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Potential capacity of *Beauveria bassiana* and *Metarhizium anisopliae* in the biosorption of Cd^{2+} and Pb^{2+}

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In this study *Beauveria bassiana* and *Metarhizium anisopliae* were used as inexpensive and efficient biosorbents for Pb(II) and Cd(II) from aqueous metal solutions. The effects of various physicochemical factors on Pb(II) and Cd(II) biosorption by *B. bassiana* and *M. anisopliae* were studied. The optimum pH for Cd(II) and Pb(II) biosorption by two fungal species was achieved at pH 6.0 for Pb(II) and 5.0 Cd(II) at a constant time of 30 min. The nature of fungal biomass and metal ion interactions was evaluated by Fourier transform infrared. The maximum adsorption capacities (q_{max}) calculated from Langmuir isotherms for Pb(II), and Cd(II) uptake by *B. bassiana* were 83.33±0.85, and 46.27±0.12 mg/g, respectively. However, the q_{max} obtained for Pb(II) uptake by *M. anisopliae* was 66.66±0.28 mg/g, and 44.22±0.13 mg/g for Cd(II). *B. bassiana* showed higher adsorption capacity compared to *M. anisopliae*. The data obtained imply the potential role of *B. bassiana* and *M. anisopliae* for heavy metal removal from aqueous solutions.

Introduction

Heavy metal contamination exists in aqueous waste streams of many industries, such as metal plating facilities, mining operations and tanneries. Some metals associated with these activities are cadmium, lead, chromium, iron, nickel and mercury. Such metals are found naturally in the soil in trace amounts, which pose few problems. Heavy metals are not biodegradable and tend to accumulate in living organisms, causing diseases and disorders. Microorganisms like bacteria, fungi, algae and yeast are known to tolerate and accumulate heavy metals. To overcome the disadvantages of accumulation in microorganisms; non-viable or dead biomass is preferred (Butter et al., 1998). The role of various microorganisms as biosorbents in the removal and recovery of heavy metal(s) has been well reviewed and documented (Gabr et al., 2008, 2009; Hassan et al., 2009; Joo et al., 2010; Oh et al., 2009).

The potential of filamentous fungi in bioremediation of industrial effluents and wastewaters containing heavy metals has been increasingly reported from different parts of the world (Gadd, 1993). Fungi, such as *Aspergillus niger* and *Mucor rouxii,* are capable of removing heavy metals from aqueous solutions (Yan and Viraraghavan, 2008). Comparatively *Rhizopus* sp. can bioadsorb higher concentration of heavy metals than *Aspergillus* sp. (Ahmad et al., 2005).

In addition, metal biosorption by non-living biomass is a metabolism-independent process and it is not ruled by physiological restriction. To test this hypothesis, two predominant fungal isolates from agricultural fields were evaluated for bioadsoption capacity of cad-

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mium and lead in laboratory experiments. The anamorphic entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin from the order Hypocreales (Ascomycota) are natural enemies of a wide range of insects and arachnids and both fungal species have a cosmopolitan distribution (Rehner, 2005; Roberts and St. Leger, 2004). Much effort has been put into research on the development of *B. bassiana* and *M. anisopliae* as biological control agents (for inundation and inoculation biological control) to be applied in agriculture and forestry in temperate regions. However, *B. bassiana* exhibits more dominance and viability than *M. anisoliae* and that may be due to some environmental causes (Hussein et al., 2010). White-rot fungi have the ability to sequester toxic levels of metal ions during growth in soil (Wiley and Sons, 2006). These metals affect growth, reproduction, metabolic activity, mycelial morphology, enzymatic activities, and so on (Baldrian, 2003). On the other hand, fungi trigger defense mechanisms to deter ill effects of metals on metabolism. More work is needed to further elucidate the exact mechanisms of metal binding by cell-wall components, so that the biosorption phenomenon can be exploited commercially.

The main objectives of this work were to:

1) Determine the possibilities of non-living biomass of the biological control fungi *B. bassiana* and *M. anisopliae* in biosorption of cadmium and lead, which are the most frequent heavy metals found in waste and mining waters.

