



Original Research Paper

Transforming Lead-Contaminated Soil into Fertile Land by Recycling Phosphogypsum

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Abstract

The presence of lead (Pb) in soil affects the environment and public health and causes serious issues as it is persistent, toxic, and bioaccumulates in the food chain. Traditional soil lead remediation methods are expensive and scale poorly. In this study, we explore an integrated and sustainable approach to remediation that combines phosphate-solubilizing fungal bioremediation with chemical stabilization. The bioremediation uses *Aspergillus niger*, while the chemical stabilization uses phosphogypsum, an industrial waste product from phosphate fertilizers. A 60-day laboratory study was conducted to assess the effects of fungal inoculation and phosphogypsum amendment, and their combination, on Pb-contaminated soil (300 mg/kg Pb). Lead bioavailability was determined using diethylene triamine penta-acetic acid (DTPA) extraction and subsequent measurement by atomic absorption spectrophotometry. Treatment results showed significant lead bioavailability reduction, and the combined approach was the most effective, with an estimated 65% reduction of Pb following 60 days of treatment. Enhanced reduction is due to bioaccumulation, organic acid production of the fungus, and lead biosorption which, in combination with phosphogypsum, precipitates lead into stable minerals, such as pyromorphite. Additionally, the integrated treatment improved soil biological activity and stability. The findings highlight the potential of this bio-chemical approach as a cost-effective, scalable, and environmentally sound solution for rehabilitating lead-contaminated soils while promoting circular economy principles through industrial waste reutilization, particularly in regions affected by heavy metal pollution such as Iraq.

Keywords: Lead contamination, soil bioremediation, phosphate-solubilizing fungi, phosphogypsum amendment, metal immobilization, biomineralization processes, pyromorphite formation, sustainable remediation, waste valorization, pollution in Iraq.

1. Introduction

Due to its cost and complexity concerns, bioremediation has received the most attention among other remediation techniques, such as physical and chemical methods (Gul *et al.*, 2024). Most other remediation techniques require large amounts of energy and other resources and, after a relatively short time, lose their effectiveness (Yin *et al.*, 2019). In contrast, bioremediation establishes a long-term, self-sustaining process, as it relies on the capabilities of microorganisms to sequester and/or transform harmful contaminants. Of these, bioremediation is the best technique to address soil contamination with heavy metals (e.g., lead). Soil bioremediation employs soil microorganisms, predominantly fungi, to eliminate heavy metals and their deleterious effects, making it one of the most

effective technologies available for soil remediation (Maqsood *et al.*, 2022).

Lead pollution has been and continues to be an environmental risk due to its extreme toxicity, adverse effects on human health, and persistence in the ecosystem (Ali *et al.*, 2019). Lead is toxic and contaminates the soil, and subsequently accumulates and is transported via the food chain to humans. With the soil pollution in Iraq, lead is one of the major heavy soil pollutants. Its clogging of the soil leads to environmental pollution via its direct and indirect impacts on the soil's physical, chemical, and biological properties (Delgado and Gómez, 2024).

The most serious and recent public health and environmental challenge is the distribution of lead in agricultural soil (Meena *et al.*, 2019). Lead is present in soil due to human activities such as mining, industry, the reckless throwing away of medical and hazardous waste, and the frequent use of lead-containing products, which all contribute to lead soil pollution (Sani and Amanabu, 2021). Highly

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elevated levels of lead, in addition to posing serious threats to the safety of food and the health of humans due to bioaccumulation, severely damage soil microbial communities, inhibit central soil enzymatic activities, and stunt the growth of soil and plants (Gupta *et al.*, 2024).

One of the most important consequences of lead's accumulation in the soil is the alteration of soil acidity, as well as the increase of other heavy metals. This affects the disrupt the ecological balance of plant nutrients and destabilizes the soil, which holds important cations such as calcium, magnesium, and potassium together (Malvi, 2011). From the biological perspective, lead's accumulation most challenges the the critical soil microbial populations, such as bacteria and fungi, and in turn lowers the function of the urease and dehydrogenase enzymes that are major contributors to soil metabolism (Satya *et al.*, 2025).

Phosphate fertilizer industry, however, as an example, generates, as a secondary byproduct, millions of tons of phosphogypsum each year (Awad *et al.*, 2024). The storage of such a byproduct, however, poses significant risks to the environment (Maina *et al.*, 2025). On the other hand, increasing studies show that, due to the high calcium sulfate and phosphate residue, phosphogypsum can be used to improve soil quality, and thus it has started to be considered a byproduct with value rather than a waste product (Cernisch *et al.*, 2021).

