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BIOTRANSFORMATION OF INSOLUBLE LEAD COMPOUND INTO THE NON-TOXIC FORM BY SOIL FUNGUS

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ABSTRACT
Nowadays, due to industrialization and extraction of natural resources, soil and water pollution is one of the major global concerns. During the recent era of environmental protection, the use of microorganisms for the recovery of heavy metals from soil, sediments and water as well as employment of plants for landfill applications has generated growing attention. The role of microorganisms and plants in biotransformation of heavy metals into nontoxic forms is well-documented in this work. The objective of this research was to investigate the ability of fungi to solubilise and immobilize insoluble lead carbonate ($PbCO_3$). Soil fungi were isolated from contaminated soil in Mathur Industrial Estate, Trichy, Tamil nadu. The isolated fungus was plated on PDA medium supplemented with 0.5% (w/v) of insoluble lead carbonate. *Aspergillus niger* showed the highest activity in solubilising insoluble lead carbonate with clear zone appearance more than 40 mm. in diameters. Precipitation of lead biomineral crystals was observed in agar medium underneath colonies of *A. niger*. The crystals were purified, and analyzed by scanning electron microscope (SEM), mass spectrum and X-ray powder diffraction (XRPD), and the results revealed that they were lead oxalate (PbC_2O_4). Biotransformation by microbes could be efficient method to reduce the lead contaminants of soil. It is suggested that soil fungus have potential application in heavy metal biotransformation.

KEYWORDS: Biotransformation, soil fungus, lead oxalate, XRD.

INTRODUCTION
The increase global population, industrialization and urbanization are the some major reasons to contaminate the environment. The release of heavy metals from industries causing the serious health problem to human and other animals and also pollutes the environments due to their persistence and bio-accumulative nature. In this regards, biotransformation process provides and effective innovative measures for treatment of a wide variety of contaminants.[1] Lead is a ubiquitously distributed in nature in biologically inactive form. The use of lead in batteries, bearing metals, cable covering, gasoline additives, explosives, manufacture of pesticides, antifouling paints and analytical reagents are major source of contamination.[2] Lead may cause the toxic effects on gastrointestinal, muscular, reproductive, neurological systems. It may enter into our body through ingestion of food or through the blood stream. Klassen and Watkins (2003) reported that lead impairs communication between cells and modification of neuronal circuitry.[3] In lead-contaminated soils, biota and vegetation influence the transformations of lead together with environmental characteristics such as soil pH, organic matter content, texture, redox-potential and presence of other elements.[4] However, sometime it is also possible that lead forms a complex with dissolved organic matter and migrate from the surface soil layer to mineral soil and possibly contaminated the underground water. A number of micro-organisms inhabiting soil and water can transform inorganic and organic lead compounds into volatile forms, which diminishes their toxic effect.[5] Microorganisms can interact with metals and radionuclides via many mechanisms, some of which may be used as the basis for potential bioremediation strategies.[6] Mechanisms by which microorganisms act on heavy metals includes biosorption (metal sorption to cell surface by physico chemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilizatio n through the formation of insoluble sulfides or polymeric complexes) intracellular accumulation and enzyme catalyzed transformation.[7] 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).[8] Oxalate crystallization, immobilizes heavy metals and may limit

bioavailability.[9] Metal oxalate complexes and crystal formation is a process of environmental significance in connection with fungal survival, biodeterioration, pathogenesis, soil weathering, and metal detoxification.[10] 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.[11] 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 from Mathur Industrial Estate, Trichy, TN, India. 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.[12] The selected fungus was identified according to their macro and microscopic structures.

Preparation of heavy metal and culture condition

Commercial $PbCO_3$ was used and the final concentration of the heavy metals of 0.5% (w/v) was applied in PDA.[13] Fungal inoculations were carried out with 7 mm. diameter discs which were then placed on the heavy metal amended plates. The plates were incubated at 25 ºC for 7 days. Selected strain was cultured in 250 ml Erlenmeyer flask containing 100 ml potato dextrose broth (PDB, pH 7). An appropriate amount of lead carbonate was added to the liquid media to give the desired final concentration. The pH value was measured after seven days.[8]

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.[14] The mycogenic crystals were examined using a scanning electron microscope (using VEGA3 TESCAN machine, Czech Republic). Crystals were identified by Mass spectrum (JEOL GCMATE II GC-MS) and X-ray powder diffraction (RigaKu Smart lab).

