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Research Article

Biosorption of Lead by Biomass of Resistant *Penicillium oxalicum* Isolated from Industrial Effluent

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Abstract

Background and Objective: Eco-friendly biological removal of heavy metal pollution from aqueous solution is a recent approach which using the biosorbent. It has lots of advantages over chemical approach like its low cost, more efficient, more effective, multiple uses etc. The fungi are one of the most desirable absorbents because of its cell wall characteristics. The present work, therefore, aimed to use *Penicillium oxalicum* isolated from lead-contaminated industrial wastewater to remove lead from aqueous solution. **Materials and Methods:** Its tolerance towards lead metal ions was studied by growing fungus in different metal concentration. Biosorption of lead by live fungal biomass was determined and different parameters like pH, temperature and incubation period were taken to observe maximum absorption. Physically and chemically retreated biomasses of the fungus were also used for lead biosorption. Lead metal concentration was determined by PAR methodology in all procedures. **Results:** Tolerance index of *P. oxalicum* was recorded 67.44 and relationship between tolerance by fungus and lead concentration was found $R^2 = 0.99$. The lead metal ion was absorbed by live biomass which is affected by the incubation period, pH and temperature. Maximum 89.82% lead was absorbed by pretreated biomass of fungus. Among the different pretreatment procedure, detergent pretreatment was found most efficient. **Conclusion:** *Penicillium oxalicum* biomass is now one alternate to remove lead from aqueous solution. The industrial effluent was found an important source of potential micro-organism.

Key words: Lead pollution, industrial wastewater, lead metal, biomass of fungus, detergent pretreatment, biosorption, Raipur, *Penicillium oxalicum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biosorption is the removal of substances from the solution of biological materials, the substances may be organic or inorganic and the soluble or insoluble form. Shumate and Strandberg[1] defined biosorption as "A non-directed physic-chemical interaction that may occur between metal/radio nuclide species and the cellular compartment of biological species". The natural source of lead in earth included volcanic eruptions, geochemical weathering and emission from sea spray etc. The lead concentration in the earth was expanded from quick industrialization amid during the 18th and 19th centuries[2]. The assessed general anthropogenic outpouring of lead noticeable all around consolidates smoldering of coal in power industries, burning of oil, mining, lead generation, steel and iron assembling, metropolitan and sewage ooze, phosphate composts, wood ignition and battery work[3]. Coal contains little amount of lead, which can be amassed in fly fiery debris from coal-burning[4].

The entire globe is facing man-made lead metal pollution. Many African countries like Algeria, Namibia, Tunisia, Zambia and Nigeria[5-8] Asian, China and Korea[9,10] European, Sweden, Switzerland and Scotland were found contaminated with lead metal ions[11-13]. In another part of the world, the atmosphere of Brisbane, Australia and water and soil of Ontario, Canada were found polluted with lead metal[14,15].

While challenging this global issue, removal of the lead metal ion from contaminated water can be a better preference. In this concern, conventional technology such as ion exchange, chemical precipitations and reverse osmosis are often inefficient and very expensive. While using biological method for adsorption of heavy metals has many advantages over conventional treatment methods including its low cost, high efficiency, minimization of chemical and biological sludge, no additional nutrition requirement, regeneration of biosorbent and also the possibility of high metal recovery[16].

Penicillium is an important group of fungi belongs to the Ascomycota. The potential use of *Penicillium* as a biosorbent is achieved to remove not only the lead but also some any other heavy metals like copper, cadmium, zinc etc.[17,18]. AlgaSORB™ and AMT-Bioclim™ are biosorption based process remove heavy metal from aqueous solution[16]. Therefore scientific communities are focusing to find out some potential micro-organism that could remove metal pollution from infected water. In this light the present study focused to analyze the ability of tolerance of lead metal ion and biosorption of lead by live and pretreated biomass of *P. oxalicum*. The findings of the present study may help us to understand the source based potentiality of micro-organisms and a new option for biosorption of lead.

MATERIALS AND METHODS

The methodology of sample collection, isolation and identification of fungus was specified in previous study[19]. The isolated fungus was obtained from lead metal ion contaminated industrial effluent[19].

