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# Removal of Pb by Fungus Aspergillus Fumigatus

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**Abstract—***Bioremediation of lead from industrial wastewater using metal resistant microbes offers a promising means to reclaim the waste in an economical and eco-friendly way. In the present study, we focused on isolation, screening and characterization of lead resistant microorganisms from sewage, sludge and industrial effluent. The highly resistant organisms were optimized under different processed conditions. A total of 15 fungal isolates showed tolerance up to 500 ppm of lead and could remove >80 % of Pb (II) from batch cultures. Out of these 15 isolates, IF-3 had the greatest removal potential >90 %. Upon molecular identification the isolate IF-3 was identified as Aspergillus fumigates. Removal efficacy of Aspergillus fumigatus was further enhanced under optimized conditions with maximum Pb (II) removal of ~92% was attained at pH 5 and concentration of 100mg/l after 120 hrs. Further the removal was highest when supplemented with lactose and beef as carbon and nitrogen source respectively and could be effectively employed as a potential biosorbent removing Pb (II) form contaminated waters.*

**Keywords***: Bioremediation, Microbial, Lead, Resistance, Effluent*

## 1. INTRODUCTION

The pollution of the environment with toxic heavy metal is spreading globally along with industrial progress [1]. Industrial waste and sewage are primarily responsible for heavy metal contamination. However traces of these heavy metal are used as cofactors for enzymatic reactions but its elevated level may be toxic to human and animals inhibiting the metabolic reactions [2-3]. Various conventional physiochemical processes like ion exchange, precipitation, membrane technologies, electrochemical treatments, activated carbon adsorption, etc can be used for removal of heavy metals but they are not adequate to clean up environmental waste that has been contaminated by diluted metal solutions. Furthermore these are expensive in terms of chemical process consumption and energy use [4-6]. Bioremediation as such provides an alternative treatment strategy for the removal of heavy metals from waste water. It employs the use of either microorganisms or plants to detoxify the prone area largely by transforming or degrading the pollutants [7].

Lead is an obnoxious heavy metal whose presence in alleviated amounts confer toxicity and hinders normal functioning by interacting with nucleic acid, respiratory proteins and resulting in oxidative damage. Lead contamination in the environment is a widespread problem throughout the world and results from industrial use and processing of lead ore. Lead (Pb), is highly toxic to human, animal and plants and is found in soil, water and air [8-9]. There are various mechanisms by which microorganisms act on heavy metals. These include biosorption (metal sorption to cell surface by physiochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes) intracellular accumulation, and enzyme-catalyzed transformation (redox reactions) [10]. A large number of fungi are under study or are already in use as biosorbents for heavy metal remediation [11-12]. In comparison to other microbes, fungi show a greater affinity for metal ions. By means of biological and physiochemical mechanisms, they could accumulate metal ions from their external environment [13-15]. Cell wall of most fungi contains large amounts of polymer of N-acetyl, chitin and chitosan, and deacetylated glucose-amine. Therefore, large amounts of potential binding sites are showed by free hydroxyl groups, amine and carboxyl. The present study attempts to isolate, screen and evaluate Pb tolerant fungi to enhance their metal removal efficacy and will be helpful for further assessment and management of natural biosorbent (fungus) for treating industrial effluents with toxic metallic ions.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Samples

Sewage sludge and industrial effluent samples were collected in sterilized containers from various industries and sewage treatment plants located at Chandigarh, Panipat, Sonipat, Karnal, Yamunanagar, Assam, Delhi These samples were brought to laboratory and kept in refrigerator at $4^0$C for further processing.

### 2.2 Isolation and Screening of Pb tolerant Fungal Isolates

These sewage sludge and industrial effluent samples were processed for the isolation of fungi by enrichment culture technique. Potato dextrose broth (Hi-Media, Mumbai, India) supplemented with 100 ppm of Pb was used as enrichment media for the isolation of Pb tolerant fungal isolates. A 1000

ppm stock solution and 100 ppm working solution of Pb was made in double distilled water using $Pb(NO_3)_2$, (Hi-Media, Mumbai, India). The primary screening of Pb tolerant isolated was done on PDA with increased concentrations from 100 to 1000 ppm of Pb. The colonies of predominant genera of fungi were picked up and purified by pour plate method and further subjected to a secondary screening in batch cultures in PDB.