RESULTS AND DISCUSSION

The selected fungus was identified as *Aspergillus niger*, according to their macro and microscopic structures. The pH of fungal growth media decreased (from pH 7.0 to pH 3.12) after 7 days growth of *A. niger* 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.[15] Fungi in taxonomic groups such as *Aspergillus sp.*, *Penicillium sp.* are common in contaminated soil. They can also produce citric and oxalic acid which were directly involved in the metal transformation. Citric acid usually results in the release of metal ion while oxalic acid results in immobilization.[16]

Analysis of mycogenic crystals

The mycogenic crystals formed under the fungal colony were purified from the agar medium and examined under scanning electron microscopy (SEM). The morphologies of lead oxalate crystals produced by *A. niger* are shown in Fig.1. According to SEM images, the crystal surface seen to be uneven, which is indicates that the crystal is not perfectly separated from the agar. SEM images showed platy morphology of lead oxalate crystals (Fig.1c & Fig. 1d). The mycogenic crystals were observed not only as the purified crystals but also in the fungal mycelium. Scanning electron microscopy showed the lead biomineral crystals formed by *A. niger* were associated with fungal mycelia (Fig.2a-Fig.2d). Subsequent examination of the samples from *A. niger* by X-ray diffraction also revealed that lead biomineral crystals extracted from lead carbonate was lead oxalate (PbC_2O_4). XRD pattern of crystals extracted from lead carbonate are shown in Fig.3. These results are in agreement with results obtained for *A. niger*, which could also transform pyromorphite into lead oxalate.[17] XRD pattern of extracted crystals further confirmed by previous study reported by Khunur et al., (2011).[18] Mass spectrum was used to confirm the identity of the compound produced by the fungi. The mass spectrum (Fig. 4) that displayed a molecular ion (M^+) peak at *m/z* 295 indicates the molecular weight of the compound corresponds to the molecular weight of lead oxalate and also corresponds to molecular formula PbC_2O_4.

