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Biological and organic remediation of heavy metal-contaminated soil and its effects on soil biological activity and L-glutaminase enzyme activity

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Abstract

Objective

Soil pollution is one of the most serious problems facing the scientists and agriculturists, which in addition to cause health problem for human and livestock, it reduces the yield quality and quantity of plants. One of the important reasons for reducing plant yield is the harmful effect of pollutants (e.g. heavy metals) on biological activities of soil microorganisms, responsible for C and nutrients cycle in soil. Therefore, the aim of this study was to evaluate the combination of biological (fungal inoculation) and organic amendments. Because with this evaluation, their effectiveness in reducing heavy metal toxicity and restoring soil biological functions can be compared.

Materials and methods

An incubation experiment was conducted to examine the role of bioremediation (*Aspergillus niger*) or organic amendments (Humic acid and cow manure) to reduce the negative effect of some heavy metals (Cd, pb, and Zn) on biological indices (total number of *A. niger*, CO_2 evolution and L-glutaminase activity) of silty loam soil collected from Basrah province, south of IRAQ. *Aspergillus niger* inoculant added to soil at population density of 50×10^3cfu; humic acid and cow manure added at rates of 20L ha^{-1} and 4% respectively.

Results

Results showed that Cd, Pb and Zn inhibited all the biological parameters and the highest effect were recorded for Cd with a decreasing percentage of 30.67, 13.93 and 45.34% for total number of *A. niger*, CO_2 evolution and L- glutaminase activity, respectively. However, the addition of *A. niger*, humic acid, or cow manure significantly reduced the available heavy metal concentrations from soil and then reduced their negative effect on biological indices. Using of the *A. niger* inoculant was the more effective overcoming among other strategies with 89.02% reduction in

DTPA-extractable heavy metals (%) compared with 63.48 and 48.71% for HA and cow manure, respectively. Consequently, the *A. niger* inoculant increased the number of total *A. niger*, CO_2 evolution and L- glutaminase activity in polluted soil by 164.71, 35.31 and 89.32%, respectively.

Conclusion

A. niger inoculation was the most effective strategy for reducing heavy metal bioavailability and restoring soil biological activity under controlled incubation conditions. However, Further research is needed to understand how heavy metals and soil amendments affect plant growth under real field conditions.

Keywords: bioremediation, fungi, heavy metals, humic acid, L-glutaminase

Paper Type: Research Paper.

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Introduction

Soil pollution by heavy metals such as cadmium (Cd), lead (Pb) and zinc (Zn) is a serious global environmental problem (Vodyanitskii, 2016). Because these heavy metals cause their persistence, toxicity and accumulation in agricultural ecosystems (Kumar et al., 2014; Odika et al., 2020). These metals also disrupt the physicochemical properties of the soil. Therefore, they can pose significant risks to plant productivity, food safety and human health (Gui et al., 2023; Sparks, 2005). The most important consequences of heavy metal pollution are that they inhibit the biological activity of the soil (Rajendran et al., 2022; Al-Jaberi, 2024). Because they can prevent the activity of microorganisms responsible for nutrient cycling and enzymatic processes essential for soil fertility. In Basra province (southern Iraq), intensive oil industry activities have been carried out for a long time and continue to be carried out. Being a port city, intense port activities are ongoing. Also, agricultural inputs are used a lot in this province. In addition, there have been long-term military conflicts in this province. All this has caused the level of heavy metals in the soils to increase. Although there are many environmental concerns, unfortunately, limited research has been conducted to explore sustainable strategies to reduce heavy metal toxicity and restore soil biological functions in this region. Among biological remediation strategies, fungal-based approaches have attracted increasing attention. This is because they have high tolerance to heavy metals and are able to immobilize or aggregate metal ions. The fungus *Aspergillus niger (A. niger)* was chosen in this study because its documented capacity for biosorption and bioaccumulation of heavy metals through cell wall functional groups such as chitin, glucans, and proteins has been proven and reported (Odeniyi and Turaki, 2022; Al-Malaky, 2024). In addition, this fungus is a producer of organic acids. This acid enhances the complexation of metals and reduces their bioavailability in soil. On the other hand, organic amendments were chosen because of their complementary mechanisms (Al- Hadrawi and Al-Jaberi, 2023). Humic acid (HA) and cow manure were the two options chosen in this study. Humic acid contains several active functional groups (carboxyl, phenolic, and carbonyl groups). These functional groups can chelate and immobilize metal ions, thereby reducing their bioavailability (Singh et al., 2018). Cow manure primarily acts as a nutrient source. Because it provides carbon and essential elements that stimulate microbial growth and enzymatic activity. It can also help immobilize metals. Because it can adsorb and complex with these metals (Al-Jaberi, 2023; Sharma et al., 2021; Gopinath et al., 2020). The effects of heavy metals on soil microbial activity and the potential of fungi or organic amendments have been investigated in various studies. However, there is limited information on the combined assessment of biological indicators such as fungal population, soil respiration and L-glutaminase activity under simultaneous contamination with cadmium, lead and zinc. Furthermore, very few studies have been conducted on soils of Basra province, which are exposed to unique pollution sources such as oil industry emissions and war residues. Therefore, for the first time, we evaluate the comparative efficacy of a locally isolated *A. niger* strain, humic acid and cow manure in reducing DTPA-extractable heavy metals and also the relationship of metal detoxification with changes in key soil biological indicators, especially L-glutaminase activity, which has rarely been evaluated under polymetal stress conditions. Therefore, the aim of this study was to evaluate the combination of biological (fungal inoculation) and organic amendments. Because with this evaluation, their effectiveness in reducing heavy metal toxicity and restoring soil biological functions can be compared.

Materials and methods

Soil sampling: A random composite sample (0-30 cm depth) was collected from agricultural soil in Al-Qurna District, located in the northern part of Basra province. The soil was placed in sterile polyethylene bags. Part of the sample was air-dried, ground, and sieved through a 2 mm mesh to analyze its physical, chemical, and biological properties. The remaining portion was stored at 4°C to preserve it for biological experiments.