### 2.3. Removal of Pb by fungal isolates in liquid medium

The most Pb tolerant fungal isolates were assessed for the metal removal potential. Potato dextrose broth medium containing 100 ppm of Pb inoculated with 1 ml of freshly prepared spore suspension ($10^6$–$10^7$ spores/ml) of each fungal isolate was used for metal removal studies. The flasks were kept under shaking conditions for 120 hrs at $30^0$C at 100 rpm. Un-inoculated flasks containing potato dextrose broth of 100 ppm concentration of Pb served as control. Fungal biomass was separated by using Whatman filter No. 42 and Pb concentration in the filtrate was estimated by Atomic Absorption Spectrophotometer (GBC932, Semiautomatic). All the experiments were conducted in triplicate to minimize variations.

### 2.4 Removal of Pb in liquid medium by fungal isolate at different process conditions

The fungal isolate with highest tolerant potential was evaluated for removal of Pb in PDB medium containing 100ppm Pb under different parameters. A pH gradients from 2 to 7 was used for evaluating the optimal pH at which fungus achieves maximum removal efficiency. Similarly other parameters like incubation time (48, 72, 96, 120, 144 and 196 hrs), inoculums size (1%, 2%, 4%, 6%, 8%, 10%) and initial metal ion concentration were optimized for maximum removal efficiency of fungal isolate. Various carbon sources (sucrose, Lactose, Mannose, Maltose, Dextrose, Cellulose, Starch) and nitrogen sources (Peptone, Beef, Urea, Ammonium chloride, Ammonium sulphate, Potassium nitrate, Sodium nitrate) were also optimized for achieving maximum removal efficiency. The fungal biomass was harvested at end of each experiment and Pb concentration in the filtrate was analyzed by Atomic Absorption Spectrophotometer. The percentage removal of Pb by fungal biomass was calculated by the following equation:

$$\% \text{ Removal} = \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}} \times 100$$

### 2.5 DNA Extraction, PCR Amplification and Sequencing of Fungal ITS

The genomic DNA was extracted by ZR Fungal/Bacterial DNA MiniPrep™ kit according to manufacturer's protocol. PCR amplification of ITS1-5.8S rDNA-ITS2 regions was performed by using universal primers ITS5: 5´-GGAAGTAAAAGTCGTAACAAGG-3´ and ITS4: 5´-TCCTCCGCTTATTGATATGC-3´ [16]. PCR amplification was performed according to the method of Devari *et al*. (2014) [17]. The PCR product was purified by PCR purification kit (AXYGEN) according to manufacturer's protocol. The amplified ITS region was sequenced in both directions by ABI 377 DNA sequencer using the Big Dye Terminator v3.1 cycle sequencing kit, following manufacturer's instruction.

## 3. RESULT AND DISCUSSION

### 3.1 Isolation and Screening of microbes

A total number of 7 fungal isolates (IF1, IF2, IF3, IF4, IF5, IF6, IF7) were isolated from different samples. These isolates were subjected to primary screening on agar plates and were tolerant up to 800 ppm of lead. Upon secondary screening out of these 17 isolates four isolates named IF3, IF4, IF6, IF7 were tolerant up to 800 ppm of Pb while IF3 was tolerant up to 1000 ppm. Isolate IF 3 was identified as *Aspergillus fumigates* by 18 S rRNA and on basis of cultural and biochemical characteristic by Indian Culture Collection, Department of Pathology, Indian Agricultural Research Institute, PUSA, New Delhi.

### 3.2 Removal of Pb by fungal isolates in liquid medium.

Removal studies of the fungal isolate IF3 were carried out in PDB medium containing 100ppm of Pb. IF3 exhibited a maximum removal of 90% of Pb at 100 ppm in batch culture. The results were in accordance with the earlier reports on lead removal by fungi such as Mucor rouxii which effectively removed about 90% of lead from aqueous solution (Shrabani S et al, 2010) [18]. A fungal isolate belonging to the aspergillus family *Aspegillus fumigatus K3* has been reported to remove ~80 % of Pb from the batch culture [15].

