to the conventional fertilizer. Nano fertilizers cause lower environmental pollution as compared to the conventional. There are several types of Zn nanofertilizers such as Zn sulphide nanoparticles (ZnS NPs) which are less commonly used but has high bio availability as it enhances photosynthesis, nutrient uptake and overall plant growth. Zn nano composites in the form of ZnO or ZnS combined with carriers like silica, clay or polymer which improves stability. Nano encapsulated Zn, in which Zn is enclosed into the bio-degradable polymers or the organic molecules which prevents rapid degradation, ensures targeted delivery, and reduces leaching. Zn-Based Metal-Organic Frameworks (MOFs) has the porous, high-surface-area structures with Zn ions, it enhances nutrient efficiency and sustained Zn release. Zn Oxide Nanoparticles (ZnO NPs) are insoluble or partially soluble nanoparticles; which slow-release Zn source; improves plant growth, enzyme activity, and resistance to stress. Zn oxide nanostructures (ZnO NS) are tiny forms of ZnO with special properties that make them useful in agriculture, medicine, electronics, and the environment. They come in various shapes, like nanoparticles, nano rods, and nanotubes. In farming, ZnO NS act as an efficient Zn source, releasing nutrients slowly for better absorption and minimal waste. They also support microbial activity and root growth. Since they are more bioavailable than regular ZnO, they work effectively in smaller doses, lowering the risk of toxicity in plants (Nie Z et al 2010, Maqbool Q et al 2017, Wang X. et al 2016). The field of fungal biosorption for heavy metal remediation, particularly Zn, is rapidly evolving with several promising research directions (Xingjie Li, 2023). A key emerging area is the use of nanotechnology, where fungal biomass is employed to synthesize functional nanoparticles such as Zn oxide or magnetite, enhancing sorption capacity due to increased surface area and reactivity. Another forward-looking approach is the genetic modification of fungi using tools like CRISPR/Cas9 to overexpress metal-binding proteins such as metallothioneins and phytochelatins, thereby improving both metal specificity and tolerance. Additionally, immobilization of fungal biomass in biodegradable carriers such as alginate, chitosan, or plant-based polymers has gained attention for its potential to improve stability, reusability, and ease of deployment in large-scale systems. Future studies are also expected to focus on the simultaneous biosorption of multiple HMs, reflecting the complexity of real-world contaminated environments (Sara L, 2021). Importantly, integrating biosorption technologies into the circular bioeconomy—such as recovering valuable metals and repurposing metal-loaded fungal biomass for agricultural or industrial use—represents a sustainable and economically viable path forward. These innovative approaches not only enhance the practical application of fungal biosorption but also align with global goals for eco-friendly and resource-efficient remediation strategies.

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AUTHOR CONTRIBUTIONS

Authors P.G., S.D., V.J. wrote the original draft and prepared the figures and J.K. has prepared the tables and added references. S.B. has done proof reading of the original draft. J.K. has suggested changes in the original draft as well as approved corrected version of the manuscript.

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