

Immobilization of Heavy Metals in Contaminated Soils Using Phosphate-Solubilizing Fungi: Mechanisms and Advantages

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Abstract With rapid industrialization, excessive use of agrochemicals, and improper waste disposal systems, the contamination of soil and water by toxic heavy metals such as cadmium, lead, arsenic etc., is increasing spontaneously and has become a global menace in recent times. It is attracting global attention due to their deleterious impact on humans and ecosystems. Little achievements have been made with regards to effectively containing or reversing the dangerous levels of heavy metal pollution of the environment. Therefore, there is an urgent need for effective and sustainable remediation methods. Several remediation strategies have been proposed to tackle the problem of continuous heavy metal contamination of soil. However, most of the strategies have some limitations of ecological concerns ranging from the high cost of operations, generation of sludge and wastes, inefficiency, and unsustainability. This review discusses the various advantages and mechanisms involved in the in-situ immobilization of heavy metals in contaminated soils by exploiting the interactions between phosphate-solubilizing fungi and phosphate minerals and fertilizers. Phosphate-solubilizing fungi (PSF) are beneficial microbes capable of solubilizing inorganic phosphorus compounds or phosphate-containing organic compounds to release phosphorus (P). The activities of PSFs in the soil ensure the availability of much-needed P to plants and microorganisms in the soil.

Keywords Bioremediation, Cadmium, Organic Acids, Phosphorus, Solubilization, Bioavailability

1. Introduction

Non-essential heavy metals, which do not have any known biological function in living organisms, have continued to contaminate the environment majorly through various anthropogenic activities such as industrialization, mining, use of phosphate fertilizers and agrochemicals, wastewater irrigation, and improper waste disposal [1, 2]. They are toxic, non-biodegradable and persist long in the environment [3]. They pose significant risks to human and ecosystem health because of its toxicity, mutagenicity, and carcinogenicity. Cadmium (Cd), for instance, has been associated with different cancers, renal and bone damage in humans [4]; disrupts protein metabolism and chlorophyll synthesis [5] in plants, and causes oxidative stress and cell death in microbes [6].

Many microorganisms have developed intrinsic (active and passive) mechanisms of resistance or tolerance to toxic heavy metals. Of a particular interest are the phosphate-solubilizing fungi, which have been studied several times for their roles in plant growth promotion and bioremediation of soil and water polluted by heavy metals.

Phosphate-solubilizing fungi (PSF) are beneficial microbes capable of solubilizing inorganic phosphorus compounds to release inorganic phosphorus (P). The dissolution of phosphate minerals e.g., fluorapatite (FAP) in soils, is crucial for the bioavailability of phosphorus to plants.

With regards to heavy metals immobilization in soil, the most commonly applied genera of phosphate-solubilizing fungi include *Aspergillus* (phylum: Ascomycota, order: Eurotiales), *Penicillium* (phylum Ascomycota: order Eurotiales) [7, 8], and *Trichoderma* (phylum: Ascomycota, order: Hypocreales) [9]. Primarily, the mechanism of solubilization of inorganic phosphates to release P is by the sequestration action of low molecular weight organic acids, which are secreted or synthesized by PSF [7, 10]. These fungi can also solubilize phosphate-containing organic compounds by first cleaving C-O-P ester bonds through the activities of phosphatase enzymes that release P [11].

Arbuscular mycorrhizal fungus (AMF) e.g., *Rhizophagus irregularis* formerly known as *Glomus intraradices* (Phylum: Glomeromycota; Order: Glomerales), which can solubilize phosphates, has also been proven to play active roles in bioremediation of soils polluted by heavy metals [12, 13]. A recent study showed that inoculation of *Rhizophagus intraradices* (RI) significantly decreased ($p < 0.05$) Cd concentration in shoot and root of rice plant (*Oryza sativa*) under Cd stress [14]. In another study, *Funneliformis mosseae* (an AMF) was proven to be effective and useful for the immobilization of Cd and Zn in polluted soil [15].

