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BIOTRANSFORMATION OF INSOLUBLE LEAD CARBONATE TO SOLUBLE LEAD OXALATE BY FUNGUS ISOLATED FROM POLLUTED SOIL

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ABSTRACT
Mining activities produce wastes which may contain heavy metals as contaminants. These residues are generally deposited on the ground and often occupy large extensions. The mining operations are the cause of heavy metal contaminated sites and the heavy metal contamination directly affects human health. The objective of this research was to investigate the ability of fungus, *Gliocladium roseum* to solubilize and immobilize insoluble lead carbonate ($PbCO_3$). Soil fungi, were isolated from contaminated soil. Isolated fungi were plated on PDA medium supplemented with 0.5% (w/v) of insoluble lead compound. Isolated fungus, *Gliocladium roseum* only showed the activity in solubilizing insoluble lead compound with clear zone appearance 46.5 mm in diameter. Precipitation of lead biomineral crystals was observed in agar medium underneath colonies of *Gliocladium roseum*. The crystals were purified and analyzed by scanning electron microscope (SEM) and X-ray powder diffraction (XRPD), and the results revealed that it was lead oxalate (PbC_2O_4). It is suggested that soil fungus have potential application in heavy metal bioremediation.

KEYWORDS: Biotransformation, Heavy metal, Lead compound, Fungus, *Gliocladium roseum.*

INTRODUCTION
Microbial communities are of primary importance in bioremediation of metal contaminated soil and water because microbes alter metal chemistry and mobility through reduction, accumulation, mobilization and immobilization. There is current interest in the use of microorganisms for the removal of nitrogen, phosphorus, and metals from commercial and municipal waste. Several species of microorganisms are capable of accumulating metal ions up to concentrations several orders of magnitude higher than the background concentration. The contamination of heavy metal represents an important environmental problem because of toxic effects to the organisms and their accumulation throughout the food chain that can lead to human health and ecological problems.[1] Lead is an important heavy metal and is a major concern because of it wide usage in many industries and it is globally distributed on a large scale as a metal and its compounds. It is known as to be a toxic metal in ecological systems and its occurrence in nature is a widespread environmental problem resulting in negative effects on human health when exposure to an amount that cannot be processed by the organism. Damage may cause adverse reactions in different organs and biological functions, including neurobehavioral problems, nausea, bone pain and vomiting.[2] Heavy metal compounds contamination in the environment generally induces physiological changes in the microbial communities resulting in the development of heavy metal resistant microorganisms. [3] Naturally, soil fungi play an importance role in the leaching of mineral rocks and are involved in transformation of insoluble metal compounds. Fungi are responsible for secretion of many metabolites such as chelating and sequestering agent (e.g. citric acid, siderophores), precipitating agent (e.g. oxalic acid), and pigments with metal binding ability (e.g. melanins).[4] Oxalate crystallization, immobilizes heavy metals and may limit bioavailability.[5] Metal oxalate complexes and crystal formation is a process of environmental significance in connection with fungal survival, biodeterioration, pathogenesis, soil weathering, and metal detoxification.[6-8] These fungi can be used in the clean up of heavy metal from the contaminated site with low cost application in bioremediation and recovery of metals.[9] The objective of this research was to study the ability of fungus to transform insoluble lead compound into the non-toxic form.

MATERIALS AND METHODS
Fungal isolation and identification
Polluted soil sample was collected in and around NLC, Neyveli, Tamil Nadu. Soil sample was stored in sterile polythene bags. The soil dilution plate method was used for fungal isolation. The isolated fungi were maintained

on potato dextrose agar (PDA) at 25 ºC.[10] The selected fungi were identified according to their macro and microscopic structures. The taxa were assigned to genera following Von Arx[11] and Barnett and Hunter.[12]

Preparation of heavy metal and culture condition

Commercial $PbCO_3$ was used and the final concentration of the heavy metal of 0.5% (w/v) was applied in PDA.[13] Fungal inoculations were carried out with 7 mm. diameter discs of mycelium cut from the leading edge of an actively growing colony which were then placed on the heavy metal amended plates. The plates were incubated at 25ºC for 7 days.[13]

Investigation of solubilization ability and pH measurement

The magnitude of solubilizing ability was assessed by the diameter of solubilizing clear zones in agar medium.[14] The degree of solubilizing clear zones was measured every day until the end of incubation period (7 days).