### 3.3 Effect of pH

Pb removal and uptake capacity of fungus increases with increase in pH from 2-5, followed by a slight decrease at pH 6 and 7. Highest removal 91% was observed at pH 5.0 which caused metals precipitation (Fig. 1).

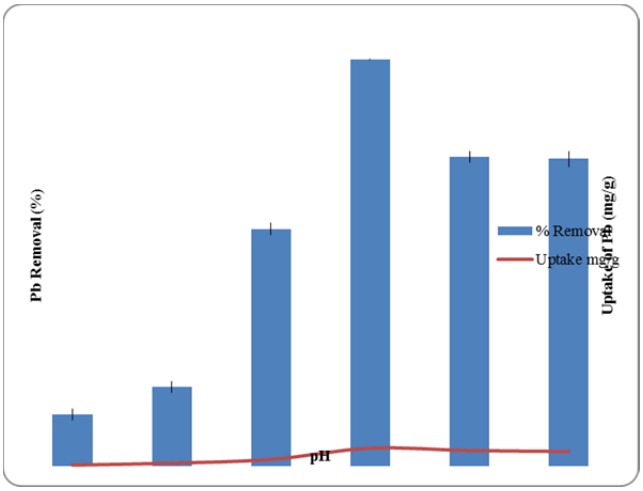


**Fig. 1: Effect of pH on the removal and uptake capacities of *Aspergillus fumigatus***

It was found that there was very low or negligible removal of lead at pH 2-3 which could be contributed to the lower adsorption of lead at this pH. Poor adsorption at lower pH could be attributed to the fact that at low pH $H^+$ or $H_3O$ competes with Pb II ions for the same negatively charged binding site [19-21]. A reduction in metal removal at higher pH could be probably due to the precipitation of metal ions in the cells or the intrafibular capillarity of the cell walls [22].

### 3.4 Effect of metal concentration

The metal removal capability of the fungus decreased with increase in metal concentration. The decrease in removal capability was due to higher toxicity of metal to fungus at higher concentrations. It was found that the percentage of removal decreases with increasing initial lead concentration which could be due to the saturation of all metal binding active sites at a certain concentration (Fig. 2) [23].

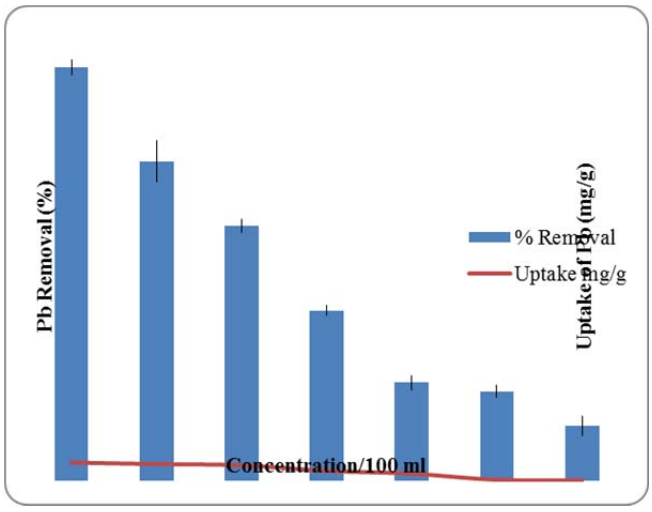


**Fig. 2: Effect of initial concentration on the removal and uptake capacities of *Aspergillus fumigatus***

### 3.5 Effect of time duration and Inoculums size

PDB with optimum fungal inoculums adjusted at optimum pH was incubated for different time intervals starting from 24hrs, 48hrs, 72hrs, 96hrs, 120hrs, 144hrs, and 160 hrs. There was an increase in fungal biomass with increased time duration. Maximum biomass growth and removal was observed at 120 hrs (Fig. 3). After 120 hrs there was a slight decrease in the fungal biomass growth and uptake capability which could be probably due to the depletion of nutrients and saturation of fungal binding sites by metal ions. *Aspergillus* showed a maximum removal of 92% after 120 hrs of incubation. Fungal biomass with inoculums size ranging from 1%, 2%, 4%, 6%, 8% and 10% was analyzed for the biosorption of Pb (Fig. 4). It was found that there was an increase in the removal efficiency with increase in fungal biomass up to a certain biomass dose which is in accordance with earlier reports [24]. After that there was no further increase in removal efficiency. This could be explained that after the saturation of all the metal ion binding sites the removal remains constant with no further increase in removal potential. Fungal biomass of 4% inoculums size exhibited maximum Pb removal efficiency of ~92 % [24].

