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# Bioleaching of toxic elements by *Paecilomyces lilacinus*

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**ABSTRACT**

Industrialization has led to the production of different kinds of pollutants and toxic elements. Conventional methods for the removal of heavy metals are not economically and environmental friendly. Fly ash was collected from a cement plant from Chandrapur, to investigate the presence of heavy metals. It was found to contain Al, Li and Mn in higher concentrations approaching toxic limits. Physio-chemical techniques for treating fly ash are expensive and time consuming. Naturally fungi have a large variety of extracellular proteins, organic acids and other metabolites. Fungi can adapt and survive in several ecosystems and under different environmental conditions. Most of the soil fungi, especially the filamentous fungi are of great interest in bioremediation. Biological treatment methods are economical and allow the cyclisation of the sediment after treatment. In present investigation *P.lilacinus* was employed as a bioleaching agent, experimentally. The concentrations of metal in its elemental form of Al, Li, Mn and Zn were analyzed by ICP -AES. The formation of oxalates in the culture filtrates bioleached by *P.lilacinus*, after treatment was confirmed using FTIR analytical technique.

**Key words**: Fly ash, *Paecilomyces lilacinus*, FTIR, Oxalates

## INTRODUCTION

The soil and water are frequently contaminated by toxic heavy metals and organic pollutants. As a consequence of human activities become a key concern in environmental and health problems. Several toxic metals (Cd, Cu, Hg, Pb, Mn, As, Ni, Zn etc.) from industrial wastewater and other human activities are directly or indirectly release into the environment. Unlike organic contaminants, these pollutants from heavy metals are non-biodegradable and able to enter the food chain via bioaccumulation.

Rapid developments of industrialization and urbanization have led to direct impact on the environment. Globally, open water and aquatic ecosystem are contaminated with several heavy metals through various human activities that directly and indirectly led to pollutions. High concentrations of heavy metals can change the physical and chemical properties of the water and thereby the profile of water. (Siddiquee *et al*., 2015).

Fly ash is produced worldwide in vast quantities as an incineration waste. Fly ash is hazardous due to the volatile toxic metals that concentrate and accumulate in the ash. Treatment of fly ash may lead to both detoxification and reuse. Fly ash includes substantial amounts of silicon dioxide (amorphous and crystal-line), aluminum oxide ($Al_2O_3$) and calcium oxide (CaO), are the chief components of the fly ash.

Biological treatment methods are the methods of choice because they are natural, economically viable, and attractive and because they allow the reuse of the sediments after their treatment. Present paper focuses on detoxification of pollutants using filamentous fungi *Paecilomyces lilacinus*.

Fungal leaching of heavy metals is an interesting biological treatment method. It is based on the principle on catalytic conversion of organic acids by various fungi to organic esters such as oxalates, citrates, maleates, tartarates, etc. thereby increasing the solubility of metals in the form of water soluble complexes. Formation of complexes is also associated with substantial decrease in toxicity fungal production of weak organic acids that solubilize metals by forming water soluble complexes with them (Shannon *et al*., 2014).

## MATERIALS AND METHODS

Fly ash is collected from Chandrapur cement plant, Chandrapur district is located in Maharashtra near Nagpur. Sampling was done randomly. The fly ash was digested then analyzed for their total heavy metal content by inductively coupled plasma atomic emission spectroscopy (ICP AES). for the digestion 0.5 g of fly ash sediments were digested in 10 ml of concentrated nitric acid for 10 minutes.

*Paecilomyces lilacinus* was isolated from the soil collected from forest. Six days old culture of *P.lilacinus*was inoculated in Czapek dox broth and examined for its Metal Tolerance Capacity (MTC).Grades of fly ash were prepared by adding 1, 5, 10 and 15 gramsof fly ash in 100 ml of Czapek dox in duplicates. Three control flasks, one with media, media with culture and media with fly ash were incubated for 15 days. pH was checked before and after the treatment. On the 15$^{th}$ day pH was checked, and the culture filtrate was filtered with Whatman's No.42 filter paper and the fresh and dry weight of the biomass was recorded. Culture filtrate and the fungal mats were separately digested and analyzed for the presence of metals. Analysis was done using ICP AES. Culture filtrate was then extracted with Ethyl acetate and vacuum dried. The residue was reconstituted in methanol. Sample was used for the further investigations. The samples of all the flasks were analyzed for presence of organic acid.