Table 1. Physicochemical properties and baseline heavy metal concentrations of the experimental soil (0–30 cm depth, Al-Qurna District, Basra, Iraq)

Property	Value	Method
Texture	Silty clay	Hydrometer method
Sand (%)	7.8	
Silt (%)	42.7	
Clay (%)	49.5	
pH (1:2.5 soil:water)	7.7	Glass electrode
EC (dS m^{-1})	2.6	EC meter
Organic matter (%)	7.6	Walkley–Black
$CaCO_3$ (%)	34.8	Calcimeter
CEC (cmolc kg^{-1})	12.3	Ammonium acetate
Field capacity (%)	29.2	Gravimetric method
Bulk density (g cm^{-3})	1.23	Core method
DTPA-extractable Cd (mg kg^{-1})	0.27	AAS
DTPA-extractable Pb (mg kg^{-1})	1.38	AAS
DTPA-extractable Zn (mg kg^{-1})	0.81	AAS

Experimental treatments: Calculated amounts of $CdSO_4$, $PbSO_4$ and $ZnSO_4$ were used to artificially contaminate the soil. Each of these was dissolved separately in distilled water to prepare aqueous solutions. The appropriate volume of each solution was gradually added to 500 g of air-dried soil to reach the desired concentration. This concentration was 3 mg/kg for cadmium and 100 mg/kg for lead and zinc (Kloke, 1980). To ensure uniform and homogeneous distribution of the metals in the soil, the treated soils were thoroughly mixed. These soils were prepared to cover 70% of the field capacity. Each metal was used separately in separate treatments. To establish equilibrium, the soils were incubated at 28°C for two weeks after spiking. This allowed for equilibrium to be established before the application of biological and organic amendments. Potato Dextrose Agar (PDA) was used for cultivation. *A. niger* was grown on the medium for 5 days at 25°C. The plates were then filled with sterile distilled water containing 0.01% Tween-80 and the surface was gently scraped to remove the spores. Filtration of the spore suspension was performed (Klich, 2002). Using serial dilution and plate counting, its concentration was adjusted to approximately 5×10^4 CFU/mL. Ten mL of this suspension was added to every 100 g of soil. This was done to obtain a final density of 50×10^4 CFU/mL of soil. To eliminate the indigenous microorganisms of the used cow manure, we sterilized it (121°C, 1 time, 20 min). This was done to ensure that any observed changes in microbial activity were attributable to the experimental treatments rather than to the microbial population introduced with the manure. Then it added to another soil samples at level of 4% based on soil dry weight. The remain soil samples were treated with 20 L ha^{-1} humic acid (HA) extracted from above cow manure by using 0.1 *N* NaOH at pH+=6.5 (Page, 1982). A control treatment (soil without fungi, cow manure or HA) was subjected. Each treatment was repeated three times. Based on the soil depth of 0 to 30 cm and the bulk density of 1.3 mg/m3, the field application rate of 20 liters/ha was converted to the laboratory scale. This was approximately equivalent to 3,900,000 kg of soil/ha. The equivalent amount was then calculated for 500 g of soil. It was then diluted proportionally in distilled water. This was done to ensure uniform distribution. The application rates of humic acid (20 l/ha) and cow manure (4% w/w) were based on previous studies. These studies have shown that organic amendments such as humic acid can reduce the bioavailable fraction of heavy metals in contaminated soils and increase soil biological activity (Lwin et al., 2018; Zhao et al., 2023; Tang et al., 2025; Zhang et al., 2025). They have also shown that cow manure and its derivatives, when applied at comparable rates, affect the distribution of heavy metals in the soil and can improve microbial communities. Therefore, these proposed rates were considered sufficient to produce measurable changes in soil microbial activity and heavy metal availability under controlled incubation conditions. Soil samples were adjusted to equivalent moisture of 70% field capacity and kept constant throughout adding distilled and sterilized water regularly, then incubated at 25±2°C for 30 days. After pre-incubation, a set of soil samples were taken after 10, 20 and 30 days to assay the following parameters. The number of total fungi was determined by dilution and plate count method using PDA media at 25±2°C incubation for 5 days (Page, 1982). The soil respiration was determined by measuring CO_2 evaluated in closed system by trapping in 1*N* NaOH solution and then titrated the remain NaOH in standard HCl using phenolphthalein indicator (Ciardi and Nannipieri,1990). The total CO_2 was calculated by formula:

meq CO2 = meq (NaOH) - meq (HCl)

mg CO2 = meq CO2 × eq. wt. of CO_2

L- glutaminase enzyme activity was assayed according to Frankenberger and Tabatabai (1991). The reduction in DTPA-extractable heavy metals (%) was determined using the following formula:

Reduction of DTPA-extractable heavy metals (%) = [(Initial DTPA-extractable Concentration - Final DTPA-extractable Concentration) / Initial DTPA-extractable Concentration] × 100

The DTPA-TEA-$CaCl_2$ extraction method based on the method presented by Lindsay and Norwell (1978) was used to measure the fractions of cadmium, lead, and zinc present in the soil. This is because the goal was to estimate the bioavailable forms of metals in the soil, not the total metal content. In other words, our goal was to reduce the concentration of DTPA-extractable metals (biodegradable), not to remove the total metal from the soil.

Statistical analysis: The experiment was conducted using a Completely Randomized Design (CRD). $SPSS_{11.0}$ statistics analysis software was used for analysis of variance (ANOVA). The least significant difference (RLSD) method was used to compare means at a significant level of 5%.

Results and discussion

Fungal count (cfu g $^{-1}$ soil): The application of heavy metals resulted in a noticeable reduction in fungal populations compared to the control, with decreases of 30.66%, 28.30%, and 19.82% recorded for Cd, Pb, and Zn, respectively (Figure 1A). Cadmium caused the greatest inhibition, resulting in the lowest fungal count (120.90 CFU g^{-1} soil), whereas zinc showed the least inhibitory effect (139.82 CFU g^{-1} soil). Because of its chemical nature, Cd is toxic just like Zn and can displace it in living organisms. In addition, Cd can create very stable bonds with biomolecules by attaching to proteins and peptides (Ahemad, 2012). The relatively higher fungal count in soils treated with Zn compared to those treated with Cd and Pb may be due to “zinc’s essential role at low levels", playing a key role in the synthesis of enzymatic proteins. However, at elevated levels, Zn becomes toxic, forming complex structures with DNA and RNA, which can interfere with vital cellular processes (Kozlowski et al., 2009). Lead (Pb), on the other hand, is known to inhibit catalytic activity and antibiotic functions, suppress protein synthesis in microorganisms, and disrupt both the wall of cell integrity and oxidative phosphorylation (Bruins et al., 2000; Sharma et al*.,* 2021).

