Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22, No. (3) : 2020 : 448-457

ISSN-0972-3005

REMOVAL OF LEAD (II) IONS FROM AQUEOUS SOLUTIONS BY ALKALIPHILIC BACTERIA *HALOMONAS ALKALICOLA* EXT

NARMIN ZMORROD[1], HALA S MOUDALLAL[2], HODA YUSEF[4], RANYA A AMER[1] AND NAWAL AL HAKAWATI[3]*

[1]Biological Sciences Department, Faculty of Science, Beirut Arab University, Debbieh Campus, Lebanon
[2] Faculty of Sciences (I), Lebanese University, El Hadath, Lebanon
[3] Natural Sciences Department, Lebanese American University, Beirut, Lebanon
[4] Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt

(Received 14 February, 2020; accepted 20 March, 2020)

Key words: *Lead (II), Bioaccumulation, Haloalkaliphilic bacteria, Halomonas sp.*

Abstract – Lead (II) is among the toxic pollutants that are considered a serious concern since it has adverse effects on the environment and the human health even at low concentrations. The treatment technologies dealing with the removal of this metal suggest biosorption and/or bioaccumulation as an attractive alternative since these methods are ecofriendly and efficient with low cost. In the present study, three halo alkaliphilic bacterial isolates, A1S2, A1S5 and A1Ext were isolated from wastewater effluents of tannery industry located South Lebanon. These bacterial isolates were screened for their ability to tolerate lead (II). A1S2, A1S5 and A1Ext were found to tolerate up to 500, 250 and 1000 $mg.L^{-1}$ of lead (II) respectively. The most lead (II) tolerant bacterial isolate was studied for its potential to remove lead (II) from aqueous solution. The mechanism of lead (II) removal was assessed using FT-IR measurements and transmission electron microscopy. Several parameters that influence lead (II) withdrawal were optimized. Kinetics of the process was estimated. The bacterial isolate A1Ext was able to remove 27.3 mgg^{-1} of lead (II).

INTRODUCTION

Heavy metals contamination is a serious environmental issue that has been increasing at an alarming rate in the past few decades. Many industries discard their effluents that contain heavy metals into the streams, rivers, sea, and ground water (El-Naggar *et al.,* 2018). These heavy metals have adverse effects on the environment and living biota due to their non-biodegradable nature and tendency to bio accumulate and bio magnify at different trophic levels of the food chain (Barka *et al.,* 2013).

Lead is considered among the most hazardous metals affecting the environment. It is toxic even at very low concentration (Haloi *et al.,* 2013). According to the US Environmental Protection Agency (US-EPA) the MCL (maximum contaminant level) of lead in drinking water should not exceed 0.015 mgL^{-1} (EPA, 2008 and Obuseng *et al.,* 2012). While as for the WHO the drinking-water guideline value for lead is 0.01 mgL^{-1} (WHO, 2004). India standard regulations state that the permissible limit for lead in drinking water is 0.05 mgL^{-1} (Kumar and Puri, 2012). Lead causes many detrimental effects on children and adults. Lead poisoning in children affects their mental health and neurological behaviors such as lowered intelligence, reduced hearing intensity and low attention span. Whereas in adults, lead is considered the most systemic toxicant exerting adverse effects on many organs including nervous, renal, reproductive, hematopoetic and endocrine systems along with being carcinogenic (Tchounwou *et al.,* 2012 and Tiquia-Arashiro, 2018). Thus lead detoxification from wastewater is required for the protection of the human health and well-being.

