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# Biosorption of Cadmium, Lead, Mercury, and Arsenic Ions by the Fungus *Penicillium purpurogenum*

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## ABSTRACT

The potential use of the fungus *Penicillium purpurogenum* to remove cadmium, lead, mercury, and arsenic ions from aqueous solutions was evaluated. Biosorption of heavy metal ions reached equilibrium in 4 h. Heavy metal ions binding by *Penicillium purpurogenum* was clearly pH dependent. Heavy metal loading capacity increased with increasing pH under acidic conditions, presumably as a function of heavy metal speciation and due to the $H^+$ competition at the same binding sites. The adsorption of heavy metal ions reached a plateau value at around pH 5.0. The maximum adsorption capacities of heavy metal ions onto the fungal biomass under noncompetitive conditions were 35.6 mg/g for As(III), 70.4 mg/g for Hg(II), 110.4 mg/g for Cd(II) and 252.8 mg/g for Pb(II).

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Their adsorption behavior can be described at least approximately with the Langmuir equation. The competitive adsorption capacities of the heavy metal ions were 3.4 mg/g for As(III), 15.8 mg/g for Hg(II), 13.1 mg/g for Cd(II), and 41.8 mg/g for Pb(II) at 50 mmol/L initial concentration of metal ions. The same affinity order on a molar basis was observed under noncompetitive and competitive adsorption conditions, which was as follows: Pb(II) > Cd(II) > Hg(II) > As(III). The equilibrium loading capacity of Pb(II) was greater than that of other metal ions. This fungal biomass showed a preference for binding Pb(II) over Cd(II), Hg(II), and As(III). Elution of heavy metal ions was performed using 0.5 M HCl. The fungus *Penicillium purpurogenum* could be used for ten cycles for biosorption.

*Key Words:* Cadmium(II); Lead(II); Mercury (II); Arsenic(III); Heavy metals; Fungal biomass; Biosorption; *Penicillium purpurogenum.*



## INTRODUCTION

Contamination of water sources (e.g., rivers, lakes, and underground-water) by heavy metal ions is important due to their toxic effects on human physiology and other aquatic life, even at very low concentrations. Removal of heavy metal ions from wastewaters is essential.[1] Heavy metal ions are commonly removed by chemical precipitation, ion-exchange, solvent extraction, adsorption and reverse osmosis processes.[2–5] These methods have several disadvantages, such as unpredictable metal ions removal, high material cost, and the generation of toxic sludges that are often difficult to dewater and also require extreme caution in their disposal. These methods become inefficient and expensive, especially when the concentration of the heavy metal ion is low on the order of 1 to 100 mg/L. Due to these disadvantages, there is a need for novel treatment methods for the removal of heavy metal ions from wastewater. Application of a biosorption method to the treatment of wastewaters containing heavy metal ions has been given significant attention recently by the research community.[6–10]

In the concept of biosorption, several chemical processes may be involved, such as adsorption, ion exchange, and covalent bonding with the biosorptive sites of the microorganisms including carboxyl, hydroxyl, sulfydryl, amino, and phosphate groups.[11] Fungi have been recognized as a promising class of low-cost adsorbents for removal of heavy metal ions from aqueous waste streams.[12–15] The cell walls of fungi are composed of polysaccharides, proteins, and lipids that contain reactive functional groups

with potential metal binding capacities.[16] Different species of fungi vary with respect to the chemical composition of the cell wall, resulting in significant variations between species, strains, and even different cell types of the same organism.[17–20]

The objective of our study was to determine the ability of soil fungi *Penicillium purpurogenum* to accumulate cadmium, lead, mercury, and arsenic at different concentration levels and under various pH values of the solution phase. *Penicillium purpurogenum*, which was isolated from soil, was chosed to represent common soil fungi in areable lands and forest soils. Finally, competitive adsorption of heavy metal ions and elution-reuse of fungal biomass was also evaluated.

## MATERIALS AND METHODS

### Cell Line and Medium

The fungus used was *Penicillium purpurogenum* (455), which was isolated from soil. The microorganism was maintained by subculturing on potato-dextrose-agar plates. Fungal biomass was cultivated in liquid medium using the shake flask method. Spores and mycelium from the potato-dextrose-agar spread-plate cultures were transferred to 250 mL Erlenmeyer flasks containing 100 mL of potato-dextrose broth. Once inoculated, flasks were incubated on an orbital shaker at 120 rpm for 7 days at $22 \pm 2$ °C. After incubation, the biomass was harvested from the medium by filtration and washed several times with distilled water. It was then dried at 90 °C in an oven for 24 h. The dried sample was then ground, using a blender, and sieved to pass through a 100-mesh sieve to obtain uniform particle size.