2. Integrated PSF-Phosphate Mineral Approach for Heavy Metals Immobilization

The application of PSF in combination with phosphate materials is a new integrated strategy for the remediation of soil or water contaminated by heavy metals [7, 16]. Recently, it was reported that the combination of *A. niger* with geological FAP significantly immobilized more Cd in aqueous solution than either the separate single treatment with either the fungus or FAP, and the Cd removal efficiency of the combination treatment was up to 98.2% and 87.3% in Cd solutions of 10 and 25 mg/L initial concentrations, respectively [16]. They stated that the effectiveness of the combination amendment was due to the multiple mechanisms of Cd removal produced by the interaction between the fungus and FAP.

In another study, the application of FAP and *Penicillium oxalicum* on the remediation of lead (Pb)-contaminated water was investigated and it was reported that the combination treatment performed better than the use of only the fungus. At day 7 of the experiment, they observed that the concentration of Pb (initial concentration of 1739 mg/L) had been drastically reduced to 17.4 mg/L in the solution by the combination treatment, while only the fungus reduced the Pb concentration to 30.7 mg/L [7].

Results from another study also showed that the combination of *A. niger* with organophosphate resulted in the enhanced Pb removal efficiency of the fungus from ~92% to ~97%. Consequently, the bioavailability of Pb was significantly reduced [17].

3. Possible Mechanisms of Heavy Metal Immobilization by PSFs

The mechanisms responsible for enhanced removal or immobilization of the heavy metals by the interactions of PSMs and phosphate minerals include: (i) biosorption, (ii) formation of insoluble metal-oxalate and metal-phosphate complexes by the action of organic acids, (iii) Phosphate enhanced metal adsorption on ligands, and (iv) the action of other heavy metal defence mechanisms such as siderophores and glutathione produced by the PSF.

(i) Biosorption.

Biosorption can be defined as the microbial uptake of organic and inorganic metal species, both soluble and insoluble, through physico-chemical mechanisms, such as adsorption [18]. In addition to adsorption to cellular surfaces, some metal ion species can be accumulated within cells via membrane transport systems of varying affinity and specificity. Once inside cells, metal species may be bound, precipitated, localized within intracellular structures or organelles, or translocated to specific structures depending on the element concerned and the organism [18].

Phosphate solubilizing fungi have been shown to effectively immobilize heavy metals by biosorption [19, 20]. Their cell wall structure consists of chitins, glucans, melanin, other polysaccharides, and proteins, and these compounds make the cell wall a very effective biosorbent for metals. Also, Fungal polymers and melanin contain carboxyl, phenolic, alcoholic hydroxyl, carbonyl, and methoxyl groups, which are critical metal-binding sites [20, 21].

Microorganisms produce different kinds of metal-binding metabolites such as the extracellular polymeric substance (EPS), which is composed of polysaccharides, proteins, glycoproteins, nucleic acid and humic substances [22, 23]. Extracellular polymeric substances are important biosorption components in living systems such as biofilms [20], and are known to effectively bind and immobilize heavy metal ions such as Cd^{2+} , Pb^{2+} , Cu^{2+} , and Zn^{2+} within the extracellular matrix [24, 25]. The ability of EPS to effectively bind metal ions is due to its significant content of chelating functional groups such as amino, thiol, carboxyl, and phenolic groups [26].

The EPS from red yeast, *Rhodotorula mucilaginosa*, has been shown to effectively remove Pb^{2+} from solution [23]. In another study, EPS was shown to play an important role in the biosorption of Pb^{2+} by *Aspergillus niger* PTN31. It was reported that the Pb^{2+} biosorption capacity of *A. niger* PTN31 was as high as 713.6 mg/g when its EPS was intact,

whereas the Pb^{2+} biosorption capacity of the fungus was decreased by 40.9%–66.8% after removal of its EPS [27].