2) Assess the factors affecting biosorption and the mechanisms of biosorption.

Materials and Methods

Microorganisms, medium and culture conditions. The microorganisms used for this experiment were *B. bassiana* strain (BbaAUMC3263) and *M. anisopliae* strain (ManAUMC3085), which were obtained from Assiut University Mycological Center (AUMC), Assiut, Egypt. The optimum conditions for the growth of fungi were pH 5.7 and 25°C. The fungi were cultured in Difco™ Sabouraud Agar (pH 5.7) which contains (per liter of deionized water): Peptic Digest of Animal Tissue 5 g, Pancreatic Digest of Casein 5 g, Dextrose 20 g, for 14 days at 25°C, then harvested by centrifugation for 30 min at 5,000 rpm using MF 550 (Hanil, Korea) and the suspension was rinsed three times with sterile wa-

ter then freeze dried using lyophilizer (FD5505 Ilshin, Korea).

Reagents.   Pb(II) stock solution of 1,000 mg/L was prepared by dissolving 1.61 g $Pb(NO_3)_2$ in 1,000 ml deionized water and Cd(II) stock solution of 1,000 mg/L was prepared by dissolving 2.3709 g $CdSO_4 \cdot 8/3H_2O$ in 1,000 ml deionized water. The chemicals used for this study were of analytical grade and they were supplied by Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA). The heavy metal stock solutions were sterilized by autoclaving at a pressure of 2.0 atm and a temperature of 121°C for 15 min.

Biosorption mechanism and factors affecting biosorption.   Equilibrium contact time: The fungal biosorbent (1 g/L) was suspended in 20 ml of Cd(II) or Pb(II) solution (50 mg/L) in 100 ml flasks. The cell/metal suspensions were shaken (150 rpm) at 30°C. The pH of the solution was initially adjusted to 6.0. Samples were taken from the solutions at desired time intervals from 0−60 min and were subsequently centrifuged at 10,000 rpm for 5 min. The Cd(II), or Pb(II) concentrations in the resulting supernatant were determined using ICP-AES (Perkin Elmer XL3100).

Effect of pH on biosorption: The impact of solution pH on Cd(II) or Pb(II) biosorption was investigated by using two different fungal biosorbents and conducted at different pH values (ranging from 2.0−7.0) containing 20 ml of metal solution. The pH adjustment was done with the addition of either 0.1 M NaOH or 0.1 M HCl. All the biosorption experiments were repeated three times to confirm the results. A blank experiments were also conducted to ensure that no adsorption had taken place on the walls of the apparatus used.

FT-IR spectra: Fourier Transform Infrared Spectroscopy (FTIR spectra) was performed in order to give a qualitative and preliminary characterization of the main functional chemical groups present in the fungal biomass, which are responsible for heavy metal biosorption. Raw samples of fungal biomass (*B. bassiana, M. anisopliae*) and biomass loaded with Pb(II) and Cd(II) were analyzed using FTIR (Bio-Rad, FTS, 3000 MX) adopting the KBr disk technique.

Biosorption equilibrium models—Assessment of sorption performance.   The biosorption equilibrium isotherm was obtained by the Freundlich model (Eq. 1) and the Langmuir model (Eq. 2), respectively (Volesky, 1990).

$$q = K_f C_e^{1/n} \qquad (1)$$

where K_f and n are the distribution coefficient and a

correction factor, respectively and C_e is equilibrium concentration of heavy metal (mg/L). By plotting the linear form of Eq. (1), $\ln q = 1/n \ln C_e + \ln K_f$, the slope is the value of $1/n$ and the intercept is equal to $\ln K_f$.

$$q_{eq} = \frac{q_{max} b C_e}{1 + b C_e} \qquad (2)$$

The linear form of Langmuir is

$$C_{eq}/q_{eq} = 1/q_{max} b + C_{eq}/q_{max} \qquad (3)$$

where q_{max} is the Langmuir constant (mg/g) reflecting the maximum adsorption capacity of the metal ion per unit weight of biomass to form a complete monolayer on the surface bound at high C_{eq}. Langmuir constant b (l/mg) represents a ratio of adsorption rate constant to desorption rate constant, which also gives an indication of the affinity of the metal for binding sites on the biosorbent. q_{max} and b can be determined from the linear form of Langmuir equation (3) by plotting C_{eq}/q_{eq} vs. C_{eq}.

