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Removing Lead from Iranian Industrial Wastewater

Bita Karim Salmani [1, a], Mohammad Ali Amoozegar[2, b*], Hamid Babavalian[3, c], Hamid Tebyanian [4, d], Fatemeh Shakeri[4, e]

[1]Department of Microbiology, Faculty of Science, Science and Research Campus of Islamic Azad University, Tehran, Iran.

[2]Extremophiles Laboratory, Department of Microbiology, Faculty of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran.

[3]Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

[4]Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

[a]salmani@gmail.com, [b]*amoozegar@ut.ac.ir, [c]h.babavalian@yahoo.com, [d]tebyan.hamid@yahoo.com, [e] shakeri1363@gmail.com

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Abstract. Metals and chemicals have been increased in industrial processes which they contain a high level of toxic heavy metals and cause a lot of disadvantages for the environment and human health .Biosorption of Pb (Π) ions has been studied from aqueous solutions in a batch system by using a bacterial strain isolated from petrochemical wastewaters. Strain 8-I was selected to study the impact of different factors on removal rate. According to morphological, physiological and biochemical characterizations of the strain and in comparison with other studies the strain was tentatively identified as *Bacillus sp* strain 8-I. The maximum Lead biosorption capacity of 8-I isolate was determined to be 41.58 % at pH 4.0 with 80 mg/l concentration in 48 hours equilibrium time. The comparison between the biosorption capacity of live (45.50 mg/g), heat inactivated (30.23 mg/g) and NaN_3 pretreated biomass (26.86 mg/g) were indicated that the ability of live biomass for both of active and passive uptake of lead.

INTRODUCTION

Increased use of metals and chemicals in the industrial process has resulted in the generation of large quantities of effluent that contain the high level of toxic heavy metals and their presence poses environmental–disposal problems due to their non-degradable and persistence nature [1]. Furthermore, their accumulation throughout the food chain leads to serious ecological and health problems. Lead is extremely toxic and can damage the nervous system, kidneys, and reproductive system, particularly in children. Lead can contaminate the environment from anthropogenic sources as well as natural geochemical processes [2].

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 precipitation, chemical oxidation or reduction, filtration, ion exchange and electrochemical processes [3, 4]. However, technical and economic constraints encountered in the application of these traditional methods have directed attention to the search for new technologies involving metal removal from waste streams [5, 6]. As a result of development in the field of environmental microbiology, recent studies have focused on the use of microbial-based potential bio-sorbents such as yeast, bacteria and fungi [7]. Dead as well as living cells are used in the removal of metal ions [8]. This interaction includes both bioaccumulation and biosorption. Biosorption is a term that describes the removal of heavy metals by the passive binding to non-living biomass from an aqueous solution. This implies that the removal mechanism is not metabolically controlled. In contrast, the term bioaccumulation describes an active process whereby removal of metals requires the metabolic activity of a living organism [9, 10].

The biosorption process includes physicochemical interactions between metal ions and several anionic ligands present on the biomass like carboxyl, phosphoryl, carbonyl and sulfhydryl. The efficiency of this process depends on various factors varying from the type of metal ion being studied to the type of biosorbent material used as well as pH [7].
The objective of this study was to isolate microbial strains from petrochemical waste water which contaminated with lead ions, study tolerance ability of Pb (Π) of different bacterial isolates, identify and study the biosorption potential of the appropriate strains. The metal loading capacity of bacterial biomass was determined as a function of initial pH, contact time and initial metal ion concentration and the ability of selected isolate for active uptake was investigated [11-15]. Biosorption of Pb (Π) ions has been studied from aqueous solutions in a batch system by using a bacterial strain isolated from petrochemical wastewaters.