There are many conventional methods used to eliminate lead (II) from aqueous solutions such as chemical precipitation (Safdar *et al.,* 2011), ion exchange (Srijaranai *et al.,* 2011), reverse osmosis (Peterskova´ *et al.,* 2012), electrochemical treatment (Sulaymon *et al.,* 2011), and filtration (Bessbousse *et al.,* 2008). However the use of these treatment methods have several disadvantages due to practical or cost related issues in other words being difficult

to apply, pollutant concentration dependent, expensive, ineffective in addition to being a source of secondary pollutants (Zouboulis *et al.,* 2004). A better alternative is the biological methods such as biosorption and bioaccumulation that are cost-effective, environment friendly and efficient methods that act through the employment of microorganisms in the removal of heavy metals (Ayangbenro and Babalola, 2017).Biosorption include the use of dead and living microorganisms. It was found that heavy metals uptake using metal-resistant living cells is better due to the active metabolic pathways that are continuously uptaking metals after the passive removal (Guo *et al.,* 2010, Pagnanelli *et al.,* 2013 and Feng *et al.,* 2018).

Biosorption of Pb^{2+} has been studied by many researchers (Tunali *et al.,* 2006; Oves *et al.,* 2013; Iram *et al.,* 2015; Muñoz *et al.,* 2015; Chen *et al.,* 2015; Ren *et al.,* 2015; Jin *et al.,* 2016; Li *et al.,* 2017). But these studies conducted Pb^{2+} removal under acidic or nearly neutral pH conditions. There was few previous studies reported dealing with the biosorption of Pb^{2+} under alkaline conditions. The present investigation deals with the removal of Pb^{2+} under alkaline conditions using alkaliphilic and halo alkaliphilic lead resistant bacteria isolated from tannery wastewater. Mechanism of Pb^{2+} detoxification along with the optimization of the process was investigated.

MATERIALS AND METHODS

Sample collection and analysis

Wastewater samples were collected from two leather tanneries in Saida, South Lebanon. Five Water samples were collected from five discharge points of these tanneries and were transferred to sterile plastic containers, stored at 4 ± 2 °C prior to analysis. After collection, samples were acid digested for determination of lead (Pb). Samples were acid digested and metal analysis was done using Atomic Absorption Spectrophotometer (Thermo Scientific iCE 3000 series). Electrical conductivity and pH of water samples were measured using a conductivity meter (Mi 170 Bench Meter) and a pH meter (Ohaus starter 3100).

Isolation and purification of bacterial isolates

For the isolation of alkaliphilic bacteria, the wastewater samples were enriched in HorikoshiI growth medium (pH 10.0) consisting of (g L^{-1}): glucose 10, yeast extract 5, peptone 5, K_2HPO_4 1, $MgSO_4$ $7H_2O$ 0.2 and Na_2CO_3 10 besides the addition of 20 gL^{-1} of agar for solidification of the medium (Horikoshi, 1999). One milliliter of each wastewater sample was added to 50 mL of sterile HorikoshiI broth and incubated at 35 ± 2 °C in a shaking incubator (ZHWY-2102C) with 150 rpm for 48 hours. For the purification of the bacterial isolates, 0.1 ml from each flask was spread on Horikoshi I agar plate and incubatedat 35 ± 2 °C for 48 hours. Single bacterial colonies were obtained using streaking method using the same growth medium (Horikoshi I agar). The obtained colonies of each bacterial isolate were then examined microscopically with Gram staining.

Screening of lead resistant alkaliphilic bacterial isolates

The ability of the bacterial strains to grow under increasing concentrations of lead solution (Pb^{2+}) was examined by micro broth dilution method (CLSI, M07-A9, 2012). In 96 well microtiter plate, two-fold serial dilutions of Pb^{2+} (nitrate salts) (starting concentration 1000 mgL^{-1}) were transferred to 10 wells of one row. After overnight incubation of the bacterial isolates in Horikoshi I broth medium, the inoculum size was adjusted to 0.5 Mcfarland (1.5×10^8 CFU/mL) and then diluted in sterile Horikoshi I broth to reach 1×10^6 CFU/mL, thereafter 50 µL of each dilution was added into each well (column 1-10) of one row resulting in a final required inoculum of 5×10^5 CFU/mL (Balouiri *et al.,* 2016). The plates were then incubated for 16 to 20 hours at 35 ± 2 °C. The highest concentration of heavy metal supporting bacterial growth was defined as the maximum tolerance concentration (MTC). Bacterial isolate(s) that showed high tolerance to Pb^{2+} was/were chosen for further experiments.