### Biosorption of Heavy Metal Ions

Biosorption of Cd(II), Pb(II), Hg(II), and As(III) from synthetic wastewaters containing single metal ions was investigated in batch experiments. Stock metal ion solutions (1000 mg/L) were prepared using nitrate salts of the metals obtained from Fisher Scientific Ltd. The effect of pH on the biosorption capacity of the fungal biomass with heavy metal ions was investigated in the pH range of 2.0 to 7.0 at 20 °C. A 100-mg sample of the biomass was washed twice with 0.01 M HCl using a centrifuge to remove any soluble materials or metal ions present on the biomass. Each heavy metal ion (100 mg/L) was prepared in 150 mM NaCl solution (50 mL) and dry fungal

biomass was transferred to this medium and agitated magnetically at 100 rpm. All water used in the experiments was purified using a Barnstead (Dubuque, IA, USA) ROpure LP reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731), followed by a Barnstead D3804 NANOpure organic/colloid removal and ion exchange packed-bed system. The resulting purified water had a specific resistance of 18 MΩ. After swelling the fungal biomass, the pH of the solution was adjusted with 0.1 M NaOH or 0.1 M HCl. During the biosorption experiment, the pH of the medium was controlled with a pH probe. The effect of the initial heavy metal ion concentration on the biosorption was studied at pH 5.0, as described, except that the concentration of each heavy metal species in adsorption medium was varied between 10 and 750 mg/L. After biosorption, the biomass was separated by centrifugation from the medium. Analysis for heavy metal content in the supernatants and controls were performed by graphite furnace atomic absorption spectrophotometer (AAS 5EA, Carl Zeiss Technology, Zeiss Analytical Systems, Jena, Germany). Hg(II) concentration was determined by AAS connected with a hydride generator. The instrument response was checked periodically with known metal solution standards. Each experiment was performed in triplicate for quality control and statistical purposes. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples to determine the margin of error. The amount of adsorbed heavy metal ions per gram of biomass was obtained by using the following expression.

$$Q = [(C_o - C)V]/m \quad (1)$$

Where Q is the amount of metal ions adsorbed on the biomass (mg/g). $C_o$ and C are the concentrations of the metal ions in the solution (mg/L) initially and after biosorption. V is the volume of the medium (L) and m is the amount of the fungal biomass (g).

Competitive adsorption of heavy metal ions from their mixture was also investigated in batch-wise manner. A 100-mg sample of the biomass was washed twice with 0.01 M HCl using a centrifuge to remove any soluble materials or metal ions present on the biomass. The biomass was then resuspended in 50 ml of metal solution containing 50 mmol/L of each metal ion at pH of 5.0. The adsorption experiment was carried out at room temperature, in the flasks stirred magnetically at 250 rpm for 4 h. After adsorption equilibrium, the concentration of the metal ions in the supernatant was measured by AAS.

To evaluate the reusability of the dry fungal biomass, the biosorption-elution of biosorbed metal ion-regeneration of biomass cycle was repeated ten

times by using the same fungal biomass. Elution of heavy metal ions was performed using a 0.5 M HCl solution. Fungal biomass carrying 31.8 mg Cd(II)/g; 98.1 mg Pb(II)/g; 20.8 mg Hg(II)/g; and 7.7 mg As(III)/g were placed in this desorption medium and stirred at 250 rpm for 2 hours at room temperature. After eluting, the biosorbed metal ions biomass was regenerated by washing with deionized water. The final metal ion concentration in the aqueous phase was determined by using an AAS. The desorption ratio was calculated from the amount of metal ion initially loaded on the biosorbents and the final metal ion concentration in the desorption medium.

## RESULTS AND DISCUSSION

### Biosorption Time

The biosorption times of heavy metal ion species on the fungal biomass *Penicillium purpurogenum* were obtained by following the decrease of the concentration of Cd(II), Pb(II), Hg(II), and As(III) within the adsorption medium with time. The amount of adsorbed metal ions was high at the beginning of adsorption and saturation levels were completely reached at about 4 h for all metals ions (Fig. 1). After this equilibrium period, the amount of adsorbed metals ions did not significantly change with time. This trend in binding of metal ions suggests that the binding may be through interactions with functional groups located on the surface of the fungal biomass. Exposure of a microbial biomass to metal ions results, first of all, in the rapid binding of metal ions to negatively charged sites on the cell wall. This binding process is very fast. The strength and extent of this cell wall–metal interaction will depend on the chemical constitution of the cell wall, the distribution and number of ligand groups, and the affinity of the particular metal ions for these groups. This process is described as surface binding.

