Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

2020, **21** (3), pp. 321 – 332

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER

KINETIC MODELING FOR THE BIOSORPTION OF COPPER, LEAD AND ZINC BY *PENICILLIUM CITRINUM* ISOLATED FROM POLLUTED ALGERIAN BEACHES

Ghania Bourzama[1*], Alima Rihani[1], Nadjet Ennaghra[1], Houria Ouled-Haddar[2], Boudjema Soumati[1]

[1]University of Badji Mokhtar (UBMA), Faculty of Sciences, Department of Biochemistry, 23000, Annaba, Algeria
[2]University of Mohamed Seddik Benyahia, Jijel, Laboratory of Molecular Toxicology, 18000, Jijel, Algeria

*Corresponding author: gbourzama@yahoo.fr

Received: April, 11, 2019
Accepted: September, 02, 2020

Abstract: The use of microorganisms, mainly fungi, is an alternative processing method for removing heavy metals from contaminated environments. These microorganisms have the ability to resist severe environments such as the presence of heavy metals and salinity. The aim of this study is to isolate natural fungi capable of accumulating lead (Pb), zinc (Zn) and copper (Cu) from polluted Algerian beaches. Heavy metal-resistant fungal strains were screened with different concentrations of these metals for 7 days at 25 ± 2 °C. The isolate showing the highest resistance to tested metals was selected to determine its ability to simultaneously uptake lead, zinc and copper from CYA broth medium. The kinetic models were applied on the metal tolerant *Penicillium citrinum*. Biosorption of the heavy metals was investigated under *p*H ranging from 1 to 11 and at temperature ranging from 15 to 45 °C. The screening of the heavy metal-resistant fungal strains showed one resistant strain to the three heavy metals at once. It followed the Langmuir model biosorption towards initial concentrations of the three metals showing an important affinity when compared with the earliest studies. The optimal *p*H and temperature were determined as 5.5 - 6 and 27 - 30 °C, respectively.

Keywords: *biosorption, heavy metals, Langmuir model, optimization, Penicillium*

INTRODUCTION

The pollution by heavy metal represents one of the most severe problems of the environment nowadays [1 – 3]. It is the result of various industrial sources such as mining and smelting of metalliferous, surface finishing industry, energy and fuel production, fertilizer and pesticide industry, metallurgy, iron and steel, etc. Thus, some of them are becoming shortage and also brings about serious environmental pollution. In general, toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.) affect human and animal health and ecosystem [4].

Conventional methods for removing heavy metals are expensive; they are not efficient due to their metal-binding properties and non-specificity to one metal. Therefore, alternative processing methods were proposed, using microbial biomass. There are several fungi that can grow at high concentrations of metals and they are capable to adsorb them [5 – 10]. These microorganisms can be employed for eliminating heavy metals from the contaminated ecosystems. Thus, fungi present the most important group due to their ability to resist severe high values of metal concentrations, extreme *p*H … [11].

This study aims to study the potential of natural fungi isolated from sand of the polluted Algerian eastern beaches and application of the kinetic modeling to understand the biosorption mechanism, furthermore, it aims to optimize this process for the simultaneous removal of three heavy metals, namely lead (Pb), zinc (Zn) and copper (Cu).

MATERIALS AND METHODS

Isolation of fungal strains from polluted beaches

The fungal strains isolation from sand samples of polluted eastern Algerian beaches was carried out using the decimal dilution technique on Potato-Dextrose Agar (PDA) medium from acacia tree (Sigma-Aldrich). Colonies of the isolated fungi were further purified on PDA plate by the method of spot inoculation repeatedly [12]. Purified isolates were identified after observation under light microscopy using a Paralux L1200 BINO SP 1600X microscope (France).

Screening of heavy metal-resistant fungal strains

The isolated strains were screened on Czapek Yeast agar (CYA) medium at different concentrations (200, 400, 800, 1000 mg·L^{-1}) of $PbSO_4$, $ZnSO_4$ and $CuSO_4$ for 7 days at 25 ± 2 °C. The mean of perpendicular diameter measurements was recorded [7].

All reagents were of analytical grade.

Influence of metal concentration on metal absorption

In order to determine the relative ability of the screened fungus to accumulate lead, zinc and copper, the fungi were cultured in 100 mL CYA broth in Erlenmeyer flasks (250 mL) with concentrations: 0, 500, 1000, 1500, 2000, 2500, 300 and 350 mg·L^{-1}.

The final *p*H was adjusted to 5.5. 10 mm plugs were inoculated in each flask. All cultures were incubated at 25 ± 2 °C for 7 days on a rotary shaker (KS 4000i Control, United Kingdom). The liquid phases from this fungus were separated using centrifugation at 10000×g for 30 min then washed. Fungal biomass was dried at 70 °C. The amounts of lead, copper and zinc uptake by dried biomass were analyzed by atomic absorption spectrophotometer (AA-6200 SHIMADZU, Australia) [13, 14].