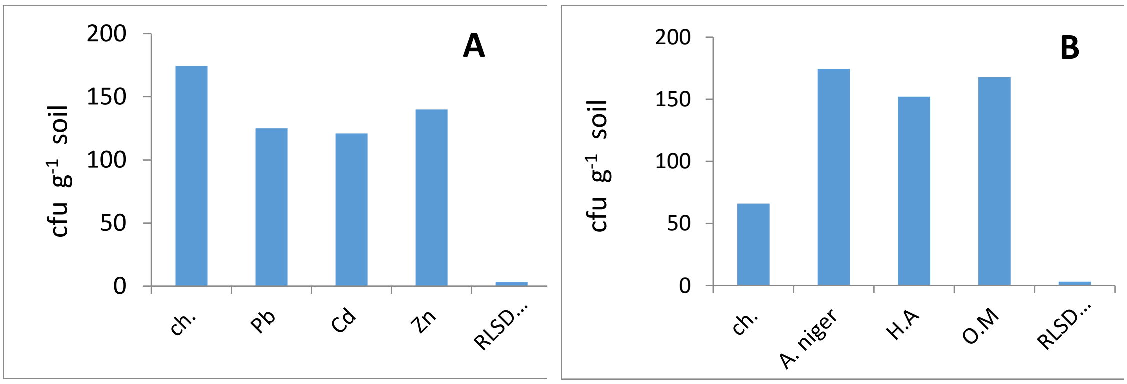

Figure 1. Effect of heavy elements, fungi and organic matter on total fungal count (cfu g $^{-1}$ × 10^3)

The effect of *A. niger*, cow manure and HA on the total number of fungi in contaminated soil is shown in fig. 1 B. The number of fungi was higher for *A. niger* treatment with an increase percent of 164.72% compared to control as well as an increase percent of 14.8 and 4.03% compared with HA and cow manure, respectively. This may be attributed to the role of fungal inoculation to improve growth and number of fungi in soil along with the presence of fungi in soil at the beginning. Furthermore, fungus used is a filamentous fungus which can absorb heavy metals from media. Its ability to bind heavy elements due to the complex structure of cell wall, it is made up of chitin, various inorganic ions, nitrogen-rich polysaccharides, glucans, lipids, and proteins which absorb the heavy metals after increasing fungus tolerance and detoxify. It was also observed that the application of organic treatments led to a significant increase in fungal populations compared to the control. The number of fungi increased by 130.58% with humic acid (HA) and by 154.48% with cow manure. This increase is attributed to the fact that organic matter serves as a vital source of energy and nutrients for microorganisms. It supplies key elements like carbon (C), nitrogen (N), phosphorus (P), and other nutrients that are essential for fungal growth and the biosynthesis of fungal tissues biosynthesis (Sibagariang et al., 2022), in addition to improving physical and chemical properties of soil resulting in enhance the fungal growth and increase its numbers (Yami and Smariti, 2005). Figure 2 showed a significant interaction effect on the total fungal number. Data revealed that soil contaminated with heavy metals have the lower number of fungi in treated and untreated with biological or organic sources. The treatment involved inoculation with fungi and not contaminated with heavy elements gave the highest fungal count (223.3 cfu g $^{-1}$ soil), while treatment involved contaminated with Cd without treatment gave the lowest values of total fungal count (51.33 cfu g^{-1} soil), that because heavy metals inhibit fungi and spores' growth that reduce its count in soil (Ano et al., 2012).

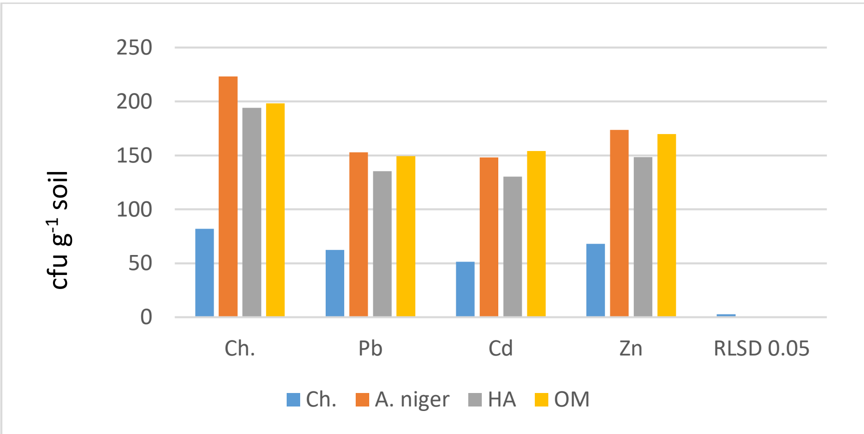

Figure 2. Interaction between treatment materials and contamination with heavy elements on the total fungal number (cfu g $^{-1}$ soil × 10 $^{-3}$)

Total CO_2 released (µg CO_2 g^{-1} soil): Figure 3A illustrates a significant reduction in the total amount of CO_2 released from soils contaminated with heavy elements compared to the control. The higher decrease was observed in the cadmium (Cd) treatment, showing a 13.92% inhibition relative to the control. Cd is recognized as one of the most toxic metals to soil microorganisms, as it negatively impacts their metabolic activity, leading to reduced respiration and slower decomposition of organic matter. This strong inhibitory effect is likely due to Cd's high mobility in the soil and its weak binding affinity to soil colloids (Zheng et al., 2019). In contrast, soils contaminated with zinc (Zn) showed the least inhibition, with CO_2 release reduced by only 7.6% compared to the control, suggesting a milder impact of Zn on microbial respiration.

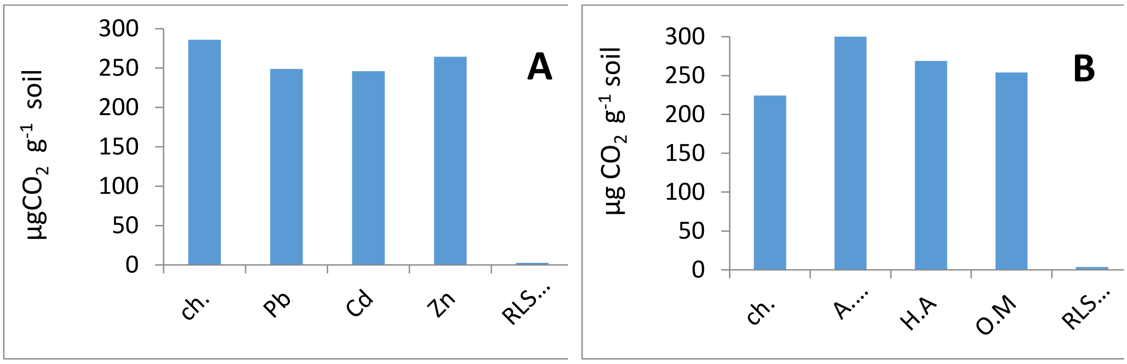

Figure 3. The effect of heavy metals and biological and organic treatment on the total amount of CO_2 evolved (µg CO_2 g^{-1} soil)

