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RESEARCH ARTICLE - BIOLOGICAL SCIENCES

Biosorption and Bioaccumulation of Copper and Lead by Heavy Metal-Resistant Fungal Isolates

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Abstract Microorganisms play an important role in the bioremediation of heavy metal-contaminated wastewater and soil. In this research, isolation of heavy metal-resistant fungi was carried out from wastewater-treated soil samples of Hudiara drain, Lahore. The purpose of the present investigation was to observe fungal absorption behavior toward heavy metal. The optimum pH and temperature conditions for heavy metal removal were determined for highly tolerant isolates of *Aspergillus* spp. along with the initial metal concentration and contact time. Biosorption capacity of *A. flavus* and *A. niger* was checked against Cu(II) and Pb(II), respectively. The optimal pH was 8–9 for *A. flavus* and 4–5.4 for *A. niger*, whereas optimal temperature was 26 and 37 °C, respectively. Moreover, the biosorption capacity of *A. flavus* was 20.75–93.65 mg g^{-1} for Cu(II) with initial concentration 200–1400 ppm. On the other hand, biosorption capacity of *A. niger* for Pb(II) ranged from 3.25 to 172.25 mg g^{-1} with the same range of initial metal concentration. It was also found that equilibrium was maintained after maximum adsorption. The adsorption data were then fitted to Langmuir model with a coefficient of determination >0.90. The knowledge of the present study will be helpful for further research on the bioremediation of polluted soil.

Keywords *Aspergillus* spp. · Biosorption · Copper · Heavy metals · Lead

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1 Introduction

Soil is one of the most important components of urban and rural environments, and its quality is maintained through the processes of land management. All over the world, heavy metals soil contamination of agricultural sites is a core problem on defense-related and industrial sites [1]. Human actions like mining industries and metal manufacture with storage, transportation and disposal problems are the main sources of pollution [2]. Plant and soils get accumulated with heavy metals which could have a negative effect on the physiological activities of plants, and these activities determine the reductions in plant growth, dry matter accumulation and yield [3]. Excessive accumulation of heavy metal in soil is poisonous to living organisms including the human beings.

As pollution by heavy metals is a serious environmental issue with severe health effects, its remediation is essential. The physical and chemical methods of remediation are time-consuming and expensive; hence, a biological approach provides an alternative solution of the problem [4]. Microbes are naturally capable of degrading wastes and have a capability to survive under stress conditions. Microbes like bacteria, algae and fungus have been effectively used for heavy metals removal as adsorbing agents [5–8]. In heavy metal-polluted environments, microbial populations become resistant to toxic concentration of heavy metals by adapting to high concentrations. Different species of *Aspergillus* have been reported as efficient nickel and chromium reducers [9]. Recently, several filamentous fungi (FF) species are found to be useful for biological treatment of the sludge (settling, dewatering, organic compound degradation) under controlled operating conditions [10]. For the removal of heavy metals from solutions, fungi have identified as potential biomass that belong to the genera *Penicillium* and *Rhizopus*. Zafar et al. [11] reported promising biosorp-

tion for Cd and Cr by two filamentous fungi, *Aspergillus* sp. and *Rhizopus* sp., isolated from agricultural soil which was contaminated with metal.

For the reclamation of polluted sites, the microorganisms' response toward heavy metals is very important. The present research involved the characterization of bioaccumulation of copper and lead by the isolated fungal strains, thus evaluating the remediation of these metals. Effect of temperature, pH, contact time and initial metal concentration was evaluated.

2 Materials and Methods

2.1 Sample Collection

For the present investigation, soil samples were collected from peri-urban agricultural areas along the Hudiara drain, Lahore. Eight random soil samples were collected from eight different agricultural fields of study area. Sampling was done at well-defined depth not exceeding 5 cm. Later samples were mixed together to form the composite sample. Spatula and digging tools were used to gather adequate amount of soil which was stored in plastic bags for transportation to the laboratory for further analysis.

