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SHORT COMMUNICATION

Biosorption of mercury (II) from aqueous solution onto biomass of *Aspergillus niger*

Bioadsorción de mercurio (II) en solución acuosa por la biomasa de *Aspergillus niger*

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ABSTRACT

Mercury (II) removal capacity in aqueous solution by *Aspergillus niger* biomass was analyzed by the atomic absorption spectrometry method. The fungus grew in 2000 ppm of the metal (20.3%). Biosorption was evaluated at different pH (3.5, 4.5, and 5.5) at different times. In addition, the effect of temperature in the range of 28°C to 45°C and removal at different initial concentrations of Hg (II) from 100 to 500 mg/L were also studied. The highest biosorption (83.2% with 100 mg/L of the metal, and 1 g of biomass) was 24 h at pH of 5.5 and 28°C. With regard to temperature, the highest removal was to 28°C, with an 83.2% removal at 24 h, and at higher biomass concentrations, the removal was most efficient (100% in 12 h with 5 g of biomass). Fungal biomass showed good removal capacity of the metal *in situ*, 69% removal in contaminated water, after 7 days of incubation and 5 g of biomass (100 mL water), so it can be used to remove industrial wastewater.

Keywords: *Aspergillus niger*, biomass, mercury (II), removal.

RESUMEN

Se analizó la capacidad de remoción de mercurio (II) en solución acuosa por la biomasa de *Aspergillus niger* por el método de espectrometría de absorción atómica. El hongo crece hasta 2000 ppm del metal (20.3%). Se evaluó la bioadsorción a diferentes valores de pH (3.5, 4.5 y 5.5) a diferentes tiempos. También se estudió el efecto de la temperatura en el intervalo de 28°C hasta 45ºC y la remoción a diferentes concentraciones iniciales de Hg (II) de 100 a 500 mg/L. La mayor bioadsorción (83.2% con 100 mg/L del metal y 1 g de biomasa) fue a las 24 h, a pH de 5.5 y 28ºC. Con respecto a la temperatura, la más alta remoción fue a los 28ºC, con un 83.2% de remoción a las 24 h, y a mayores concentraciones de biomasa, la remoción fue más eficiente (100%, 12 h y 5 g de biomasa). La biomasa natural mostró una excelente capacidad de remoción del metal *in situ,* 69% de remoción en aguas contaminadas, a los 7 días de incubación y 5 g de la biomasa (100 mL de agua), por lo que se puede utilizar para eliminarlo de aguas residuales industriales.

Palabras clave: *Aspergillus niger,* biomasa, mercurio (VI), remoción.

1. INTRODUCTION

At present, there is a great concern in the world, due to the considerable increase in the levels of contamination of industrial effluents by heavy metals such as chromium, nickel, cadmium, lead and mercury (Stefanescu *et al.,* 2017). These toxic substances tend to persist indefinitely in the environment, compromising the well-being and balance not only of the fauna and flora existing in the ecosystem but also the health of people living in the surrounding communities, through their accumulation and entry into the food chain (Cabral *et al.,* 2014). Among the various effects produced by heavy metals in plants are necrosis at the tips of leaves, inhibition of root growth, and at worst the total death of the plant. In humans, heavy metals can become very toxic when introduced into the organism. At high concentrations, these can cause: skin rashes, stomach upset (ulcers), respiratory problems, weakening of the immune system, damage to the kidneys and liver, hypertension, alteration of genetic material, cancer, neurological disorders and even death (Nava Ruiz and Mendez Armenta, 2011). The World Health Organization (WHO) established that the maximum concentration of heavy metal ions in water should be in the range of 0.01-1 ppm (WHO, 2003), however, concentrations of heavy metal ions up to 450 ppm have been found in effluents (Srivastava & Anil Dwivedi, 2015). Among the main industrial sectors that are sources of contamination of heavy metals are mining, cement industry, dyes, tanning, electroplating, steel production, photographic material, corrosion paints, energy production, textile fabrication, wood preservation, aluminum anodizing, water cooling and others (Abass *et al.*, 2014; Stefanescu *et al.,* 2017). In Cedral, San Luis Potosí, México the Hg (II) contamination in areas of historical silver amalgamation was evaluate. The total concentrations of Hg in mining residues varied between 8 and 548 mg/kg, while in soils the concentrations were in a range of between 1 and 116 mg/kg. It was found that 80% of the soil samples analyzed exceeded the maximum permissible limit of Hg for land for residential use established in Mexican regulations (Leura Vicencio *et. al*., 2017). Too, Giangrosso *et. al*., (2016), studied the hair mercury levels detection in fisherman from Sicily (Italy).