Kinetic modeling

The model of cell metal binding sites of each metal was determined by application of the Langmuir–Freundlich absorption model [15], the following equation describes the Langmuir model (1916) [16]:

$$qm = \frac{qm \cdot b \cdot ce}{1 + b \cdot ce} \quad (1)$$

Rearrangement of the linear form of the above equation may be:

$$\frac{ce}{qe} = \frac{1}{bqm} + \frac{ce}{qm} \quad (2)$$

where: *Ce* is the equilibrium concentration (mg·L^{-1}) and *qe* is the adsorbed amount of metal ion per gram of biomass at equilibrium (mg·L^{-1}). *qm* is the maximum amount of metal ion per unit weight of biomass to form a complete mono layer on the surface bound at high *Ce* (mg·L^{-1}), *b* is a constant related to the affinity of the binding sites (L·mg^{-1}). A plot of *Ce/qe* versus *Ce* should indicate a straight line of slope *1/qm* and an intercept of *1/bqm*.
The Freundlich model (1906) [17] equation is of the form:

$$qe = k \cdot ce \cdot \left(\frac{1}{n}\right) \quad (3)$$

and the linear equation is:

$$ln\,qe = k \cdot \left(\frac{1}{n}\right) ln\,ce \quad (4)$$

Influence of *p*H and temperature on metal biosorption

The metal uptake by the dry biomass of *Penicillium citrinum* was investigated under *p*H ranging from 1 to 11 (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11). The *p*H of the medium was adjusted using 0.1 N HCl and 0.1 N NaOH solutions. For each tested *p*H, the cultures were incubated at 25 ± 2 °C for 7 day [18].
The optimal temperature for biosorption efficiency was determined by incubation of the fungal culture at different temperatures (15, 17, 20, 23, 25, 27, 30, 33, 37, 40, 43 and 45 °C) [18]. All tests were carried out in triplicate, in presence of each tested metal with 3500 mg·L^{-1}.

RESULTS AND DISCUSSION

Isolation of fungal strains from polluted beaches

The strains were isolated, purified and identified. The fungal cultures were identified based on macroscopic (colonial morphology, color, texture, shape, diameter and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium) [19]. This identification was achieved according to the literature [20, 21].

Screening of heavy metal-resistant fungal strains

The growth of all strains was tested at different heavy metal concentrations. This screening showed a resistant strain, which was identified as *Penicillium citrinum* (Figure 1 and Figure 2).

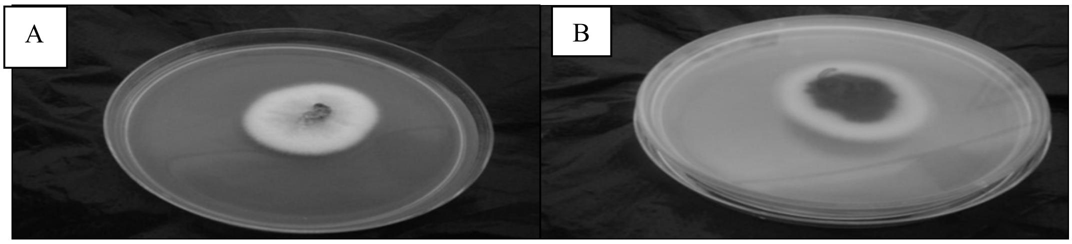

Figure 1. *Growth of Penicillium citrinum on CYA without metals at 25 ± 2 °C for 7days (A: surface, B: back)*

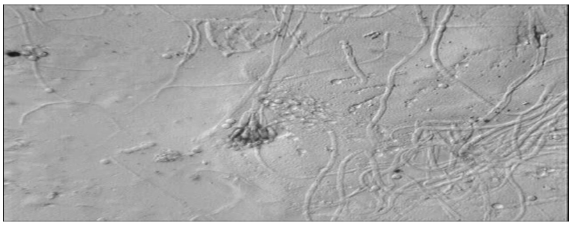

Figure 2. *Microscopic observation (60X) of Penicillium citrinum on CYA without metals at 25 ± 2 °C for 7 days*

This strain was able to resist the three heavy metals (Pb, Zn and Cu) at once (Figure 3). On the basis of the mycelial diameter, the isolated *Penicillium citrinum* showed a good colony growth on medium at different concentrations of the three metals (Table 1) when compared to the control (3.5 cm) (Figure 1). This strain can consequently grow easily in presence of these heavy metals even at high concentrations.

Table 1. *Mycelial growth diameter of Penicillium citrinum at different concentrations of the three metals (mean ± SD)*

Concentration [mg·L^{-1}] / Metal ions	200	400	600	800	1000
Lead (Pb)	6.1 ± 0.7	7 ± 0.1	8.1 ± 1.3	8.2 ± 0.7	7.9 ± 0.7
Zinc (Zn)	5.1 ± 1.1	5.7 ± 1.3	7.1 ± 0.8	7.7 ± 0.9	8.1 ± 0.3
Copper (Cu)	5.3 ± 0.9	5.6 ± 0.7	6.2 ± 0.9	6.3 ± 1.2	6.1 ± 1.2