Phosphate-solubilizing fungi are preferred over bacteria as biosorbent materials for heavy metal-contaminated aqueous solutions, because of the ease with which they are grown e.g., *A. niger* and *Saccharomyces cerevisiae* [21]. Besides, fungi can effectively tolerate toxic heavy metals even at high concentrations more than bacteria [28, 29]. Dead biomass of *A. niger* has also been reported to be a very effective biosorbent of heavy metals [30].

(ii) *The action of organic acids.*

Phosphate-solubilizing fungi are known to produce organic acids, which are well-known heavy metal chelating agents [13, 31, 32]. Low molecular weight organic acids such as oxalic, citric and tartaric acids have been identified as metal chelating agents, thereby can solubilize heavy metals [33]. Oxalic acid is the dominant organic acid secreted by PSFs [7, 32], and it is the primary organic ligand implicated in the immobilization of heavy metals. Oxalic acid production by PSF, such as *A. niger* [16] and *Beauveria caledonica* [32] is one of the mechanisms by which fungi tolerate the toxicity of Cd^{2+} and other heavy metals. Organic acids immobilize metals through the following mechanisms:

(a) Formation of metal-oxalate complexes.

It has been recorded that oxalic acid secreted by PSF immobilizes heavy metals by reacting directly with the metal ions to form insoluble metal-oxalates. For example, oxalic acid reacts with Cd to form insoluble Cd-oxalate (CdC_2O_4) [16], while the acid reacts with Pb to form Pb-oxalate [7].

(b) Precipitation of metal phosphates.

Organic acids, chiefly oxalic acid, secreted by PSFs dissolve phosphate minerals and fertilizers in soils to liberate P anions, which react with heavy metals to form insoluble metal-phosphate complexes e.g., pyromorphite [7] and cadmium phosphate [16]. The precipitation of cadmium phosphate is one of the main mechanisms of immobilization of Cd by P materials especially in soil or water with high Cd concentration [34, 35]. Cadmium phosphates ($Cd_3(PO_4)_2$) has a relatively low solubility ($\text{Log } K_{sp} = -38.1$) over a wide pH range [35]. Meanwhile, $Cd_3(PO_4)_2$ starts precipitating from the neutral pH range in soils [36].

(iii) *Metal adsorption on ligands enhanced by phosphates.*

Phosphate anions released by organic acids also enhance metal adsorption on ligands present in soil. Studies have shown positive results of phosphate enhancement of Cd adsorption onto inorganic ligands or minerals such as aluminium oxide (Al_2O_3) [37], iron oxyhydroxide or goethite [38], manganese dioxide ($\beta\text{-MnO}_2$) [39]. The results of the investigation of Zn and Cd adsorption to Al_2O_3 nanoparticles as influenced by phosphate (PO_4), citrate, and humic acid (HA), showed that Cd reached near-complete adsorption at 2 mmol L^{-1} PO_4 in the mono-metal and

binary-metal systems [37]. Comparing the Cd sorption enhancement by phosphate, citrate, and HA, it was reported that complete adsorption of Cd was not observed in any of the systems with HA concentrations. With HA, the most substantial increase in adsorption was for Cd in the mono-metal system [from 5% (46 mmol kg^{-1}) to 58% (583 mmol kg^{-1})], at HA concentrations ranging from 0 to 240 mg L^{-1} HA. In the citrate systems where adsorption of Cd was enhanced, adsorption did not exceed 33% (33 mmol kg^{-1}) of the total initial concentration. Therefore, phosphate was the most effective ligand in comparison with HA and citrate for promoting Cd adsorption [37].

