Anadolu Üniversitesi Bilim ve Teknoloji Dergisi C - Yaşam Bilimleri ve Biyoteknoloji
Anadolu University Journal of Science and Technology C - Life Sciences and Biotechnology

2016 - Volume: 5 Number: 1
Page: 13 - 22
DOI: 10.18036/btdc.01000
Received: 21 March 2016 Accepted: 04 April 2016

LEAD BIOSORPTION BY A MODERATELY HALOPHILE *PENICILLIUM* SP. ISOLATED FROM ÇAMALTI SALTERN IN TURKEY

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ABSTRACT

Owing the importance of biosorption of heavy metals by different organisms, a moderately halophilic fungus isolated from Çamalti saltern was first time investigated for its potential for biosorption. Different heavy metals namely, lead [$(Pb(NO_3)_2$], nickel ($NiCl_2$), chromium (K_2CrO_4), zinc ($ZnCl_2$), cadmium ($CdCl_2.H_2O$), copper ($CuSO_4$) and cobalt ($CoCl_2.6H_2O$) were screened for resistance and the most tolerated heavy metal by *Penicillium* sp. was chosen in biosorption assay.

The heavy metal tolerance of *Penicillium* sp. was observed in order of lead>cadmium >chromium>copper>nickel>zinc>cobalt. Different concentrations (145 mg/l, 644 mg/l and 1388 mg/l) of lead biosorption was investigated and increasing the metal ion concentration resulted in decreased uptake for lead. Freundlich isotherm was more effective than Langmuir isotherm for lead biosorption by *Penicillium* sp. The binding sites for lead attributed to the amine groups on the biomass surface were verified by Fourier Transform Infra Red (FTIR) analysis.

A halotolerant *Penicillium* sp. having high resistance to lead, could be suggested for use as an agent for abatement of lead pollution in hypersaline conditions or in waters of fluctuating salinity, as well as in non-saline environments after further studies of optimization.

Keywords: Biosorption, Lead, Penicillum sp., Moderately halophile

TÜRKİYE ÇAMALTI TUZLA'SINDAN İZOLE EDİLMİŞ ILIMLI HALOFİL *PENICILLIUM* SP. İLE KURŞUN BİYOSORPSİYONU

ÖZET

Ağır metallerin farklı organizmalarla biyosorbsiyonunun önemi sebebiyle Çamaltı tuzlasından izole edilen tuza toleranslı (halotolerant) fungusun biyosorbsiyon potansiyeli bu çalışma ile ilk kez araştırılmıştır. *Penicillium* sp. izolatının kurşun [$(Pb(NO_3)_2$], nikel ($NiCl_2$), krom (K_2CrO_4), çinko ($ZnCl_2$), kadmiyum ($CdCl_2.H_2O$), bakır ($CuSO_4$) ve kobalt ($CoCl_2.6H_2O$) ağır metallerine dirençlilikleri araştırılmış ve *Penicillium* sp. nin en yüksek tolerans gösterdiği ağır metal biyosorpsiyon çalışmaları için seçilmiştir.

Penicillium sp. izolatının ağır metal toleransı kurşun > kadmiyum > krom > bakır > nikel > çinko > kobalt şeklinde gözlenmiştir. Biyosorpsiyon çalışmalarında 3 farklı kurşun derişimi (145 mg/lt, 644 mg/lt ve 1388 mg/lt) kullanılmış ve metal iyon derişimi yükseldikçe kurşun tutma kapasitesi düşmüştür. *Penicillium* sp. izolatının kurşun biyosorpsiyonunda Freundlich izotermi Langmuir izotermine göre daha uygundur. Kurşun bağlayıcı bölgelerin biyokütle yüzeyindeki amin grupları olduğu FTIR analizi ile doğrulanmıştır.

Kurşuna yüksek direnç gösteren halotolerant *Penicillium* sp. yüksek oranda veya değişken oranlarda tuz içeren ortamlardaki kurşun kirliliğinin azaltılmasında kullanım için olduğu kadar optimizasyon çalışmalarından sonra tuz içermeyen ortamlarda da kullanım için önerilebilir.

