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Bioremoval of Lead (Pb) Heavy Metal by Resistant Aspergillus Terreus Isolated from Industrial Effluent Polluted Soil

Usman U. Zango[1], Anil K. Sharma[2]

[1,2]*Maharishi Markandeshwar University, Mullana-Ambala, (Haryana)*
[1]*uumarzango@gmail.com*

Abstract: *Industrial effluent causes serious problem to the environment, it contain toxic chemicals such as heavy metals, dyes and other substances which affect water bodies, soil and other parts of the ecosystem. Heavy metals such as Lead (Pb), Cadmium (Cd), Chromium (Cr) and Copper (Cu) are very hazardous to human life, when released through human anthropogenic activities. Methods of removal by physicochemical techniques were found inadequate. Bioremediation procedures can be employed to remove waste products from the environment which is eco-friendly and less cost-effective.*

Pb-resistant fungus was isolated from the polluted soil from petrol pump site in from Chandigarh, Punjab, India. It was grown on Sabouraud Dextrose Broth (SDB) by enrichment method and plated on Sabouraud Dextrose Agar (SDA) and then screened at various concentrations of Pb ions concentrations up to 700mg/L. It was identified as Aspergillus terreus. The uptake of the Pb ions was also carried out and enhanced in the liquid medium by optimization of the conditions which includes pH, temperature, inoculums size and incubation time. The highest Pb removal was found to be 84%, with dried biomass of 1.78 mg. The isolate could be used for the removal of contaminants especially hazardous chemicals and heavy metals from the environment.

Keywords*: Bioremoval, Heavy metal, Lead, Aspergillus terreus, Industrial effluent, Toxic.*

1. INTRODUCTION

Industrialization leads to the rapid increased in production in many areas, which in other hand caused anthropogenic impact to the environment due to some activities carried out in the environment, release of hazardous chemicals and heavy metals affect the essential parts of the ecosystem. Most of the industries released their effluents which contain heavy metals higher than permissible limit by the World Health Organization [1]. As such the environment turn to be dumping ground for deposition of industrial wastes beyond imagination, which cause serious threat to farm land, water bodies and human life. It was also reported that heavy metals in the underground water have caused various diseases in human beings and also affect metabolic functions negatively Bernard *et al*. [2]; Ademorotti *et al*. [3]; Umar *et al*. [4]. Physicochemical methods of removing heavy metals such as filtration, ion exchange, chemical precipitation; electrochemical treatment, membrane technologies, adsorption on activated carbon and evaporation are found ineffective, difficult and very costly. Also these methods cannot be carried out at large scale [5].

The past two decades have seen a tremendous upsurge in the search for cost effective and environmentally friendly, alternatives to the conventional method for dealing with wastes. The technologies that have emerged as most promising are those that closely mimics the time tested, natural system that have restored environments to their original status following undesirable perturbation. Of all the technologies that have been investigated, bioremediation has emerged as the most desirable approach for cleaning up many environmental pollutants in effluent [6]; Tobin *et al.* [7]; Leusch *et al*. [8]; Chaudhry *et al*. [9].

Bioremediation is a pollution control technology that uses biological system to catalyze the degradation of or transformation of various toxic chemicals to less harmful forms [10]. The general approaches to bioremediations are to enhance natural biodegradation by natural organisms (Intrinsic bioremediation), to carry out environment modification by applying nutrients or aeration (biostimulation), or though addition of micro-organisms (bioaugmentation) [11].

The ability of microorganisms to transform a variety of chemicals has led to their use in bioremediation process. It is now an exact science and includes processes such as intrinsic bioremediation, biostimulation and bioaugmentation [12]. Intrinsic bioremediation is the process whereby natural indigenous micro flora and environmental conditions interact to bring about the natural attenuation of pollution to safe levels, within an acceptable time frame Elshanshoury *et al*. [13].