A wide range of equilibrium adsorption times are reported in the research literature with various biosorbent systems. For example, Kapoor et al found an 8 h equilibrium biosorption time in their lead, cadmium, copper, and nickel binding kinetic studies, in which they used the fungus *Aspergillus niger.*[21] Sar and D'Souza investigated uranium biosorption with a living and lyophilized form of *Pseudomonas* strain. They reported that equilibrium was achieved in about 2 h.[22] Saglam et al studied the biosorption of mercury on *Phanerochaete chrysosporium* immobilized carboxymethylcellulose. They reported that the biosorption equilibria were established in approximately 1 h.[23] To determine how the exposure time affects the amount of chromium

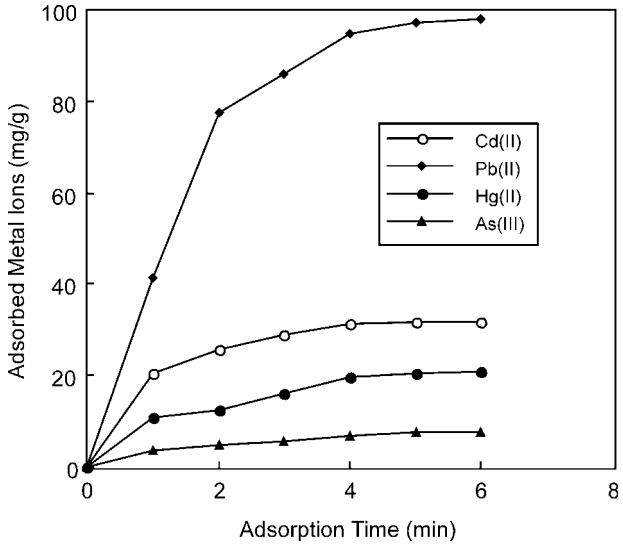


***Figure 1.*** Biosorption times of metal ions on the fungal biomass from aqueous solutions. Initial concentration of heavy metal ion, 100 mg/L; pH, 5.0; temperature, 20°C.



binding by an oat biomass, time-dependent experiments were performed.[24] Chromium bound rapidly to the oat biomass within a 5-min period, binding of Cr(III) increased as a function of time up to 120 min. Pagnanelli et al have studied copper and cadmium adsorption in single and multimetal systems on *Arthrobacter sp.* biomass. They reported that the system reached equilibrium conditions in about 30 min.[25] Tsezos et al studied uranium biosorption by *Rhizopus arrhizus.*[26] They showed that adsorption is rather slow; the biomass appear to be attained equilibrium only after 10 h. Sakaguchi and Nakajima studied the accumulation of uranium by immobilized persimmon tannin. They reported a 6 h equilibrium adsorption time.[27] Note that there are several parameters that determine the equilibrium adsorption time, such as agitation rate in the aqueous phase, physical properties of the adsorbent (e.g., protein and carbohydrate composition, surface charge density, topography, porosity, and surface area, etc.), amount of adsorbent, properties of the ions under investigation, initial concentration of ionic species, and the presence of other metal ions that may compete with the ionic species interest for the active binding sites. Therefore it is difficult to compare the adsorption rates reported.

## Effect of pH on the Biosorption Capacity

The pH is a critical parameter in biosorption because it influences the equilibrium by affecting the speciation of the metal ion(s) in solution, the concentration of competing hydrogen ions, and the chemistry of the active binding sites on the biomass. The fungal cell wall contains amino, carboxyl, thiol, sulfydryl, and phosphate functional reactive groups. The carboxyl and phosphate groups carry negative charges that allow the fungal cell wall components to be potential scavengers of metal ions. The maximum biosorption of heavy metal species on the biomass was observed at around pH 5.0. The amounts of adsorbed heavy metal ions on the dry fungal biomass at pH 5.0 were found to be 31.8 mg/g for Cd(II), 98.1 mg/g for Pb(II), 20.8 mg/g for Hg(II), and 7.7 mg/g for As(III) (Fig. 2). There was an increase in the adsorption amount of heavy metal ions per unit weight of fungal biomass with increasing pH from 2.0 to 5.0, but it seemed to reach a constant value at a pH greater than 5.0. At acidic pH (pH 2.0), protonation of the cell wall components adversely effected the biosorption capacity of the fungal biomass, but its effect became minor with increasing pH in the medium. With an