It is renowned from Figure 3B that treating soil with *A. niger*, HA or cow manure caused a significant increase in the amount of CO_2 evolved from contaminated with heavy metals with percent 35.31, 19.72, and 13.14 %, respectively. Fungal inoculation showed the highest amount of CO_2 evolved (224.373 µg CO_2 g^{-1} soil) due to role of fungal inoculation, to increase the total numbers of fungi (Figure 2), subsequently led to an increase in their activities and increase total amount of CO_2 evolved. Furthermore, *A. niger* is able to adapt to environment contaminated with heavy metals (Kumar et al., 2014), by absorbing heavy metal ions in a wide pH range (Wasay et al., 1998). Fig.3B illustrated adding HA also increasing microbial activity in contaminated soils since HA contains many active groups such as carbonyl, carboxyl, and amide (Nardi et al., 2021), which to adsorbed ions of heavy elements and reducing their negative effect on microbial activities. This was true for cow manure, but with less effect because it needs more time for mineralization and decomposition. The role of organic materials and its active groups represented in adsorption / chelation of heavy metals and remove their negative effect from soil organisms (Sibagariang et al., 2022), HA and cow waste are also considered among the most important sources of carbon, energy, and nutrients which microorganisms need for growth and activity. The increase in CO_2 release after *A. niger* inoculation is mostly due to the increase in microbial respiration. This is also attributed to the higher fungal biomass and enzymatic degradation of organic substrates. In addition, fungal cell walls can biologically absorb and intracellularly sequester heavy metals. Therefore, it may reduce metal toxicity. It can therefore restore microbial metabolic activity. Humic acid can chelate bioactive metal ions. By doing so, it increases the release of CO_2. Therefore, it can reduce toxicity and improve microbial respiration. In addition, functional groups such as carboxyl and phenolic moieties can stimulate microbial activity. Therefore, it can improve the physicochemical conditions of the soil. Cow manure primarily provides organic carbon and degradable nutrients. Therefore, it can stimulate heterotrophic microbial respiration, thereby helping to increase CO_2 emissions.

Effect of organic amendments on CO_2 emission: In contrast to heavy metal stress, the application of organic amendments like humic acid (HA) and cow manure led to a noticeable increase in CO_2 emissions. This indicates increased microbial activity along with a better-organic matter decomposition. The organic materials not only provide the necessary nutrients and carbon sources but also promote the microbes' respiration process by acting as energy sources. Besides this, the organic materials contribute to the soil's physical properties, such as its moisture holding capacity and air circulation, thus making it more suitable for the microbes to grow. The results obtained strongly support the idea that organic amendments can effectively counteract the negative influence of heavy metals and at the same time support the growth of healthier soil microbial communities. Under the controlled incubation conditions of the present study, the increase in CO_2 emission due to the use of organic amendments is due to the fact that the addition of organic carbon and nutrients increases microbial respiration. Cow manure provides degradable carbon substrates. Therefore, it can stimulate heterotrophic microbial activity. It is worth noting that organic amendments can improve soil structure under field conditions. However, in short-term laboratory incubations using sieved soil, the improvement in soil structure is probably very minor and minimal. Therefore, it can be said that the dominant mechanism of increased CO_2 emission is probably nutrient supply rather than structural improvement.

Effect of organic amendments on fungal populations: The use of organic treatments had a very positive effect on the fungal populations when compared to the untreated control. The number of fungi changed to 130.58% more with humic acid and 154.48% more with cow manure. This increase is due to the fact that organic matter is rich in carbon, nitrogen, phosphorus, and other essential elements for fungi's cell division and growth (Sibagariang et al., 2022). In addition, the introduction of organic materials changes the soil's physical and chemical conditions, thereby increasing the fungi's ability to thrive and reproduce more quickly (Yami and Smariti, 2005). The analysis of the binary interaction presented in Figure 4 indicated the highest CO_2 emission of 305.73 $\mu g\ CO_2\ g^{-1}$ soil from the soil treatment contaminated with zinc (Zn) and inoculated with A. niger. This result is in line with the study of Tahir et al. (2017), who mentioned that *A. niger* can remove heavy metals by both the absorption and adsorption of their ions. The detoxification of the metals helps to counteract the poisonous effect of the heavy metals on the soil microorganisms' growth and activity. On the other hand, the lowest CO_2 emission was found in the lead (Pb)-contaminated soil that was not treated, which amounted to 200.16 µg CO_2 g^{-1} soil. The presence of metals like Pb is known to decrease the number of fungi and bacteria through inhibiting spores' germination and making the latter grow more slowly, causing a drop in microbial respiration and consequently, lower CO_2 release.

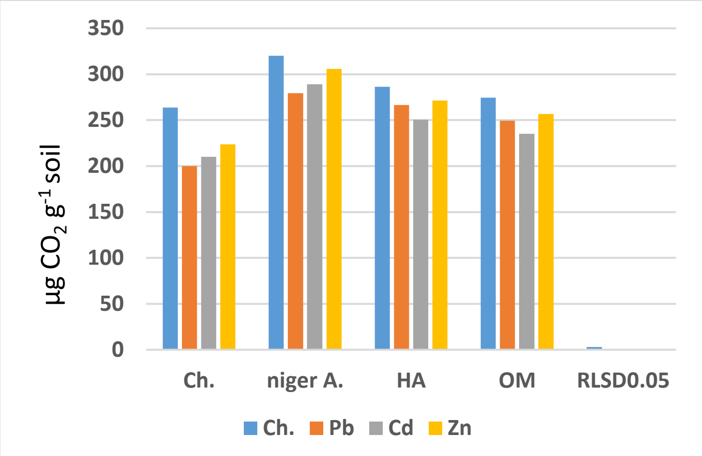

Figure 4. interaction Effect between heavy metal contamination in the total amount of CO_2 evolved (µg CO_2 g^{-1} soil)

Reduction in DTPA-extractable heavy metals (%): The different treatments and their respective reduction in DTPA-extractable heavy metals (%) are depicted in Figure 5A. *A. niger* inoculation resulted in the highest reduction in DTPA-extractable heavy metals rate of 89.04%, thus it was a 40.20% and 82.77% increase over HA and cow manure treatments, respectively. Fungi have always been regarded as the best bioremediation agents, due to their mycelium having great capacity to hold a great number of different elements (Sanyaolu, 2018). Moreover, the fungi form pores likened to those that bind metal ions through chelation and produce melanin and other pigments that help with the active ion transport outside the cell, thus formation of complexes by associating heavy metals with the intracellular components takes place and detoxification is facilitated (Singh et al., 2018). The highest reduction in DTPA-extractable heavy metals of 99.34% could be seen in the soil previously contaminated with zinc (Zn) and inoculated with *A. niger* as per Figure 5B. The high efficiency is primarily due to the combination of several microbial strategies that they can use to survive and even prosper in heavy metal contaminated environments. These include absorption, adsorption, ion exchange, complex formation, trapping metals in extracellular capsules, precipitation, and active transport across cell membranes (El-Hameed et al., 2015). After inoculation with A. niger, a decrease in DTPA-extractable metals occurred. This is probably due to the effect of biosorption mechanisms. The fungal cell wall contains functional groups such as carboxyl, hydroxyl, amino and phosphate moieties. Therefore, it can bind divalent metal ions. It does this through electrostatic adsorption and ion exchange. In addition, *A. niger* can produce organic acids (such as oxalic and citric acids). These acids can complex or precipitate metal ions, especially lead. In doing so, they can reduce their bioavailability. Although intracellular accumulation can contribute to metal detoxification, the main mechanisms under soil incubation conditions are: surface adsorption and extracellular complexation.