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND ISOLATION

The soil samples were collected from the soils contaminated with industrial effluent and pump petrol site, which was contaminated at Chandigarh, Punjab, India. The sample was obtained from few inches below the soil surface and transported to the Laboratory.1g of the soil sample was weighed and put in the 9ml of sterilized distilled water and 10^4 serial dilution were carried out and put into sterilized 100 mg/L of Pb-ion concentration working solution, enriched with Sabouraud Dextrose Broth (SDB) in the 250 ml Erlenmeyer flask and put in the orbital shaker at 100 rpm for 24 h as described by Lee *et al*. [14]. 1 ml of the aliquot was taken and inoculated on the Sabouraud Dextrose Agar by spread method and incubated for 96 h Clausen *et al* [15].

2.2 SCREENING OF PB RESISTANT ISOLATE AND IDENTIFICATION

Different concentration of 100 ml of Pb-ion concentrations were prepared of (100, 200, 300, 400, 500 and 700 mg/L) were put into 250 ml of Erlenmeyer flasks enriched with Sabouraud Dextrose Agar (SDA), which was sterilized at 15 lbs/psi, then poured into the plates and allowed to solidify. The fungal isolate was inoculated on the plates; control was made then incubated for 96 h. The resistant fungal isolate that grown at high concentrations of Pb was selected as described by (Price *et al*. [16]. Lactophenol cotton blue staining technique was carried out to identify the fungal isolate. Also, further identification was done and confirmed the isolate as *Aspergillus terreus* at Department of Pathology, Indian Agricultural Research Institute Pusa, New Delhi.

2.3 DETERMINATION OF BIOREMOVAL OF PB FROM LIQUID MEDIUM

100 mg/L of concentration Pb ion enriched with SDB medium volume 100 ml in the 250 Erlenmeyer flask was sterilized. The culture of *A. terreus* was grown on SDA to the end of exponential phase, in which sterilized distilled water was poured into the test tube and vertex for 2 min and 1 ml was taken, the spores were counted in the haemocytometer to be 1.6 x 10^7 spores/ml then dispensed into the flask, and placed in the orbital shaker at 30^0C under 100 rpm for 96 h. Control was made without inoculation of the fungal spores and kept under the same condition, as described by Clausen *et al.* [15]. The fungal biomass grown were filtered with No1 Whattman filter paper and acidified with 1ml of 95% H_2SO_4. The residual of Pb was determined by Atomic Absorption Spectrophotometer (model GBC 932) as reported by Abou Zeid *et al*. [17]. The percentage of Pb removal of biomass was obtained [18].

% Removal = Initial concentration – Final concentration / Initial concentration x 100

2.4 OPTIMIZATION OF BIOREMOVAL OF PB UNDER DIFFERENT CONDITIONS IN THE LIQUID MEDIUM

2.4.1 EFFECT OF DIFFERENT PH VALUES

Different pH values of 100 mg/L of Pb solutions containing Sabouraud Dextrose containing were taken 100 ml of flask, the pH values (4, 5, 6, 7 and 8). Sterilized and inoculated with ml suspension of spores of *Aspergillus terreus* at 30^0C in the orbital shaker under 100 rpm for 96 h.

2.4.2 EFFECT OF DIFFERENT INCUBATION TEMPERATURE

100 ml of SDB having 100 mg/L of Pb solution was adjusted to pH 6 sterilized and inoculated with 1 ml of suspension of the spores into the flask. Incubated at different temperature conditions (25, 30, 35 and 40 ^{0}C) in the orbital shaker at 100 rpm for 96 h, to ascertain the bio removal of Pb by the fungal isolate.

2.4.3 EFFECTS OF INOCULUMS SIZE AND INCUBATION TIME

The medium solutions of SDB containing 100 mg/L of Pb were made sterilized and inoculated with different inoculums sizes of *Aspergillus terreus* suspensions (2, 4, 6, 8 and 10 ml) and kept at 30^0C and pH 6 in orbital shaker at 100 rpm for 96 h. The same set up were made with inoculums size of 8 ml of *A. terreus* for the incubation periods (72, 96, 120 and 144 h) at 100 rpm at 30^0C. The fungal biomass obtained was filtered with Whatman filter paper No. 1, dried the biomass in the oven and weight.