2.2 Heavy Metals Analysis of Soil Samples

In the collected soil samples, mean concentration of copper and lead was analyzed. For heavy metals analysis, each soil sample (1 g) was taken in the conical flask (50 ml), 10 ml of HNO_3:$HClO_4$ (1:2) solution (50 ml) added and heated for half an hour. Solutions were filtered through Whatman 1 filter paper, and distilled water was added to make volume up to 50 ml. Soil samples were digested in triplicate and analyzed for Cu and Pb. The blank was prepared for quality assurance of samples. The blank sample contained 10 ml of HNO_3:$HClO_4$ (1:2) solution and heated for half an hour, and volume was made 50 ml by adding distilled water. For the determination of heavy metals, the atomic absorption spectrophotometer PerkinElmer (Analyst 700) was powered on and warmed up for 30 min. After the heating of cathode lamp, the air acetylene flame was ignited and instrument was calibrated or standardized with different working standards [12].

2.3 Isolation of Fungi

Potato dextrose agar (PDA) media (1 l) were used for the isolation of fungi. From selected soil samples, *Aspergillus flavus* and *Aspergillus niger* were isolated by using soil dilution method [12] and preserved in the laboratory of Environmental Mycology and Ecotoxicology, Fatima Jinnah Women University, Rawalpindi, for further detail investigation of heavy metal biosorption analysis.

2.4 Biosorption Analysis

The *A. flavus* and *A. niger* biomass was prepared in potato dextrose broth (PD broth). The flasks were agitated on a rotatory shaker for 3–4 days at 150 rpm and at 30 °C. After 3–4 days, thick bed of fungal biomass was developed which was further used for biosorption experiments. To explore the biosorption of the *Aspergillus* isolates to the heavy metals, varying initial heavy metal concentrations with optimal culture conditions were used. Each growth medium was amended with $CuSO_4$ and Pb $(NO_3)_2$ with different concentrations ranging from 200 to 1400 ppm.

2.5 Effect of pH and Temperature on Fungal Biomass Adsorption

The fungal isolates were inoculated into a series of 250-ml conical flasks containing metals solution. The pH was varied from 4 to 9. The pH of the medium was adjusted using dilute HCl or NaOH [13]. To simultaneously search for optimal temperature, for each pH, the represented cultures were incubated at different temperatures (22–37 ° C). The cultures were shaken in a rotary shaker (120 rpm) in a temperature-controlled water bath. After 20 hours, the fungal biomasses were filtered in order to separate the biomasses from media. The biomass on the filter paper was then dried by the process of drying on hot plate in order to absorb the moisture content at temperature of 120 °C for about 3–4 days.

2.6 Removal of Copper and Lead at Different Concentrations

Dried and powdered dead biomass (0.5 g) was inoculated into 100 ml of metal solution containing 200–1400 ppm $CuSO_4$ and Pb $(NO_3)_2$ in distilled water for single-metal system. The flasks were kept on the rotary shaker at 150 rpm at 30 °C with contact time of 50 h. The content of supernatant was determined by using atomic absorption spectrophotometer. The experiment was done in duplicate, and the amount of metallic ion biosorbed per gram of biomass (q) and the efficiency of biosorption (E) were calculated using Eqs. 1 and 2 [14].

$$q = \left(\frac{C_\mathrm{i} - C_\mathrm{f}}{m}\right) V \tag{1}$$

where $\mathbf{q}$ = mg of metal ions uptake per gram biomass (mg g^{-1}); $\mathbf{C_i}$ = initial concentration of the metallic ions (mg l^{-1}); $\mathbf{C_f}$ = final concentration of metallic ions (mg l^{-1}); $\mathbf{m}$ = dried mass of the biosorbent in the reaction mixture (g); and $\mathbf{V}$ = volume of reaction mixture (ml).