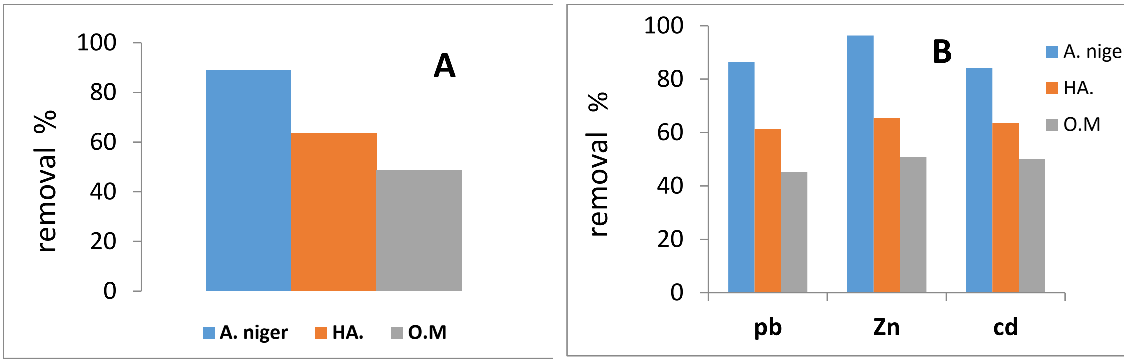

Figure 5. The bioremediation materials effect on the percentage of reduction in DTPA-extractable heavy metals (%)

L-Glutaminase enzyme activity (mg NH_4^+-N Kg^{-1} 2hr): L-glutaminase activity significantly decreased in soils contaminated with heavy metals compared to the control, with reductions of 45.34%, 31.92%, and 39.60% observed for Cd, Pb, and Zn, respectively (Figure 6A). The lowest enzyme activity was recorded in Cd-contaminated soil (41.24 mg NH_4^+-N Kg^{-1} 2hr), while the highest was associated with Zn contamination. These results correlate with the fungal population data presented in Figure 1A. Meng et al. (2018) reported a negative effect of heavy elements on soil enzyme. The negative effect of heavy elements on enzyme including catalytic groups destroying, changing the nature of Previous studies have shown that heavy metals negatively affect soil enzyme activities by damaging catalytic groups, denaturing enzymatic proteins, and interfering with substrate binding during enzyme-substrate complex formation (Meng et al., 2018; Bottomley et al., 2020). Krajewska (2009) explained that heavy metals inhibit enzymes by binding to sulfhydryl (-SH) groups at their active sites, altering the enzyme's structure and reducing functionality. Among the metals tested, Cd had the strongest inhibitory effect, followed by Zn and then Pb. Additionally, Pb disrupts nucleic acid structure and function, impairs cell wall integrity, then inhibits oxidative phosphorylation (Bruins et al., 2000; Alloway, 2013).

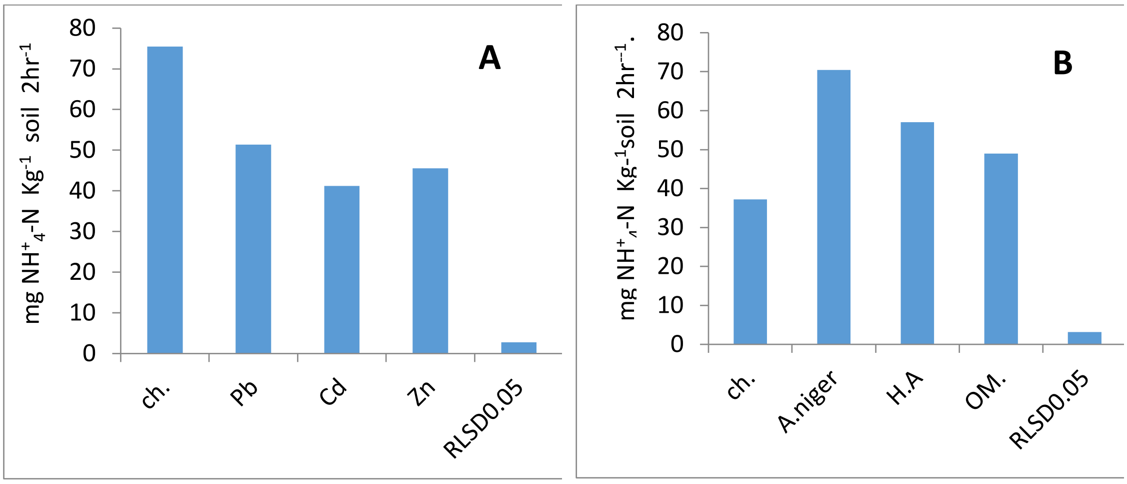

Figure 6. The effect of biological and organic treatment on the activity of L-Glutaminase enzyme in contaminated soil with heavy metals

The addition of *A. niger*, humic acid (HA), or cow manure to lead-contaminated soils led to an increase in the activity of the soil L-glutaminase enzyme, thus exerting a positive effect on the environment. Enzyme activity increased by 89.32% with *A. niger*, 53.30% with HA, and 31.68% with cow manure, compared to the control. The *A. niger* treatment resulted in the highest enzyme activity among the treatments, highlighting the important role fungi play in removing heavy metals by accumulating them within their cells (Utobo and Tewari, 2015). Furthermore, *A. niger* is known as one of the most prolific enzyme-producing in agricultural environments (El-Said et al., 2016). This, however, does not diminish the contribution of organic amendments, which also enhance enzyme activity in metal-contaminated soils. These organic matters are rich in active ionized aggregates that adsorb heavy elements, thereby protecting the enzyme's active sites and reducing inhibition of enzymatic activity. Figure 7 showed that the binary interaction of bioremediation with all heavy metals gave the highest values of enzyme. This may be due to the fact that fungi are a main source of most enzymes (Tabatabaie, 1994). The highest enzyme activity (66.62 mg $NH^+{}_4$-N Kg^{-1} $2hr.^{-1}$) was obtained in treatment involved Zn and inoculated with *A. niger*. This is supported by the results of figures 3, 5 and 6B which indicated that increasing in the total fungal count associated with high value of CO_2 evolved, and the largest reduction in DTPA-extractable heavy metals (%). The high reduction in DTPA-extractable heavy metals (%) observed indicate that the bioavailability of metals has decreased. This is due to immobilization, adsorption or complexation by fungal biomass and organic amendments and is not related to the complete reduction in DTPA-extractable all heavy metals from the soil. In this study, each heavy metal (cadmium, lead, and zinc) was studied separately to assess the separate effects of each metal on soil biological parameters. In other words, we did not have a treatment that combined all three metals so that all effects could be examined together in one treatment. Therefore, the investigation of interactions between metals (e.g., synergistic, additive, or antagonistic interactions) was not performed to report possible interactions. It is important to note that the decision to analyze metals separately was intentional to isolate the specific response of soil biological activity to each metal.