(iv) *The action of other heavy-metal defence mechanisms produced by PSF.*

(a) Siderophores

Siderophores are low molecular weight ligands with a high affinity for iron (Fe^{3+}). They are produced by many microorganisms to facilitate the uptake of iron which is an essential nutrient for most living organisms [40]. Toxic metals induce microbial production of siderophores, and these siderophores are known to play an important role in microbial heavy metal tolerance [41]. In addition to the supply of essential iron to microbes and plants, siderophores can chelate, bind and complex toxic heavy metals such as Cd, Pb, Zn and Cu [40]. The binding of heavy metals to siderophores in the extracellular medium reduces the heavy metals concentration and affects the mobility, speciation, bioavailability, and long-term fate of heavy metals in the environment [41]. In this way, siderophores help to alleviate the stress of toxic heavy metals in soils. The most common genera of PSF studied for the production of siderophores are *Aspergillus* [42], and *Trichoderma* [43, 44].

(b) Glutathione

Glutathione (GSH) is very vital in heavy metal tolerance, immobilization, and detoxification in living organisms. Glutathione, a tripeptide (L- γ -glutamyl-L-cysteinyl-glycine), is the most abundant non-protein thiol present in living organisms [6, 45, 46]. *Aspergillus niger* [47] and *Penicillium* sp. [46] are notable PSF with significant glutathione production in response to oxidative stress. It is a key component in metal scavenging and detoxification by living organisms, due to its high affinity of metals for its thiol ($-SH$) group and as a precursor of phytochelatin [6]. Exposure to Cd has been reported to cause the accumulation of GSH in fungi, suggesting the role of GSH in Cd detoxification [48]. Glutathione readily binds Cd^{2+} in cells [49] and has been reported to constitute the first line of defence or protective barrier against Cd^{2+} in many cells [45].

Cadmium is complexed by GSH, resulting in the formation of the $Cd(GS)_2$ complex. Once it is formed, this transport active and stable $Cd(GS)_2$ complex is usually transported into the vacuole compartment in the cytoplasm or outside the cells via specific protein transporters [45, 49].

(c) Glomalin

Arbuscular mycorrhizal fungi are known to produce glomalin, a glycoprotein, which can bind and immobilize heavy metal cations in the soil [50-52]. In a recent study in soil with high Cd concentrations, it was revealed that the inoculation of sunflower plants with AMF, *Funneliformis mosseae* significantly lowered Cd and Zn concentrations in the shoot of the plant and the biological concentration factor (BCF) values than in the non-inoculated plants [15]. This result was partly attributed to the secretion of glomalin in the hyphae of the AMF and glomalin release to the plants by the AMF.

4. Conclusions

Phosphate solubilization by fungi is an eco-friendly and sustainable process that can be employed effectively in heavy metal immobilization in soil. Combining PSF and PRs also improves soil fertility by enhancing the mobility and availability of phosphorus to plants and microorganisms in the soil. This review provides extensive background knowledge on the several mechanisms involved in the application of phosphate solubilization by microbes for bioremediation of heavy metal-contaminated soil or water. Those mechanisms also serve as defence mechanisms of the fungi against the toxicity of heavy metals such as Cd. Based on our review, the multiple mechanisms of this PSF-phosphate combined approach were found to be a more effective and sustainable alternative for in situ immobilization of heavy metals in soils.

This strategy is advantageous since both materials are cheap and can be obtained easily. Phosphate-solubilizing fungi such as *A. niger*, *P. oxalicum*, *Trichoderma* spp. and AMF are ubiquitous in the soil and can be easily grown using simple laboratory culture techniques. *Aspergillus niger* is also generated as a waste product of fermentation industries. Phosphate minerals are common and abundant on earth. Combining PSF and FAp should be exploited on a largescale because of sustainability.

5. Future Research Considerations

Considering the fact that pH affects the solubility and availability of heavy metals and metalloids in soil, it becomes imperative that future research should be directed towards ascertaining the efficiency of combining PSFs with phosphate materials for remediation of polluted soils under varying pH levels (acidic, alkaline and neutral) over long terms. In addition, future research should be targeted on the interactions between PSF and bacterial commensals, and the effect of those interactions on heavy metal immobilization in soils.

Conflict of Interest

Authors declare no conflict of interests.

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