Evaluation of culture medium acidification

Selected strains were cultured in 250 ml Erlenmeyer flasks containing 100 ml potato dextrose broth (PDB, pH 7). An appropriate amount of lead compound was added to the liquid media to give the desired final concentration (**Figure.1**). The pH value was measured after seven days and measurements were taken in triplicate using pH electrode.[15]

Figure.1: Liquid broth containing fungus and lead compounds before purification

Analysis of mycogenic crystals

The mycogenic crystals formed under the fungal colony were purified from the agar medium according to the procedure described by Sayer and Gadd.[16] The mycogenic crystals were examined using a scanning electron microscope (using VEGA3 TESCAN machine, Japan). Crystals were identified by X-ray powder diffraction.

RESULTS AND DISCUSSION

Investigation of solubilizing ability

Nine fungal isolates were screened from polluted soil, and among these isolates, four isolates showing different morphotaxa were selected for further study. Four fungal isolates were tested for the solubilization of lead compounds (**Table 1**). *Gliocladium roseum* showed the largest halo zone diameter for lead carbonate (46.5±1.0 mm). Final pH of lead carbonate was 3.24. The pH of fungal growth media decreased after 7 days growth of *Gliocladium roseum* which indicated that they can acidify the lead compound-containing medium during growth. The acidification had a strong effect on solubilization. Fungal organic acid secretion during growth decreases the pH of the system and increases heavy metal solubility.[17]

Table 1.Solubilizing clear zone diameters produced by fungi grown on insoluble lead compounds

Isolate	Fungal strains	Insoluble $PbCO_3$ 0.5% (w/v)
1	*Fusarium spp.*	No clear zone
2	*Trichoderma spp.*	No clear zone
3	*P. chrysogenum*	No clear zone
4	*Gliocladium roseum*	46.5±1.0 mm

Analysis of mycogenic crystals

Following incubation period, lead biomineral crystals were found in the agar medium after growing *Gliocladium roseum* on lead carbonate. The crystals were purified and examined under scanning electron microscopy (SEM). The morphologies of lead oxalate crystals produced by *Gliocladium roseum* are shown in **Figure 2**.