Anahtar Kelimeler: Biyosopsiyon, Kurşun, *Penicillum* sp., Ilımlı halofil

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1. INTRODUCTION

Increasing industrial development has led to continuously increasing production of toxic substances, discharged into the environment with risk for living organisms, and potentially constituting serious hazards to public health [1]. Especially heavy metal pollution has become one of the serious environmental problems of worldwide concern [2].

Lead is widely used in industry and their accumulation in the living tissues may cause serious health problems [3] while lead is extremely toxic and can demage the nervous system, kidneys and reproductive system, particularly in children [4].

The severe toxic effects imposed by heavy metals on living tissues and environment directed the research at investigating alternative technologies for wastewater purification systems. Conventional separation techniques applied to the treatment of industrial effluents include chemical precipation, chemical oxidation or reduction, filtration, ion exchange and electrochemical processes. However, technical and economical constraints encountered in the application of these methods have directed attention to the search for new technologies involving metal removal from waste streams [5].

As a result of development in the field of environmental microbiology, recent studies have focused on the use of microbial-based potential biosorbents such as yeast, bacteria and fungi [6]. This biologial phenomenon defined as biosorption seem to be a good alternative to the existing methods since it does not produce chemical sludges, it could be highly selective, more efficient, easy to operate and hence cost effective for the treatment of large volumes of wastewaters [7]. Biosorption utilizes the ability of various biological materials to bind and sequester heavy metals from aqueous solutions [8]. Biosorption processes are based on the ability of a microorganism to adsorb a metal in an aqueous solution by several phsical-chemical processes through the cell's wall; this process does not always involve biomass metabolism, and that is why it does not matter if the microorganisms are dead or alive for the development of such processes [2]. A variety of natural materials of biological origin including bacteria, fungi, algae, mosses, macrophytes and higher plants can decrease the concentration of heavy metal ions from aqueous solution from ppt (part per trillion) to ppb (part per billion) level [2, 9, 10].

Compared to bacteria, lead resistant fungi are much less investigated and fungal biosorption of heavy metals are mostly investigated with dead biomass by far. However, bioaccumulation with living cells is recognized to have irreplaceable advantages in the removal of heavy metals [11].

In recent years, the number of biotechnological uses of halophilic microorganisms has increased and additional applications are under development. The uses of halophiles in biotechnology can be divided into a number of categories. First, the halotolerance of many enzymes derived from halophilic microorganisms can be exploited wherever enzymatic transformations are required to function at low water activities, such as in the presence of high salt concentrations. Second, some organic osmotic stabilizers produced by halophiles have found interesting applications. Third, some halophilic microorganisms may produce valuable compounds that can also be found in non- halophiles, often without any direct connection with their halophilic properties. But halophiles may present distinct advantages for the development of biotechnological production processes [12]. Studies on the microbial diversity in hypersaline environments revealed the presence of melanized fungi, 'considered as a new group of eukaryotic halophiles', halotolerant black yeast and several other filamentous fungi, including *Penicillium* spp. Although isolates of halophilic penicillia are reported from hypersaline environments [13-17] there has been only a report about heavy metal resistance of this group of fungi [18].

Therefore, the aim of present study was to investigate heavy metal tolerance and biosorption of lead by a halotolerant *Penicillium* sp. isolated from Camalti Saltern in Turkey. In addition, the biosorption equilibrium was evaluated using the Langmuir and Freundlich isotherms and functional groups of adsorbtion on fungal cell surface was investigated by using Fourier Transform Infrared (FTIR) Spectrometer.

2. MATERIALS AND METHODS

2.1. Microorganism and Screening to Resistance to Heavy Metals

Halotolerant *Penicillium* sp. strain isolated from soil of Çamalti Saltern located in the west cost of Turkey. The isolate was identified with molecular and conventional methods is deposited in Anadolu University, Department of Biology. Fungal growth occurred on plates of isolation medium (Rose Bengal Chloramfenicol Agar) supplemented with 15% NaCl after 10 days incubation.