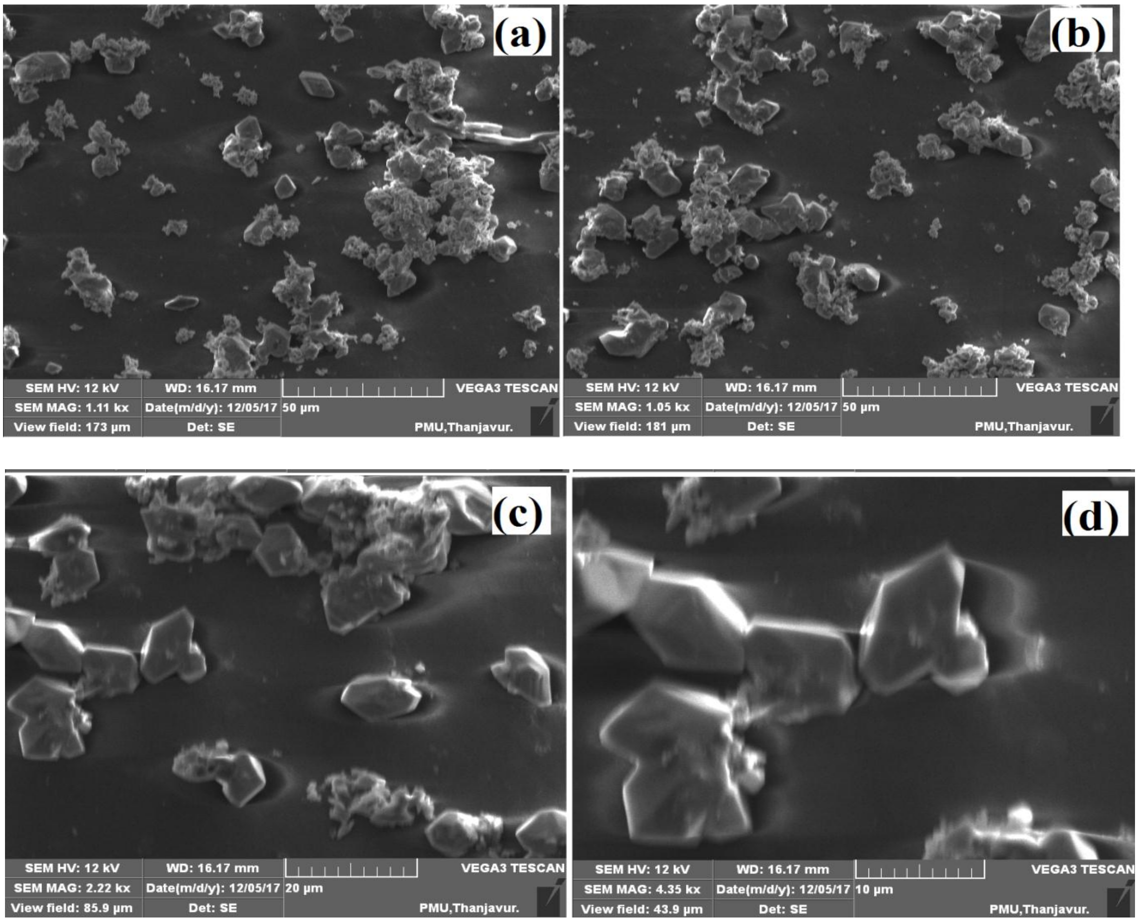

Fig. 1: Different maginifications of Scanning electron micrographs of lead oxalate crystals produced by *Aspergillus niger.*

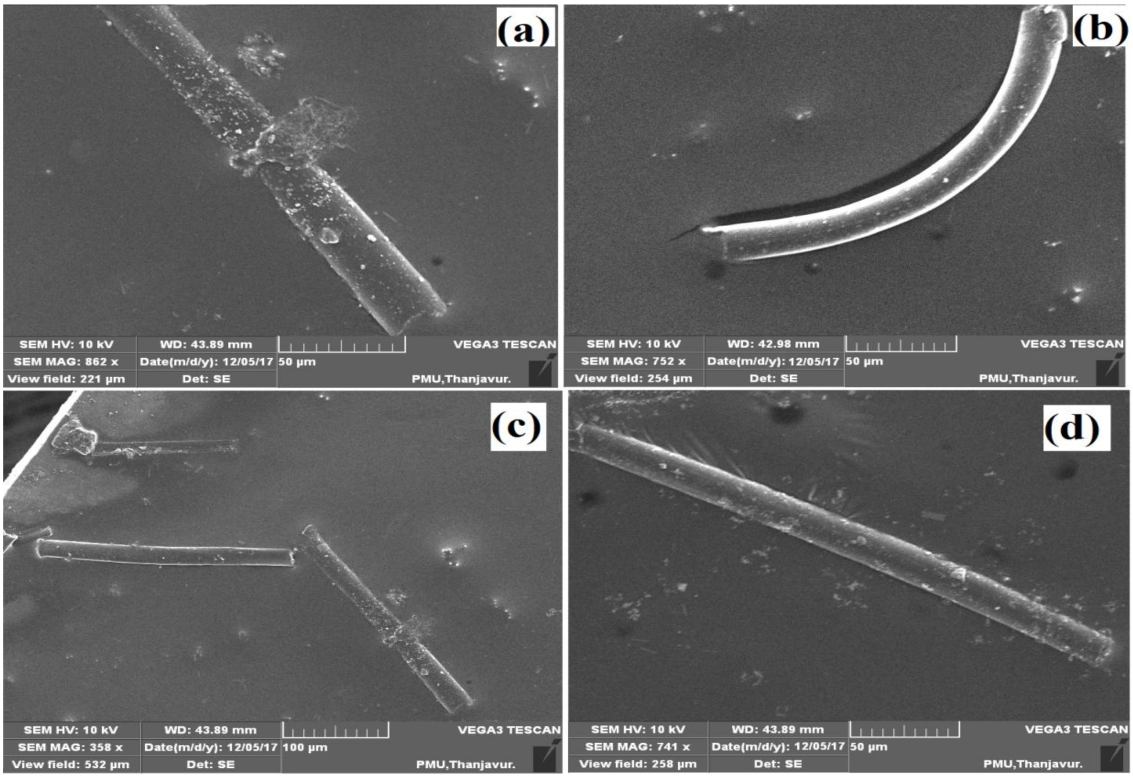

Fig. 2: Different magnifications of SEM images of lead oxalate crystals associated with the mycelium of *Aspergillus niger*