Tolerance and biosorption of lead by live biomass of *P. oxalicum*: Tolerance of lead metal ions by *P. oxalicum* was also done according to previous study[19]. Biosorption of lead metal ions by living biomass of *P. oxalicum* was performed according to Patil *et al.*[20]. Triplicate flasks containing 50 mL dextrose broth medium were used up to grow the fungus. Preliminary the broth medium was supplemented with the known concentration of lead metal ions. The fungus was inoculated with 1 mL of culture broth (72 h old) and incubated. After incubation mycelium was filtered and the broth was analyzed for lead metal ion by Spectroquant Nova 60 (developed by Merck, Germany) using PAR methodology. The different parameter like incubation period, pH and temperature were taken for better absorption of lead. The percentage of biosorption of lead by living biomass of fungi was calculated with the formula[21]. Modification in the formula was done in which, the initial lead concentration is taken as concentration of lead in control and final lead concentration is taken as the concentration of lead in the test:

$$x = \frac{C - T}{C} \times 100$$

Where:

x = Biosorption (%)
C = Concentration of lead in control
T = Concentration of lead in test

Biosorption of lead by pretreated biomass of *P. oxalicum*: The fungus is also used for biosorption of lead with modification of its biomass using the physical and chemical method. The modification of biomass is known as pre-treatment. Peptone dextrose liquid medium containing Dextrose 20 g, Peptone 10 g, NaCl 0.2 g, $CaCl_2.2H_2O$ 0.1 g, KCl 0.1 g, KH_2PO_4 0.5 g, $NaHCO_3$ 0.05 g, $MgSO_4$ 0.25 g, $FeSO_4.7H_2O$ 0.005 g in 1,000 mL of distilled water was employed to grow the fungus[22]. The fungus was grown in large 500 mL flasks containing 300 mL medium. Inoculums (2 mL, 72 h old cultures in same the medium) was added to the medium and flasks were then kept into the shaking incubator at 125 rpm for 7 days. After 7 days of incubation, fungi were filtered and washed twice with distilled water. Thirty gram wet control biomass were then treated with physical and chemical methods. Physical pre-treatment was done by autoclaving of control biomass for 30 min at 15 lbs. Chemical modification of biomass was done with the treatment of sodium hydroxide, formaldehyde and detergent according to Kapoor and Viraraghavan[22]. After treatment biomasses were dried and fine powder was obtained by grinding into mortal pestle. Powdered biomasses were then used as biosorbent for absorption of lead present in aqueous solution. A known amount of lead solution was taken in 100 mL of distilled water and 1 g of mycelium powder was supplemented. Later on a 15 h contact time, mycelium was filtered and concentration of lead was recorded using Spectroquant Nova 60 based on PAR methodology.

Biosorption capacity, i.e., the amount of lead (mg) absorbed by per gram of biomass was calculated with the formula:

$$Q = \frac{Ci - Cf}{M} \times V$$

Where:

Q = Milligram of lead ion, absorbed by per g of biomass
Ci = Initial metal ion concentration (mg L^{-1})
Cf = Final metal ion concentration (mg L^{-1})
M = Weight of biomass used in reaction mixture
V = Volume of the reaction mixture in liter

RESULTS AND DISCUSSION

Tolerance of lead metal ion by *P. oxalicum*: Tolerance is defined by the ability to grow against stress condition. *Penicillium oxalicum* was found tolerant against lead nitrate. Maximum tolerance was found in low concentration while it is decreased by increasing metal concentration. Thus, the highest concentration is toxic to the fungus but is able to live against 1,000 mg L^{-1} concentration with a tolerance of 67.44 (Table 1). The fungus is therefore useful for biosorption of lead. The value of R^2 (coefficient of determination), for the relationship between tolerance by a fungus and the concentration of lead nitrate ($R^2 = 0.99$) indicates that 99.00% of the variation in tolerance is explained by the concentration of lead nitrate (Fig. 1). *Penicillium* species were