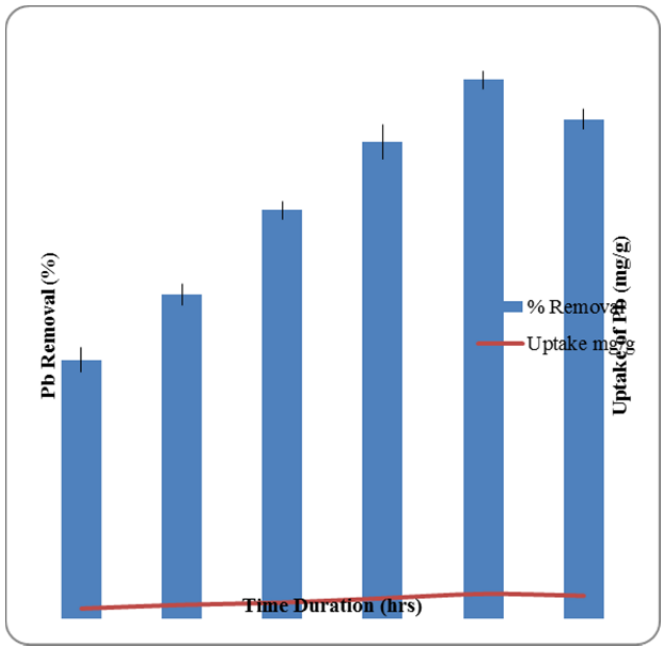


**Fig. 3: Effect of time duration on the removal and uptake capacities of *Aspergillus fumigatus***

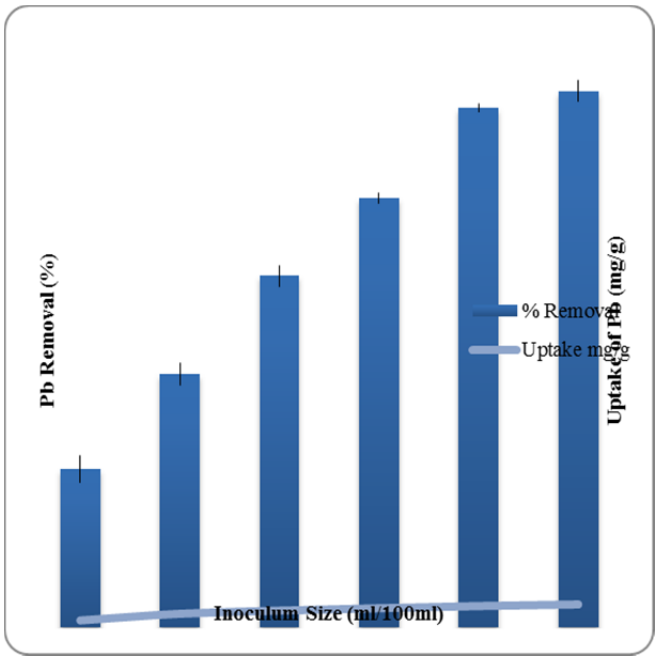


**Fig. 4: Effect of inoculums size on the removal and uptake capacities of *Aspergillus fumigatus***

### 3.6 Carbon and Nitrogen source

The fungal biomass was also optimized for maximum growth on various carbon and nitrogen sources. It was found that *Aspergillus fumigatus* showed maximum biomass growth when supplemented with lactose as carbon source as compared to other sources like maltose, sucrose, mannose, dextrose, cellulose and starch (Fig. 5). Maximal biomass growth was also obtained for peptone as nitrogen source compared to other sources like ammonium sulphate, ammonium chloride, beef, yeast, potassium nitrate and urea (Fig. 6).