For screening of resistance to heavy metals, a general fungal growth medium (SDA, BBL B11584) was prepared and amended with various amounts of heavy metals lead [($Pb(NO_3)_2$], nickel ($NiCl_2$), chromium (K_2CrO_4), zinc ($ZnCl_2$), cadmium ($CdCl_2.H_2O$), copper ($CuSO_4$ and cobalt ($CoCl_2.6H_2O$) to achieve the desired metal concentration ranging from 0.05 to 10 mg/ml. Each heavy metal plate was subdivided into equal sectors and an inoculum of test fungus (10^6 CFU ml) was spotted in duplicate on heavy metal containing plates. Control plates included only SDA medium without heavy metals. The plates were incubated at 26 ± 1° C for 2–5 days to observe the growth of fungus on the spotted area. Heavy metal tolerance was determinated as of the heavy metal that inhibited visible growth of test fungus [19]. The most tolerated heavy metal by *Penicillium* sp. was chosen in following biosorption assay.

2.2. Biyosorption Assay and Isotherms

All glassware was acid washed in 5% nitric acid for a minimum of 5 hours and rinsed in deionised water overnight and dried before use [20]. Biomass of *Penicillium* sp. was grown on YMS (yeast extract, 10 g/l; malt extract, 10 g/l; sucrose, 10 g/l) medium with a pH adjusted to 4.5. The fungus was cultured in filamentous form under aerobic conditions for 3 days in shake flasks (125 rpm). The biomass was harvested by filtration through a 150 µm steril filters. The biomass was thoroughly washed with distilled deionized water to remove residual growth medium. The washed biomass (live biomass) was used immediately thereafter. Briefly, 2.5 g of biomass of the fungus were added to 50 ml of metal solution containing three different concentrations (145, 644, 1388 mg/l) of $Pb(NO_3)_2$ in sterile distilled water, at an initial pH of 5.0 in a 250 ml conical flask. The flasks were agitated on a rotatory shaker at 25 °C and samples were withdraw at pre-determined time intervals (1, 2, 4, 8, 24, 28, 32, 48, 52, 56, 72, 96 and 120 h). The solution was then centrifuged at 8000 rpm for 30 min to separate the biomass.

Elemental analyses were carried out on the supernatant of each sample with use of inductively coupled plasma optical emission spectroscopy (ICP-OES) (VARIAN 720 ES) in the Center for Applied Environmental Research at Anadolu University.

Metal removal by *Penicillium* sp. was determined as:

$$R = \frac{A_0 - A_t}{A_0} \text{ x } 100 \quad (1)$$

where R is the percentage of lead biosorption by biomass in percentage, A_0 is the initial concentration of metal ion in mg/l and A_t is the final concentration of metal ion in mg/l [2].

Freundlich and Langmuir models was used for biosorption isotherm models. The Freundlich model is based on the relationship between the metal uptake capacity “q_e” (mg/g) of biomass and the residual (equilibrium) metal ion concentration “Ce” (mg/l). The general Freundlich equation is as:

$$q_e = K_f Ce^{1/n} \quad \log q_e = \log K_f + \frac{1}{n} \log C_e \quad (2)$$

where intercept ln k (lnK_f) is a measure of adsorption capacity and the slope $1/n$ is the intensity of adsorption. The general Langmuir equation is commonly presented as:

$$\frac{Ce}{qe} = \frac{1}{Qb} + \frac{Ce}{Q} \quad (3)$$

where qe is the amount of metal ion removed (mg/g), Ce the equilibrium concentration (mg/l), Q and Qb are the Langmuir constants related to adsorption capacity and adsorption energy, respectively [21].