MATERIALS AND METHODS

Isolation and screening of microorganism

Three different wastewater samples from one of Iranian petrochemical industry were collected. Each sample was placed in sterilized tubes and 5 ml of each sample centrifuged (3500 rpm) for 20 min, then supernatant and pellet inoculated to 10ml nutrient broth and after 5 days growth in 37 °C, then 10 µl of solution inoculated to nutrient agar medium and finally 99 bacterial isolates by using of nutrient agar (pH adjusted in 7) were obtained. The agar dilution method was used to determine the tolerance of bacterial isolates. Examination of these isolates for Pb^{2+} tolerance were carried out using PYT agar medium (it contained 10 gr peptone, 2 gr yeast extract, 0.8 gr tryptone, 15 gr agar per liter and MES buffer 10 mM, pH was adjusted to 7) supplemented with different concentrations of Pb $(NO_3)_2$ (4,5,6,6.5,7,8,10,12 and 15 mM). Volumes of 20 ml of this medium was poured into 8 cm plates. Then 10µL of bacterial suspensions (1.5×10^8CFU ml^{-1}) were inoculated on each plate and incubated at 37 °C for 48 h. Bacterial isolates were able to grow in a high concentration of Pb $(NO_3)_2$ and were selected (one isolate from each group) [16, 17].

Estimation of Lead uptake by tolerant isolates

To 20 mL of PYT-broth medium supplemented with 80 mg/l of Lead, 200µL of bacterial suspension (1.5×10^8 CFU/ml) from each of tolerant isolate, was inoculated and incubated at 34 °C and 150 rpm for 48 h and 96 h. Additionally, a control without bacterial isolate was supplied. At the end of incubation, bacterial cultures were centrifuged at 5000 rpm for 20 min, and the supernatant was analyzed by AAS to determine residual Pb (Π) concentrations [18, 19].

Characterization of tolerant isolates

Microscopic and biochemical tests were applied to effective bacterial isolate (strain 8-I) according to Bergey's Manual of Systematic Bacteriology. The genus to which this isolate belongs was detected [20].

Preparation of wet biomass

PYT-broth medium containing no Pb ions was used for the production of isolated 8-I biomass. After 48h incubation at 37˚C and 150 rpm, viable biomass was harvested by centrifugation at 5000 rpm for 20 minutes at 4 °C and washed twice with deionized water. The experiments were carried out with wet biomass of the selected strain, but results were calculated using a dry biomass basis. According to Volesky, for scientific interpretations, the sorbent material dry-weight basis is preferred [18].

Preparation of dry cells

Washed biomass (wet) from a measured amount of whole-cell broth was placed in a previously weighed Glass cup and dried in an oven at 80 °C overnight. It was weighed again, the weight of the dry cell mass was calculated by finding the difference [18].

Bio-sorption experiments

The batch bio-sorption experiments were carried out with 20 g wet cells l−1 (3g/l dry weight) of bio-sorbent at 34 °C to elucidate the optimum operating conditions which enhance the Pb (Π) uptakes. The effect of pH on the biosorption capacity of the bacterial biomass for Pb (Π) was investigated in the pH range of 2.0–6.0 by using solutions containing 100 mg l^{-1} of metal ions. The solutions were adjusted to required pH values using 1N HCl and 1N NaOH. Biomass of selected strain was added (2%) to medium and the reaction mixture was shaken at 150 rpm and was incubated at 34˚C for 90 min. Experimental control tests were also carried out in the absence of the biosorbent. Similarly, metal solutions with varying concentrations of Pb ion (ranging from 50 to 200 mg l^{-1}) were used to assess the effect of initial metal ion concentration. In these experiments, the pH of the Pb solutions was adjusted to 4.0 (optimum pH value) [7, 18].

The effect of contact time on the bio-sorption was investigated in the time range of 30–360 min with metal solutions containing 150 mg/l of Pb (Π) ions and pH was adjusted to 4.0. These samples were incubated at 34˚C and shaken at 150 rpm. For this purpose 10ml of samples were taken from the bio-sorption media at different time intervals (30, 60, 120, 240, 360 min) and tested for residual lead-ion concentration [7, 8, 18].

Metal analysis

The concentrations of unabsorbed Pb (Π) in solutions were determined after the separation of bio-sorbent by centrifugation at 5000 rpm for 20 min using flame atomic absorption spectrophotometer. All the experiments were carried out in duplicate and the arithmetical average values were used in calculations. The amount of adsorbed metal ions [2] per gram biomass was calculated using the general definition (Eq. 1):

$$q= (C_i-C_f).V/M \quad \text{(Eq. 1)}$$

Where q is the metal uptake (mg metal per g bio-sorbent), V the liquid sample volume (l), C_i the initial concentration of the metal in the solution (mg/L), C_f the final (equilibrium) concentration of the metal in the solution (mg/L) and M the amount of the added bio-sorbent on the dry basis (g) [7, 21]. The quantity of heavy metal ion uptake was determined by comparing the amount of heavy metal ion sequestered by the biosorbent (q from Eq. 1) with the initial concentration of heavy metals in the refinery effluent before bio-sorption studies. The percentage was calculated as follows (Eq. 2):

$$Q = (q\ max/Co) \times 100 \quad \text{(Eq. 2)}$$

where Q is the adsorption efficiency (%), q max is the maximum uptake capacity (mg/g dry wt) and Co is the concentration of the metal ions before bio-sorption (mg/l) [22].