The environmental impact generated by these toxic substances has led the scientific community to develop different methods for the treatment of industrial effluents contaminated with these substances, among which are: precipitation, oxidation-reduction, exchange ionic, filtration, electrochemical treatment, membrane technologies and recovery by evaporation. However, these methods have been quite costly and inefficient, especially when the metals concentration is very low, as well as the formation, disposal and storage of sludge and wastes, originated during the processes, which becomes a major problem to solve (Tejada-Tovar *et al*., 2015; Caviedes Rubio *et al*., 2015). Therefore, arise emerging technologies such as biosorption, the process of attracting various chemical species by biomass (live or dead), by physicochemical mechanisms as adsorption or ion exchange (Tejada Tovar *et al*., 2015). Recently, varieties of low cost materials have been studied for their ability to removal Hg (II) from aqueous solution and promising results are shown. Among these low cost adsorbents are dead microorganisms, clay minerals, agricultural wastes, industrial wastes and various other low cost materials (Alimohammadi *et al*., 2017; Duygu Ozsoy, 2010; Hoque and Fritscher, 2016; Martínez-Juárez *et al.,* 2012; Shekhawat *et al*., 2017; Stefanescu *et al.,* 2017; Tejada Tovar *et al*., 2015). Thus, there is a need to develop or find innovative low cost adsorbents with an affinity towards metal ions for the removal of Hg (II) from aqueous solution, which leads to high adsorption capacity (Caviedes Rubio *et al*., 2015). The objective of this study was to analyze biosorption of Hg (II) by *Aspergillus niger* biomass, which also remove other metals such as chromium (VI), and arsenic (III, and V).

2. MATERIALS AND METHODS

2.1. Biosorbent used and resistant to mercury test

It worked with a strain of *A. niger* that grows in 200 ppm of As (III) (Santos *et al*., 2017) and that was isolated from the environment from a zone adjacent to the Faculty of Chemical Sciences of the UASLP, San Luis Potosí, S.L.P., Mexico; and was identified based on its morphological characteristics, both macro and microscopic (López Martínez *et al.,* 2004). The fungal strain was routinely maintained on potato dextrose agar. For the obtain the biomass, 1 x 10^6 spores/ mL of the fungus were inoculated in 1 L Erlenmeyer flasks, containing 600 mL of thioglicolate broth, incubating for 5 days at 28ºC and 100 rpm. Later, the biomass was obtained by filtration, washed with 200 mL of trideionized water, and was dried at 80ºC, for 12 hours in bacteriological oven. The product was ground in blender and stored in amber vials until use.
Mercury-resistant tests of the isolated strain, filamentous fungus *A. niger*, were performed on liquid LMM (Lee´s medium minimum) containing the appropriate nutritional requirements and different concentrations of Hg (II) (as mercury chloride), and the dry weight was determined.

2.2. Biosorption studies and determination of divalent mercury

They prepared a series of solutions of mercury of 100 mg/L, pH was adjusted with sulfuric acid 1M and the quantity of biomass added to each flask was of 1 g/100 mL for the mercury´s solution. They were taken samples at different times, the biomass is eliminated for centrifugation (3000 rpm/5min) and the supernatant was analyzed to define the ion metal concentration. The concentration of mercury ions in solution was determined by Atomic Absorption Spectrometry for generation of Hydrides (Atomic Absorption Spectrometer Varian, model Spectra AA- 20), according to the procedure indicated by the Official Mexican Standard (SSA, 1994). The experiments were performed a minimum of 3 times and in triplicate.