A

B

C

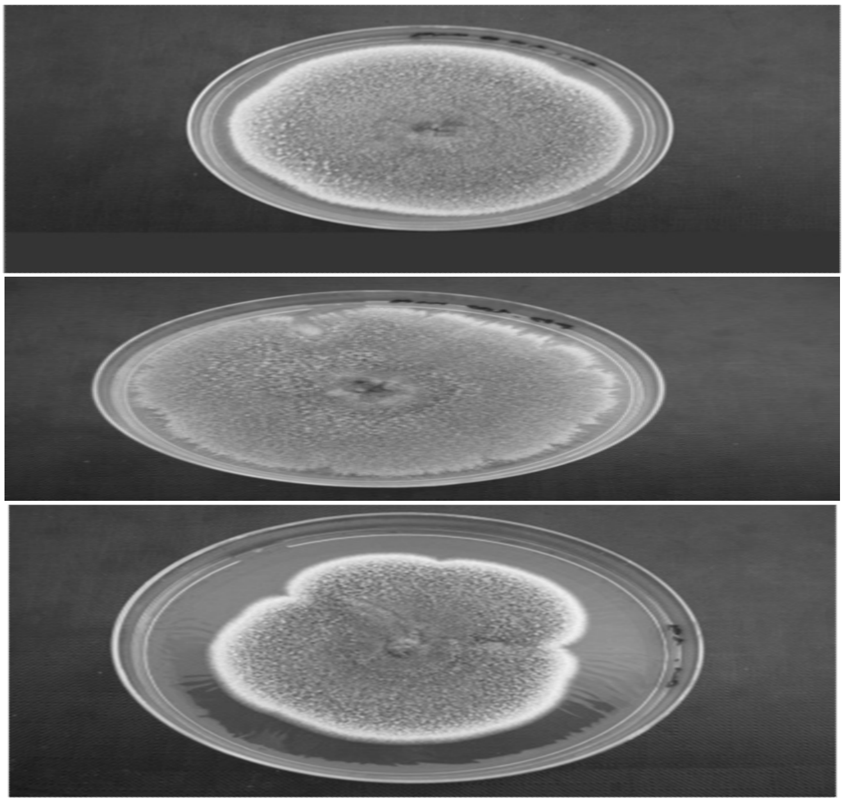

Figure 3. *Growth of Penicillium citrinum after exposure to 1000 mg·L^{-1} concentration of heavy metals on CYA at 25 ± 2 °C for 7 days (A: CYA + 1000 mg of $PbSO_4$, B: CYA + 1000 mg of $ZnSO_4$, C: CYA + 1000 mg of $CuSO_4$)*

Influence of metal concentration on metal absorption

Figure 4 indicated that the concentration of lead affects strongly the uptake ability. When the metal level increased (from 100 up to 3500 mg·L^{-1}), biosorption capacity increased also (from 1.7 up to 402.8 mg·g^{-1}) and it reached a saturation value at 3000 mg·L^{-1}.

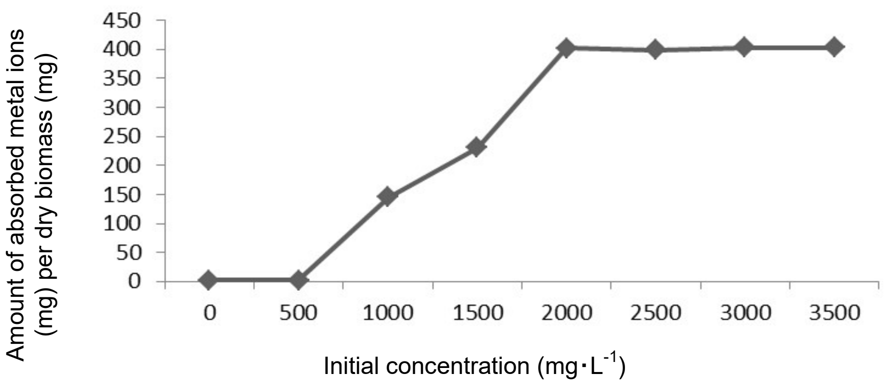

Figure 4. *Effect of initial metal ion concentration on the Pb^{2+} biosorption on dry Penicillium citrinum biomass (biomass concentration = 1 mg·L^{-1}, agitation rate = 150 rpm, temperature = 25 ± 2 °C, pH = 5.5, contact time = 7 days)*

From Figure 5 it can be seen that the initial zinc ions concentration had a strong effect on biosorption capacity. The amount of adsorbed Zn^{2+} per mass unit was increased (from 0.01 up to 620 mg·g^{-1}) while amount of zinc ions rises (from 100 up to 3500 mg·L^{-1}). The maximum Zn^{+2} uptake capacity of dry *Penicillium citrinum* biomass was determined at 3000 mg·L^{-1} initial Zn^{2+} concentration.

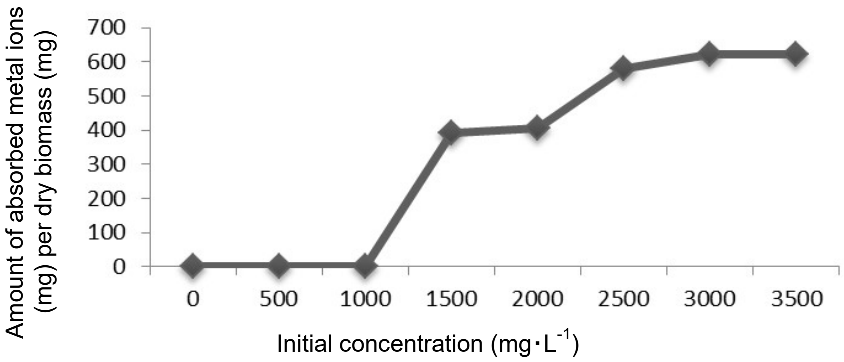

***Figure 5.** Effect of initial metal ion concentration on the Zn^{2+} biosorption on dry Penicillium citrinum biomass (biomass concentration = 1 g·L^{-1}, agitation rate = 150 rpm, temperature = 25 ± 2 °C, pH = 5.5, contact time = 7 days)*

In case of Cu^{2+} ion, the tested fungus exhibited an uptake capacity of 381 mg·g^{-1} at 3000 mg·L^{-1} (Figure 6).