Pretreatment experiments

An amount of live biomass (0.5 g wet weight) was subjected to pretreatment with two different methods as listed below in an effort to study the effect of pretreatment on Pb (Π) uptake capacity of isolated 8-I biomass: autoclaved for 15 min at 121±2 °C, biomass was added to 0.4 g/l solution of sodium azide (NaN_3) for 210 min and was shaken at 150 rpm. After 210 min, biomass was collected by centrifugation (5000 rpm, 20min). The subsequent bio-sorption experiments using pretreated biomass were performed in initial Pb (Π) ion concentration of 150 mg/l under the optimum pH of 4.0 and contact time of 240 min [21].

RESULTS

A total 78 Gram-positive and 18 Gram-negative bacterial cultures were recovered from the petrochemical waste water. The results of tolerance experiments showed that 63, 29, 2, 3, 2 and 1 percent of strains were resistant in order to less than 4 , 4, 5, 6, 6.5 and 15 mM of Pb $(NO_3)_2$ (Table 1). The results of experiments showed that the highest tolerance of isolates 8-I, 34-II and 13-III were 15, 6.5 and 6 mM of Pb $(NO_3)_2$, respectively. The absorption of Pb (Π) by growing cells of 8-

I, 34-II and 13-III isolates, was detected after 48 and 96 hours of incubation using AAS technique. After 48h, the highest removal percentage of Pb from PYT broth medium contain 80mg/l of lead, was achieved by isolate 8-I (41.58%). After 96h, isolate 8-I and 13-III showed the highest removal percentage of Pb 36.58% and 45.79%, respectively (Table 2).

Table 1: Comparison of MIC of $Pb(NO_3)_2$ between isolates of each samples

Samples	**Isolates**	**Concentration[23]**									
		>4	4	5	6	6.5	7	8	10	12	15
I	$29G^+$	$17G^+$	$11G^+$	-	-	-	-	-	-	-	$1G^+$
	$5G^-$	$1G^-$	$4G^-$								
II	$28G^+$	$18G^+$	$7G^+$	$1G^+$	$1G^+$	$1G^+$	-	-	-	-	-
	11G	$11G^-$									
III	$24G^+$	$14G^+$	$6G^+$	$1G^+$	$2G^+$	$1G^+$	-	-	-	-	-
	$2G^-$	$1G^-$	$1G^-$								

Table 2: Comparison of lead uptake by 48h and 96h cultures in PYT broth medium contain 80 mg/l of Pb (Π) between 3 resistant isolates.

Bacterial Strains	48h	96h
Control	0%	0%
8-I	41.58%	36.58%
34-II	32.45%	27.7%
13-III	30.17%	45.79

Sorption of Pb (Π) of isolated 34-II was decreased after 48 hand production of spores might be the reason for this reduction. 48h and 96 h cultures had a high absorption of Pb (Π) because isolated 8-I tolerated 15 mM of Pb $(NO_3)_2$. Biochemical tests performed for identification and microscopic properties of effective isolate are shown in Table 3. The bacterium was belonging to *Bacillus* genus according to these tests. In this article, the bacterial strain is named as *Bacillus* sp. (8-I).