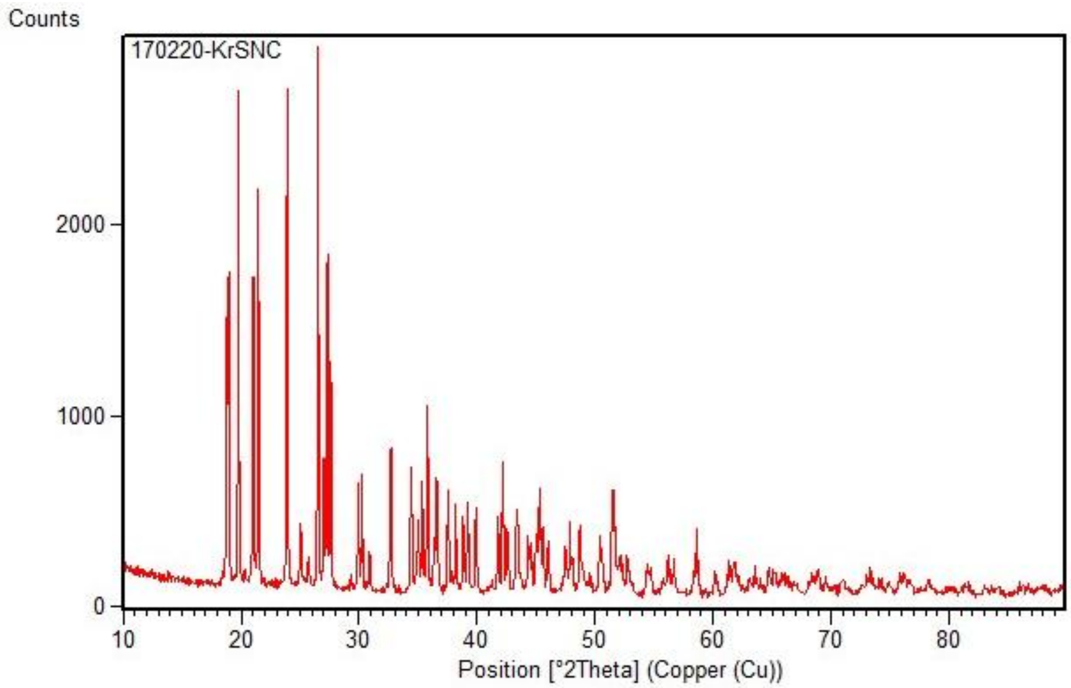

Fig. 3: XRD pattern of lead oxalate crystals transformed by *Aspergillus niger.*

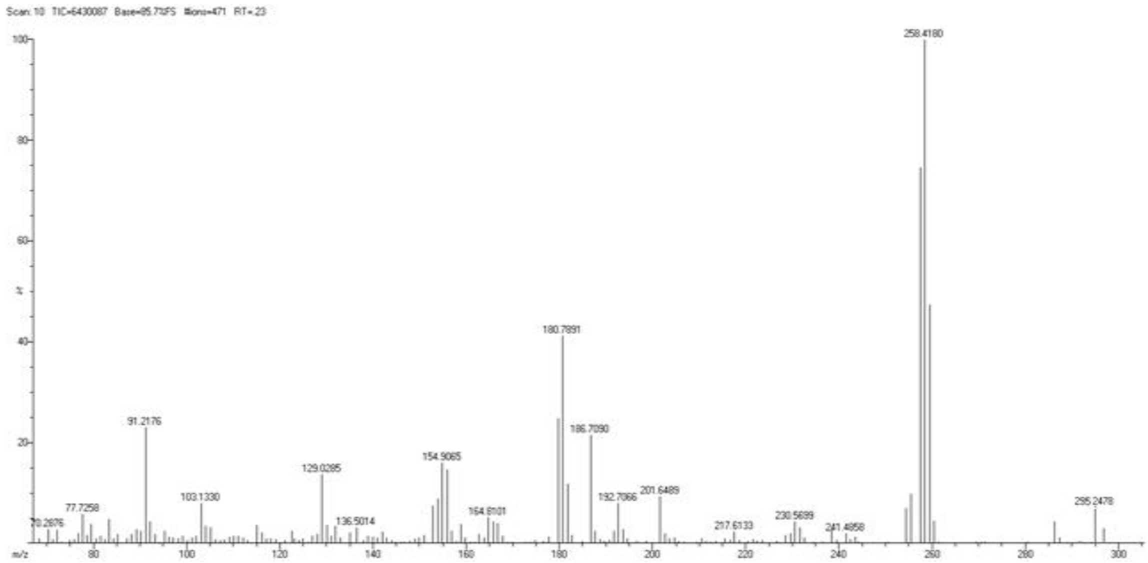

Fig. 4: Mass spectrum of lead oxalate crystals transformed by *Aspergillus niger.*

Soil bacteria could directly or indirectly interact with lead present in the contaminated soil and reduced it into non-toxic forms. Some of bacteria responsible for the bioremediation of lead may reduce the lead in the contaminated source through biosorption of lead by functional groups on the cell surface or by interaction and complex formation between lead and acidic sites in the cell wall.[19] The possible mechanisms includes exclusion by forming a permeable barrier, intra- and extracellular sequestration, active transport, enzymatic detoxification, dissolution of lead by acid production, chelation of lead, precipitation of lead through the production of organic bases, extracellular metal precipitation, and biotransformation reactions, such as methylation, volatilization, oxidation, and reduction.[1]