2.3. FTIR Spectral Analysis

Another investigation related to the fungal biosorption of lead-loaded *Penicillium* sp. was carried out by Fourier transform spectroscopy (FTIR). Fungal biomass was dried by vacum evaporator at room temparature. About 1 mg of biomass was encapsulated in 100 mg of KBr in order to prepare translucent sample disk and was analysed by Fourier Transform Infrared spectroscopy (Perkin Elmer Spektrum 100). The absorption spectrum of unadsorbed dry fungal biomass was used as control for comparison with lead adsorbed biomass to investigate the functional groups of *Penicillium* sp. in relation to biosorption of lead.

3. RESULTS AND DISCUSSION

The heavy metal tolerance of *Penicillium* sp. was observed in order of lead [(Pb $(NO_3)_2$]→ 3.17 mM> cadmium ($CdCl_2.H_2O$)→ 0.223 mM > chromium (K_2CrO_4)→ 0.15 mM > copper ($CuSO_4$)→ 0.056 mM > nickel ($NiCl_2$)→ 0.06 mM> zinc ($ZnCl_2$)→ 0.055 mM > cobalt ($CoCl_2$.6 H_2O)→ 0.01 mM. *Penicillium* sp. showed relatively high tolerance to lead in comparison to other heavy metals.

Maximum biosorption of 145 mg/l concentration of lead by *Penicillium* sp. was obtained at 120th hour and absorption rate was determined as 95.24% (Figure 1). Maximum biosorption of 644 mg/l concentration of lead was obtained at 28th hour and the rate of the adsorption was 59.31% (Figure 1). However, maximum biosorption of 1388 mg/l initial concentration of lead was obtained at 96th hour and adsorption rate was 40.30% (Figure 1). Increasing the metal concentration resulted in decreased uptake for lead.