3. RESULTS AND DISCUSSION

The fungus specie isolated from the soil samples collected by enrichment method was screened at various concentrations of Pb up to 700 mg/L. It was found to be tolerant to that concentration. The cultural morphology fungus was stained, observed and identified as *Aspergillus terreus.*

100 mg/L of Pb concentration in the liquid containing SDB medium was used to determine the bioremoval of Pb which was found to remove 74% of Pb; this corresponds to the findings of (Abou Zeid *et al.* [17] .

The optimizations of bioremoval of Pb under different conditions (pH, temperature and inoculums size and incubation time) were studied. Different pH values were taken (4, 5, 6, 7 and 8), the maximum removal was obtained at pH 6 with percentage of removal of 75.6%. The bioremoval of Pb from the liquid started to increased significantly with the pH, it produced higher activity at pH 6, then decreased slowly with increased of the pH value. These correspond with [19] that the biosorption of *Aspergillus* spp increased significantly with increase of pH 2 to 6 because of osmotic concentration. For the temperature

the maximum removal was observed at 30^0C with value of 78.4% then the activity started to come down at 35^0C and 40^0C respectively. Also similar to the Strandbergy *et al*. [20] that demonstrated the optimum temperature of uptake of Uranium increased with the temperature in the range 20 – 30^0C and decreased at higher temperature because of it effect of the integrity of the cell membranes and hinder compartmentalization of metal ions, which lead to reduced in the removal activity.