Table 3: Biochemical and microscopic characteristics of *Bacillus* sp. (8-I)

Properties	**result**	**Properties**	**result**
Gram reaction	+	Acid from D(+)Glucose in aerobic condition	+
Cell shape	Rod	Acid from D(+)Glucose in Anaerobic condition	+
Width	0.7μm	Acid from xylose	-
Length	2.9	Acid from arabinose	-
Spore shape	Swollen	Acid from manitol	-
Spores state	Sub terminal and central	Acid from sucrose	+
		Acid from maltose	+
Size of spores	Greater than cells	Acid from terhalose	+
Catalase	±	Acid from lactose	-
Oxidase	+	Utilization of L-lysine	-
Anaerobic growth	+	Utilization of L-phenylalanin	-
Motility in wet lam	+	Utilization of L-histidine	-
Methyl red test	+	Utilization of L-argenine	-
Voges-proskauer test	-	Utilization of D-glutamic acid	-
Nitrate reduction	+	Utilization of D-manitol	-
Hydrolysis of gelatin	+	Utilization of D-xylose	-
Hydrolysis of starch	-	Utilization of D-glucose	-
Hydrolysis of casein	+	Utilization of L- arabinose	-
Hydrolysis of esculin	+	Growth in NaCl 2%	+

Properties	result	Properties	result
Hydrolysis of tween 80	-	Growth in NaCl 5%	+
lecithinase	+	Growth in NaCl 7%	+
Utilization of citrate	-	Growth in NaCl 10%	-
Urease	-	Growth at 4˚C(after 2 weeks)	+
DNase	-	Growth at 25˚C	+
Motility in SIM	+	Growth at 34˚C	+
Formation of indole	-	Growth at 37˚C	+
H_2S production	-	Growth at pH 3-4, nutrient broth	-
Phenylalanin deaminase	-	Growth at pH 5-11, nutrient broth	+
Hemolytic activity	β - hemolysis		

This property of strain 8-I in bioremediation of wastewaters with living cells is useful. An analysis of experimental results indicates the influence of operating conditions on the biosorption process:

Effect of pH

With the increase in pH (between 2.0 and 4.0), bifunctional groups on the cell walls with negative charges increase due to deprotonation of the metal binding sites which promote metal uptake. Pb (П) ions become precipitate in the form of Pb $(OH)_2$, because of increasing concentration of OH^- ions after the pH values 6.0 for Pb (П) (Table 4).

Table 4: Effect of pH on the metal bio-sorption by *Bacillus* sp. (8-I); 25 ml metal solution (100 mg/L) of Pb (П) was contacted with 0.5g of biomass for 90 min.

pH	2	3	4	5	6
Bio-sorption of Pb (mg/l)	0	32.5	32.5	32.5	32

Effect of initial Pb ions

The effect of initial metal ion concentration on the bio-sorption capacity of *Bacillus* sp. (8-I) was studied at optimum pH value 4.0. These experiments were carried out using metal ion solutions (50–200 mg/L). Amount of adsorption of Pb (П) ion per unit mass of bio-sorbent increased with increasing in initial lead ion concentration (range 50 –200 mg/L) (Table 5), but percent adsorption increased first with increasing in concentration (range 50-150 mg/L), then decreased to a small extent (range 150-200 mg/l) (Table 5) due to rapid saturation of the metal binding sites of the bio-sorbent. The observed enhancement of metal uptake could be due to an increase in electrostatic interactions (relative to covalent interactions) which involve sites of progressively lower affinity for metal ions[18, 21].

Table 5: Effect of initial Pb ion concentration on the amount of bio-sorption of Pb by *Bacillus* sp. (8-I).

Pb concentration (mg/l)	Bio-sorption of Pb (mg/l)	Bio-sorption of Pb (%)
50	9	62%
100	28	86%
150	45	98%
200	54	90%

Effect of time

Figure 1 shows the effect of time course profiles for the adsorption of Pb (П) by biomass of *Bacillus sp.* 8-I. The figure showed that rapid sorption rate (35 mg/g) was observed within the first 30 min, then Pb (П) sorption increased with contact time and reached equilibrium at about 240 min (48.25 mg/g).

In this study, long contact time of 240 min was required for equilibrium and this can be related to active uptake of Pb that need longer time (Figure 1) of metal loading capacity for Pb (Π) which it was found as 48.25 Pb (Π) mg/g biomass at the initial concentration of 150 mg/l with pH 4.0 after 240 min.