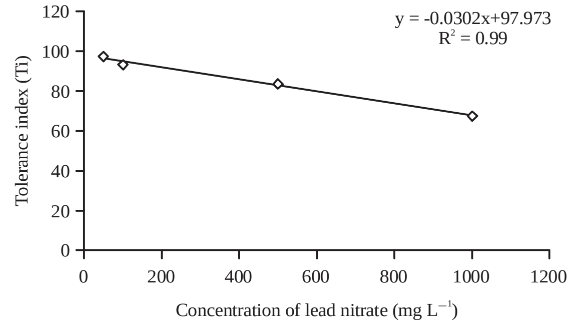

Fig. 1: Linear regression analysis between concentrations of lead metal ion and tolerance of *P. oxalicum*

Table 1: Tolerance of lead metal ions by *P. oxalicum*

Concentration of lead nitrate in medium (mg L^{-1})	Weight of mycelium, average of triplicate (Mean±SE)	Tolerance index (Ti)
50	0.42±0.00	97.67
100	0.40±0.00	93.20
500	0.36±0.01	83.72
1,000	0.29±0.02	67.44

studied for tolerant against many heavy metals. The *Penicillium chrysogenum* was found tolerant to copper, cadmium[23], lead[23,24]. Similarly, *P. simplicissimum* and *Penicillium digitatum* were also found to tolerate lead metal ions[24,25]. The *P. simplicissimum*, *P. chrysogenum* and *P. digitatum* were able to survive at 8,00-1,000 mg L^{-1} concentration of lead metal ions. *Penicillium* sp. MRF-1 tolerate more than 1,000 mg L^{-1} lead concentration in the medium [26] while *Penicillium* sp., *P. chrysogenum*, *P. oxalicum* and *P. citrinium* even survive at 2,000 mg L^{-1} [21,27]. In the higher concentration of lead metal ions, *P .oxalicum* survives against toxic effect but their biomass was decreased. The similar observation was obtained by Acharya *et al.*[28] and Siham[29].

Biosorption of lead by live and pretreated biomass of *P. oxalicum*: Living biomass of *P. oxalicum* has been investigated for biosorption of lead. A 10.11 (%) of the leads were absorbed by *P. oxalicum* at day one of the incubations which was increased until day 9. The biosorption (%) at day 3, 5, 7 and 9 was recorded in 27.45, 45.08, 52.89 and 56.35 (Fig. 2). Day 7 was selected for the study of pH and temperature parameter. The study with the effect of pH on the biosorption of lead by *P. oxalicum*, 37.93 (%) of lead was absorbed by the fungus on pH 3.0 which was increased to pH 7.0. Biosorption was then decreased to pH 8.0 and pH 7.0 with 62.35% of biosorption has been found optimum for absorption of lead by *P. oxalicum* (Fig. 3). The temperature was also affecting the biosorption of lead by *P. oxalicum*. A 40.17% of the leads were absorbed by the fungus at 20°C

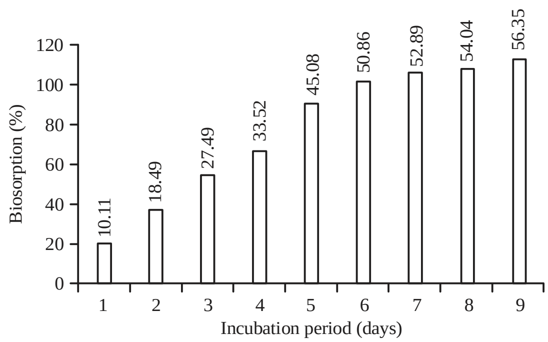

Fig. 2: Effect of incubation period on biosorption of lead by living biomass of *P. oxalicum*

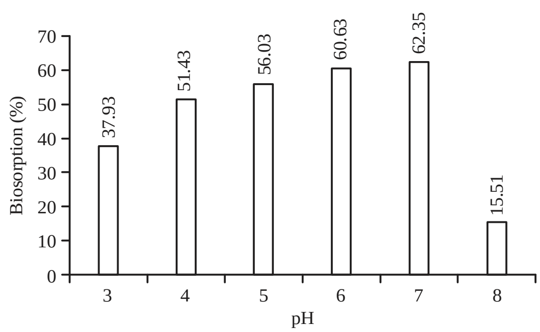

Fig. 3: Effect of pH on biosorption of lead by living biomass of *P. oxalicum* (Incubation period 7 days)