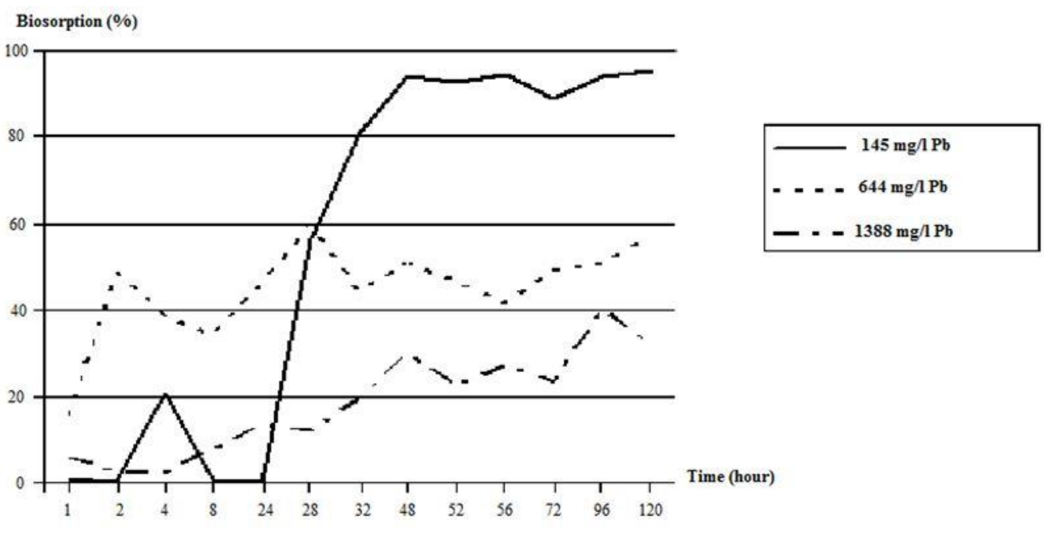


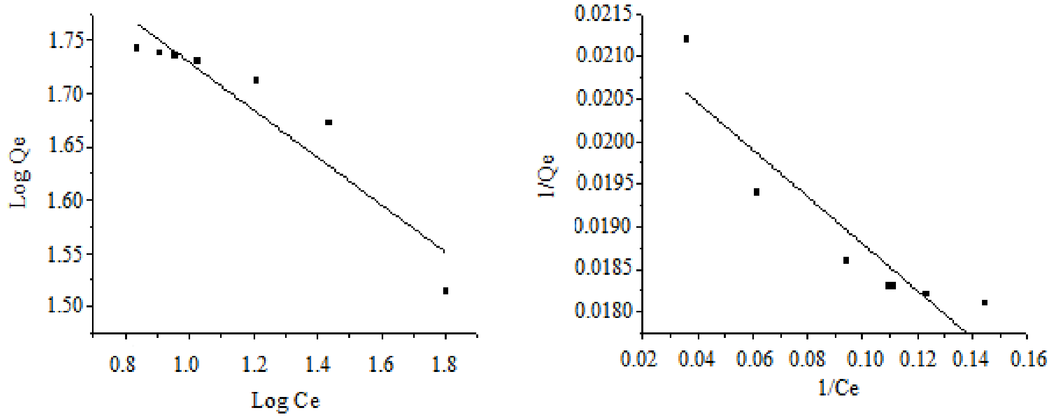
Figure 1. Percentage of lead biosorption by moderately halophile *Penicillium* sp.

The Freundlich and Langmuir biosorption isotherms of *Penicillium* sp. for different concentrations of lead are given Figures 2-4. Values of constant obtained from isotherms are compared in Table 1.

Table 1. Isotherm model constants for biosorption of lead by *Penicillium* sp.

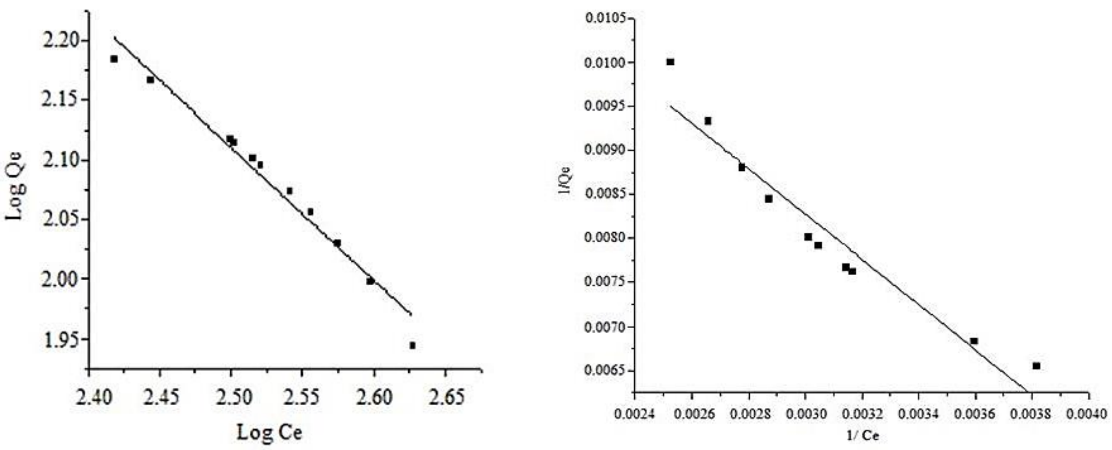
Lead Concentration (mg/l)	Langmuir Isoterm parameters			Freundlich Isoterm Parameters		
	Q	b	R^2	n	K_f	R^2
145	46.3821	-0.7805	0.820	-4.4830	89.571	0.872
644	62.5390	$-6.21x10^{-3}$	0.925	-0.8911	82.444.17	0.969
1388	29.7530	$-1.29x10^{-3}$	0.744	-0.2522	$1.11x10^{14}$	0.922

K_f and n illustrate the seperation metal ion and the high adsorption capacity of fungi. Q and b are the Langmuir constants related to adsorption capacity and adsorption energy, respectively. Freundlich isotherm was more effective than Langmuir isotherm for lead biosorption by *Penicillium* sp. when comparation of the square of correlation coefficients (R^2) at different concentrations of lead (Figures 2-4).