3. RESULTS

The cells of the fungal strain grew on LMM supplemented with 2 g/L of Hg (II); about 20.3% of growth relative to control (85.4 mg of dry weight without metal) was obtained (Fig. 1) and, therefore, probably is resistant to the metal. We do not know why, however, at 600 p.m. there is more growth so we can say that there is more resistance.

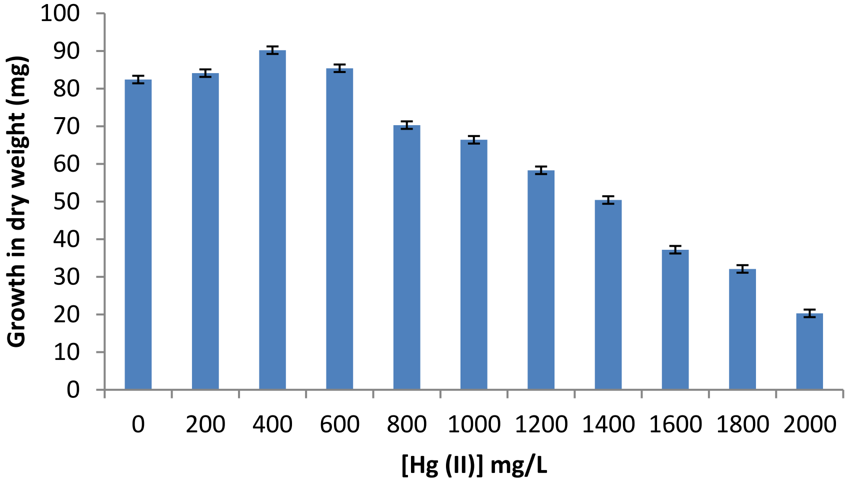

Fig. 1. Final dry weight of *A. niger* with different initial concentrations of Hg (II), 1 × 10^6 spores/mL, 28°C, 7 days of incubation, and 100 rpm.

The optimum time and pH for Hg (II) removal for *A. niger* biomass was 24 h and pH 5.5, at constant values of biosorbent dosage (1 g/100 mL), with an initial metal concentration (100 mg/L), and temperature of 28°C (Fig. 2). Adsorption efficiency of Hg (II) was observe a maximum at pH 5.5 with the biomass analyzed. As the initial pH values decreased from 5.5 to 3.5, the removal efficiencies of Hg (II) with the biomass decreased from 83.2% to 36.1%, respectively (Fig. 2).

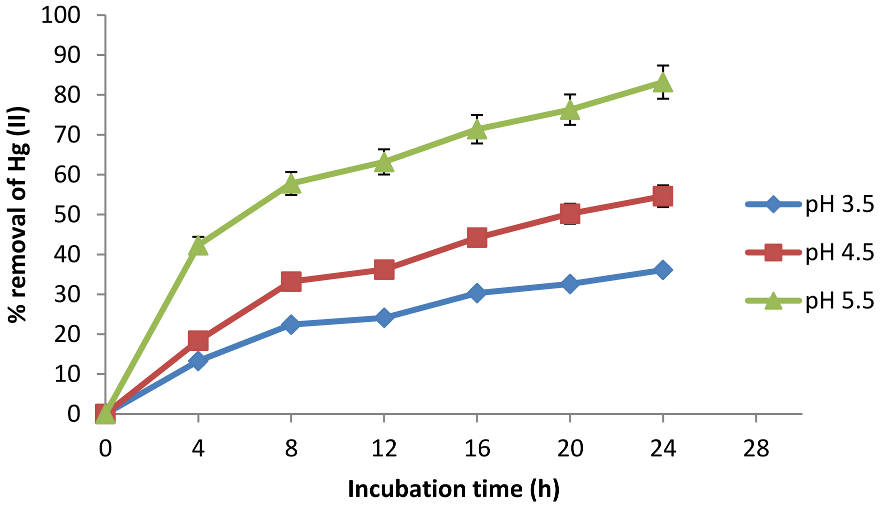

Fig. 2. Effect of incubation time and pH on mercury (II) removal by the biomass of *A. niger*.100 mg/L Hg(II), 100 rpm, 28°C, 1 g of biomass.