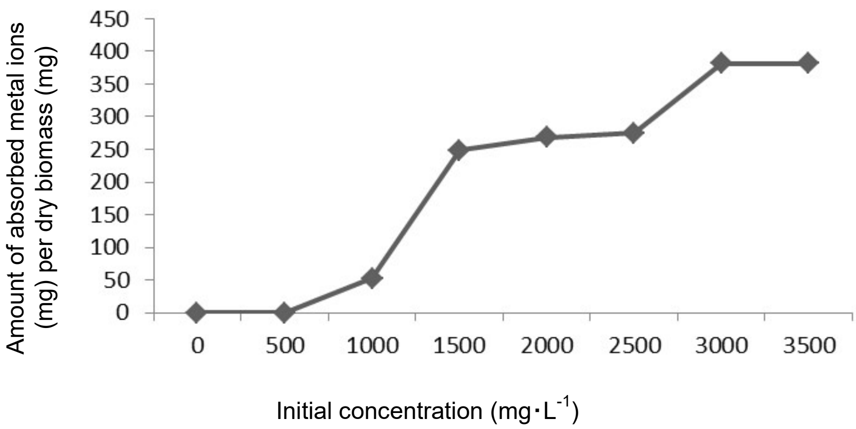

***Figure 6.** Effect of initial metal ion concentration on the Cu^{2+} biosorption on dry Penicillium citrinum biomass (biomass concentration = 1 g·L^{-1}, agitation rate = 150 rpm, temperature = 25 ± 2 °C, pH = 5.5, contact time = 7 days)*

Kinetic modeling

Using the equations (2) and (4), the linear plots of each model (Langmuir and Freundlich) were presented in Figure 7, Figure 8 and Figure 9.

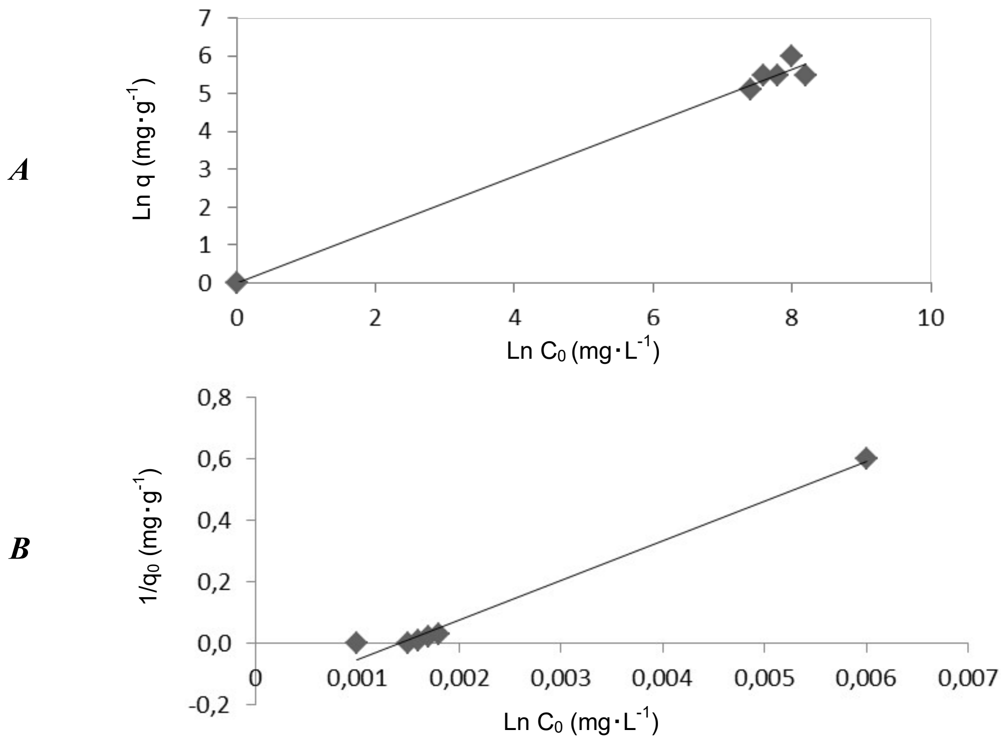

Figure 7. *Linear plot of lead ions uptake: A - Freundlich model, B - Langmuir model*