Lead has adverse effects on the rate of seed germination and root development, as well as on the absorption of water and nutrients by the roots. The most serious danger of lead accumulation in the tissues of plants is in how it can be taken up by people through agricultural crops. Lead poisoning causes malfunctions of the nervous system, especially the system's neurotransmitters, which is particularly harmful to children. It also causes hemolytic anemia due to membrane damage from lipid peroxidation, and impacts the the urinary system and the kidneys (Debnath *et al.*, 2019). Mycorrhizal bioremediation is a new and holistic approach to the remediation of heavy metals contaminated soils that has been developed and increased in popularity in the last several years (Thakur *et al.*, 2023). Mycofungi resistant to lead, particularly *Aspergillus niger*, develops excellent adaptive strategies that allow it to metabolize and grow in the presence of metal stress (Li *et al.*, 2024). Some of these strategies are the secretion of certain biosynthesis of cell wall functional groups, bioaccumulation in the fungal biomass, secretion of certain organic acids (i.e., oxalic and citric), and transformation of lead from a mobile ionic form to a more stable, less bioavailable form (Šebesta *et al.*, 2022).

In overcoming these challenges, phosphogypsum can be used in a positive way as a supportive and synergistic modifier (Maina *et al.*,

2025). Apart from improving soil cohesion and balancing soil pH, the calcium and sulfate in phosphogypsum further reinforce the fixation of lead through lead-precipitation and lead-ion exchange (Shruthi *et al.*, 2024). Moreover, phosphogypsum does less harm than other alternatives.

Another advantage of phosphogypsum is that it provides and maintains a microenvironment that is suitable for the growth and activity of fungi, thereby promoting bioremediation (Shruthi *et al.*, 2024). Additionally, the circular economy concept of repurposing phosphogypsum in treatment systems addresses the combined challenge of industrial waste and soil contamination in a sustainable manner (Cui *et al.*, 2024).

A synergistic treatment approach combining fungal bioremediation and the chemical catalyst phosphogypsum (a key component of synergistic treatment for lead (Pb) contaminated soil was employed in this study (Ben Mefteh *et al.*, 2019). *Aspergillus niger* was selected as the fungal species for this bioremediation process. Furthermore, phosphogypsum is often the only solution available to most polluted sites due to its capacity to integrate into contaminated ecosystems and to biogenerate organic acids which facilitate the dissolution and stabilization of heavy metals (Meng *et al.*, 2022).

Phosphogypsum is also of great importance because it is a by-product of several industries, particularly the phosphate fertilizer sector. It is also one of the largest by-products generated annually (Pliaka, and Gaidajis, 2022).

However, the increased interest in its use and application in rehabilitating and agro-related activities is due to its formulation-calcium sulfate with several micro-nutrients, which consistent with improving the physical and chemical properties of the soil, aids in moisture retention, and creates a microbially favorable environment (Usharani *et al.*, 2019).

The combination of fungal bioremediation and phosphogypsum use is a novel and holistic approach, integrating biological detoxification with chemical stabilization (Gao *et al.*, 2025). This system improves the fungal activity by complexing and transforming lead, while phosphogypsum hinders the lead from becoming unstable in a mineral form; phosphogypsum aids the long-term stabilization of lead (Gadd, 2017). Apart from the metal detoxification, this holistic approach enhances the restoration of soil functions by improving the quality indicators of pH, electrical conductivity, microbial biomass, and activity of the dehydrogenase enzyme and increases the soil functions (Sarivastava *et al.*, 2017)

The fungi's bioremediation mechanism entails generating citric, oxalic, and gluconic acids, which both sequester lead and transform it into a soil solution, making the lead less detrimental and fixable more easily. Then, the added

phosphogypsum further provides the phosphate and sulfate ions that precipitate the dissolved lead (Pb^{2+}) into the less soluble and environmentally acceptable pyromorphite and lead sulfate (Arwidsson *et al.*, 2010).