Temperature was found to be a critical parameter in the bioadsorption of Hg (II) (Fig. 3). The higher removal was observed at 28°C with 24 h of incubation.

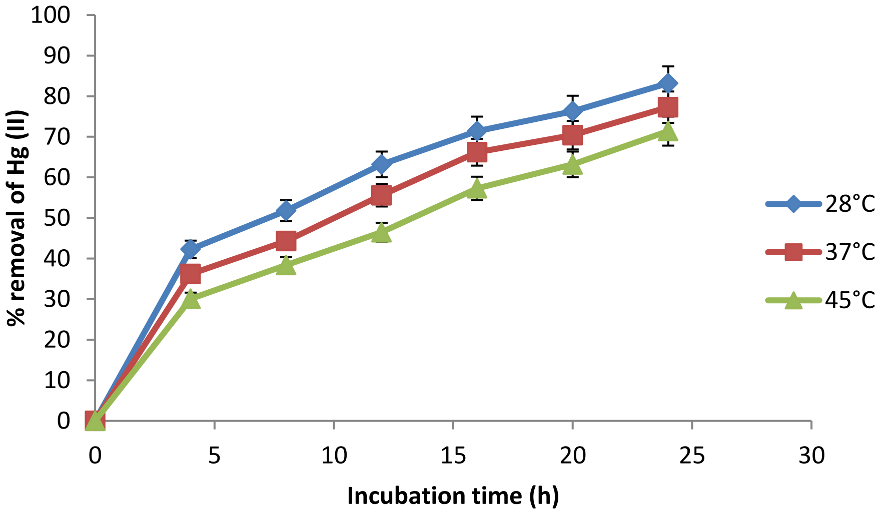

Fig. 3. Effect of temperature on mercury (II) removal by the biomass of *A. niger*. 100 mg/L Hg (II), 100 rpm, 28°C. 1 g of biomass.

Otherwise, at low metal concentrations (100 mg/L), we observe the best results for removal, with the biomass analyzed, at 28°C, respectively (Fig. 4).

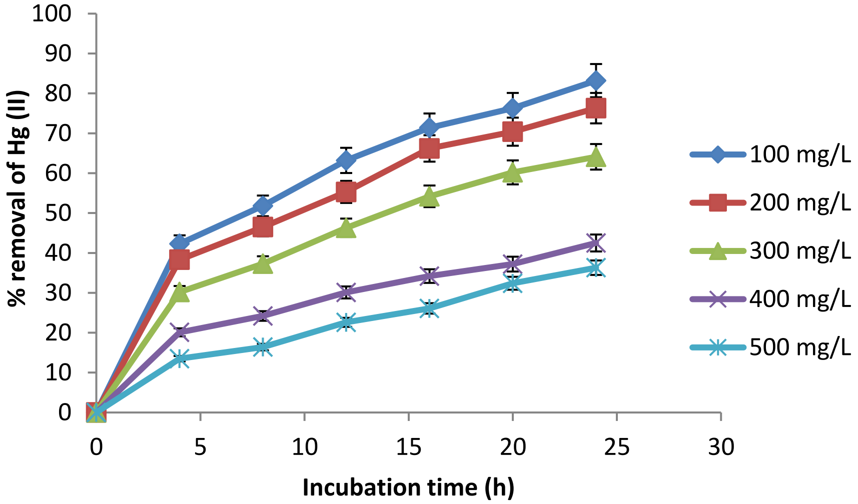

Fig. 4. Effect of initial concentration of metal on mercury (II) removal by the biomass of *A. niger*. 100 rpm, 28°C. 1 g of biomass.

The influence of biomass concentration on the removal capacity of Hg (II) is depict in Fig. 5. If we increase, the amount of biomass it also increases the removal of the metal in solution, with more biosorption sites of the same, because the amount of added biosorbent determines the number of binding sites available for metal biosorption (Cervantes *et al.*, 2001).