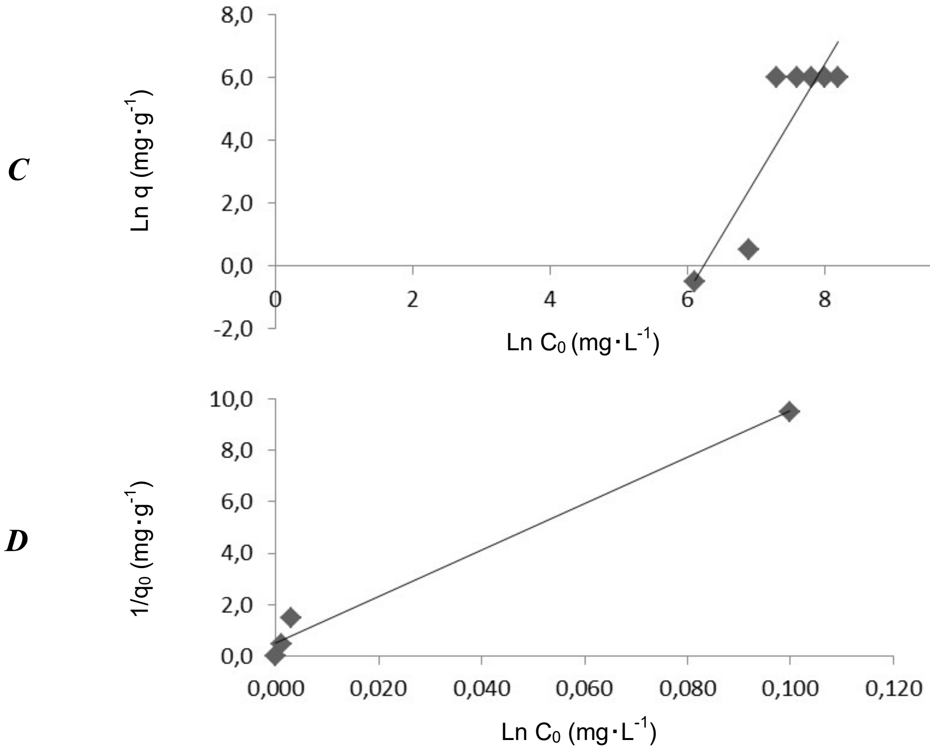

Figure 8. *Linear plot of zinc ions uptake: C - Freundlich model, D - Langmuir model*

The absorption of the three metals by the *Penicillium citrinum* followed the kinetic Langmuir model. The amount of absorbed Zn^{2+} (620 $mg \cdot g^{-1}$) was greater than the absorbed Pb^{2+} and Cu^{2+} but the affinity of the biosorption of Cu^{2+} was the lowest, as the linear regression coefficient was calculated.

E

F

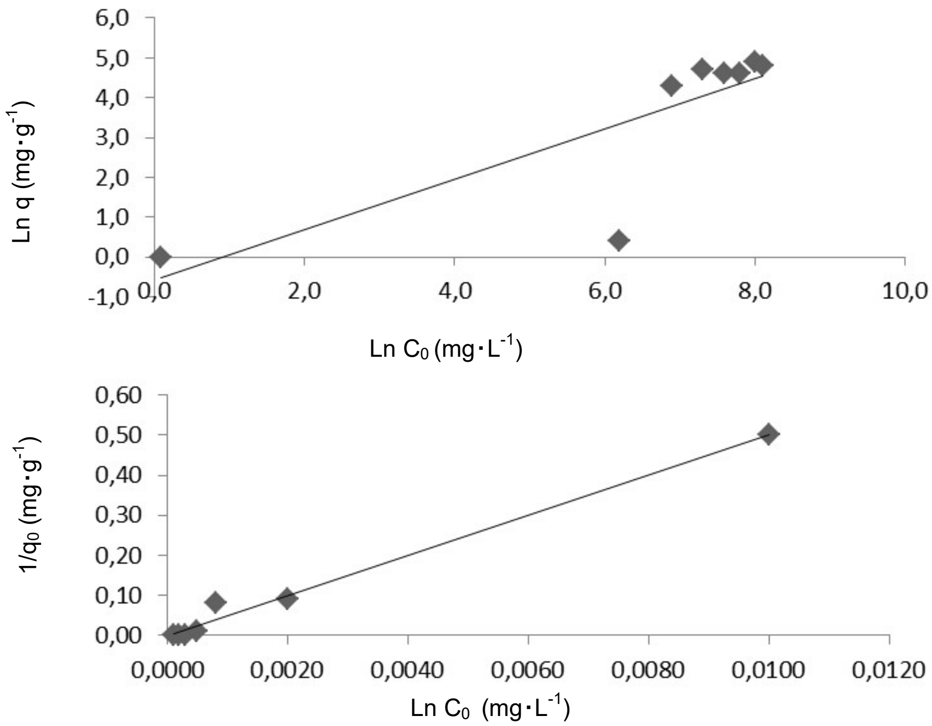

Figure 9. *Linear plot of copper ions uptake: E - Freundlich model, F - Langmuir model*

Effect of pH and temperature

The effect of *p*H on Pb^{2+}, Zn^{2+} and Cu^{2+} uptakes by the dry biomass of *Penicillium citrinum* is shown in Figure 10.