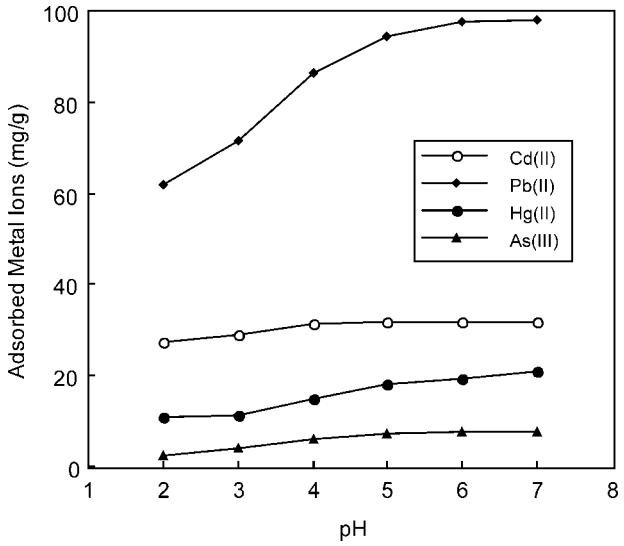


***Figure 2.*** Effect of pH on biosorption of heavy metal ions on the fungal biomass from aqueous solutions: initial concentration of heavy metal ion, 100 mg/L; temperature, 20°C.

increase in pH, the negative charge density on the cell surface increases due to the deprotonation of the metal binding sites and thus increases biosorption. Several researchers investigated the effect of pH on biosorption of heavy metals by using different kinds of microbial biomass. For example, the biosorption of Cu(II) by nonliving *Saccharomyces cerevisiae* was pH dependent and the maximum biosorption was obtained in the pH range of 5.0 to 7.0.[8]

## Adsorption Isotherms

The biosorption capacities of Cd(II), Pb(II), Hg(II), and As(III) onto the fungal biomass *Penicillium purpurogenum* are shown in Fig. 3. The biosorption capacity of the biomass first increased with increasing the equilibrium concentration of metal ions and reached a saturation value. These saturation values are around 500 mg/L for all heavy metal species. The fungal cell walls have a negative charge due to the arrangement of the carboxyl and phosphate groups of the cell walls. The phosphate-containing teichoic acid in the cell wall of the fungi is primarily responsible for metal binding.[28] The maximum adsorption capacities of the dry fungal biomass in the studied range

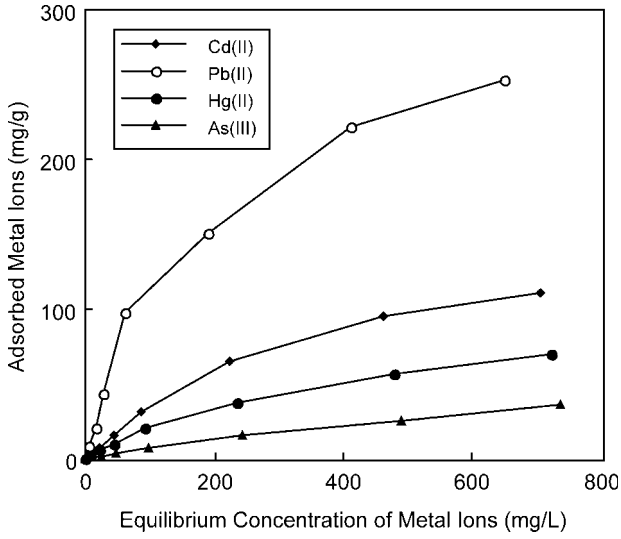


***Figure 3.*** Effect of initial metal ion concentration on biosorption of metal ions on biosorbents from aqueous solutions. pH, 5.0; temperature, 20°C.

were 35.6 mg/g for As(III), 70.4 mg/g for Hg(II), 110.4 mg/g for Cd(II), and 252.8 mg/g for Pb(II). The affinity order on mass basis was as follows: Pb(II) > Cd(II) > Hg(II) > As(III).