(a) (b)

Figure 2. Freundlich (a) and Langmuir (b) biosorption isotherms of lead at 145 mg/l concentration



(a) (b)

Figure 3. Freundlich (a) and Langmuir (b) biosorption isotherms of lead at 644 mg/l concentration

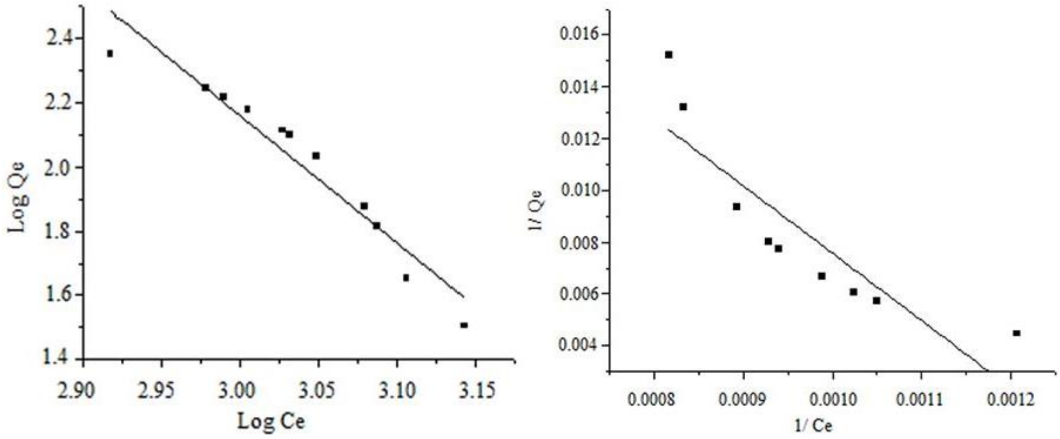


Figure 4. Freundlich (a) and Langmuir (b) biosorption isotherms of lead at 1388 mg/l concentration

The FTIR analysis (Figure 5) had eventually confirmed the difference between functional groups in relation to biosorption of lead. The absorption spectrum of lead-loaded fungal biomass was compared with that of control biomass. A change of absorption bands can be seen when comparing the FTIR spectra of control biomass. On the basis of the change of the band, the structure of the metal bound functional groups at *Penicillium* sp. were determinated at amide [N-imonosubstituted amide (1650-1700 cm^{-1}), N,N-disubstituted amide (1640-1680 cm^{-1}), N-unsubstituted amide (1650-1700 cm^{-1}), N-alkyl aromatic amide (1595-1670 cm^{-1}) ve N-unsubstituted aromatic amide (1595-1670 cm^{-1})].

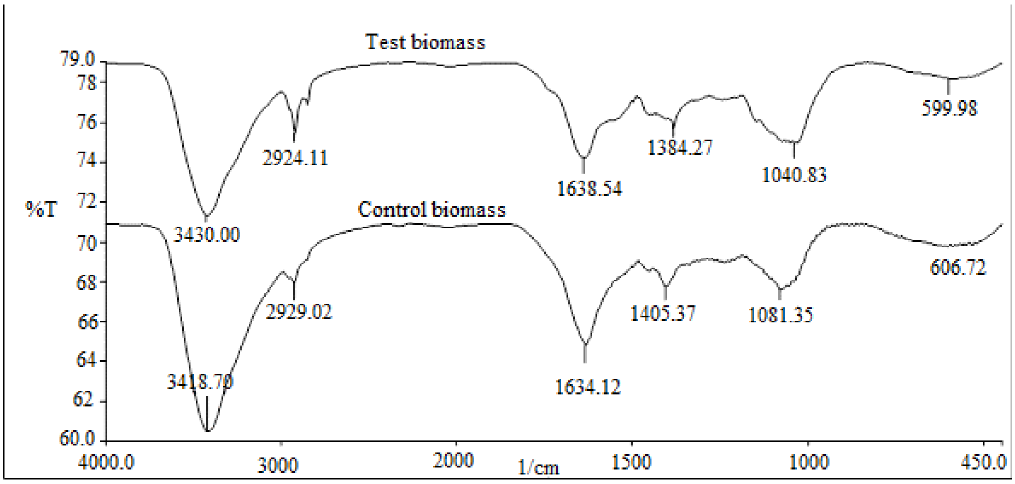


Figure 5. FTIR spectrum of *Penicillium* sp. before (control biomass) and after (test biomass) biosorption of lead (644 mg/l Pb, 120 hours)