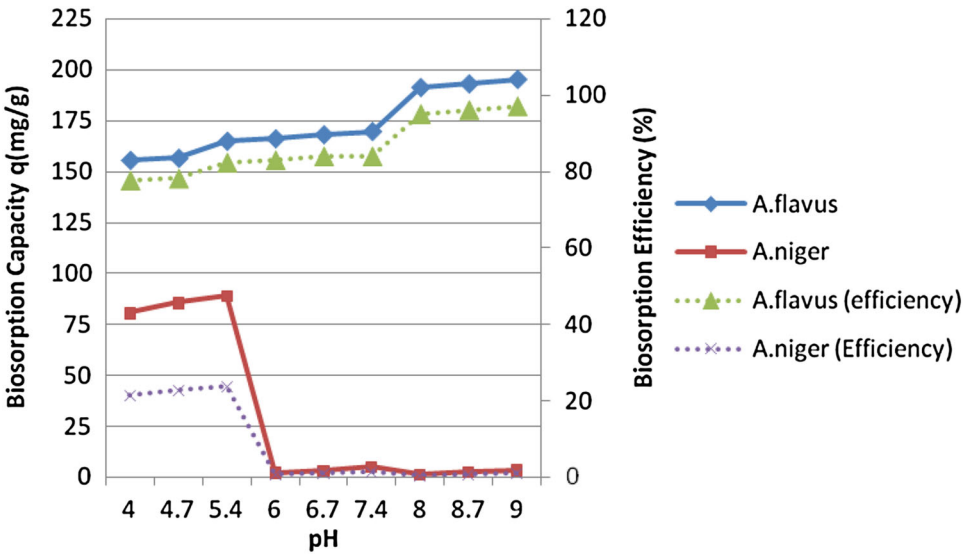

Fig. 1 Effect of pH on the biosorption of Cu(II) and Pb(II) with efficiency

$$E = \left(\frac{C_i - C_f}{C_i}\right) \times 100 \tag{2}$$

2.7 Isotherm Assessment

The uptake of heavy metals ion by inactive cell of fungi was analyzed by the adsorption isotherm Langmuir model, and the amount of metal bound by the biosorbents was calculated as follows (Eq. 3).

$$q_e = \frac{bQ_m C_{eq}}{1 + bC_{eq}} \tag{3}$$

where $\mathbf{q}$ = metallic ions adsorbed per unit of weight of adsorbents (mg g^{-1}), $\mathbf{Q_m}$ = maximum possible amount of metallic ions adsorbed per unit of weight of adsorbents (mg g^{-1}), $\mathbf{b}$ = constant related to the affinity of binding sites for metal ions, $\mathbf{C_{eq}}$ = equilibrium concentration (mg l^{-1}).

3 Results and Discussion

In the collected soil samples, the copper and lead mean concentration was 94.5 and 68.4 mg kg^{-1}, respectively. The results of biosorption analysis with the effect of pH, temperature, initial metal concentration and contact time are given below.

3.1 Effect of pH

Biomass of *A. flavus* and *A. niger* was exposed to Cu(II) and Pb(II), respectively. *Aspergillus flavus* followed an increasing trend and exhibited maximum sorption capacity for Cu(II) in the pH range 8–9 with maximum efficiency 97 %, while *A. niger* exhibited maximum sorption capacity for Pb(II) at pH range 4–5.4 with biosorption efficiency 21.5 %, and above this pH, substantial decline in metal uptake was evidenced which represented the pH factor being highly sensitizing element (Fig. 1).

Hasan et al. [15] reported maximum nickel removal in the pH range of 4.5–5.5. The sorbent surface chemistry and metal chemistry in solution formed the basis of variation in nickel adsorption at various pH. Low pH limits biosorption of Cu(II) ions on the surface of fungal biomass, most probably due to competition effects with oxonium (hydronium) ion to some extent in the biosorption mechanism and the ion exchange between metallic species [16]. In similar findings by earlier investigators, it has been attributed to protonation or poor ionization of acidic functional group of cell wall at low pH, inducing a weak complexation affinity between the cell wall and the metal ions [17]. At higher pH, the reduced uptake of metal ions by fungus might be due to the accumulation of metal ions inside the intrafibular capillarities of the cell walls or inside the cells by a combined sorption microprecipitation mechanism; therefore, at higher pH, biosorption experiments are meaningless [18].