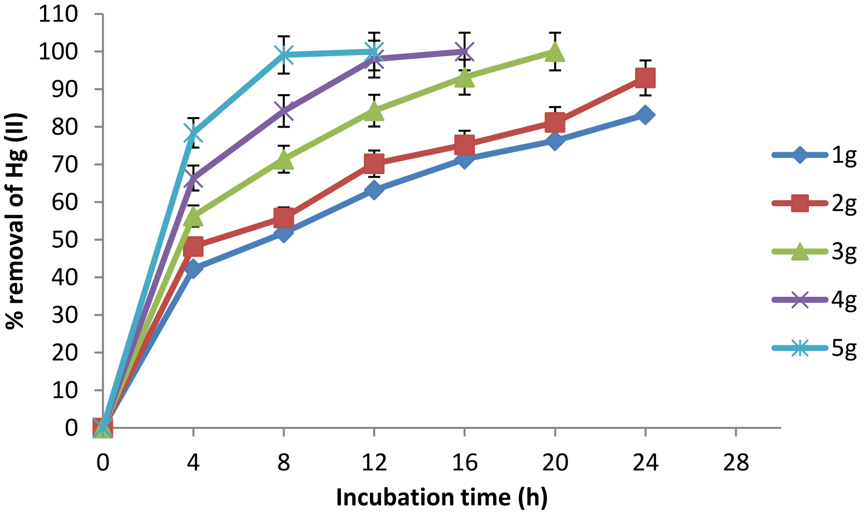

Fig. 5. Effect of biomass concentration on the removal of 100 mg/L of mercury (II) by the biomass of *A. niger*. 100 rpm, 28°C.

Finally, we adapted a water-phase bioremediation assay to explore possible usefulness of this biomass for eliminating Hg (II) from industrial wastes, the biomass (5 g), was incubate with non-sterilized contaminated water containing 183 mg Hg (II)/L. It was observe that in 7 days of incubation with the biomass, the Hg (II) concentration of water samples decrease 69% (Fig. 6).

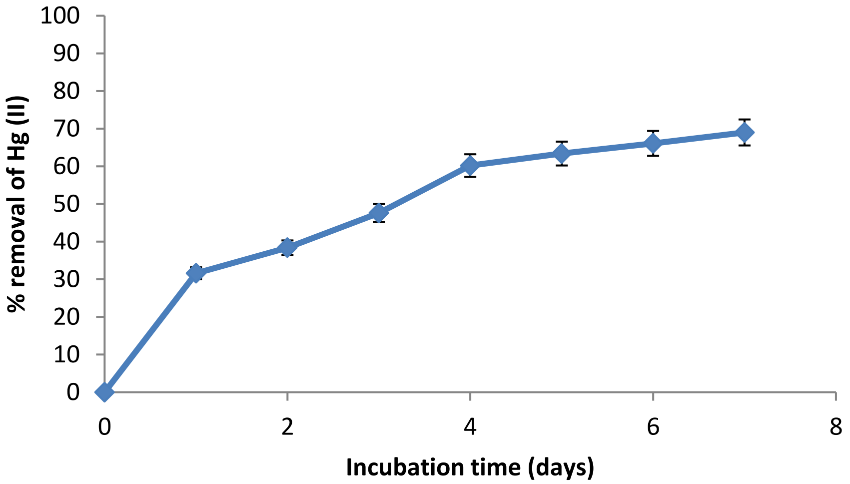

Fig. 6. Removal of Hg (II) in industrial wastes incubated with 5 g of biomass. 28°C, 100 rpm, 100 mL of contaminated water, (183 mg Hg (II)/L.

The adsorption capacities of Cr (VI) with different biosorbents are showed in Table 1.

Table 1. Comparison of adsorption capacities of Cr (VI) with other biosorbents.