Assessment of data. The specific metal biosorption q was calculated using the following equation [4]:

$$q_e \text{ (mg/g)} = \left[\frac{C_i - C_e}{M} \right] * V \qquad (4)$$

where q_e is the specific metal biosorption (mg metal/g biomass), V is the volume of metal solution (l), C_i and C_e are the initial and equilibrium concentration of metal (mg /L), respectively, and m is the dry weight of the biomass (g).

Results and Discussion

Effect of time

Fungal biosorbents can adsorb heavy metals such as Cu, Al and Sb from aqueous solution and the process is dependent on the fungal species, biosorbent size, metal concentration, solution pH and ionic strength (Tomko et al., 2006). Contact time is one of the important parameters for successful biosorption application. Figure 1 shows biosorption of Pb(II) and Cd(II) on a fungal biomass of *B. bassiana* and *M. anisopliae* depending on time. The rate of metal uptake increased rapidly in the first 5 min of contact. After that, the rate decreased until it reaches a constant value of metal concentration after 30 min. Therefore, we can conclude that the appropriate equilibrium time for biosorption is 30 min. This represents the equilibrium time at which an equilibrium metal ion concentration is presumed to have been attained. The data obtained from this experiment was further used successfully to
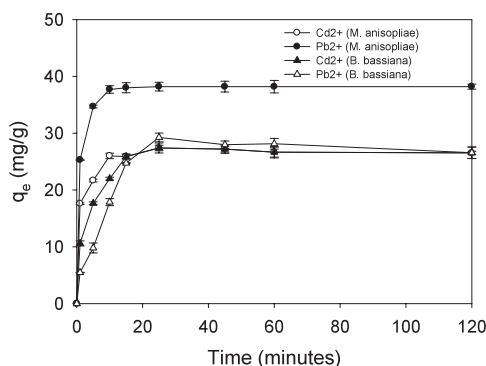

Fig. 1. Effect of time on biosorption of Pb^{2+} and Cd^{2+} by *Beauveria bassiana,* and *Metarhizium anisopliae,* pH 5.0 at initial concentration 50 mg/L, and 30°C.

evaluate the kinetics of the adsorption process. This short time required for biosorption is in accordance with the results given by other authors (Gabr et al., 2009; Joo et al., 2010; Sar et al., 1999; Tomko et al., 2006; Volesky, 1990; Zouboulis et al., 2004). Gabr et al. (2008) showed that the maximum biosorption of lead and nickel by *P. aeruginosa* ASU 6a. The temperature range of 20−45°C apparently exhibited no significant influence on the biosorption process of the tested fungal species (Javaid et al., 2010).