This study presents the comprehensive framework to remediate lead-contaminated soil, which is one of the major heavy pollutants in Iraq, for which the complex environmental and health effects are caused by the continuous soil and environmental components. This study aimed to explore soil remediation techniques whereby contaminated soil could be transformed into fertile and biologically active land, which was made possible through the synergistic application of *A. niger* and phosphogypsum. By primarily minimizing the bioavailability of lead and, secondarily, restoring the soil's biological activities, this research develops a model for soil rehabilitation that is sustainable, inexpensive, and ecologically safe, addressing treated soils in both industrial and agricultural wastelands. From a sustainability point of view, the mixing of industrial byproducts with biological systems for treatment is environmentally sound and rehabilitates the ecosystem.

With regard to the aforementioned studies, it can be stated that the potent myco-remediating properties of the fungi will be a valuable asset to the conversion of lead to be non-hazardous; the lead, which exists in an insoluble and environmentally damaging state due to the acidic actions of the fungi, to lead that has been converted to be environmentally safe solid compounds due to the presence of phosphogypsum compounds.

2. Materials and Methods

The main goal of this study is to find out whether lead can be fixed using fungal myco-remediation, in conjunction with the phosphogypsum-remediation processes of fungal biomineralization, to lead's environmentally immobile forms in soil solution, thus rendering it unusable by plants.

To this end, a study was planned and executed that involved the construction of a study model looking at soil that is free of lead contamination, and this soil was obtained from agricultural regions which are also lead free and free of industrial activities. The soil was verified to contain lead within the universally accepted levels of contamination, and the noted levels were in accordance with the levels noted in uncontaminated soil. The lead soil was also made in the laboratory to complete the model as it was soil of internationally accepted levels of lead contamination.

The lead concentrations of the different treatments were modified within a closed system and controlled conditions, where the moisture, pH and temperature were closely monitored. Changes in lead concentrations were taken using an analytical

balance, as were differences in the other treatments, which were monitored during the defined interval.

2.1 Experimental Design and Procedures

Soil samples were collected from sites far from industrial activity to ensure they were free of lead or contained trace amounts, indicating the absence of contamination.

Samples were taken from the ground surface and allowed to dry at room temperature. They were then sieved using a 2 mm sieve to remove impurities. The soil samples were sterilized in an autoclave at 121°C and 15 psi for 15–20 minutes to prevent the effect of endogenous fungi on the integrity of the added inoculum. 500 g of soil samples (for three replicates) were placed in special cylindrical plastic tanks, 15 cm in diameter and 12 cm in height, with a capacity of 1 liter, and equipped with mesh covers for soil aeration. A fungal treatment was used in the first replicate, phosphogypsum in the second, and a synergistic treatment of both in the third. The soil was experimentally contaminated by adding a 240 mg lead nitrate solution ($Pb(HNO_3)$) to achieve a final lead concentration of 300 mg/kg. The soil was then left for 72 hours to ensure homogeneity of the element within its structure.

2.2 Preparation of Treatments

First: Preparation of the Fungal Inoculum

Aspergillus niger was chosen for its ability to secrete abundant quantities of organic acids that can react with heavy metals.

The fungal inoculum is cultured on potato dextrose agar at an incubation temperature of 28°C for 5 to 7 days. Afterward, the biomass is collected, and fungal suspensions are prepared. One gram of biomass is weighed and added to 100 ml of water, then sprayed onto the soil being treated for heavy metals, specifically free lead.

Second: Preparation of Phosphorousgypsum as an industrial byproduct. It is dried, ground, and sieved before being added to the soil. The optimum rate of addition of phosphogypsum is established as 4%. In previous studies, 1%- 10% concentration ranges have been outlined, with 4% being optimal to trigger chemical reactions that fix lead contaminants while being minimally invasive to soil functionality. An increasing concentration of phosphogypsum in soil may lead to pronounced salinity and acidity. Phosphogypsum serves as a source of calcium, sulfates and phosphates, all of which are critical in lead stabilization as a result of the creation of insoluble mineral compounds (Mu *et al.*, 2024).

The synergistic reaction can be summarized as converting lead from its insoluble and environmentally harmful state, due to the acidic activity of the fungi, to soluble lead, and then to stable, harmless lead compounds, thanks to the presence of phosphogypsum compounds. As shown in the Figure 1.

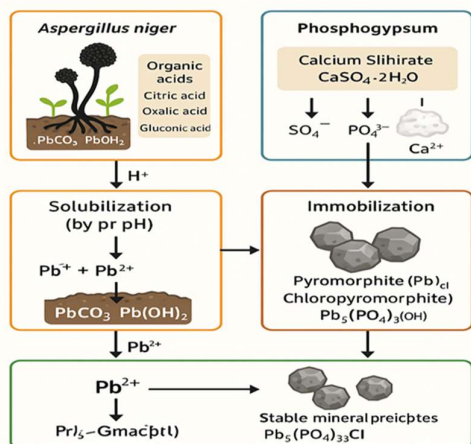


Figure 1: Synergistic interaction by bioremediation of Pb contaminated soil.