Adsorbent	pH	Adsorption capacity mg/g	Reference
Aspergillus niger	5.2	3.2	Karunasagar, *et. al.*, 2003
Aspergillus versicolor	5.0-6.0	75.6	Das *et. al.*, 2007
Different fungal biomasses	5.5	78.3-95.3	Martínez-Juárez *et. al.*, 2012
Eucaliptus leaves	5.0-6.0	129.87	Alimahammadi *et. al.*, 2017
Impregnated chitosan	3.0	43.3	Shekawat *et. al.*, 2017
Phanerochaete chrysosporium	6.0	72.46	Bashardoost *et. al.*, 2010
Pleurotus sapidus	6.0	207.89	Yalcinkaya *et. al.*, 2002
Rhizopus oligosporus	6.0	33.33	Duygu Ozsoy, 2010
Silica gel modified	3.0-7.0	0.20*	Chaves *et. al.*, 2011
Aspergillus niger	5.5	83.2	Present work

4. DISCUSSION

The fungal strain grew about 20.3% with 2 g/L of Hg (II). Different microorganisms that are Hg (II) resistant have been isolated from different contaminated sites: Giovanella *et. al*., 2017) reported the bacteria: *Pseudomonas putida* C50A, *Pseudomonas* sp. B50D, *Alcaligenes faecalis* U21 and *Brevundimonas* sp.U22, which were isolated from metal contaminated residues and effluents, and they growth with 10 μM of Hg; Mahbub *et. al*., (2016) isolated *Sphingobium* SA2, a highly mercury resistant bacterial strain from contaminated soil, with estimated EC_{50} values of 4.5 mg/L and 44.15 mg/L and MIC values of 5.1mg/L and 48.48 mg/L; Koushalshahi *et. al*., (2012), reported the isolation of *Streptomyces* from marine sediments in different regions of the Caspian Sea, resistant to 20 and 40 mg/L of Hg. However, the mechanism of tolerance in this strain of *A. niger* fungus is not investigated.

On the other hand, among the most important environmental problems involving water and soil contamination of the country, is the direct dumping of heavy metals highly pollutants, which constitutes a danger to balance and natural functioning of aquatic ecosystems. One of these metals is mercury, which is used in artisan gold extraction and purification processes. Bioadsorption, to through the use of microorganisms and agroindustrial waste, has proven to be an alternative for the treatment of aquatic matrices contaminated by metals, is a clean technology, efficient and economical for environmental remediation processes. The main factors associated with the process of adsorption of Hg (II) by fungal biomass of *A, niger*, established that this biomaterial has a high potential in the removal of this metal, achieving a maximum removal of 83.2% (100 mg/L of the metal, and 1 g of biomass) at 24 h, pH of 5.5 and 28ºC.The experimental evidence shows a strong effect of the experimental conditions. Maximum biosorption capacity values showed that this biosorbent used are very effective in recovery or removal of mercury ion from aquatic systems, like to others biosorbents (Table 1).

Finally, it was observing that in 7 days of incubation with the fungal biomass, the Hg (II) concentration of wastewater samples decrease 69%. In the literature was reported the ability of the ethylhexadecyldimethyl ammonium bromide impregnated chitosan for removal of cadmium and mercury (Shekhawat *et. al.,* 2017). Two different synthetic effluents containing Cd (II) in first while Hg (II) in second were prepared having similar composition to that of the real effluents and adsorption efficiency was determined for these metal ions. The results show a removal of 86.4%, and 73.8%, for Cd (II) and Hg (II), respectively (Shekhawat *et. al.,* 2017). The widespread presence of biogenic selenium nanoparticles and goethite could significantly removal (81.2%) mercury of contaminated groundwater (Wang *et. al*., 2018). The removal of 99% of the metal from water within 10–48 h after initial exposure to Hg (II), from Munich groundwater, with a new mercury-accumulating *Mucor hiemalis* strain EH8 (Hoque and Fritscher, 2016). These results suggest their potential applicability for the remediation of Hg (II) from polluted water and soils in the fields. Otherwise, in mercury detoxification process, work is still necessary to illustrate the distribution and diversity of the microbial communities under heavy metals stress in order to employ them for the bioremediation of these toxic pollutants, singly or in combination for greater efficiency. Moreover, some mercury biosorbent fungi cannot only detoxify mercury but also remove other metals such as chromium (VI), and arsenic (III, and V) (Acosta-Rodríguez *et. al*., 2017; Santos *et. al*., 2017).