3.2 Effect of Process Temperature

Figure 2 shows that the effect of temperature on the biosorption of heavy metal ions was significant. The heavy metal caused a decline in the uptake of Cu(II) by *A. flavus*. The maximum biosorption capacity was 81.6 mg g^{-1} and efficiency 40.8 % at 26 °C, while *A.niger* followed the declining trend with maximum biosorption capacity and efficiency of Pb(II) 91mg g^{-1} and 45.5 %, respectively, at 37 °C.

Temperature can cause chemical moieties ionization and affects the cell wall's stability and its configuration. The binding sites on isolated bacterial and fungal species may get

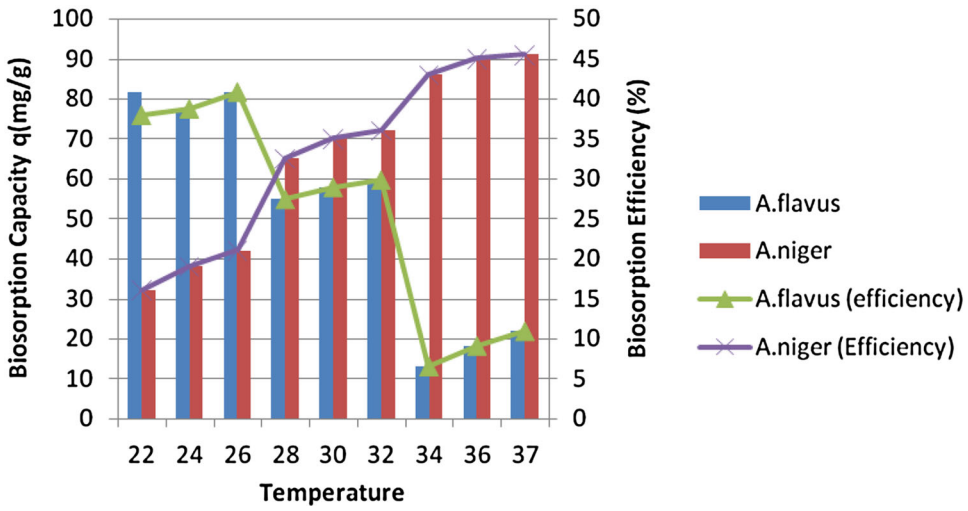

Fig. 2 Effect of temperature on the biosorption of Cu(II) and Pb(II) with efficiency

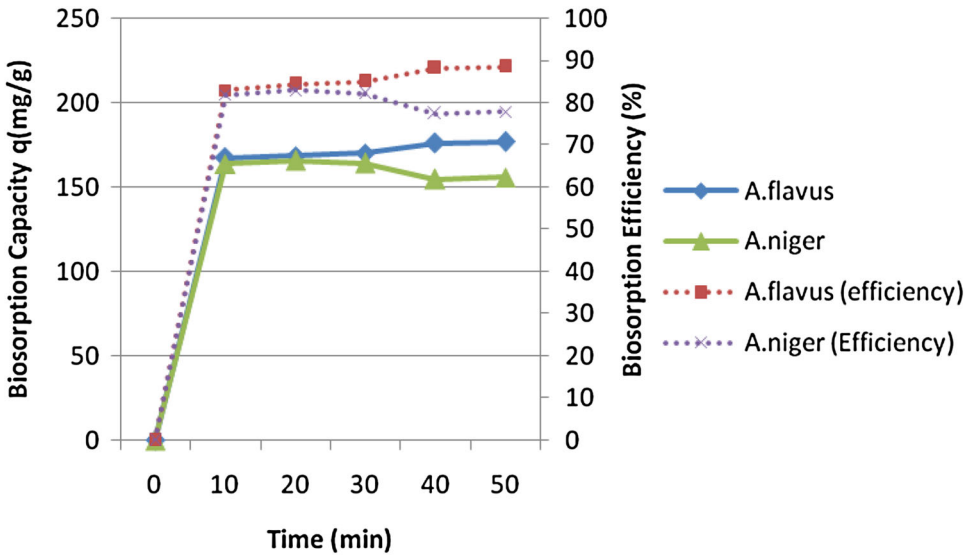

Fig. 3 Effect of contact time on the biosorption of Cu(II) and Pb(II) with efficiency