The adsorption capacities of the fungal biomass obtained with heavy metal species are comparable with the values reported in the previous studies. The biosorption capacity of the NaOH pretreated *Aspergillus niger* was 7.24 mg for lead, 3.43 mg for cadmium, and 2.66 mg for copper per g dry biomass.[14] The adsorption capacity of *R. arrhizus* was 78 mg for Fe(III), 71 mg for Pb(II), and 62 mg for Cd(II) per g dry biomass.[29] Sağ and Kutsal used *Zoogloea ramigera* microorganisms for heavy metal adsorption.[30] The maximum amounts of adsorption capacity achieved was 86.9 mg Pb(II) per g dry weight of microorganisms. It should be noted that the initial lead concentration was 200 mg/L. Sağlam et al studied the bisorption of inorganic mercury and alkylmercury species onto *Phanerochaete chrysosporium* mycellium. They obtained a maximum adsorption capacity for Hg(II) ions of 61 mg/g.[31] Say et al investigated the biosorption of heavy metal ions on the filamentous fungus *Phanerochaete chrysosporium*. Maximum adsorption capacities were found to be 23 mg/g for Cd(II), 69.7 mg/g for Pb(II), and 20.2 mg/g for Cu(II).[32] The comparison of the biosorption capacities of dried fungal biomass used in this work with those reported in previous research shows that these microorganisms are suitable for this purpose.



## Langmuir Adsorption Model

An adsorption isotherm is used to characterize the interaction of the each metal ion with the adsorbents. This provides a relationship between the concentration of metal ion in the solution and the amount of metal ion adsorbed on to solid phase when the two phases are at equilibrium. The Langmuir adsorption model assumes that the molecules are adsorbed at a fixed number of well-defined sites, each of which can only hold one molecule. These sites are also assumed to be energetically equivalent, and distant from each other so that there are no interactions between molecules adsorbed to adjacent sites.

During the batch experiments, adsorption isotherms were used to evaluate adsorption properties. For the systems considered, the Langmuir model was found to be applicaple for interpreting metal ion biosorption by fungal biomass. The Langmuir adsorption isotherm is expressed by Eq. 2. The corresponding transformations of the equilibrium data for metal ion gave rise to a linear plot, indicating that the Langmuir model could be applied in these

systems and described by the equation:

$$Q = Q_{max} \cdot b \cdot C_{eq} / (1 + b \cdot C_{eq}) \quad (2)$$

Where Q is the concentration of bound metal ion in the biosorbent (mg/g), $C_{eq}$ is the equilibrium metal ion concentration in solution (mg/L), b is the Langmuir constant (L/mg) and, $Q_{max}$ is the maximum biosorption capacity (mg/g). This equation can be linearized so that

$$1/Q = [1/(Q_{max} \cdot b)][1/C_{eq}] + [1/Q_{max}] \quad (3)$$

The plot of $1/C_{eq}$ vs. 1/Q was employed to generate the intercept of $1/Q_{max}$ and the slope of $1/Q_{max} \cdot b$. The maximum capacity ($Q_{max}$) data for the adsorption of metal ion was obtained from experimental data. The correlation coefficient ($R^2$) was 0.99 for all metal ions studied here, indicating that the Langmuir adsorption model can be applied in this biosorbent system. The parameters $q_{max}$ and $K_d$ were determined by nonlinear regression with commercially available software and are shown in Table 1. It must be noted that the standard deviation of the values determined by regression analysis is comparatively low. For the fungal biomass used in the adsorption tests, the order of $q_m$ value for both cases is as follows: Pb(II) > Cd(II) > Hg(II) > As(III). It must be pointed out that the measured adsorption capacities observed are lower, according to the calculated adsorption capacities. This difference is due to the accessibility of the metal ions through the binding sites on the surface of fungal biomass.



## Competitive Biosorption

Competitive biosorption of Cd(II), Pb(II), Hg(II), and As(III) ions were also studied. The medium containing 0.5 mmol/L of each metal ion was incubated with the biomass in batch system. The competitive biosorption

***Table 1.*** Adsorption parameters of heavy metal ions on fungal biomass.

| Metal ions | $(q_m)_{measured}$ (mg/g biomass) | $(q_m)_{calculated}$ (mg/g biomass) | $K_d$ (mg/mL) |
|---|---|---|---|
| Pb(II) | 252.8 | 285.0 | $4.57 \times 10^{-3}$ |
| Cd(II) | 110.4 | 125.1 | $2.85 \times 10^{-3}$ |
| Hg(II) | 70.4 | 80.2 | $2.75 \times 10^{-3}$ |
| As(III) | 35.6 | 50.4 | $1.65 \times 10^{-3}$ |