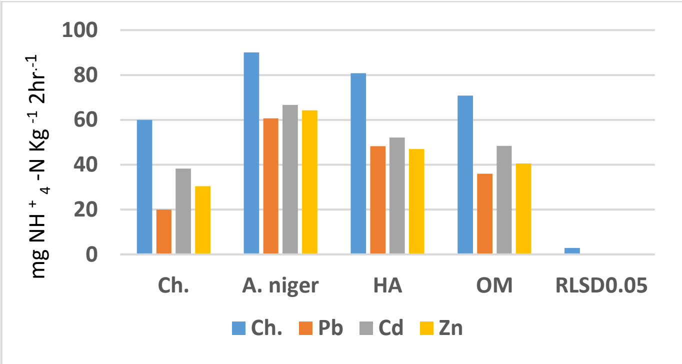

Figure 7. Binary effect of bioremediation and heavy metals on the activity of L-Glutaminase enzyme (mg NH_4^+- N Kg^{-1} 2hr.$^{-1}$)

It should be acknowledged, however, that under real field contamination conditions, mixed metal interactions may occur. Therefore, further studies are recommended to investigate such combined effects. As mentioned, the metals tested have inhibitory effects on fungal populations. This could be due to their distinct biochemical interactions with fungal cells. Cadmium (Cd) is a non-essential and highly toxic metal. It binds to the sulfhydryl (-SH) groups of proteins and enzymes, thereby exerting its toxicity. This binding inactivates enzymes and induces oxidative stress. By disrupting membrane integrity, this metal can impair nutrient uptake. This allows it to inhibit spore germination and mycelial growth. Lead (Pb) toxicity is mainly due to its ability to displace essential cations such as Ca^{2}+ and Mg^{2}+, disrupt cell wall stability, and disrupt ATP synthesis and nucleic acid function. It can also alter membrane permeability, inhibit key metabolic pathways, and reduce microbial growth. Unlike cadmium and lead, zinc acts as an essential cofactor for many enzymatic functions at normal concentrations. However, at high concentrations it competes with other divalent cations and disrupts protein structure. Therefore, it is toxic at high concentrations. The fact that we observed a relatively lower inhibition under zinc contamination compared to cadmium could be due to the fact that fungal cells may have mechanisms of relative tolerance to zinc. This is also likely due to its physiological role at low concentrations.

Conclusion: It can be concluded that the fungal count, CO2 evolution and the L- glutaminase enzyme activity in soil were negatively affected by contamination of soil with Cd, Pb or Zn. However, treating soil with *A. niger* (bioremediation), humic acid or cow manure (organic amendments) can recover the negative effect of heavy metals and significantly increase the mention biological indices. *A. niger* inoculation was the most effective strategy for reducing heavy metal bioavailability and restoring soil biological activity under controlled incubation conditions. However, Further research is needed to understand how heavy metals and soil amendments affect plant growth under real field conditions.

Author contributions

M. A. L. is fully responsible for all activities related to this paper.

Data availability statement

Data supporting the findings of this study will be available from the author upon reasonable request.

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Ethical considerations

This research does not involve human or animal subjects, thus it does not require formal ethical approval.

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Conflict of interest

The author declares that there is no conflict of interest related to this research.

References

Abd El Hameed, A. H., Eweda, W. E., Abou-Taleb, K. A., & Mira, H. I. (2015). Biosorption of uranium and heavy metals using some local fungi isolated from phosphatic fertilizers. *Annals of Agricultural Sciences*, *60*(2), 345-351. https://doi.org/10.1016/j.aoas.2015.10.003

Ahemad, M. (2012). Implications of bacterial resistance against heavy metals in bioremediation: A review. *Journal of Institute of Integrative Omics and Applied Biotechnology*, *3*(3). https://api.semanticscholar.org/CorpusID:13232689

Al-Hadrawi, W. B., & Al-Jaberi, M. M. (2023). Effect of the substrate and inoculation of phosphate-dissolving bacterial and fungal with humic acids in the activity of the alkaline phosphatase enzyme in cadmium contaminated soil. *International Journal of Agriculture and Animal Production*, *4*(1), 10-23. https://doi.org/10.55529/ijaap.41.10.23

Al-Jaberi, M. M. (2023). Effect of paper mill sludge (furfural) on soil respiration, urease activity and ammonia volatilization of calcareous soils. *Journal of Wildlife and Biodiversity*, *7*(Special Issue), 217-229. https://doi.org/10.5281/zenodo.10212150

Al-Jaberi, M. M. (2024). Effect of bioremediation or organic treatments of soil contaminated with some heavy metals on the biological and L-glutaminase activity. *Journal of Global Innovations in Agricultural and Social Sciences*, *12*(1), 243-250. https://doi.org/10.22194/JGIAS/12.1143

Alloway, B. J. (2013). Sources of heavy metals and metalloids in soils. In B. J. Alloway (Ed.), *Heavy metals in soils* (pp. 11-50). Springer. https://doi.org/10.1007/978-94-007-4470-7_2

Al-Malaky, I. A. Y., Abdulkareem, M. A., & Al-Jaberi, M. M. (2024). Effect of humic acids extracted from different organic sources and inoculation with *Bacillus subtilis* or *Aspergillus niger* on urease activity in calcareous soil. *Edelweiss Applied Science and Technology*, *8*(3), 326-335. https://doi.org/10.55214/25768484.v8i3.1658

Ano, A. O., Ubochi, C. I., & Nwokocha, C. C. (2012). Effect of heavy metals (Cd, Ni and Pb) on soil productivity: Organic carbon mineralization. *International Journal of Physical Sciences*, *7*(4), 573-577. https://doi.org/10.5897/IJPS11.044

Bottomley, P. J., Angle, J. S., & Weaver, R. W. (Eds.). (2020). *Methods of soil analysis, Part 2: Microbiological and biochemical properties* (Vol. 12). John Wiley & Sons. https://www.wiley.com/en-us/Methods+of+Soil+Analysis%2C+Part+2%3A+Microbiological+and+Biochemical+Properties-p-9780891188650

Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety*, *45*(3), 198-207. https://doi.org/10.1006/eesa.1999.1860

Ciardi, C., & Nannipieri, P. (1990). A comparison of methods for measuring ATP in soil. *Soil Biology & Biochemistry*, *22*(5), 725-727. https://doi.org/10.1016/0038-0717(90)90022-R

El-Said, A. H., Shebany, Y. M., Hussein, M. A., & El-Dawy, E. G. (2016). Antimicrobial and L-asparaginase activities of endophytic fungi isolated from *Datura innoxia* and *Hyoscyamus muticus* medicinal plants. *European Journal of Biological Research*, *6*(3), 135-144. https://doi.org/10.5281/zenodo.56056