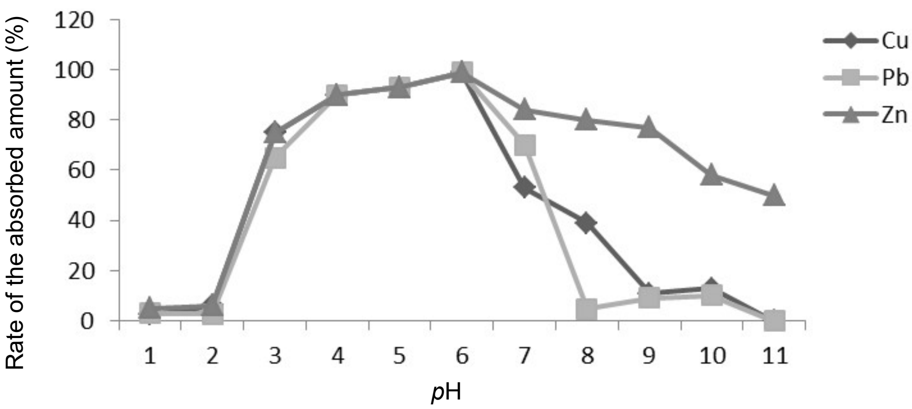

Figure 10. *The effect of initial pH of the medium on the metals (Pb, Zn, and Cu) uptake by dry Penicillium citrinum biomass (agitation rate = 150 rpm, temperature = 28 °C, contact time = 7 days)*

In accordance to other reported results metals biosorption efficiency is strongly *p*H sensitive. A decrease in the absorbed three metals (Pb^{2+}, Zn^{2+}, Cu^{2+}) was observed at lower *p*H (*p*H = 1) and mainly at higher *p*H values (*p*H = 11) indicating that the optimum *p*H for absorption was around 5.5 for Cu^{2+} absorption and was 6 for Pb^{2+} and Zn^{2+} absorption. The comparison of *p*H sensitivity of the three metal ion uptakes investigated in this study showed that the copper uptake was the most sensitive to *p*H change; however the zinc uptake was the least affected by *p*H change.
The coefficients of determination (R^2) were more or less greater than 0.90, indicating that models adequately describe the experimental data of all metal ions biosorption (Table 2). Langmuir model fits better than the Freundlich (R^2 = 0.80) model in describing the experimental data.

Table 2. *Linear regression parameters for Langmuir and Freundlich models of three metals onto Penicillium citrinum dry biomass*

Kinetic model / Metal ions	Langmuir model			Freundlich model		
	R^2	q_m	b	R^2	n	k
Pb^{2+}	0.999	47.61	0.78	0.847	0.793	0.083
Zn^{2+}	0.982	4.56	0.046	0.828	0.247	0.509
Cu^{2+}	0.977	27.77	0.47	0.858	0.564	0.303

Iram and Abrar (2015) [18] found that the maximum biosorption of copper and lead by *Aspergillus niger* and *Aspergillus flavus* at 1400 mg·L^{-1} were 170 and 90 mg·g^{-1}, respectively. In comparison with these previous investigations, this study suggested that the isolated *Penicillium citrinum* was the most effective to uptake and to remove the three heavy metals.
The results revealed that the temperature (°C) plays a key role in biosorption of heavy metals. The maximum lead biosorption was observed at 30 °C with a percentage of 98.04 % (Figure 11).

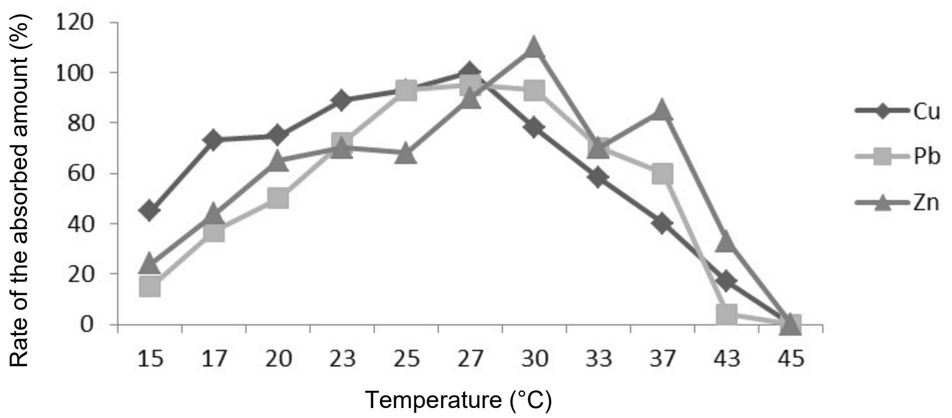

Figure 11. *The effect of the incubation temperature on the metals (Pb, Zn, and Cu) uptake by dry Penicillium citrinum biomass (agitation rate = 150 rpm, pH = 5.5, contact time = 7 days)*

The reported results indicated the optimal temperature for zinc biosorption by *Penicillium citrinum that was* 30 °C, whereas biosorption percentages of the three metals decreased mainly at the high temperatures (0 % at 45 °C) except for zinc uptake.