CONCLUSION

The formation of biogenic lead oxalate crystals has been demonstrated in this paper. The insoluble lead carbonate, can be solubilised by *Aspergillus niger*, with simultaneous generation of lead oxlate, a non-toxic form. Analysis of this biomineral, by mass spectrum, XRD with scanning electron miscroscopy revealed the presence of lead oxalate. Microbial transformations of metals and minerals are a vital part of natural biosphere processes and can also have beneficial for human society. Increasing our understanding of this important area of microbiology and exploiting it in applications such as biotransformation and other areas of biotechnology will clearly require a multidisciplinary approach, this multidisciplinary approach between biotechnologist and metallurgists to bring lab scale biotransformation process to land scale technology that will acceptable for industrialists.

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REFERENCES

1. Akhtar, M.S.., B. Chali and T. Azam, 2013. Bioremediation of Arsenic and Lead by Plants and Microbes from Contaminated Soil. Res. Plant Sci, 1: 68-73.
2. Johnson, F.M., 1998. The genetic effects of environmental lead. Mutation Res, 410: 123-140.
3. Klaassen, C.D. and J.B.Watkins, 2003. Absorption, distribution and excretion of toxicants. Toxicol, 8: 512-519.
4. Van der Sloot, H.A., R.N.J.Coomans and O. Hjelmar, 1996. Similarities in the leaching behaviour of trace contaminants from waste, stabilized waste, construction materials and soils. Sci. Tot. Environ, 178: 111-126.
5. Thayer, J. S., 2002. Biological methylation of less-studied elements. Appl. Organomet. Chem, 16: 677–691
6. Meenambigai, P., R. Vijayaraghavan, R. Shyamala Gowri, P. Rajarajeswari and Prabhavathi, P. 2016. Biodegradation of Heavy Metals – A Review. Int. J. Curr. Microbiol. App. Sci, 5: 375-383.
7. Lloyd, J.R., 2002. Bioremediation of metals: The application of microorganisms that make and break minerals. Microbiol. Today, 29: 67–69.
8. Sutjaritvorakul, T., A.J.S. Whalley and P. Sihanonth, 2015. Biotransformation of insoluble lead compounds by fungi isolated from polluted soil. KMITL Sci. Tech. J, 15: 55-61.
9. Gadd, G.M., 2000. Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr. Opinion Biotech, 11: 271-279.
10. Wei, Z., S. Hillier and GM. Gadd, 2012. Biotransformation of manganese oxide by fungi: solubilization and production of manganese oxalate biominerals. Environ. Microbiol, 14, 1744-1752.
11. Singh, H., 2006. Mycoremediation: fungal bioremediation. New Jersey, Wiley-Inter Science.
12. Sayer, J.A., S.L. Raggett and G.M. Gadd,, 1995. Solubilization of insoluble metal compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. Mycological Research, 99: 987-993.
13. Fomina, M.A., I.J.Alexander, J.V. Colpaert and G.M. Gadd, 2005. Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. Soil Biol. Biochem, 37: 851-866.
14. Sayer, J.A. and G.M. Gadd, 1997. Solubilization and transformation of insoluble metal compounds to insoluble metal oxalates by Aspergillus niger. Mycological Res, 101: 653-661.
15. Martino, E., S. Perotto, R. Parsons and G.M. Gadd, 2003. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. Soil Biol. Biochem, 35: 133-141.
16. Gadd, G.M., 1999. Fungal productions of citric and oxalic acid: importance in metal speciation, physiology and biochemical process. Adv. Microbial Physiol, 41: 47-92.
17. Sayer, J.A., J.D. Cotter-Howells, C. Watson, S. Hillierand and G.M. Gadd, 1999. Lead mineral transformation by fungi. Curr. Biol, 9: 691-694.
18. Khunur, M.M., D.T. Wahyuni and Y.P. Prananto, 2011. Synthesis of Lead(II) Oxalate Hydrate (PbC2O4.xH2O) Crystal In Silica Gel. Adv. Nat. Appl. Sci, 5: 467-472.
19. Cabuk, A., T. Akar, S. Tunali and O. Tabak, 2006. Biosorption characteristics of Bacillus sp. ATS-2 immobilized in silica gel for removal of Pb. J. Hazard. Mat, 136: 317-323.