Frankenberger, W. T., Jr., & Tabatabai, M. A. (1991). L-glutaminase activity of soils. *Soil Biology and Biochemistry*, *23*(9), 869-874. https://doi.org/10.1016/0038-0717(91)90099-6

Gopinath, A., Krishna, K., & Karthik, C. (2020). Adsorptive removal and recovery of heavy metal ions from aqueous solution/effluents using conventional and non-conventional materials. In M. Oves, M. Ansari, M. Zain Khan, & M. Shahadat (Eds.), *Modern age waste water problems* (pp. 265-293). Springer. https://doi.org/10.1007/978-3-030-08283-3_15

Gui, H., Yang, Q., Lu, X., Wang, H., Gu, Q., & Martín, J. D. (2023). Spatial distribution, contamination characteristics and ecological-health risk assessment of toxic heavy metals in soils near a smelting area. *Environmental Research*, *222*, Article 115328. https://doi.org/10.1016/j.envres.2023.115328

Klich, M. A. (2002). *Identification of common Aspergillus species*. Centraalbureau voor Schimmelcultures.

Kloke, A. (1980). Guideline data for tolerable total levels of some elements in cultivated soil. *Mitteilungen der VDLUFA*, *1*, 9-11.

Koller, M., & Saleh, H. M. (2018). Introductory chapter: Introducing heavy metals. In *Heavy metals* (pp. 3-11). IntechOpen. https://doi.org/10.5772/intechopen.74783

Kozlowski, H., Janicka-Klos, A., Brasun, J., Gaggelli, E., Valensin, D., & Valensin, G. (2009). Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation). *Coordination Chemistry Reviews*, *253*(21-22), 2665-2685. https://doi.org/10.1016/j.ccr.2009.05.011

Krajewska, B. (2009). Ureases I. Functional, catalytic and kinetic properties: A review. *Journal of Molecular Catalysis B: Enzymatic*, *59*(1-3), 9-21. https://doi.org/10.1016/j.molcatb.2009.01.003

Kumar, R., Singh, P., Dhir, B., Sharma, A. K., & Mehta, D. (2014). Potential of some fungal and bacterial species in bioremediation of heavy metals. *Journal of Nuclear Physics, Material Sciences, Radiation and Applications*, *1*(2), 213-223. https://europub.co.uk/articles/-A-593454

Lindsay, W. L., & Norvell, W. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, *42*(3), 421-428. https://doi.org/10.2136/sssaj1978.03615995004200030009x

Lwin, C. S., Seo, B. H., Kim, H. U., Owens, G., & Kim, K. R. (2018). Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality—A critical review. *Soil Science and Plant Nutrition*, *64*(2), 156-167. https://doi.org/10.1080/00380768.2018.1440938

Meng, X., Ai, Y., Li, R., & Zhang, W. (2018). Effects of heavy metal pollution on enzyme activities in railway cut slope soils. *Environmental Monitoring and Assessment*, *190*(12), Article 712. https://doi.org/10.1007/s10661-018-6567-9

Nardi, S., Schiavon, M., & Francioso, O. (2021). Chemical structure and biological activity of humic substances define their role as plant growth promoters. *Molecules*, *26*(8), Article 2256. https://doi.org/10.3390/molecules26082256

Odeniyi, O. A., & Turaki, L. (2022). Soil-borne alkaline phosphatase-producing *Bacillus* and *Penicillium* species as growth promoters of the *Corchorus olitorius* and *Amaranthus hybridus* plants. *Novel Research in Microbiology Journal*, *6*(3), 1557-1582. https://researcherslinks.com/current-issues/Soil-borne-alkaline-phosphatase-producing/44/5/10708

Odika, P., Anike, O., Onwuemesi, A., Odika, N. F., & Ejeckam, R. B. (2020). Assessment of environmental geochemistry of lead-zinc mining at Ishiagu area, Lower Benue Trough, Southeastern Nigeria. *Earth Science Research*, *9*(1), 31-43. https://doi.org/10.5539/esr.v9n1p31

Page, A. L. (Ed.). (1982). *Methods of soil analysis: Part 2. Chemical and microbiological properties* (2nd ed., Agronomy Monograph No. 9). American Society of Agronomy; Soil Science Society of America. https://doi.org/10.2134/agronmonogr9.2.2ed

Rajendran, S., Priya, T. A. K., Khoo, K. S., Hoang, T. K. A., Ng, H.-S., Munawaroh, H. S. H., Karaman, C., Orooji, Y., & Show, P. L. (2022). A critical review on various remediation approaches for heavy metal contaminants removal from contaminated soils. *Chemosphere*, *287*(Part 4), Article 132369. https://doi.org/10.1016/j.chemosphere.2021.132369

Sanyaolu, A. A. A. (2018). Verification of *Aspergillus niger* as a myco-remediation agent of lambda-cyhalothrin and associated heavy metals in *Lactuca sativa* (L.) leaf. *Journal of Applied Sciences and Environmental Management*, *22*(5), 621-624. https://doi.org/10.4314/jasem.v22i5.2

Sharma, N., Sodhi, K. K., Kumar, M., & Singh, D. K. (2021). Heavy metal pollution: Insights into chromium eco-toxicity and recent advancement in its remediation. *Environmental Nanotechnology, Monitoring & Management*, *15*, Article 100388. https://doi.org/10.1016/j.enmm.2020.100388

Sibagariang, N. A., Bertham, Y. H., Widiyono, H., Anandyawati, & Utami, K. (2022). The effect of humic acid and micro compound fertilizer on soil microorganism population and upland rice yield in coastal land. *TERRA: Journal of Land Restoration*, *5*(2), 58-64. https://ejournal.unib.ac.id/terrajournal/article/view/23405

Singh, P. C., Srivastava, S., Shukla, D., Bist, V., Tripathi, P., Anand, V., & Srivastava, S. (2018). Mycoremediation mechanisms for heavy metal resistance/tolerance in plants. In R. Prasad (Ed.), *Mycoremediation and environmental sustainability* (pp. 233-255). Springer. https://doi.org/10.1007/978-3-319-77386-5_14

Sparks, D. L. (2005). Toxic metals in the environment: The role of surfaces. *Elements*, *1*(4), 193-197. https://doi.org/10.2113/gselements.1.4.193

Tabatabai, M. A. (1994). Soil enzymes. In R. L. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai, & A. Wollum (Eds.), *Methods of soil analysis: Part 2. Microbiological and biochemical properties* (pp. 775-833). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.2.c37

Tahir, A., Lateef, Z., Abdel-Megeed, A., Sholkamy, E. N., & Mostafa, A. A. (2017). In vitro compatibility of fungi for the biosorption of zinc (II) and copper (II) from electroplating effluent. *Current Science*, 839-844. https://api.semanticscholar.org/CorpusID:53476883