2.3 Practical Application of the Experiment

Prior to the start of the experiments, the samples were separated into four groups with three replicates each, with each soil sample having an exact weight of 500 grams, and each sample was placed in a plastic container. In the case of biological treatment experiments with fungi, the fungal suspensions were 1% of the soil weight and so 5 grams of biomass were added to samples labeled T1 and T3. In the case of experiments involving the use of phosphogypsum, so in the case of T2 and T3, gypsum was added to samples at 4% of the weight of the soil so 20 grams for every 500 grams of soil were added. Using a sterile mixing stick, the components were mixed with the soil. In T3, both the fungal suspension and the phosphogypsum were added. The moisture was maintained between 40 and 60% as per requirement. The ambient temperature of the incubator was set to $28 \pm 2^\circ\text{C}$ for a period of 60 days. Sample turning and moisture evaluation were performed weekly to facilitate aeration and to maintain the moisture content. Moisture content was determined by weighing the prepared soil sample before and after drying in a drying oven at $105\text{--}110^\circ\text{C}$ for 24 hours. Fungal viability was confirmed by periodic biological tests to verify fungal activity. Fungal cultures were prepared using Potato dextrose agar medium at 28°C for 3–7 days. Colonies were clearly visible, and their fine morphological characteristics were confirmed using a light microscope.

2.4 Sampling and Testing

Soil samples are taken monthly to determine the effectiveness of each treatment method. A specific sample about 10 g weight of soil is collected which taken and dried at 40°C , ground, and sieved. Then, 20 ml of DTPA (diethylene triamine penta-acetic acid) solution, prepared according to standard protocols, were added. This solution includes 0.005

M of DTPA and 0.1 M Triethanolamine (TEA) and 0.01 M CaCl_2 was added, ensuring the pH was controlled between 7.2–7.7 (Lindsay and Norvell, 1978).

The sample was exposed to a shaking incubator for 2 hours at 180 rpm. The solution was then filtered through filter paper, and the resulting samples were examined using an AA-7000 Shimadzu Atomic Absorption spectrophotometer, by Pb hollow cathode lamp – single element lamp at 283.3 nm, and Air- C_2H_2 flame atomization was used.

2.5 Statistical Analysis

Results were expressed as significant differences between means estimated using two-way analysis of variance (ANOVA) to ensure comparable differences between treatments vs control over time. It was determined that the probability p value ≤ 0.01 highly statistically significant. Statistical analyses were performed using the statistical package IBM SPSS Statistics version 25 (Armonk, NY: IBM Corp) and Graph-Pad Prism version 8 (San Diego, CA, USA) (Morgan *et al.*, 2019).

3. Results and Discussion

The results of the experiment, which included the application of three treatment methods to reduce the bioavailability of lead in the soil, showed a clear and gradual decrease in lead concentration after the application of the treatments. The variation between these treatments indicates that the synergistic treatment method achieved the highest reduction rate after a treatment period of 60 days. The Table 1 and Figure 2 shows the lead concentrations achieved in each sample measured throughout the treatment period.

Table 1: Treatment of lead concentration in soil.

Treatment Time (days)	T1 Fungal Treatment (mg/kg) Mean $\pm$ SE	T2 Phosphoric Gypsum (mg/kg) Mean $\pm$ SE	T3 Synergistic Treatment (mg/kg) Mean $\pm$ SE
0	300 $\pm$ 2.89	300 $\pm$ 2.31	300 $\pm$ 3.46
15	276 $\pm$ 2.31	270 $\pm$ 2.89	259 $\pm$ 2.89
30	254 $\pm$ 2.31	248 $\pm$ 2.89	200 $\pm$ 2.89
45	220 $\pm$ 2.89	195 $\pm$ 4.04	162 $\pm$ 4.04
60	180 $\pm$ 2.89	150 $\pm$ 2.89	105 $\pm$ 2.89
p-value	<0.001**		