DISCUSSION

The contaminated sites are the most important sources of metal resistant micro-organisms [22]. Some fungi have the ability to accumulate and bind many heavy metal ions like *Penicillium citrinum,* isolated in this study, while others are specific to only one metal [23]. This specificity of bioaccumulation depends on the capacity of the biosorbent for several types of metal ions with specific affinity for particular metals using the sites with high and more covalent affinity towards resisted metal ions [24].
In this context, biosorption of the tested metals increased with the increase of the initial metal ion concentration, this is explained by the biosorption through extracellular mechanisms implicating cell wall components, this last exhibits excellent metal binding properties as it is negatively charged due to the presence of various anionic structures, such as glucan and chitin, this gives molds the ability to bind metal cations [25]. The second mechanism is intracellular, with transport proteins of metal compounds that may be involved in metal tolerance: efflux out of the metal ion cell or by compartmentalization in vacuoles or also by complexation with cytoplasmic proteins called metallothioneins [26]. However metal bioaccumulation efficiency of metals decreased at high metal concentrations due to saturation of the biosorbent sites [27].
In addition, Ezzouhri *et al.* (2010) [12] reported that Pb^{2+} uptake was passively and actively carried out by *Penicillium* sp. cells. At low metal ion concentrations, lead ions are perfectly tolerable by the fungus and lead uptake occurs as a result of simple ion exchange between metal and functional groups present on cell wall surface of cells. Whereas, at higher metal concentrations, the amount of biosorbed metal ions was more than at low metal concentrations, where more binding sites were free for interaction. Moreover, the higher Pb^{2+} adsorption in cells of *Penicillium citrinum* was expected to contribute to the intracellular uptake of Pb^{2+} occurring in metabolically active cells in combination with extracellular adsorption as a mechanism of metal detoxification. A plausible mechanism of Zn^{2+} uptake was already explained by living cells of *Penicillium* sp. with extracellular adsorption and intracellular uptake as zinc phosphate precipitates/complexes within the cell [28]. Despite copper is known to show toxicity to fungal strain, Soares *et al.* (2002) [29] isolated highly copper-tolerant strain. In this study, the isolated *Penicillium citrinum* resists and accumulates copper probably by using the binding of Cu (II) onto the cell wall surface, as suggested by Anand *et al.* (2006) [11].
At low *p*H of 2.0-3.0, the surface ligands are closely associated with the hydronium ions (H_3O^+) and the uptaken metal cations. As a result, a repulsive force causing decreased metal sorption takes place at low *p*H. At low *p*H, the carboxylic groups cannot bind these metal ions to the fungal cell wall [30]. While, at high *p*H value metals get precipitated; therefore, there is a biosorption decrease [31, 32]. The dependence of metal uptake on *p*H is related to both surface functional groups present on biomass and metal chemistry in solution. Generally, the optimal *p*H for biosorption of heavy metals by fungal biomass is between 5.0 - 5.2 [10]. Temperature also affects the stability of the

cell wall; it can also cause ionization of chemical groups which represent the binding sites on isolated fungal species causing reduction in heavy metal removal. The energy of biosorption mechanisms is affected by temperature in nature [33].

CONCLUSION

As conclusion, some microorganisms provide answers to resolve environmental pollution as *Penicillium citrinum* in this study affirmed its potential ability for removing lead, zinc and copper. This strain may be used as an efficient biological agent for the simultaneous removal of several metals from saline contaminated environments.

ACKNOWLEDGEMENTS

The authors acknowledge Department of Biochemistry, University of Annaba for providing research facilities and Dr. Boudouen Zoulikha for her help in the field of chemistry.

REFERENCES

1. Dube, A., Zbytniewski, R., Kowalkowski, T., Cukrowska, E., Buszewski, B.: Adsorption and migration of heavy metals in soil, *Polish Journal of Environmental Studies*, **2001**, **10** (1), 1-10;
2. Urbaniak, M., Zieliński, M., Wesołowski, W., Zalewski, M.: PCB and heavy metal contamination in bottom sediments from three reservoirs of different catchment characteristics, *Polish Journal of Environmental Studies*, **2008**, **17** (6), 941-949;
3. Kuklová, M., Kukla, J., Hnilička, F.: The soil-to-herbs transfer of heavy metals in spruce ecosystems, *Polish Journal of Environmental Studies*, **2010**, **19** (6), 1263-1268;
4. Wang, J., Chen, C.: Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review, *Biotechnology Advances*, **2006**, **24** (5), 427-451;
5. Kapoor, A., Viraraghavan, T., Cullimore D.R.: Removal of heavy metals using the fungus *Aspergillus niger*, *Bioresource Technology*, **1999**, **70** (1), 95-104;
6. Bhanoori, M., Venkateswerlu, G.: In vivo chitin-cadmium complexation in cell wall of *Neurospora crassa, Biochimica et Biophysica Acta (BBA) - General Subjects*, **2000**, **1523** (1), 21-28;
7. Price, M.S., Classen, J.J., Payne, G.A.: *Aspergillus niger* absorbs copper and zinc from swine wastewater, *Bioresource Technology*, **2001**, **77** (1), 41-49;
8. Liu, H., Ramnarayanan, R., Logan, B.E.: Production of electricity during wastewater treatment using a single chamber microbial fuel cell, *Environmental Science & Technology*, **2004**, **38** (7), 2281-2285;
9. Moon, H., Chang, I.S, Kim, B.H.: Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell, *Bioresource Technology*, **2006**, **97** (4), 621-627;
10. Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., Thamaraiselvi, K.: Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates, *Journal of Hazardous Materials*, **2007**, **146** (1-2), 270-277;
11. Anand, P., Isar, J., Saran, S., Saxena, R.K.: Bioaccumulation of copper by *Trichoderma viride*, *Bioresource Technology*, **2006**, **97** (8), 1018-1025;
12. Ezzouhri, L., Castro, E., Moya, M., Espinola, F., Lairini, K.: Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco, *African Journal of Microbiology Research*, **2009**, **3** (2), 035-048;