Tang, T., Zeng, P., Wang, J.-Y., Gu, J.-F., Liu, C.-F., Mu, Y.-S., Liu, Y.-F., Zhou, H., & Han, Z.-Y. (2025). Remediation of multi-metal contaminated soil using cow dung and its biochar: Effects on heavy metal uptake and soil microbiome in *Triticum aestivum* L. *Environmental Technology & Innovation*, *39*, Article 104243. https://doi.org/10.1016/j.eti.2025.104243

Utobo, E. B., & Tewari, L. (2015). Soil enzymes as bioindicators of soil ecosystem status. *Applied Ecology and Environmental Research*, *13*(1), 147-169. https://api.semanticscholar.org/CorpusID:52885121

Vodyanitskii, Y. N. (2016). Standards for the contents of heavy metals in soils of some states. *Annals of Agrarian Science*, *14*(3), 257-263. https://doi.org/10.1016/j.aasci.2016.08.011

Wasay, S. A., Barrington, S. F., & Tokunaga, S. (1998). Using *Aspergillus niger* to bioremediate soils contaminated by heavy metals. *Bioremediation Journal*, *2*(3-4), 183-190. https://doi.org/10.1088/10889869809380376

Yami, K. D., & Shakya, S. (2005). Effect of *Rhizobium leguminosarum* biovar phaseoli inoculation alone and in combination with organic fertilizers on bean (*Phaseolus vulgaris* L.). *Nepal Journal of Science and Technology*, *6*(1), 1-8. https://api.semanticscholar.org/CorpusID:55756637

Zhang, X., Yu, Q., Gao, B., Hu, M., Chen, H., Liang, Y., & Yi, H. (2025). Organic amendments enhance the remediation potential of economically important crops in weakly alkaline heavy metal-contaminated bauxite residues. *Agriculture*, *15*(1), Article 15. https://doi.org/10.3390/agriculture15010015

Zhao, P., Wang, A., Wang, P., Huang, Z., Fu, Z., & Huang, Z. (2023). Two recyclable and complementary adsorbents of coal-based and bio-based humic acids: High efficient adsorption and immobilization remediation for Pb(II) contaminated water and soil. *Chemosphere*, *318*, Article 137963. https://doi.org/10.1016/j.chemosphere.2023.137963

Zheng, L., Li, Y., Wenqin, D., Xianglin, D., Quan, T., & Hua, C. (2019). The inhibitory effect of cadmium and/or mercury on soil enzyme activity, basal respiration, and microbial community structure in coal mine-affected agricultural soil. *Annals of Microbiology*, *69*(8), 849-859. https://doi.org/10.1007/s13213-019-01478-3

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زیست‌پالایی و اصلاح آلی خاک آلوده به فلزات سنگین و تأثیر آن بر فعالیت زیستی خاک و فعالیت آنزیم ال-گلوتامیناز

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چکیده

هدف: آلودگی خاک یکی از جدی‌ترین مشکلات پیش روی دانشمندان و کشاورزان است که علاوه بر ایجاد مشکلات بهداشتی برای انسان و دام، موجب کاهش کمّی و کیفی عملکرد گیاهان می‌شود. یکی از دلایل مهم کاهش عملکرد گیاهان، اثرات زیان‌بار آلاینده‌ها (مانند فلزات سنگین) بر فعالیت‌های زیستی میکروارگانیسم‌های خاک است که مسئول چرخه کربن و عناصر غذایی در خاک می‌باشند. بنابراین، هدف این مطالعه ارزیابی ترکیب زیست‌پالایی (تلقیح قارچی) و اصلاح‌کننده‌های آلی و مقایسه کارایی آن‌ها در کاهش سمیت فلزات سنگین و بازگرداندن عملکردهای زیستی خاک بود.

مواد و روش‌ها: یک آزمایش انکوباسیون به‌منظور بررسی نقش زیست‌پالایی با استفاده از قارچ *Aspergillus niger* و همچنین اصلاح‌کننده‌های آلی (اسید هیومیک و کود دامی گاوی) در کاهش اثرات منفی برخی فلزات سنگین (کادمیم Cd، سرب Pb و روی Zn) بر شاخص‌های زیستی شامل تعداد کل *A. niger*، میزان آزادسازی CO_2 و فعالیت آنزیم ال-گلوتامیناز در خاک سیلتی-لوم جمع‌آوری‌شده از استان بصره (جنوب عراق) انجام شد. تلقیح *A. niger* با تراکم جمعیتی 50×10^3 واحد تشکیل‌دهنده کلنی (cfu)به خاک افزوده شد. اسید هیومیک و کود گاوی نیز به‌ترتیب با مقادیر ۲۰ لیتر در هکتار و ۴ درصد به خاک اضافه گردیدند.

نتایج: نتایج نشان داد که Cd، Pb و Zn تمامی شاخص‌های زیستی را مهار کردند و بیشترین اثر مربوط به کادمیم بود که باعث کاهش ۳۰/۶۷، ۱۳/۹۳ و ۴۵/۳۴ درصدی به‌ترتیب در تعداد کل *A. niger*، آزادسازی CO_2 و فعالیت آنزیم ال-گلوتامیناز شد. با این حال، افزودن *A. niger*، اسید هیومیک یا کود گاوی به‌طور معنی‌داری غلظت فلزات سنگین قابل‌دسترس در خاک را کاهش داده و در نتیجه اثرات منفی آن‌ها بر شاخص‌های زیستی را کم کرد. استفاده از تلقیح *A. niger* مؤثرترین راهکار در مقایسه با سایر تیمارها بود و موجب کاهش ۸۹/۰۲ درصدی فلزات سنگین قابل استخراج با DTPA شد، در حالی که این میزان برای اسید هیومیک و کود گاوی به‌ترتیب ۶۳/۴۸ و ۴۸/۷۱ درصد بود. در نتیجه، تلقیح *A. niger* موجب افزایش ۱۶۴/۷۱، ۳۵/۳۱ و ۸۹/۳۲ درصدی به‌ترتیب در تعداد کل *A. niger*، آزادسازی CO_2 و فعالیت آنزیم ال-گلوتامیناز در خاک آلوده گردید.

نتیجه‌گیری: تلقیح *A. niger* مؤثرترین راهبرد برای کاهش زیست‌دسترسی فلزات سنگین و احیای فعالیت زیستی خاک در شرایط کنترل‌شده انکوباسیون بود. با این حال، انجام تحقیقات بیشتر برای درک تأثیر فلزات سنگین و اصلاح‌کننده‌های خاک بر رشد گیاهان در شرایط واقعی مزرعه ضروری است.

کلمات کلیدی: اسید هیومیک، ال-گلوتامیناز، زیست‌پالایی، فلزات سنگین، قارچ‌ها

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