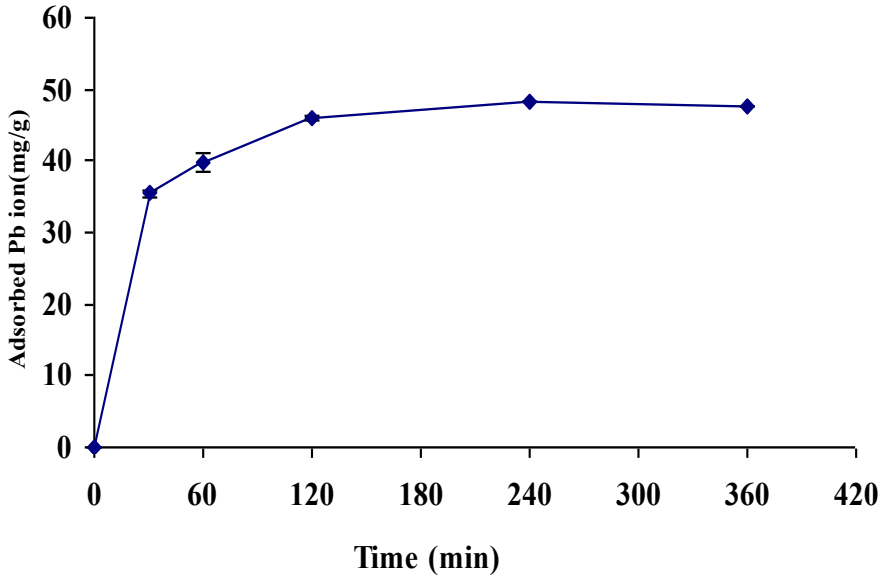

Figure 1: Effect of contact time on bio-sorption of Pb (Π) by *Bacillus sp*. (8-I); 75ml metal solution (150 mg l−1) of Pb (Π) was contacted with 1.5g of biomass at pH4.0 for 240 min. The bars represent the standard error of the mean.

Effect of biomass pretreatments on Pb sorption

The subsequent bio-sorption experiments using pretreated biomass were performed in initial Pb (Π) ion concentration of 150 mg/l under the optimum pH of 4.0 and contact time of 240 min. It has been determined that the amount of Pb (Π) removal by the living biomass was higher than the amount removed by the dead biomass (Table 6). Significant change was not observed between the bio-sorption capacity of autoclaved biomass and NaN_3 pretreated biomass which was 30.23 mg/g and 26.86 mg/g, respectively. Regard to the results of experiments, equilibrium was achieved after 4 hours of contact time and Pb (Π) uptake capacity of inactivated biomass was lesser than active biomass, thus the ability of live biomass was demonstrated for both of active and passive uptake.

Table 6: Effect of pretreatments on the Pb (Π) uptake of *Bacillus* sp. Initial Pb (Π) ion concentration= 150 mg/l, the bio-sorbent concentration= 20 g/l of wet biomass, pH 4.0.

	Adsorbed Pb ion (mg/g)
Live Biomass	45.50
Inactivated Biomass	30.23
NaN_3 Pretreated Biomass	26.86

The obtained results showed that is higher than many of corresponding bio-sorbents reported in the literature (Table 7).

Table 7: Bio-sorption results of Pb (Π) ions from the literature by various bio-sorbent and operating conditions

Biosorbent type	Biosorption capacity (mg/g)	Operating conditions				Refrences
		pH	T(˚C)	Initial Pb concentration range (mg/l)	Biomass (g/l)	
Phanerochaete chrysosporium (formaldehyde and alkali pretreated)	12.34	4.5	27	50	2	[24]
Streptomycess longwoodensis	100	3.0	28	50-200	0.3	[25]
Saccharomyces cerevisiae	2.7	5.0	25	10.4	2	[26]
Rhizopus arrhizus	15.5	5.0	25	0-300	1	[27]
Rhizopus arrhizus	55.6	5-7	-	10-600	3	[28]
Streptomyces noursei	36.5	6.1	30	2-207	3.5	[29]
Mucor rouxii	17.13	5.0	-	10	-	[30]
Pinus sylvestris	11.38	4.0	25	10-100	4	[15]
Streptomyces rimosus (NaOH treated)	135	2-12	-	500	3	[31]
Zooglea ramigera	10.4	4.5	25	0-200	1	[27]
Aspergillus niger	16.8	4.5	30	50	-	[32]
Polyporus squamosus	26.52	4-6	-	-	-	[21]
Botrytis cinerea (heat inactivated)	107.10	4.0	25	0-500	2	[20]
Bacillus sp .(ATS-1)	92.27	3.0	25	250	2	[33]
**Bacillus Cereus M116*	25	6.0	30	50	1.8	[34]
**Bacillus* sp. (OGUB 001)	88	4.5	27	200	20 (wet cells)	[35]
***Bacillus* sp. (8-I)	48.25	4.0	34	150	3 (20 wet cells)	In this study
***Bacillus* sp. (8-I) heat inactivated	30.23	4.0	34	150	3 (20 wet cells)	In this study
***Bacillus* sp. (8-I) NaN_3 pretreated	26.86	4.0	34	150	3 (20 wet cells)	In this study