Oxalic acid was estimated by titrating culture filtrate with 0.02 N Potassium permanganate solution. The oxalates in the samples were detected and confirmed with the UV spectroscopy and FTIR.

Fungal mats from each concentration were examined to observe the modification of mycelia due to the effect of metals.

## RESULTS AND DISCUSSION

**Detection of metals by ICP AES**

Culture filtrate and the fungal mats of the control and treated flasks were digested with concentrated nitric acid for analysis. It was observed that fly ash showed the presence of Li, Al, Zn and Mn. Fly ash is as such not hazardous but it causes ground water pollution. Filamentous fungi *Paecilomyces lilacinus* was grown in various concentrations of fly ash amended media. Before treatment the pH was 6 and it changed to pH 10 after treatment. Culture mat was weighed and digested with concentrated nitric acid for analysis. (**Table. 1**)

**Table. 1. pH and Biomass of the culture**

| Sample | pH | | Weight of biomass in grams | |
|---|---|---|---|---|
| | before inoculation | after 15 days incubation | (fresh) | (dry) |
| Control (Media) | 6 | 6 | - | - |
| Control (M+C) | 6 | 9 | 17.982 | 4.228 |
| Control (M+FA) | 6 | 6 | - | - |
| 1% FA | 6 | 10 | 16.522 | 3.821 |
| 5% FA | 6 | 10 | 12.632 | 2.112 |
| 10% FA | 6 | 10 | 11.222 | 2.290 |
| 15% FA | 6 | 10 | 11.082 | 2.082 |

**Table. 2. Concentration of the elements before and after treatment**

| Sample | Al(ppm) | Li (ppm) | Mn (ppm) | Zn (ppm) |
|---|---|---|---|---|
| Control (Media) | 0 | 0 | 0 | 0 |
| Control (M+C) | 0 | 0 | 0 | 0 |
| Control 1% | 2.80 | 0.20 | 1.28 | 5.04 |
| 1%FA | 0.15 | ND | 0.11 | 0.285 |
| 1%BF | 2.23 | 0.11 | 1.08 | 4.36 |
| Control 5% | 3.21 | 0.31 | 1.98 | 6.87 |
| 5%FA | 0.31 | ND | ND | 0.85 |
| 5%BF | 2.84 | 0.23 | 1.78 | 5.90 |
| Control 10% | 4.01 | 1.21 | 2.01 | 7.59 |
| 10%FA | 0.28 | 0.34 | ND | 2.40 |
| 10%BF | 3.82 | 0.85 | 1.95 | 5.10 |
| Control 15% | 6.83 | 2.33 | 2.58 | 8.58 |
| 15% FA | 3.60 | 0.53 | 0.723 | 3.28 |
| 15% BF | 3.12 | 1.79 | 1.657 | 5.25 |

FA=Culture filtrate, BF=Biomass of culture mat

In control 1% it was observed elemental Al was 2.80 ppm, Li 0.20 ppm, Mn 1.28ppm and Zn 5.04 ppm.In the culture filtrate after treatment Al was 0.15 ppm , Li was not detected , Mn 0.11 ppm and Zn 0.28 ppm. In the culture mat Al was 2.23 ppm, Li 0.11 ppm, Mn 1.08 ppm and Zn 4.36 ppm. (**Table. 2**)

In control 5% it was observed elemental Al was 3.21 ppm, Li 0.31 ppm, Mn 1.98ppm and Zn 6.87 ppm.In the culture filtrate after treatment Al was 0.31 ppm , Li was not detected , Mn not detected and Zn 0.85 ppm. In the culture mat Al was 2.84 ppm, Li 0.23 ppm, Mn 1.78 ppm and Zn 5.90 ppm. (**Table. 2**)