Effect of pH

The effect of different pH on the biosorptive capacity of Pb(II) and Cd(II) by *B. bassiana* (BbaAUMC3263) and *M. anisopliae* strain (ManAUMC3085) was evaluated at an initial concentration of 50 mg/L and equilibrium time of 30 min, at room temperature as shown in Fig. 2. Biosorption capacities for metal ions increased with an increase in pH until reaching the optimum at pH 5.0 for cadmium and pH 6.0 in the case of lead. However, at a pH higher than 5.0 the metal cations begin to precipitate in case of cadmium, similarly for lead at a pH higher than 6.0. At a low pH, cell wall ligands of the fungal biosorbent were closely associated with the hydronium ions H_3O^+ and restricted the approach of metal cations as a result of the repulsive force. In similar findings by earlier investigators, this 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 (Chergui et al., 2007). As the pH increased, more ligands such as carboxyl, phosphate, imidazole and amino groups would be exposed and carried negative charges with a subsequent attraction of metallic ions with a positive charge and biosorption onto the
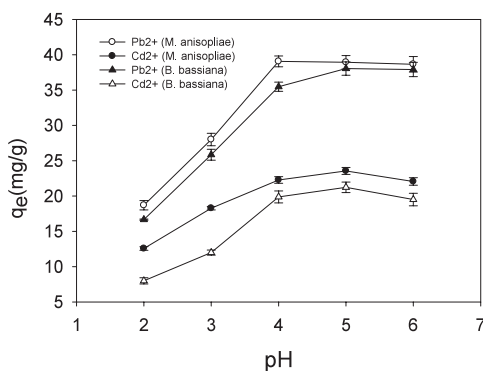
Fig. 2. Effect of pH on biosorption of Pb^{2+} and Cd^{2+} by *Beauveria bassiana,,* and *Metarhizium anisopliae,* equilibrium time 30 min at initial concentration 50 mg/L, and 30°C.

Fig. 3. Biosorption isotherm of Pb(II) and Cd(II), by *Beauveria bassiana,* equilibrium time 30 min at initial concentration 50 mg/L, pH 5.0 and 30°C.

cell surface (Joo et al., 2010; Pardo et al., 2003). Previous research has shown that most living microorganisms have a limited absorption for heavy metals at a lower pH, due to physiological properties (Fowle and Fein, 1999; Sar et al., 1999). A similar result was observed by using lyophilized cells of *P. stutzeri,* where the adsorption capacity for Cd(II) decreased steeply at low pH (Oh et al., 2009).

Biosorption equilibrium models— Assessment of sorption performance

The biosorption isotherm curve represents the equilibrium distribution of metal ions between the aqueous and solid phases. The equilibrium distribution is important in determining the maximum biosorption capacity. Several isotherm models are available to describe this equilibrium distribution. The two most common types for describing biosorption isotherm are the Langmuir and Freundlich models (Ahmad et al., 2010; Arief et al., 2008; Wang and Li, 2009). The Cd (II) and Pb (II) biosorption performance by lyophilized cells of *B. bassiana* and *M. anisopliae* biomass was achieved by measurements at initial concentrations of 0−300 mg/L, 30 min contact time, and pH 5.0 and 6.0 for Cd(II) and Pb(II) biosorption, respectively, as shown in Figs. 3 and 4. The figure indicate the relationship between the amount of Cd(II) or Pb(II) adsorbed (mg/g) by *B. bassiana* and *M. anisopliae* versus the concentration of Cd(II) or Pb(II) ions remaining in solution (C_{eq}, mg/L). The biosorption rate increases proportionally with the equilibrium concentration of the sorbent. Figures 3 and 4 show that the equilibrium biosorption isotherm of metal uptake by two fungal biomasses was a chemically equilibrated and saturable
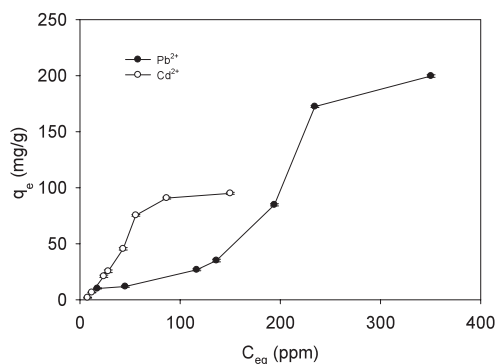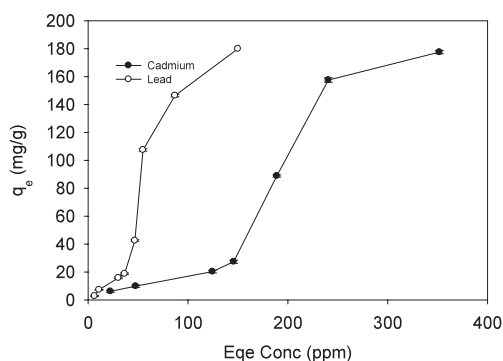