13. López-Errasquín, E., Vázquez, C.: Tolerance and uptake of heavy metals by *Trichoderma atroviride* isolated from sludge, *Chemosphere*, **2003**, **50** (1), 137-143;
14. Senthamizhlselvan, P., Sujeetha, J.A.R.P., Jeyalakshmi, C.: Growth, sporulation and biomass production of native entomopathogenic fungal isolates on a suitable medium, *Journal of Biopesticides*, **2010**, **3** (2), 466-469;
15. Diels, L., Van der Lelie, N., Bastiaens, L.: New development in treatment of heavy metal contaminated soils, *Reviews in Environmental Science and Biotechnology*, **2002**, **1** (1), 75-82;
16. Langmuir, I.: The constitution and fundamental properties of solids and liquids Part I. Solids, *Journal of the American Chemical Society*, **1916**, **38**, 2221-2295;
17. Freundlich, H.M.F.: Over the adsorption in solution, *The Journal of Physical Chemistry*, **1906**, **57**, 385-470;
18. Iram, S., Abrar, S.: Biosorption of copper and lead by heavy metal resistant fungal isolates, *International Journal of Scientific and Research Publications*, **2015**, **5** (1), 5 pages, http://www.ijsrp.org/research-paper-0115/ijsrp-p3732.pdf;
19. Zafar, S., Aqil, F., Ahmad, I.: Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil, *Bioresource Technology*, **2004**, **98** (13), 2557-2561;
20. Domsch, K.H., Gams, W., Anderson, T.H.: *Compendium of Soil Fungi*, Academic Press, London, **1980**;
21. Barnett, H.L., Hunter, B.B.: *Illustrated Genera of Imperfect Fungi* (4th ed), APS Press, St. Paul (Minnesota), **1998**;
22. Gadd, G.M.: Interactions of fungi with toxic metals, *New Phytologist*, **1993**, **124** (1), 25-60, https://doi.org/10.1111/j.1469-8137.1993.tb03796.x;
23. Ahluwalia, S.S., Goyal, D.: Microbial and plant derived biomass for removal of heavy metals from wastewater, *Bioresource Technology*, **2007**, **98** (2), 2243-2257;
24. Akar, T., Tunali, S.: Biosorption characteristics of *Aspergillus flavus* biomass for removal of Pb(II) and Cu(II) ions from an aqueous solution, *Bioresource Technology*, **2006**, **97** (15), 1780-1787;
25. Javaid, A., Bajwa, R., Javaid, A.: Biosorption of heavy metals using a dead macro fungus *Schizophyllum commune* fries: evaluation of equilibrium and kinetic models, *Pakistan Journal of Botany*, **2010**, **42** (3), 2105-2118;
26. Liu, Y., Fan, T., Zeng, G., Li, X., Tong, Q., Ye, F., Zhou, M., Xu, W., Huang, Y.-E.: Removal of cadmium and zinc ions from aqueous solution by living *Aspergillus niger*, *Transactions of Nonferrous Metals Society of China*, **2006**, **16** (3), 681-686;
27. Rao, K.R., Rashmi, K., Latha, J.N.L., Mohan, P.M.: Bioremediation of toxic metal ions using biomass of *Aspergillus fumigatus* from fermentative waste, *Indian Journal of Biotechnology*, **2005**, **4** (1), 139-143;
28. Sintuprapa, W., Thiravetyan, P., Tanticharoen, M.: A possible mechanism of Zn^{2+} uptake by living cells of *Penicillium* sp., *Biotechnology Letters*, **2000**, **22** (21), 1709-1712;
29. Soares, E.V., De Coninck, G., Duarte, F., Soares, H.M.V.M.: Use of *Sacchromyces cerevisiae* for Cu^{2+} removal from solution: the advantages of using a flocculent strain, *Biotechnology Letters*, **2002**, **24** (8), 663-666;
30. Choudhary, S., Sar, P.: Characterization of a metal resistant *Pseudomonas* sp. isolated from uranium mine for its potential in heavy metal (Ni^{2+}, Co^{2+}, Cu^{2+}, and Cd^{2+}) sequestration, *Bioresource Technology*, **2009**, **100** (9), 2482-2492;
31. Pino, G.H., de Mesquita, L.M.S., Torem, M.L., Pinto, G.A.S.: Biosorption of heavy metals by powder of green coconut shell, *Separation Science and Technology*, **2006**, **41** (14), 3141-3153;
32. Rani, M.J., Hemambika, B., Hemapriya, J., Kannan, V.R.: Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: A biosorption approach, *African Journal of Environmental Science and Technology*, **2010**, **4** (2), 77-83;
33. Bayramoğlu, G., Bektaş, S., Arica, M.Y.: Biosorption of heavy metal ions on immobilized white-rot fungus *Trametes versicolor*, *Journal of Hazardous Materials*, **2003**, **101** (3), 285-300.