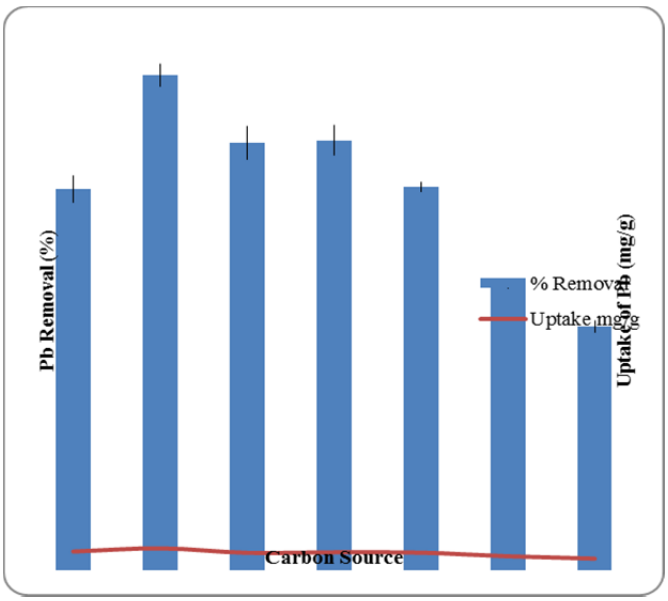


**Fig. 5: Effect of carbon source on the removal and uptake capacities of *Aspergillus fumigatus***

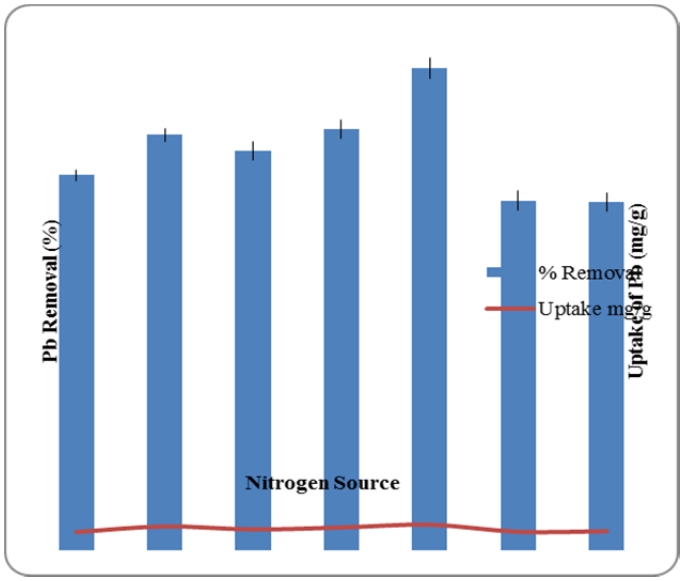


**Fig. 6: Effect of nitrogen source on the removal and uptake capacities of *Aspergillus fumigatus***

## 4. CONCLUSION

Heavy metal biosorption by fungi represents an effective methodology for the removal of toxic metals from contaminated water bodies. In the present investigation a filamentous fungus belonging to the species *Aspegillus fumigatus* has shown considerable potential in removing Pb (II) ions from contaminated waters. The biosorption potential of this fungus was further removed by optimizing the fungus under different processed conditions. Overall the fungus holds a tremendous potential as a biosorbent and could be envisaged as a economically good source in treating contaminated effluents.

## REFERENCES

[1] Gavrilescu M, Chisti Y (2004). Biotechnology-a sustainable alternative for chemical industry. Biotechnol Adv 23: 471-499.

[2] Huang SS, Liao QL, Hua M, Wu XM, Bi KS, Yan CY, Chen B, Zhang XY (2007) Survey of heavy metal pollution and assessment of agricultural soil in Yangzhong district, Jiangsu Province, China. Chemosphere 67: 2148–2155.

[3] Veglio F, Beolchini F (1997) Removal of metals by biosorption: A review, Hydrometallurgy, 44 (1997) 301-316

[4] Haq R, Shakoori AR (2000). Microorganisms resistant to heavy metals and toxic chemicals as indicators of environmental pollution and their use in bioremediation. Folia Biol (Krakow) 48: 143-147.