Fig. 4. Biosorption isotherm of Pb(II) and Cd(II), by *Metarhizium anisopliae,* equilibrium time 30 min at initial pH 5.0 and 30°C.

mechanism. Thus, there was an increase in metal uptake as long as metal binding sites were free. The ability of the basidiomycetes to adsorb and accumulate metals together with excellent mechanical properties of fungal mycelia provides an opportunity to utilize such candidates in selective sorption of industrial heavy metal ions from polluted waters (Bayramoglu et al., 2005; Razmovski and Šćiban, 2008). The data show that the metal biosorption decreased with an increase in metal ion concentration. The decrease in percentage of biosorption may be attributed to a lack of sufficient free sites for metal biosorption. In addition, biosorption generally decreased with increase in biosorbent particle size and its concentration (Zhou, 1999). At lower concentrations, all metal ions present in the solution could interact with the binding sites and thus the biosorption percentage was higher than those at higher ion concentrations. At higher concentrations, lower adsorption may have occurred due to the saturation of adsorption sites. The sorption capacity increased and reached a saturation value as the metal

ion concentration increased in an aqueous medium (Zouboulis et al., 2004). Similar results were obtained by many researchers (Gabr et al., 2008; Kadukova and Vircikova, 2005; Lu et al., 2006; Pardo et al., 2003; Tunali et al., 2006). The values of Langmuir and Freundlich parameters are summarized in Table 1. These data showed that the q_{max} obtained for lead uptake by *B. bassiana* was 83.33±0.85 mg/g, and for cadmium uptake was 46.27±0.12 mg/g. The *b* values of lyophilized cells of *B. bassiana* for Cd(II) and Pb(II) were 0.042 and 0.017 L/mg, respectively. However, the q_{max} obtained for lead uptake by *M. anisopliae* was 66.66±0.28 mg/g and cadmium uptake was 44.22±0.13 mg/g and the *b* values of lyophilized cells of *M. anisopliae* for Cd(II) and Pb(II) were 0.076 and 0.039 L/mg, respectively.

Freundlich parameters can be determined from the linear form of the equation (5) by plotting the ln *q* versus ln C_e; the slope is the value of $1/n$ and the intercept is equal to ln K_f. The linear form of the Freundlich equation for the biosorption of Cd(II) and Pb(II) by lyophilized cells of *B. bassiana* and *M. anisopliae* is shown in Figs. 5 and 6. The values of Freundlich parameters are summarized in Table 1. The magnitudes of K_f and

n show a higher uptake of Pb(II) and using *B. bassiana* and *M. anisopliae* compared to Cd(II). The highest K_f for Pb(II) values were 9.02 and 5.12 while those for Cd(II) were 3.51 and 2.83 for *B. bassiana* and *M. anisopliae*, respectively.

From Table 1, very high regression correlation coefficients (>0.94) were found for all Cd(II) and Pb(II) biosorption studied. The high correlation coefficients show that the Freundlich model describes the biosorption equilibrium of Cd(II) and Pb(II) by lyophilized cells of *B. bassiana* and *M. anisopliae* in the studied concentration range.

Generally, the data presented here indicate that the biosorption capacity increased with increasing initial Cd(II) and Pb(II) concentration. This biosorption characteristic indicates that the surface saturation is dependent on the initial metal ion concentration; at low concentration, adsorption sites took up the available metal more quickly. However, at higher concentration, metals need to diffuse into the fungal biomass surface by intraparticular diffusion and greatly hydrolyzed ions will diffuse at a slower rate (Sar et al., 1999).

Table 1. Langmuir and Freundlich isotherm parameters for Pb(II) and Cd(II) biosorption by *Beauveria bassiana* and *Metarhizium anisopliae*.