16S rRNA gene sequencing and molecular analysis

Molecular characterization of the selected bacterial strains was performed using 16S rRNA sequence analysis. From the pure bacterial culture, the total genomic DNA was extracted and purified according to Ausubel *et al.* (2003). The 1500 bp fragment of the bacterial 16S rDNA was amplified from the total genomic DNA using universal eubacteria specific primers. The forward primer was: 8F (AGA GTT TGA TCC TGG CTC AG) and the reverse primer was: U1492R (GGT TAC CTT GTT ACG ACT T). The PCR product was purified using Gene JET™

PCR Purification Kit (Thermo K0701). The purified product was sequenced and aligned with known 16S rDNA sequences in the Genbank database using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). Based on the percent homology scores, the bacterial isolate was identified. Phylogenetic tree was constructed using MEGA version 3 software (Kumar *et al.,* 2004).

Conductivity and FT-IR measurements

Overnight bacterial culture was harvested by centrifugation at 6000 rpm for 15 minutes and washed three times with deionized distilled water to get the bacterial pellet. Pb^{2+} (nitrate salts) solution was prepared in deionized distilled water at pH 10. Ten milliliters of Pb^{2+}solution was transferred to the bacterial pellet (0.1 g) and vortexed for 5 minutes then incubated overnight at 30 ± 2 ^{0}C. During incubation period, conductivity of the Pb^{2+} solution was measured at different time intervals (0, 60, 120, 240, 1080 and 1440 minutes) using conductivity meter (Mi 170 Bench Meter).

After the incubation period, FT-IR analysis was done for these bacterial pellets. Dried bacterial cells were analyzed using Fourier Transform Infrared (FT-IR) spectrometer (Thermo Scientific Nicolet iS5 FT-IR) (Jin *et al.,* 2016). The variation in the vibration frequency of metal-free (control) and Pb^{2+} loaded bacterial biomass was detected. Spectra were recorded in the region of 400-4000 cm^{-1} with 32 scans.

Transmission electron microscopy imaging

Transmission Electron Microscope (TEM) (JEM-1400 Plus) was used to determine the location of Pb^{2+} within the bacterial cells, TEMwas at the Electron Microscope Unit, Faculty of Science, Alexandria University, Egypt. Bacterial cells were fixed using universal electron microscope fixative. Series dehydration steps were made to the pellets using ethyl alcohol and propylene oxide. The pellets were then embedded in labeled beam capsules and polymerized. Thin sectionswere obtained using LKB 2209-180 ultra-microtomestained with a saturated solution of uranyl acetate for 30 minutes and lead acetate for 2 minutes (McDo well and Trump, 1976). The same procedure was applied to control bacterial cells (metal-free cells). The magnification used for the investigated samples was 15000x, and the accelerating voltage was 80 Kv.

Factors influencing lead uptake by the investigated bacterial isolate

Further experiments were done on the identified bacterial isolateto study factors affecting its ability to remove Pb^{2+} (nitrate salt) from aqueous solutions. These parameters include the biomass dose, pH of the solutions, temperature, Pb^{2+} initial concentration and kinetics of the process. A batch equilibrium method was used to determine the removal of lead (II) by the bacterial isolate. All set of experiments was done in fixed volume (10 mL) of Pb^{2+} metal ion solution inpolypropylene tubes. Bacterial pellets were obtained from the centrifugation of bacterial culturesat 6000 rpm for 15 minutes.