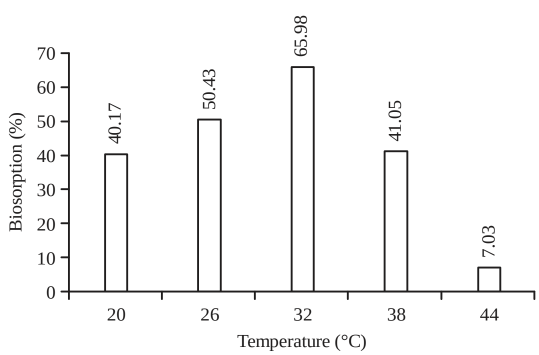

Fig. 4: Effect of temperature on biosorption of lead by living biomass of *P. oxalicum* (Incubation period 7 days and pH 7.0)

which was increased to 32°C by 65.98. In 38 and 44°C, biosorption of lead was decreased to 41.04 and 07.03% absorption (Fig. 4). Optimum temperature was recorded at 32°C for biosorption of lead by *P. oxalicum*.

The absorption of lead and other heavy metals by the genus of *Penicillium* was previously considered. In a mixture of metal ions, *P. oxalicum* absorbed the 27% lead from a live biomass and 23% lead from dead biomass[30]. While in the present study, only lead metal ions were treated with a live

Table 2: Effect of physical and chemical pretreatment on *P. oxalicum* for biosorption of lead

Pretreatments	Biosorption (%)
Untreated control biomass	65.75
Autoclaved	66.99
NaOH	87.59
Formaldehyde	86.35
Detergent	89.82

and pretreated dead biomass of *P. oxalicum* and maximum biosorption was recorded 65.98% (alive biomass) and 89.82% (dead biomass). The biosorption using living biomass of *P. austurianum* was found 75.57-94.21% while in case of dead biomass[31] it was 44.47-98.85. Apart from the genus of *Penicillium and Aspergillus* are also the excellent tool for heavy metal removal. Living biomass of *Aspergillus niger* RH17 and *A. niger* RH18 absorbed 92.04 and 93.09%, respectively[32]. The growth conditions of microbes are important to study for the better absorption of metal ions to the absorbent. In the present study incubation time, pH and temperature were found to affect the absorption of lead. pH is the factor which strongly affects the activity of biomass to absorb or adsorb the metal ion. In the present study when pH was increased from 3-7 the biosorption simultaneously increase after the pH biosorption was decreased. So the favorable pH was found 7 which is also recorded by Oso *et al.*[21]. It is found with *Penicillium* sp. MRF-1 that biosorption was increased from pH 2-6 and after that biosorption was stable[26]. pH 5 was found suitable with *P. purpurogenum*[33] and *P. simplicissimum*[17] too. For the *P. austurianum* pH 4 was found favorable for the living biomass and pH 7 for the dead biomass to absorb lead from aqueous solution[31]. Similarly, temperature also influences the activity of biomasses to absorb metal ions. In the present study, biosorption was increased from 20-32°C and after that lead biosorption was decreased. Biosorption was increased by increasing the temperature and it was recorded up to 60°C too[26]. Studied with the *Aspergillus niger* RH17 and *A. niger* RH18 28°C was found perfect[32].

The biosorption of lead by untreated *P. oxalicum* was recorded 2.65 mg g^{-1} biomass. While, 2.70, 3.53, 3.48 and 3.62 mg g^{-1} biomass of lead was absorbed biomass treated with autoclaving, NaOH, formaldehyde and detergent (Table 2). The maximum biosorption was found with detergent treated biomass and least biomass was recorded with untreated fungal biomass (Fig. 5). Highest Biosorption of lead (89.82%) was observed with detergent as shown in Table 2. The fungal cell wall contains chitin, glucans, mannans some polysaccharides and proteins. Chitin of fungal cell wall is a good biosorbent for heavy metals and pollutants, apart from this fungal cell wall also contain some functional groups which help to absorb pollutants[34]. Biosorption of heavy metals by fungi can be change by various pre-treatment methods,