Fungus	Metals	Langmuir			Freundlich		
		q_{max}	b	r^2	n	K_f	r^2
Beauveria bassiana	Pb	83.33±0.85	0.017	0.98	0.63	9.02	0.96
	Cd	46.27±0.12	0.042	0.70	0.92	3.51	0.85
Metarhizium anisopliae	Pb	66.66±0.28	0.039	0.79	0.85	5.12	0.94
	Cd	44.22±0.13	0.076	0.93	0.96	2.83	0.83

K_f sorptive capacity, *n* sorptive intensity, r^2 correlation coefficient, *b* Langmuir constant, q_{max} maximum adsorption capacity.
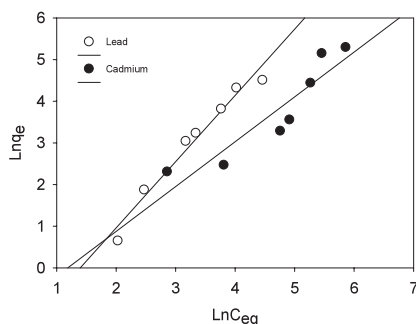

Fig. 5. Freundlich isotherm of Pb(II) and Cd(II), by *Beauveria bassiana,* equilibrium time 30 min at initial pH 5.0 and 30°C.
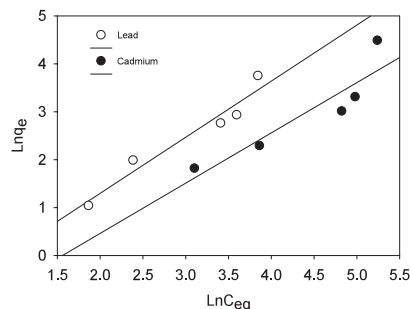

Fig. 6. Freundlich isotherm of Pb(II) and Cd(II), by *Metarhizium anisopliae,* equilibrium time 30 min at initial pH 5.0 and 30°C.

FTIR spectra of Pb^{2+} and Cd^{2+} loaded fungal species

In biosorption research, there is a lack of information about the sorption sites and mechanisms responsible for the capture of metallic ions by the biomasses (de Carvalho et al., 2003). Therefore, a preliminary and qualitative analysis of the main functional groups present on the cell wall was done by the FTIR technique, which may be helpful to understand Cd(II) and Pb(II) biosorption on the fungal biomass. Fungi offer a wide range of chemical groups that can attract and sequester the metals in biomass. Cell walls are composed of structural polysaccharides, proteins and lipids that offer metal-binding functional groups (Veglio and Beolchini, 1997). Figures 7 and 8 illustrate the FTIR spectrum for the fungal biomasses before the biosorption (native biomass) and the metal loaded biomass with
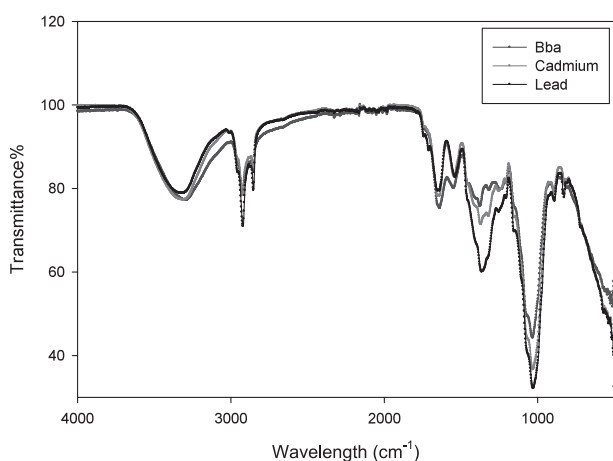