** highly statistically significant at $p \leq 0.01$.

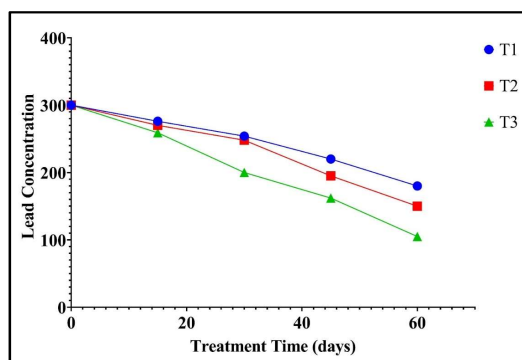


Figure 2: A diagram representing the activities of the three treatments: T1: innate (fungal), T2: chemical (phosphoric gypsum), and T3: synergistic.

The results indicate that among the treatments the synergistic treatment achieved the most considerable drop in lead concentration to 65%, while the fungal treatment and the treatment with phosphogypsum achieved lead concentrations of 40% and 50%, respectively. One of the possible reasons for the decreasing concentration of lead during fungal treatment is due to the activities of the fungus *A. niger*. Removal of lead can happen as a result of biosorption which is the result of the cell walls containing chitin and polysaccharides. There is also bioprecipitation and secretion of some organic acids (Gul *et al.*, 2024).

The results align with those of Refaey *et al.* (2021), who noted that fungi could be used to remediate heavy metal contamination from soils and water. Rigoletto *et al.* (2020) further noted that soils fungi can absorb and decrease the bioavailability of lead. The chemical treatment using phosphogypsum that led to the greatest decrease in lead concentration shows the great importance of phosphogypsum in the retention of lead and thus the reduction of lead in soils. This is because the phosphates and sulfates present in phosphogypsum create some of the insoluble lead containing compounds and thus decrease the solubility and translocation of lead (Endovitsky *et al.*, 2017). These results are in agreement with those of Shao *et al.* (2020) who also stated that lead combined with a source of phosphorus in lead poisoned soils precipitates as a stable mineral, pyromorphite. Also, Basta and McGowen (2004) stated that phosphorus amendments are among the most effective materials for decreasing the bioavailability of lead in soil. The focus is on the results achieved through a synergistic treatment technique with the combination of fungi and phosphogypsum, where the concentration of lead was diminished to 105 mg/kg after 60 days. The success of this treatment is ascribed to the synergistic effect of the biological processes of the fungi and the chemical processes of phosphogypsum. Phosphoric gypsum, chemically stabilizes lead and diminishes its solubility, and the

fungi, on the other hand, assist in the biological retention of lead and, therefore, its re-releasing into the soil solution is prevented (Meng *et al.*, 2024). According to Khan *et al.* (2015), one of the most effective techniques in the treatment of soils polluted with heavy metals is the combination of biological and chemical processes. Also, Wu *et al.* (2018) showed that, in contrast to the use of each method separately, synergistic treatment lowers long-term ecological impacts. One of the most important objectives of this treatment is to reduce the bioavailability of lead and thus limit its uptake by plants; reduce the risk of lead transfer through the food chain; improve soil quality and reduce toxic effects on microorganisms; and promote the sustainable use of soil in agricultural and environmental systems. The results of this study confirm that synergistic treatment represents a promising and environmentally friendly option for managing lead-contaminated soils, especially in sensitive agricultural environments.

Conclusion

This study exemplifies that the combined use of fungal bioremediation employing *A. niger* and phosphogypsum amendment is sustainable and effective in reducing the bioavailability of lead in contaminated soils. While both phosphogypsum and fungal treatments aided in the immobilization of lead, the combined application of both was able to yield greater remediation results. The effectiveness of combined systems performed is attributed to the dual-action of biological sequestration of the fungus and organic acid secretion, in conjunction with the chemical fixation and mineral precipitation of the phosphogypsum.

The integrated treatment of soils also restored the microbial functions of the soils. This treatment enhances the soil's functionality and the biological processes of the soil by improving the activity of the microbes. This shows the potential of the integrated lead immobilization to be used in the agricultural and ecological rehabilitation. Also, incorporating phosphogypsum in lead immobilization helps in recycling the industrial waste, thus increasing the sustainable remediation of the integrated method.

The research offers a promising, cost-effective, and environmentally safe remediation method for lead-contaminated soils, especially in areas of high agricultural and industrial pollution. Future research should be directed toward assessing long-term field-scale applications, plant uptake, and immobilized lead under a range of environmental conditions to complete the assessment of the method's practicability.

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