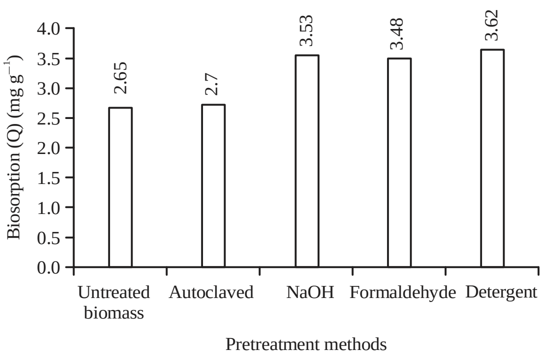

Fig. 5: Biosorption of lead in mg g^{-1} biomass of physically and chemically pretreated *P. oxalicum*

these methods added functional groups to fungal cell wall which may able to absorb more metal ions as compared to untreated biomass[35]. In the present study physical treatment such as heat treatment by autoclaving, chemical treatment such as treatment with sodium hydroxide, detergent and formaldehyde were applied. Detergent pretreatment (3.62 mg g^{-1} biomass) followed by sodium hydroxide (3.53 mg g^{-1} biomass) treatment biomass were found as maximum biosorption capacity while untreated biomass (2.65 mg g^{-1} biomass) showed the least capacity. Studied performed with the *Penicillium lanosa-coeruleum*, heat, sodium hydroxide and detergent pretreatment increased the biosorption capacity of fungal biomass[36]. The fungal biomass absorbed 24.2 (heat), 24.2 (NaOH) and 24.16 mg g^{-1} lead and the maximum increase of biosorption capacity were recorded 27% as compared to the untreated biomass. Similarly, in the present study, all pretreatment methods increased the biosorption capacity and the maximum increased in the capacity with detergent pretreatment was found 34.24% as compared to the untreated biomass. Enhancement of the biosorption capacity using sodium hydroxide, detergent and formaldehyde were also observed in *Penicillium verrucosum*[37]. Apart from *Penicillium* sp., some other fungal species like *Mucor rouxii* was studied for the removal of lead using physical and chemical pretreatment[38]. The *Mucor rouxii* absorbed 16.62, 14.44 and 10.02 mg g^{-1} lead with sodium hydroxide, detergent and autoclaved treated biomass. Similarly, *Rizopus stolonifer* showed 12.10 mg g^{-1} biosorption of lead by sodium hydroxide treated biomass[39].

The fungus used in the present study was isolated from lead-contaminated industrial effluent[19]. Similarly, *Penicillium* sp. MRF-1 was isolated from mine soil having the high concentration of lead was able to absorb the lead metal ions[26]. Thus, the microbes isolated from metal contaminated sited were more efficient to tolerate and absorb the heavy metals simply due to the adaptation[40,41]. Some most recent research study also supporting present study in view of *Penicillium* species were used for removal of various heavy metals from polluted water furthermore these fungi were isolated from contaminated wastewater. *Penicillium oxalicum*, *P. chrysogenum*, *P. expansum* and *P. aurantiogrisem* has been analyzed for heavy metal resistant against cadmium and mercury. The *P. aurantiogrisem* tolerate 1000 mg L^{-1} $HgCl_2$ and $CdCl_2$ and used for the uptake of same metal ion[42]. A *Penicillium* sp. was used for the removal of lead and cadmium from wastewater in where a maximum 81.99% removal of lead was recorded in a single system where only lead metal ion was used[43]. Recently, *P. chrysogenum* XJ-1 was utilized for removal of copper and chromium from polluted water[44].

CONCLUSION

The *P. oxalicum* tolerate high concentration of lead nitrate as well as it removes the lead from aqueous solution both from live and pretreated biomass. The biosorption potentiality of living biomass was affected by the different growth parameters while lead uptake by pretreated biomass was affected by various pretreatment procedures. This finding provides an alternate of removal of lead pollution. The present procedure is safe and economic further it is eco-friendly too. The heavy metal contaminated effluent found habitat of potential fungi to control the pollution.

SIGNIFICANCE STATEMENT

This study gives a detail account about the biosorption capacity of *Penicillium oxalium* for the removal of lead metal pollution. This microbial tool was isolated from lead polluted industrial wastewater hence resistant to the metal ion. The lead resistant capacity along with the biosorption of lead from live and pretreated biomass was explore with this study. This microbial technology is an eco-friendly method also. In this way, this very important tool can be utilized for the removal of lead metal ion from aqueous solution and can be taken as a component if any microbial based process intended for the control of lead pollution.

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