[5] Resmi G, Thampi SG, Chandrakaran S (2010). Brevundimonas vesicularis: A Novel Bio-sorbent for Removal of Lead from Wastewater. Int Journal Environ Res 4: 281-288.

[6] Maldonado J, Diestra E, Huang L, Domènech AM, Villagrasa EV, Puyen ZM, Duran R, Esteve I, Solé A (2010). Isolation and identification of a bacterium with high tolerance to lead and copper from a marine microbial mat in Spain. Ann. Microbiol 60: 113-120.

[7] Bhattacharya P, Mukherjee AB, Jacks J, Nordqvist S (2002). Metal contamination experimental studies on remediation. Sci Total Environ 290: 165-180.

[8] Tchounwou PB, Yedjou CG, Patlolla CG, Sutton DJ (2012). Heavy Metals Toxicity and the Environment. EXS 101: 133–164

[9] Wuana RA, Okieimen FE (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecology 2011: 402647.

[10] Lloyd JR (2002). Bioremediation of metals: The application of microorganisms that make and break minerals. Microbiol Today 29: 67-69.

[11] Gadd GM (1993). Interactions of fungi with toxic metals. New Phytol 124: 25-60.

[12] Volesky B, Holan ZR (1995). Biosorption of heavy metals. Biotechnol Prog 11: 235-50.

[13] Cabuk A, Ilhan S, Filik C, Caliskan F (2004). $Pb2^{+}$ biosorption by pretreated fungal biomass, Turk J Biol 29: 23-28.

[14] Preetha B, Viruthagiri T (2005). Biosortion of zinc (II) by Rhizopus arrhizus: equilibrium and kinetic modeling. Afr J Biotech 4: 506-508.

[15] Shazia I, Uzma, Sadia GR, Talat A (2013). Bioremediation of Heavy Metals Using Isolates of Filamentous Fungus Aspergillus fumigatus Collected from Polluted Soil of Kasur, Pakistan. Int Res J Biological Sci 2: 66-73.

[16] White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) In *PCR protocols: a guide to methods and application.* Academic Press, San Diego, pp 315-322.

[17] Devari S, Jaglan S, Kumar M, Deshidi R, Guru S, Bhushan S, Kushwaha M, Gupta AP, Gandhi SG, Sharma JP, Taneja SC, Vishwakarma RA, Shah BA (2014) Capsaicin production by *Alternaria alternata*, an endophytic fungus from *Capsicum annum*; LC–ESI–MS/MS analysis. *Phytochemistry*; 98: 183-189.

[18] Majumdar SS, Das SK, Chakravarty R, Saha T, Bandyopadhyay TS, Guha AK (2010). A study on lead adsorption by Mucor rouxii biomass. Desalination 251: 96-102

[19] Jalali R, Ghafourian H, Asef Y, Devarpanah SJ, Sepehr S (2002). Removal and recovery of lead using nonliving biomass of marine algae. J Hazard Mater 92: 253-262.

[20] Luo F, Liu Y, Li X, Xuan Z, Ma J (2006). Biosorption of lead ion by chemically-modified biomass of marine brown algae Laminaria japonica, Chemosphere 64: 1122-1127.

[21] Deng S, Ting YP (2005) Characterization of PEI-modified biomass and biosorption of Cu(II), Pb(II), and Ni(II). Water Res 39: 2167-2177.

[22] Azouaoua N, Belmedanib M, Mokaddema H, Sadaoui Z (2013). Adsorption of Lead from Aqueous Solution onto Untreated Orange Barks. Chem Eng Transc 32: 55-60.

[23] El-Zayat SA, Rashed MN (2009). Removal of lead ions by the filamentous fungus A*spergillus niger* from polluted water. *Assiut Univ J Botany 38: 15-22.*

[24] Sani RK, Peyton BM, Jandhyala M (2003) Toxicity of lead in aqueous medium to Desulfovibrio desulfuricans G20. Environ Toxicol Chem 22:252–260.