Effect of biomass dose on Pb^{2+} removal

The effect of bacterial biomass on the removal of lead (II) ions was studied by using different biomass dose 0.05, 0.1, 0.25, 0.5 and 0.75 g of the biomass. Pb^{2+} solution (0.1 $mmolL^{-1}$) was added to each mass of the prepared bacterial pellets in polypropylene tubes. The tubeswere vortexed for 5 minutes and incubated overnight at 30 ± 2 ^{o}C. Then the bacterial biomass was separated from the metal ion solution by filtration through 0.45 μm filters. The amount of remaining lead (II) concentration in the filtered samples was measured using Atomic Absorption Spectrophotometer (Thermo Scientific iCE 3000 series). Lead (II) extraction percentages (% E) were calculated according to the following equation:

$$\% (E) = (C_i - C_f)/ C_i \times 100 \qquad \text{Eq. (1)}$$

$C_i$and C_f are initial and equilibrium Pb^{2+} concentrations ($mmolL^{-1}$) in water.

Effect of solution pH on Pb^{2+} removal

To evaluate the impact of pH on the Pb^{2+} removal, the optimum bacterial biomass was added to 0.1 $mmolL^{-1}$ of Pb^{2+} solution (nitrate salts) with solutions of different pH values ranging from 6 to 10. The pH of the solutions was adjusted by adding 0.1 M H_2SO_4or 0.1 M NaOH solutions. All samples were vortexed for 5 minutes and then incubated overnight at 30 ± 2 ^{o}C. Then the samples were filtered and analyzed for the determination of Pb^{2+} concentration left in the sample using the procedure described in the previous section. The extraction percentage (%E) was calculated using Eq. (1).

Uptake capacity of Pb^{2+} by the investigated isolate

To study the Pb^{2+} uptake capacity of the bacterial isolate, different concentrations of Pb^{2+} (0.1-5

mmolL^{-1}) were set to the most favorable pH value and transferred to the optimum bacterial mass. Then samples were vortexed for 5 minutes and incubated overnight at 30 ± 2 ^{0}C. The uptake capacity, q_{eq} (mmol.g^{-1}) amount of Pb^{2+} taken up per unit of mass of bacterial pellet was calculated using the following Eq:

$$q_{eq} = ((C_i – C_{eq}) \times V)/m \quad \text{Eq. (2)}$$

In Eq. (2), V denotes the sample volume (mL), c_i and c_{eq} are the initial and equilibrium Pb^{2+} concentrations (mmolL^{-1}) respectively, and m is the amount of the bacterial pellet used.

Kinetics of the lead removal process

To study the kinetics of lead (II) bioaccumulation process, optimal experimental conditions were applied but at different time intervals (5, 20, 30, 60, 120, and 1440 min), q_{eq} (mmol g^{-1}) was calculated using Eq. (2), half-life values for lead(II) removal were determined from the plots of the bacterial isolates uptake capacity (q_{eq}) *vs* the time in minutes.

RESULTS AND DISCUSSION

Analysis of tannery effluents

The five collected samples were analyzed for their electrical conductivity (EC), pH and lead concentration values and are shown in Table 1. All the studied parameters were found greater than the standard permissible limit (Inland Surface Water-Bangladesh Standard 1997; Indian Standard Institute–2000; National Environmental Quality Standards 2000). Thus microorganisms that were able to grow and tolerate such toxic values of lead are more likely to play a role in bioremediation of this heavy metal. Further experiments on bacterial isolation and characterization from these samples have been done and are detailed below.

Screening of lead (II) tolerant Alkaliphilic bacterial isolates

A total of ten bacterial isolates were isolated from tannery effluents and were studied for their ability to tolerate Pb^{2+}. Only three bacterial isolates showed tolerance and growth in culture medium supplemented with Pb $(NO_3)_2$. Thus these three isolates (A1S2, A1S5 and A1Ext) were checked for their maximum tolerance concentration to Pb^{2+} by broth micro dilution assay. The bacterial isolates A1S2, A1S5 and A1Ext were found to tolerate up to 500 ± 0.0, 250 ± 0.0 and 1000 ± 0.0 mg L^{-1} Pb^{2+} respectively (Fig. 1). Previous reports have stated a lead tolerance range of 100 to 2200 mg L^{-1} by microorganisms (Amoozegar *et al.*, 2012; Gupta *et al.*, 2012; Suriya *et al.*, 2013; Oves *et al.*, 2013; El Baz *et al.*, 2015 and Kalita and Joshi, 2017). Thus, A1Ext that exhibited highest tolerance to Pb^{2+} was chosen for further studies.