Fig. 7. FTIR spectra of lyophilized *B. bassiana* (BbaAU-MC3263) measured at room temperature before and after biosorption of 100 mg/L of each Pb(II) and Cd(II).
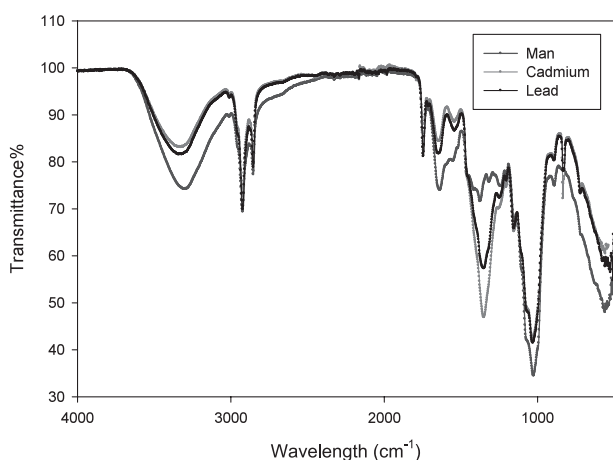

Fig. 8. FTIR spectra of lyophilized *M. anisopliae* strain (ManAUMC3085) measured at room temperature before and after biosorption of 100 mg/L of each Pb(II) and Cd(II).

lead and cadmium. The absorption band characterizing hydroxyl OH$^-$ was detected at 3,200–3,600 cm^{-1}, and C=O of carbonyl group at 1,670–1,820 cm^{-1}. The band located at 1,100–1,200 cm^{-1} may indicate the phosphate group P=O; the spectrum 1,210–1,320 cm^{-1} strong intensity was attributed to the C–O link of the acidic groups. The bands in the range between 1,034 and 1,075 cm^{-1} may indicate the organic phosphate C–PO$_2^{-3}$ group's affinity. The spectrum 1,250–1,450 cm^{-1} became broader and more intense particularly in the case of cadmium loading. Strong peaks at 2,850–3,000 cm^{-1} may be referring to alkanes C–H. The comparison between the spectrum of a native biomass and a metal loaded biomass reports the following:

I) Stretching and shifting of bands was detected at 3,200–3,600 cm^{-1} characterizing the presence of OH$^-$ may be due to Cd(II) and Pb(II) interactions with this hydroxyl group. II) The broadening of the band belonging to C–H groups in the range of 2,850–3,000 cm^{-1} indicates the heavy metal interaction with C–H on the surface of the fungal biomass either in *M. anisopliae* or *B. bassiana*. III) Broadening and stretching of the bands characterizing C=O of carbonyl groups were observed in the range of 1,670–1,820 cm^{-1} after biosorption. IV) There is a distinctive similarity in the FTIR spectra plotted between the lyophilized biomasses of *B. bassiana* and *M. anisopliae* and also in their behavior after heavy metals adsorption, that may be due to the great similarity between the two cell walls as entomopathogenic fungi. V) Stretching was observed also in the range of 1,034 to 1,075 cm^{-1}, which belongs to organic phosphate C–PO$_2^{-3}$ group, indicating possible interaction between the loaded heavy metals and the C–PO$_2^{-3}$ group. Because all indicate the involvement of the functional groups of the fungal biomass during the biosorption process of Cd(II) and Pb(II), ion exchange is another possible mechanism, Na, K, Ca, and Mg ions were released from the biomass after biosorption of Pb, Cd, Ni and Zn, indicating that ion exchange was a key mechanism in the biosorption of metal ions by *M. rouxii* biomass (Yan and Viraraghavan, 2008). These results are in conformance with findings revealed in some articles (Chhikara et al., 2010; Jin-ming et al., 2010). Since these fungal species are used widely in different agricultural fields as important biocontrol agents, they have a wide host range of insect pests, which can be attained free or at low cost from these activities.

A comparison of heavy metal uptake capacities of various microbial species

Table 2 compares the maximum adsorption capacities obtained in this study with some other values reported in the literature. The adsorption capacities for Pb(II) and Cd(II) using lyophilized cells of *B. bassiana* and *M. anisopliae* were found to be comparable with many of the reported literature values. However, a direct comparison of experimental data is not possible, due to different experimental conditions such as pH, temperature, equilibrium time, heavy metal concentration and biomass dosage.