affected simultaneously causing reduction in the removal of heavy metals. Since the processes responsible for the removal of heavy metals are mostly physiochemical in nature, the temperature impact on energy-independent mechanisms is negligible [19]. Goyal and Banerjee [20] have reported similar results for the bioaccumulation of Cr(VI) by *S. equisimilis* and *A. niger*.

3.3 Effect of Contact Time

Time course profiles for the adsorption of Cu(II) and Pb(II) by *A. flavus* and *A. niger* revealed that almost 83 and 82 % removal of heavy metal ions and saturation level of total biomass were recorded during the first 10 min (Fig. 3). The adsorption equilibrium or plateau level gradually reached within 15–20 min for both Cu(II) and Pb(II) ions. The Cu(II) uptake by *A. flavus* kept on increasing gradually after 30 min, while adsorption of Pb(II) followed a gradual declining trend after 30 min.

The findings verify two phases of biosorption, rapid initial uptake due to adsorption by the surface and subsequent slow uptake due to metal ions' membrane transport into cytoplasm of cell or slow intracellular diffusion or reduced permeability of cell wall [21]. Similar results were obtained by Chatterjee et al. [22], while in some other studies, single-step uptake has been suggested for different biosorbent [23].

3.4 Effect of Initial Concentration of Metal Ions

The graphical presentation for the effect of initial metal ion concentrations on biosorption capacity of the test fungi is depicted in Fig. 4. The temperature was maintained at about 30 °C. The batch was carried out for more than 50 min. The biosorption capacity of *A. flavus* was 20.75–93.65 mg g^{-1}

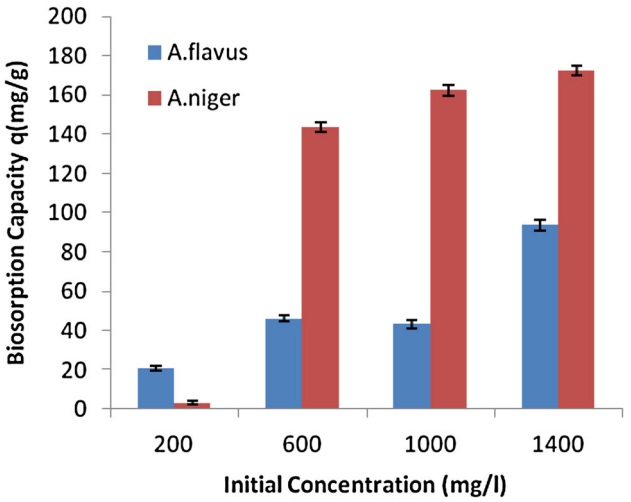

Fig. 4 Effect of different metal concentrations on biosorption capacity of *A. flavus* and *A. niger*

Table 1 Isotherm model parameters for the biosorption of metal ions

Metal ions	q_m (mg g^{-1})	b (mg l^{-1})	R^2
Cu(II)	963	0.002	0.98
Pb(II)	1142	0.001	0.926

with initial concentration 200–1400 ppm. On the other hand, the biosorption capacity of *A. niger* for Pb ranged from 3.25 to 172.25 mg g^{-1} with the same range of initial metal concentration mentioned above.

The results of present findings clearly indicate that the sorption capacity increased and reached a saturation value as the metal ion concentration increased in aqueous medium. This assessment is in line with previously reported data on metal ion sorption by many other similar studies [24–26]. At high metal ion concentration, sorption of ions is more than at low concentration, where more binding sites are free for interaction [27].