***Table 2.*** Comparison of adsorption of heavy metal ions on the dry fungal biomass: concentration of each metal ion 50 mmol/L; pH, 5.0, T, 20 °C.

| | Non-competitive adsorption | | Competitive adsorption | |
|---|---|---|---|---|
| Ions | (mg/g) | (mmol/g) | (mg/g) | (mmol/g) |
| As(III) | 3.5 | 0.047 | 3.1 | 0.041 |
| Hg(II) | 20.8 | 0.103 | 19.0 | 0.095 |
| Cd(II) | 17.0 | 0.151 | 14.2 | 0.127 |
| Pb(II) | 98.1 | 0.473 | 80.1 | 0.387 |



capacities were 80.1 mg for Pb(II), 14.2 mg for Cd(II), 19.0 mg for Hg(II), and 3.1 mg for As(III) per g dry fungal biomass. As is seen in Table 2, the competitive biosorption capacities of the fungal biomass for all metal ions were lower than noncompetitive conditions. The presence of other heavy metal ions slightly decreased the total biosorption capacity of the fungal biomass under given experimental conditions. The equilibrium loading capacity of Pb(II) was greater than that of other heavy metal ions. This biomass showed a preference for binding Pb(II) over other metal ions. The order of affinity under competitive conditions was as follows: Pb(II) > Cd(II) > Hg(II) > As(III) on a molar basis. This affinity order is the same as in the noncompetitive adsorption conditions.

## Re-use of Biosorbents

To be useful in metal ion recyling prosesses, metal ions adsorbed should be easily desorbed under suitable conditions. Adsorbents should be used many times to decrease material cost. Elution experiments were performed using 0.5 M HCl solution as the elution agent. The dry fungal biomass loaded with the respective metal ions were placed within the elution medium and the amount of metal ions eluted in 2 hours was measured. It must be pointed out that biosorption (i.e., binding of heavy metal ions onto fungal biomass) is completely reversible. More than 85% (up to 91.5%) of the adsorbed heavy metal ions was desorbed in all cases. This means that HCl breaks down the chelates between heavy metal ions and binding sites onto the surface of the fungal biomass. Table 3 shows the adsorption-elution values of heavy metal ions by dry fungal biomass after several cycles of consecutive adsorption and

***Table 3.*** Heavy metal ions adsorption capacity of dry fungal biomass after repeated adsorption-elution cycle. Initial concentration of metal ions 100 mg/L; pH, 5.0. T, 20°C.

| | As(III) | | Hg(II) | | Cd(II) | | Pb(II) | |
|---|---|---|---|---|---|---|---|---|
| Cycle number | Adsorption (mg/g) | Desorption (%) | Adsorption (mg/g) | Desorption (%) | Adsorption (mg/g) | Desorption (%) | Adsorption (mg/g) | Desorption (%) |
| 1 | 7.7 | 89.0 | 20.8 | 91.5 | 31.8 | 88.3 | 98.1 | 88.7 |
| 2 | 7.5 | 89.2 | 20.6 | 89.3 | 31.8 | 89.7 | 97.3 | 89.6 |
| 3 | 7.4 | 89.4 | 20.3 | 86.8 | 31.5 | 91.5 | 97.4 | 90.3 |
| 4 | 7.3 | 89.5 | 20.0 | 87.3 | 31.0 | 90.0 | 96.1 | 89.5 |
| 5 | 7.2 | 86.8 | 19.5 | 88.0 | 30.5 | 90.4 | 96.0 | 89.3 |
| 6 | 7.0 | 88.7 | 19.2 | 92.0 | 30.3 | 85.4 | 95.7 | 89.0 |
| 7 | 6.9 | 90.3 | 19.0 | 86.7 | 29.6 | 85.5 | 93.6 | 88.6 |
| 8 | 6.7 | 90.0 | 18.1 | 85.0 | 29.2 | 83.0 | 92.5 | 87.9 |
| 9 | 6.5 | 88.5 | 17.6 | 83.7 | 28.6 | 86.7 | 92.0 | 85.0 |
| 10 | 6.0 | 87.0 | 17.0 | 84.0 | 28.0 | 85.9 | 91.7 | 83.0 |

desorption. The adsorption capacities of As(III), Hg(II), Cd(II), and Pb(II) ions were decreased about 22%, 18%, 12%, and 7%, respectively, after ten cycles. This table clearly shows that the dry fungal biomass can be used repeatedly without significantly losing the adsorption capacity for all metal ions studied here.

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