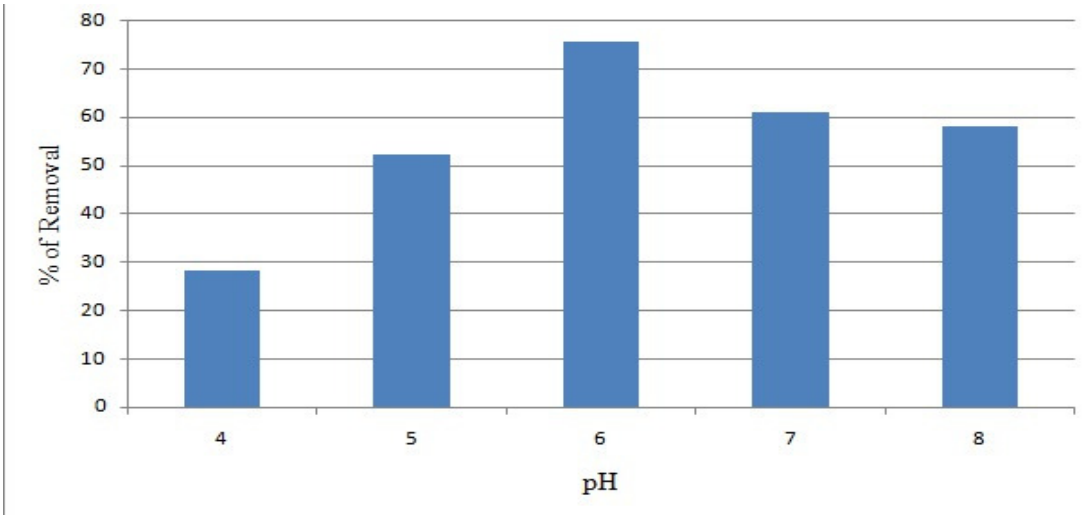

Fig. 1. Percentage of removal of Pb against Ph by Aspergillus terreus

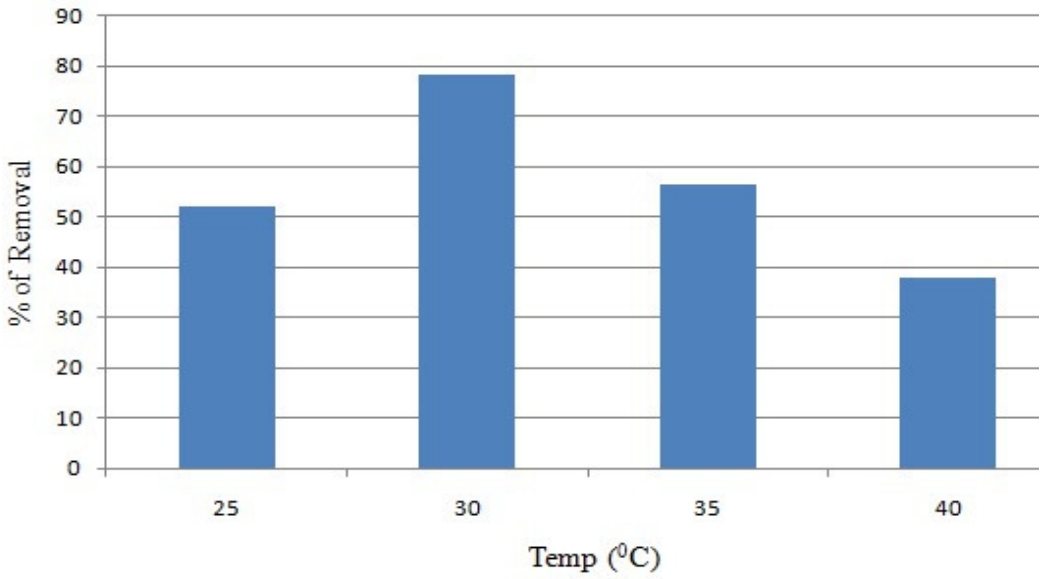

Fig. 2. Percentage of removal of Ph against temperature

While for the effect of inoculums size of Aspergillus terreus, in which different inoculum size of biomass were inoculated to ascertained the bioremoval of Pb. The highest removal was found at 8ml of the inoculums biomass found to remove 80.5% of the Pb fig. 3. This correlate with findings [21] reported that the increase of mycelia by mass of *Aspergillus niger* lead to the increase in the biosorption activities of the heavy metals due to the contacts of fungal biomass and the metals. In case of incubation time there was increased in the removal of Pb from 72 – 96 h, the highest removal was obtained at 120 h with removal of 84%, with the dried biomass obtained was 1.78 mg. It then the bioremoval started to decreased through 144 – 168 h.. This corresponds to the Badar *et al*. [22] who reported biosorption of Zn by *A. niger* and *Penicillium* spp reaching maximum limit was reached within 120 h of incubation with 98.06% of removal.

Also according to the Khan *et al.* [23] reported that contact time of fungi biomass affect the absorption of the metals in the liquid medium.