In control 10% it was observed elemental Al was 4.01 ppm, Li 1.21 ppm, Mn 2.01 ppm and Zn 7.59 ppm.In the culture filtrate after treatment Al was 0.28 ppm, Li was 0.34 ppm, Mn not detected and Zn 2.40 ppm. In the culture mat Al was 3.82 ppm, Li 0.85 ppm, Mn 1.95 ppm and Zn 5.10 ppm. (**Table. 2**)

In control 15% it was observed elemental Al was 6.83 ppm, Li 2.33 ppm, Mn 2.58 ppm and Zn 8.58 ppm.In the culture filtrate after treatment Al was 3.60ppm, Li was 0.53 ppm, Mn 0.72 ppm and Zn 3.28 ppm. In the culture mat Al was 3.12 ppm, Li 1.79 ppm, Mn 1.65 ppm and Zn 5.25 ppm. (**Table. 2**)

From the above observation there was a clear reduction of the Al, Li, Mn and Zn in the culture filtrate after treatment. There was an increase in the presence of Al, Li, Mn and Zn in the culture mat. This clearly indicates the biosorption of the elements on the mycelium of *P.lilacinus*.

**Organic Acid production**

Organic acids qualitative test was done in which oxalic acid was present in control as well treated samples. Estimation of organic acid was done by titration method. Oxalic acid is a well-known chelating agent that has been widely studied because of its ability to dissolve different minerals. Incontrast to other low molecular–weight carboxylic acids with low complexing abilities that erode minerals in acid solution by protolysis. Oxalic acid is able to mobilize metals very efficiently at neutral pH and even in basic solutions. (Fomina *et al*., 2005)

**Interpretation of IR spectra**

FTIR analysis of the samples under present investigation was performed by the NaCl cell technique. The FTIR was done on liquid extract. Equipment used was Perkin -Elmer Spectrum –GX and samples were scanned in the region of 4000-650 $cm^{-1}$to evaluate the presence of oxalate peaks in the spectra was run using air as a background.

FTIR spectra related to each studied sample are interpreted in terms of wave numbers in $cm^{-1.}$. In present investigation oxalates shows distinct peaks which are in accordance to literature survey. The oxalates in general shows strong peaks in the region of 3500-2880 $cm^{-1.}$

**Biosorption and Bioleaching**

Metal mobilization by fungi can occur as a result of several mechanisms, including acidolysis (proton promoted), complexolysis (ligand promoted), reductive mobilization and mycelium functioning as sink for soluble metal species. Solublization yield is related to the association of metalsto the acid soluble and reducible fractions. The bioleaching of heavy metals is mainly brought about by organic acids through acido-lysis and complexolysis. Toxic metals may increase oxalate excretion by fungi. The elements are tightly bound to the mat. Al, Li, Mn and Zn were the most solubilized and biosorbed metals in the fungal treatm-ent. In the current study it was