DISCUSSION

Both Gram-positive and Gram-negative bacteria are ubiquitous in nature with highly resilient cell walls that are anionic. These anionic cell walls can fix metals, and provide sites for nucleation and growth of minerals. This property has been the basis for many heavy metal removal studies using bacterial biomass [9]. The walls of gram-positive bacteria are efficient metal chelators. *Bacillus* sp. (8-I) is a gram-positive bacterium and has similar cell-wall properties as other gram-positive bacteria. Many studies exist concerning the metal tolerance of bacteria; however, it was difficult to make a meaningful comparison with the literature because of the diversity of growth media and

incubation conditions [10]. The present results showed a high incidence of Pb (Π) resistance as compared to other research [10, 16, 17, 36-39]. Bacterial cell metabolic process involved in the uptake of Pb (Π) heavy metal into the cells. Biosorption results of Pb (Π) reported by the other researchers in the literature by various bio-sorbents and operating conditions are summarized in Table 3. This rapid initial uptake is similar to the previous reports on the biosorption of heavy metals by different biosorbents [7, 10]. The uptake values obtained in this study are comparable and were found to be higher than that of many corresponding biosorbents. We selected this isolate to study the effect of different factors on removal rate. The most effective strain was identified according to Bergey's Manual of Systematic Bacteriology [20].

Earlier studies on heavy metal biosorption showed that pH was an important factor affecting the biosorption process [7]. The interaction of metal cations with the electron-rich functional groups located on the biomass may be strongly sensitive to the pH value of the environment. In this study, biosorption was not observed at pH 2.0, the greatest uptake capacity was obtained at pH 3.0 and 4.0, with an increase of pH (4-6), bio-sorption decreased but generally, there was no significant difference in Pb uptake capacity.

The pH of the adsorption medium affects the solubility of metal ions and the ionization state of the functional groups on the cell wall of biosorbent. Metal biosorption depends on the protonation or deprotonation of the functional groups on the cell wall. Because of high proton concentrations at extremely acidic conditions, cell wall functional groups are closely associated with the protons and it restricts the approach of metal ions as a result of increasing positive charge density of binding sites. Indeed, metal cations and protons compete for binding sites on the cell wall, which results in a lower metal uptake [7, 17].

Metal loading capacity greater than 15% of biomass [2] could be used as an economic threshold for practical applications of bio-sorption as compared with alternative techniques [22]. The quantity of Q was autoclaved for living and NaN_3 pretreated biomass was 32.16, 21.13 and 18.87%. Thus, the both of active and inactivated biomass of *Bacillus* sp. 8-I can be used for removal of lead from industrial waste waters.

In this study, about 62% of Pb ions uptake was related to bio-sorption process and 35 mg/g of uptake was observed in 30min of contact time. This rapid initial uptake of metals may be an important parameter for a practical application of bio-sorption in industrial wastewater treatment.

CONCLUSION

A total 99 bacterial strains were isolated from Iranian petrochemical waste waters. Strain 8-I had the highest resistance ability. The bio-sorption properties of *Bacillus* sp. (8-I) were studied for Pb ions in the present work. This result indicated that this strain may be used as an inexpensive, selective, effective and easily cultivable bio-sorbent for the removal of Pb (Π) ions from aqueous solutions. The bio-sorption process of this metal ions on *Bacillus* sp. (8-I) was found which can be dependent on experimental conditions such as the initial pH, initial metal ion concentration and contact time. *Bacillus* sp. (8-I) can remove Pb from an aqueous solution through both bioaccumulation by living cells, and bio-sorption by dead cells.

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CONFLICT OF INTEREST

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