In conclusion, the biomass analyzed, showed a good capacity of biosorption of 100 mg/L Hg (II) in solution at 24 h of incubation, at 28°C, 100 rpm with 1 g of biomass, besides this removal the metal *in situ* (7 days of incubation, 5 g of biomass), in water contaminated.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare

REFERENCES

Abbas, S.H., Ismail, I.M., Mostafa, T.M. & Sulaymon, A.H. 2014. Biosorption of Heavy Metals: A Review. Journal of Chemical Science and Technology. 3(4): 74-102.

Acosta-Rodríguez, I., Cárdenas-González, J.F., Moctezuma-Zárate, M.G., Rodríguez Pérez, A. & Martínez-Juárez, V.M. 2017. Hexavalent Chromium (VI) Removal by *Aspergillus niger*. In: "Metal-microbe interactions and bioremediation: Principles and applications for toxic metals". Editor: Surajis Das. CRS Press Book. Taylor Francis. Chapter 42, pp. 673-688. ISBN: 9781498762434 (ebook).

Alimohammadi, M., Saeedi, Z., Akbarpour, B., Rasoulzadeh, H., Yetilmezsoy, K., Al-Ghouti, M.A., Khraisheh, M. & McKay, G. 2017. Adsorptive removal of arsenic and mercury from aqueous solutions by Eucalyptus leaves. Water Air Soil Pollution. 429: 1-27.

Bashardoost, R., Vahabzadeh, F., Shokrollahzadeh, S. & Ali Reza, M. 2010. Sorption performance of live and heat-inactivated loofa-immobilized *Phanerochaete chrysosporium* in mercury removal from aqueous solution. Iran Journal of Chemical Engineering. 29(4): 79-89.

Cabral, L., Giovanella, P., Kerlleman, A., Gianello; C., Menezes Bento, F., & Oliveira Camargo, F.A. 2014. Impact of selected anions and metals on the growth and in vitro removal of methylmercury by *Pseudomonas putida* V1. International Biodeterioration & Biodegradation. 91: 29-36.

Caviedes Rubio, D.I., Muñoz Calderón, R.A., Perdomo Gualtero, A., Rodríguez Acosta, D. & Sandoval Rojas, I.J. 2015. Tratamientos para la Remoción de Metales Pesados Comúnmente Presentes en Aguas Residuales Industriales. Una Revisión. Revista Ingeniería y Región. 13(1): 73-90.

Cervantes, C., Campos, J., Devars, J.S., Gutiérrez, F., Loza, H., Torres, J.C. & Moreno, R. 2001. Interactions of chromium with microorganisms and plants. FEMS Microbiology Review. 25: 335-347.

Chaves, M.R.M., Valssaraj, K.T., DeLaune, R.D., Gambrell, R.P. & Buchler, P.M. 2011. Mercury uptake by biogenic silica modified with L-cysteine. Environmental Technology. 32(14):1615–1625.

Das, S.K., Das, A. & Guha, A. 2007. A Study on the Adsorption Mechanism of Mercury on *Aspergillus versicolor* Biomass. Environmental Science and Technology. 41: 8281–8287.

Duygu Ozsoy, H. 2010. Biosorptive removal of Hg(II) ions by *Rhizopus oligosporus* produced from corn-processing wastewater. African Journal of Biotechnology. 9(51): 8783-8790.

Giangrosso, G., Cammilleri, G., Macaluso, A.,Vella, A., D'Orazio, N., Graci, S., Lo Dico, G.M. & Galvano, F., Giangrosso, M. & Ferrantelli, V. 2016. Hair mercury levels detection in fishermen from Sicily (Italy) by ICP-MS method after microwave-assisted digestion. Bioinorganic Chemistry and Applications Volume 2016, Article ID 5408014, 5 pages.

Hoque, E. & Johannes Fritscher, J. 2016. A new mercury-accumulating *Mucor hiemalis* strain EH8 from cold sulfidic spring water biofilms. Microbiology Open. 5(5): 763–781.