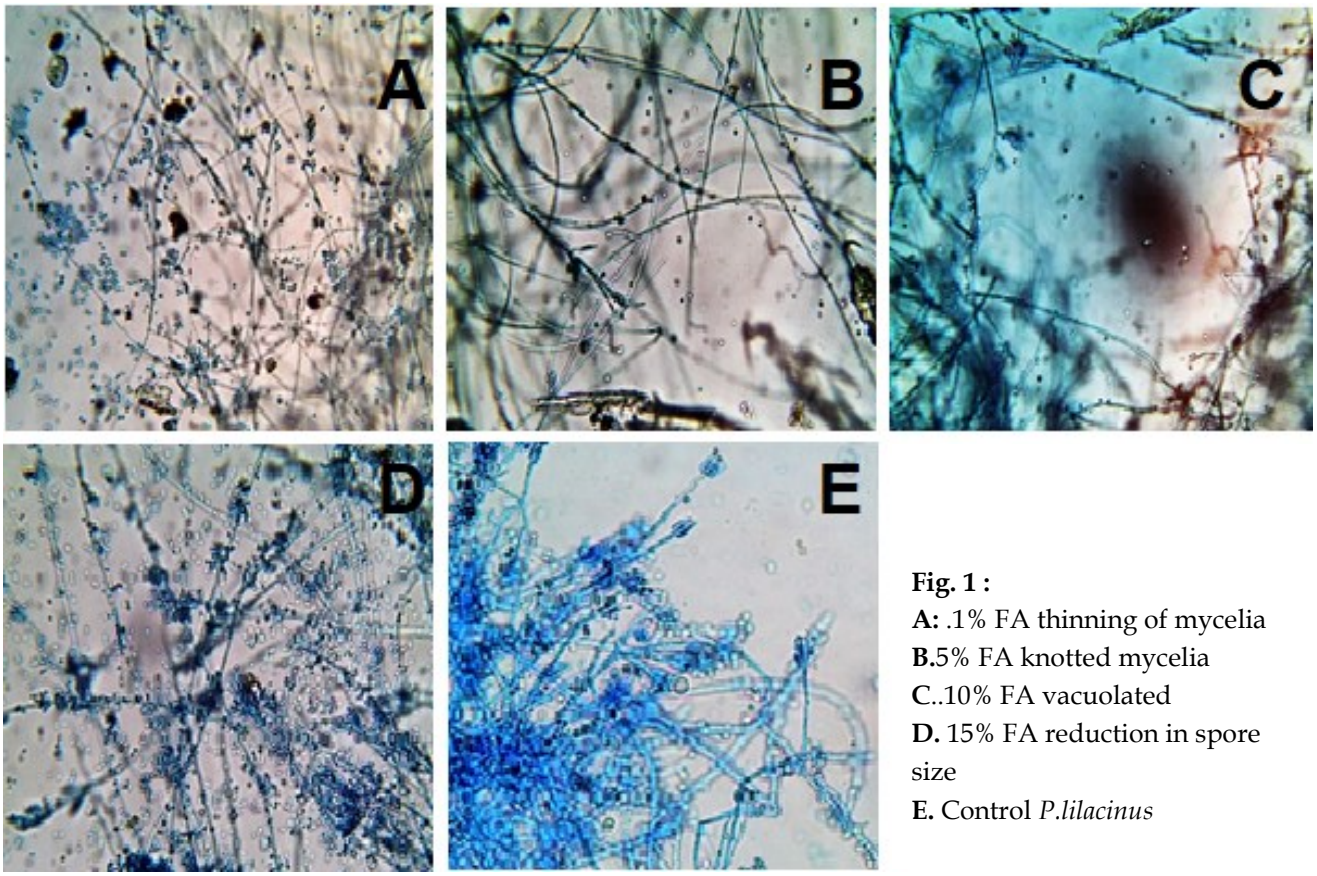


**Fig. 1 :**
**A:** .1% FA thinning of mycelia
**B.**5% FA knotted mycelia
**C**..10% FA vacuolated
**D.** 15% FA reduction in spore size
**E.** Control *P.lilacinus*

observed biosorption of Al, Li, Mn and Zn were efficiently done by *P.lilacinus.*

**Microscopic observation**

In *P.lilacinus* the changes in the mycelia were observed in the different grades of fly ash treatments. It was observed mycelia was width0.7 µm in control culture, whereas the growth with the fly ash was 0.5µm and 0.3µm in 10% and 15% concentration. It also showed changes in the mycelia as thin (**Fig. 1a**), vacuolated (**Fig.1 c**), knotted (**Fig. 1 b**) and reduction in spore size (**Fig.1 d**). Formation of mycelia covered by a thick hydrated mucilaginous sheath leading to formation of jelly like mass which provide micro environment for chemical reactions. Change in the mycelial growth indicates the aspect of metal tolerance. *P.lilacinus* tolerated the toxic metal stress but maintained a high biomass yield.

Metal toxicity may be reduced if the mobilized toxic metal forms complexes with organic ligands excreted by the fungus and especially if toxic metals are precipitated as highly insoluble oxalates. (Fomina *et al*., 2005). Therefore, overexcretion of oxalic acid probably contributed to the metal tolerance exhibited by the *P.lilacinus*

**Conflicts of interest:** The authors stated that no conflicts of interest.

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