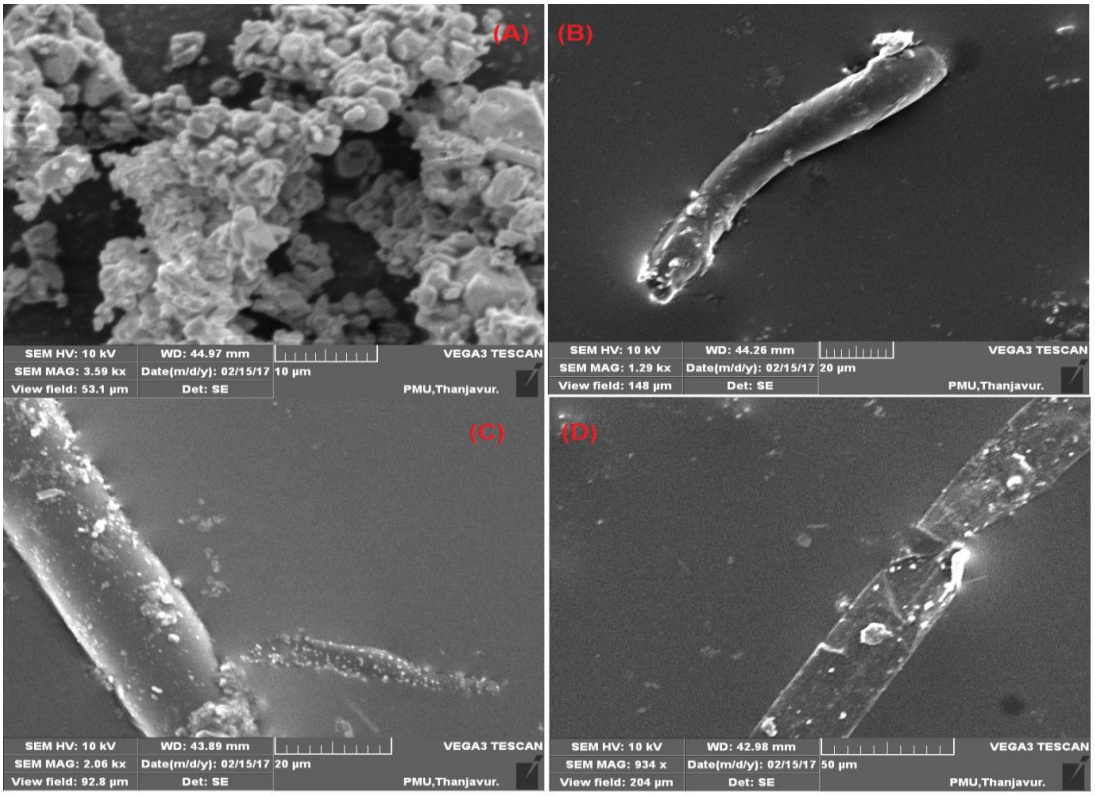

Figure: 2. Scanning electron micrographs of lead biomineral crystals produced by *Gliocladium roseum*. (A) Biotransformed Lead biomineral crystals, scale bar = 10 µm. (B)-(D) Lead biomineral crystals associated with the mycelium of *Gliocladium roseum,* scale bar 20 µm – 50 µm.

When fungi are grown under high levels of heavy metal contamination, they have to reduce heavy metal ion transport across plasma membranes, such as sorption of metal ions in the mycelium, reduced metal mobility as a result of hydrophobicity of the mycelium, secretion of fungal chelating agent e.g. oxalate and extracellular metal sequestration by exopolysacchrides and other extracellular metabolites.[18] Oxalate production was responsible for metal transformation in this case. These results are in agreement with results obtained for *Aspergillus niger*, which could also transform pyromorphite ($Pb_5(PO_4)_3Cl$) into lead oxalate dehydrate.[19] The formation of oxalates containing potentially toxic metals may provide a mechanism whereby oxalate-producing fungi can tolerate metal-rich environments.[16] The mycogenic crystals were observed not only as the purified crystals but also in the fungal mycelium (Figure 2, B-D). Scanning electron microscopy showed the lead biomineral crystals formed by *Gliocladium roseum* were associated with fungal mycelia. Fungal cell walls are complex three-dimensional structures of organic macromolecules, predominantly glucans, chitins and chitosans but also containing proteins, lipids and other polysaccharides.[20] This variety of structural components contains many different functional groups each with their own charge distribution and therefore able to bind metals ions to a greater or a lesser extent.[21] Loci on fungal cell walls can act as precipitation nuclei, with precipitation of metals occurring in and around cell wall components. It is possible that solubilization and immobilization are key fungal processes with potential for metal recovery and reclamation from contaminated soil, solid wastes, and low grade ores.[5,7]

The Identification of biomineral crystals, XRPD patterns of crystals extracted from lead carbonate are shown in **Figure 3**. Powder diffraction file revealed that lead biomineral crystals extracted from lead carbonate was lead oxalate (PbC_2O_4). The XRD pattern was in good agreement with previous study by Sayer *et. al*., (1999).[21]

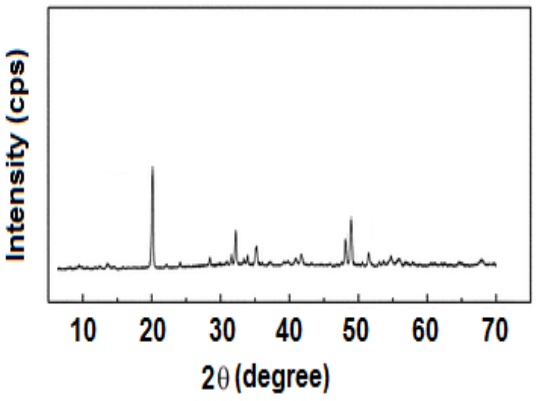

Figure: 3. XRD pattern of bio transformed Lead oxalate crystals by *Gliocladium roseum*

CONCLUSION

It is concluded that fungus with high potential to transform insoluble lead compound to soluble lead oxlate. Moreover, organic acid production has a strong effect on solubilization and can immobilize through metal oxalate complex formation. These mechanisms of lead solubilisation, or its immobilisation as lead oxalate, have significant implications for metal mobility and transfer to other environmental compartments and organisms. The importance of considering microbial processes when developing remediation techniques for toxic metals in soils is therefore emphasised.

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