Karunasagar, D., Arunachalam, J., 1, Rashmi, K., Naveena, J., Latha, L. & Maruthi Mohan, P. 2003. Biosorption of inorganic and methyl mercury by a biosorbent from *Aspergillus niger*. World Journal of Microbiology & Biotechnology 19: 291–295, 2003.

Koushalshahi, M.B., Issazadeh, K., Tehranifard, A., Khoshkholgh Pahlaviani, M.R. & Massiha, A. 2012. Isolation of Hg and Cu resistant *Streptomyces* from marine sediments in different regions of the Caspian Sea. African Journal of Microbiology Research. 6(18): 4048-4052.

Leura Vicencio, A.K., Carrizales Yañez, L. & Razo Soto, I. 2017. Mercury pollution assessment of mining wastes and soils from former silver amalgamation area in North-Central Mexico. Revista Internacional de Contaminación Ambiental. 33(4): 655-669.

López Martínez, R., Méndez Tovar, L.J., Hernández Hernández, F. & Castañón Olivares, R. (2004). Micología Médica. Procedimientos para el Diagnóstico de Laboratorio. Ed. Trillas. México. 2ª. Ed.

Mahbub, K.R., Kannan Krishnan, K., Megharaja, M. & Naidua, R. 2016. Bioremediation potential of a highly mercury resistant bacteria strain *Sphingobium* SA2 isolated from contaminated soil. Chemosphere 144: 330–337.

Martínez Juárez, V.M., Cárdenas-González, J.F., Torre Bouscoulet, M.E. & Acosta Rodríguez, I. 2012. Biosorption of mercury (II) from aqueous solutions onto fungal biomass. Bioinorganic Chemistry and Applications Volume 2012, Article ID 156190, pp. 1-5. doi:10.1155/2012/156190. ISSN: 15653633.

Nava Ruíz, C. & Méndez Armenta, M. 2011. Efectos neurotóxicos de metales pesados (cadmio, plomo, arsénico y talio). Archivo de Neurociencias Mexicanas. 16 (3): 140-147.

Norma Oficial Mexicana NOM-127-SSA1-1994, Salud ambiental. Agua para uso y consumo humano. Límites permisibles de calidad y tratamientos a que debe someterse el agua para su potabilización.

Santos, E.E., Cárdenas, J.F., Torre, M.E., Martínez, V.M., Rodríguez, A. & Acosta, I. 2017. Bioadsorción de arsénico (III) en solución acuosa por la biomasa modificada de *Aspergillus niger*. Avances en Ciencias e Ingeniería. 8(2): 1-10.

Shekhawata, A., Kahua, S., Saravananb, D. & Ravin Jugadea, R. 2017. Removal of Cd(II) and Hg(II) from effluents by ionic solid impregnated chitosan. International Journal of Biological Macromolecules. 104: 1556–1568.

Srivastava S. & Anil Dwivedi K. 2015. Biological Wastes the Tool for Biosorption of Arsenic. Journal of Bioremediation & Biodegradation. 7; 323. doi:10.4172/2155-6199.1000323.

Stefanescu, R, Butnariu, A.E., Zamfirache, M.M., Surleva, A., Ciobanui, C.I., Pintilie, O. & Drochioiu. G. 2017. Yeast-based microbiological decontamination of heavy metals contaminated soils of Tarnita. Carpathian Journal of Earth and Environmental Sciences. 12 (1): 153 – 159.

Tejada-Tovar, C., Villabona-Ortiz, A. & Garcés-Jaraba, L. 2015. Adsorción de metales pesados en aguas residuales usando materiales de origen biológico. Tecno Lógicas. 18(34): 109-123.

Wang, X., Zhang, D., Qian, H., Liang, Y., Pan, X., & Gadd, G.M. 2018. Interactions between biogenic selenium nanoparticles and goethite colloids and consequence for remediation of elemental mercury contaminated groundwater. Science of the Total Environment. 613–614: 672–678.

Yalcinkaya, Y., Arica, Y.M., Soysal, L., Den_Izl, A., Genc, I.O. & Bectas, S. 2002. Cadmium and mercury uptake by immobilized *Pleurotus sapidus*. Turk Journal of Chemical. 26: 441-452.