Fungal biosorption is considered as a biotechnological strategy of great value [1]. Major advantages of fungal biosorbent materials include their good metal uptake capacities and low anticipated price. In addition, fungal biosorbents can be regenerated for multiple uses [22].

The use of fungal biomass has been preferred in numerous studies for biosorption of toxic metal ions from aqueous solution [23, 24]. Fungal strains belonging to the taxonomic group of *Zygomycetes* are of interest due to the presence of chitin, chitosan, and glucan in their cell walls. These polysaccharides have shown to be efficient metal biosorbents. *Rhizopus oryzae* [25], *Aspergillus niger* [25, 26], *Penicillium janthinellum* [27], *Penicillium chrysogenum* [28-31], *Penicillium simplicissimum* [32], *Penicillium austurianum* [33] have already been studied as potential biomass for removal of heavy metals from aqueous solution.

Organisms able to grow under extreme environments offer good potential as indicators of pollution and as biosorbents, and in applications for bioremedial measures. Industrial processes also use salts and frequently release brine-effluent into the environment. Although isolates of halophilic penicillia are reported from hypersaline environments, there has been only a report about heavy metal resistance of this group of fungi. In the study of Marbaniang and Nazareth [18], halotolerant *Penicillium* sp. were resistant to lead at a concentration of 7.5 mM. Also most of the isolates could tolerated either Cu^{2+} or Cd^{2+} as sulphate or as nitrate salt and two halotolerant *Penicillium* isolates showed resistance to all the heavy metals tested. However in this study, *Penicillium* sp. was resistant to lead 3.17 mM concentration a level which is higher than their records.

Studies have used biosorption experiments performed over uniform time periods but with different metal concentrations, expressing results as sorption isotherms for the biosorbents, or Langmuir/Freundlich plots for the metals allowing approximate calculation of the maximum capacity for uptake of each metal per unit dry weight of biosorbent [34]. In this study it was observed that Freundlich isotherm (R^2= 0.969) was more effective than Langmuir isotherm for lead biosorption by *Penicillium* sp. when comparation of the regression coefficent value (R^2).

It was reported that [28] biosorption of lead by *P. chrysogenum* biomass was strongly affected by pH and lead sorption was higher at pH 4-5. Therefore, the biosorption of lead by *Penicillium* sp. was performed only at pH 5 in this study.

The mechanism of metal uptake by microbial cells is not yet well understood. The real attachment of the metal ions on the cell surface may include physical adsorption (biosorption), ion-exchange or chemisorption. Since fungal cell wall surface contained many functional groups of carboxyl, hydroxyl, sulfhydryl, amino groups, and phosphate group of lipids, proteins and polysaccharides having ability to bind metal ions [27]. In this study, FTIR analysis had eventually confirmed the difference between functional groups in relation to biosorption of lead. When the absorption spectrum of lead-loaded fungal biomass was compared with that of control biomass, a change of absorption bands can be seen at amide groups.

4.CONCLUSION

The halotolerant *Penicillium* sp. isolated from Çamaltı saltern which is able to grow at high concentrations of salt as well as in its absence possess high resistance to lead, could be used as an agent for abatement of lead metal pollution in hypersaline conditions or in waters of fluctuating salinity, as well as in non-saline environments. The affinity of sites for metal ions binding by the halophilic fungi may be enhanced by the application of genetic and protein engineering which could lead to the development of new peptides or biopolymers with increased metal uptake rate and stability in further studies.

ACKNOWLEDGEMENTS

The authors thanks to Anadolu University Research Foundation for financial support through project number 071018.

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