Conclusions

Fungal biomasses of *B. bassiana* (BbaAUMC3263) and *M. anisopliae* (ManAUMC3085) were used as effective biosorbents for the biosorption of Cd(II) and Pb(II) from aqueous metal solutions. The biosorption performance was strongly affected by solution pH and contact time at room temperature. The optimum pH for biosorption of Cd(II) and Pb(II) by the two fungal species were achieved at pH 6.0, at a constant time of 30 min, and at 30°C. *B. bassiana* (BbaAUMC3263) showed a higher adsorption capacity compared to *M.*

anisopliae (ManAUMC3085), which could be due to the difference in cell wall structure. The main functional groups evaluated by FTIR analysis of the two fungal biomasses revealed the presence of amino, carboxyl, hydroxyl and carbonyl groups, which are responsible for biosorption of Pb(II) and Cd(II). Biosorption of Pb(II) and Cd(II) by *B. bassiana* (BbaAUMC3263) and *M. anisopliae* (ManAUMC3085) was fitted well with the Freundlich adsorption isotherm equation in the studied metal concentration range compared to the Langmuir isotherm. In the case of *B. bassiana*, the maximum adsorption capacities (q_{max}) calculated from the Langmuir adsorption isotherm were 83.33±0.85 and 46.27±0.12 for Pb(II) and Cd(II), respectively. In the case of *M. anisopliae* the maximum adsorption capacities (q_{max}) calculated from the Langmuir adsorption isotherm were 66.66±0.28 and 44.22±0.13 for Pb(II) and Cd(II), respectively. The results obtained through this study support that both entomopathogenic fungi, *Beauveria bassiana* (BbaAUMC3263) and *Metarhizium anisopliae* (ManAUMC3085), are effective and low-cost biosorbents for Pb(II) and Cd(II) removal from aqueous solutions. More work is needed to elucidate further the exact mechanisms of metal binding by fungal cell-wall components, so that the biosorption phe-

Table 2.   The biosorption capacities of different biosorbent species in comparison with our studied fungal species.

Metal	Biosorbent	Biomass affinity	Amount adsorbed (mg/g)	References
Cd	*Ascophyllum nodosum*	Brown marine alga	215	(Holan et al., 1993)
	Sargassum natans	Brown marine alga	135	(Holan et al., 1993)
	Fucus vesiculosus	Brown marine alga	73	(Holan et al., 1993)
	Rhizopus arrhizus	Fungus Zygomycota	27	(Fourest and Roux, 1992)
	Rhizopus nigricans	Fungus Zygomycota	19	(Holan and Volesky, 1995)
	Ulva sp.	Seaweed alga	0.58	(Mattuschka et al., 1993)
	Gracillaria sp.	Red seaweed alga	0.3	(Mattuschka et al., 1993)
	Pencillium spinulosum	Fungus Ascomycota	0.4	(Sheng et al., 2004)
	Candida tropicalis	Yeast Ascomycota	60	(Townsley et al., 1986)
	Sacchromyces cervisiae	Yeast Ascomycota	20−40	(Tuzun et al., 2005)
	Penicillium chrysogenum	Fungus Ascomycota	56	(Holan and Volesky, 1995)
	Beauveria bassiana	Entomopathogen Ascomycota	46	The current study
	Metarhizium anisopliae	Entomopathogen Ascomycota	44	The current study
Pb	*Absidia orchidis*	Fungus Zygomycota	351	(Holan and Volesky, 1995)
	Penicillium chrysogenum	Fungus Ascomycota	93	(Volesky et al., 1993)
	Rhizopus nigricans	Fungus Zygomycota	166	(Holan and Volesky, 1995)
	Rhizopus arrhizus	Fungus Zygomycota	55	(Holan and Volesky, 1995)
	Chlamydomonas reinhardtii	Green alga Chlorophyta	96.3	(Volesky and May-Phillips, 1995)
	Phanerochaete chrysosporium	Fungus Basidiomycota	1.84	(Çeribasi and Yetis, 2001)
	Beauveria bassiana	Entomopathogen Ascomycota	83.33	The current study
	Metarhizium anisopliae	Entomopathogen Ascomycota	66.66	The current study

nomenon can be exploited commercially.

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