3.5 Isotherm Assessment

Isotherm studies are basic requirement to design biosorption application procedures. For this purpose, the empirical model, i.e., Langmuir [28] for single solute system was employed to describe the biosorption equilibria of the test fungus. The parameter resulted from the Langmuir plots for Cu(II) and Pb(II) ions by the test fungus is presented in Table 1.

The plot of $1/q_e$ versus $1/C_{eq}$ in various initial concentrations range (200–1400 ppm) of metal ions was found to be linear indicating the applicability of classical Langmuir adsorption isotherm to single metal ion solution (Fig. 5a, b).

The coefficients of determination (R^2) were more or less greater than 0.90, indicating that models adequately described the experimental data of all metal ions biosorption. The maximum capacity (q_m) was determined from the Langmuir isotherm point calculated by the model in function of the experimental values of q_e, which showed a linear tendency among the observed and predicted values. The Langmuir isotherm "b" the stability complex formed between metals ions and fungal cell wall under specific experimental conditions clearly demonstrated the small values, which meant greater affinity of biomass for copper and lead ions. A good metal sorbent in general should have a high q_m as well as low b value [29]. Javaid et al. [14] also observed a classical linear Langmuir adsorption isotherm with the value of coefficient of determination >0.90 for four different heavy metals. Similar results have been reported by [30].

In biosorption analysis, it has been commonly observed that pH, temperature, contact time and initial concentration of metal solution strongly influence the fungal biosorption process cumulatively [31,32]. Several researchers have also investigated the effect of these parameters on the biosorption of similar heavy metals by *Aspergillus* sp. using different biomass [12,33,34] and found similar results with the present study. According to previous researches and the present study, it is estimated that these data are appropriate for envi-

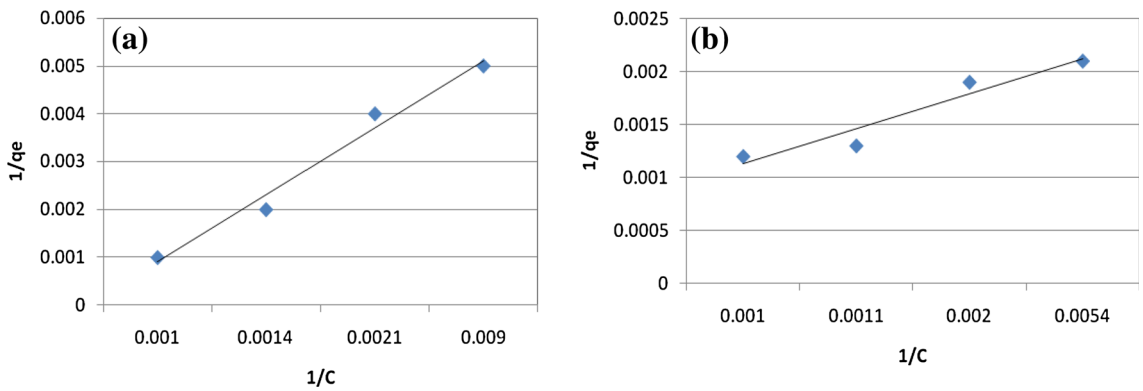

Fig. 5 Linearized Langmuir adsorption isotherm of **a** Cu(II) by *A. flavus* and **b** Pb(II) by *A. niger*

ronmental studies. The biosorption is an efficient process to remove contamination in environment.

4 Conclusion

The purpose of the present research was to find the biosorption characteristics of selected metal-resistant fungal isolates for the removal of copper and lead ions. Experiments were performed as a function of pH, temperature, initial metal ion concentration and time. Fungal strains showed a remarkable metal adsorption capacity over a wide range of temperature and basic pH. The Langmuir adsorption model was used for the mathematical description of the biosorption of copper and lead ions. These fungal isolates may be employed in future for metal remediation from wastewater and heavy metal-contaminated soils.

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