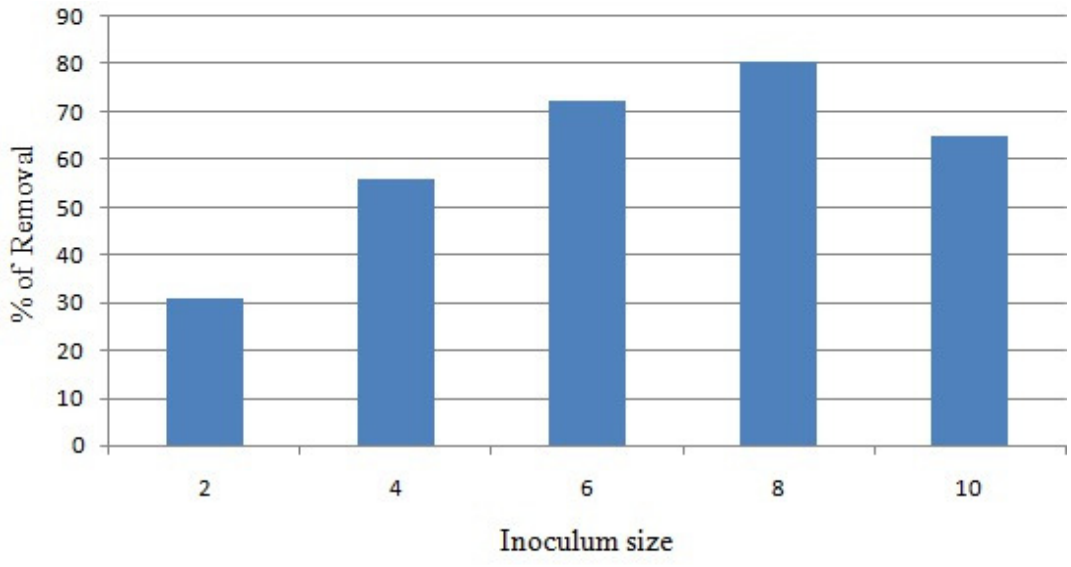

Fig. 3. Percentage of Pb Removal against Incubation size in (ml)

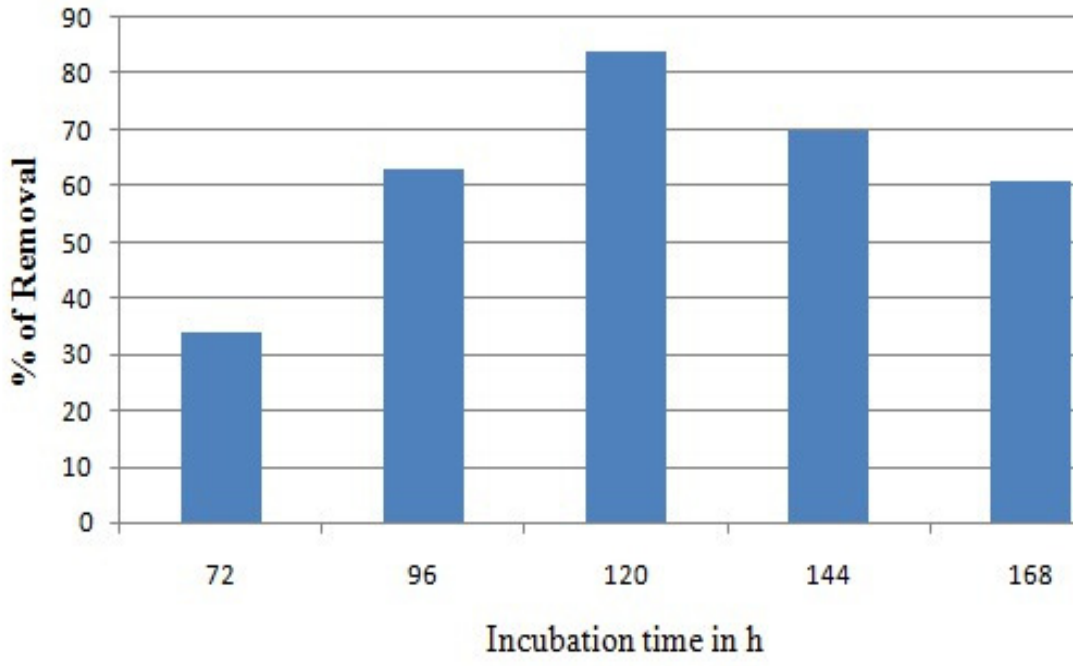

Fig. 4. Percentage of Removal of Pb against Incubation time in hour

4. CONCLUSIONS

The *Aspergillus terreus* was isolated from soil sample obtained from contaminated site at Chandigarh, Punjab, India. The isolate was screened to various Pb concentrations and found to be tolerant to the Pb up to 700 mg/L. Optimization of different conditions such as Ph, temperature, inoculum size and incubation time were also carried out in order to to increase the uptake of the Pb ion. The maximum removal obtained was 84%. This indicated that the *Aspergillus terreus* can be potential isolates for the reclaiming soils and water that are contaminated by heavy metals from industries. The technology is efficient, less costly and environmentally friendly.

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