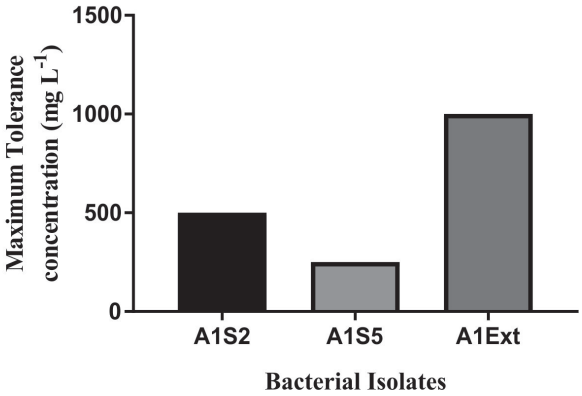


Fig. 1. Maximum tolerance concentration (mg L^{-1}) of isolated alkaliphilic bacteria to Pb^{2+}

Morphological, biochemical and molecular characterization of the Pb^{2+} tolerant bacterial isolate

The selected bacterial isolate A1Ext was characterized and identified at the morphological and biochemical levels. A1Ext isolate was gram negative, motile and rod shaped. The isolate was positive for catalase, oxidase, lipase, gelatinase, protease and amylase enzymes but wasn't able to produce cellulase and pectinase. It showed a

Table 1. Physicochemical properties of tannery effluents

Parameters	S1	S2	S3	S4	S5	Standard Permissible Limits		
						ISI (2000)	NEQS (2000)	ISW-BDS-ECR (1997)
EC (mS/cm)	39.40	22.11	84.80	67.10	53.20	0.85	0.288	-
pH	7.72	8.63	12.30	12.17	10.20	6-9	6-9	6-9
Lead (mgL^{-1})	12.10	7.50	15.87	22.45	26.00	-	0.5	0.05

ISI-2000 =Indian Standard Institute–2000, NEQS (2000) = National Environmental Quality Standards-2000, ISW-BDS-ECR =Inland Surface Water-Bangladesh Standard.

negative result for Voges-Proskauer, citrate utilization, urea hydrolysis, indole and H_2S production. Then A1Ext was further identified at the molecular level and was subjected to 16S rRNA gene sequence analysis. The bacterium showed a maximum homology with *Halomonas* species. The sequence of 16S rRNA of strain A1Ext was submitted to Gen-Bank (GenBank accession number MK 478810). A similar search was performed by using the BLAST program that indicated a 99% similarity of strain A1Ext with the rRNA sequence of *Halomonas alkalicola* strain CICC 11012s in NCBI database. A phylogenetic tree presented in Fig. 2 illustrates the maximum similarity of the isolate with the other 16S rRNA sequences of relevant *Halomonas* species. Tang *et al.* (2017) stated that *Halomonas alkalicola* is an alkaliphilic, moderate halophilic non-spore forming *Halomonas* species, catalase and oxidase positive with incubation temperature range of 15-37 °C.

This fully identified isolate was investigated for its Pb^{2+} removal mechanism along with the optimization of this mechanism in the following sections.

Conductivity and FTIR analysis

Conductivity of Pb^{2+} aqueous solution (0.1 mmol L^{-1}) was measured at t=0 before the addition of bacterial pellet and during the incubation with the haloalkaliphilic bacterium *Halomonas alkalicola* Ext at pH 10. The conductivity value decreased within 18 hours of incubation and after that no change was observed (Fig. 3). This decrease in conductivity can be explained as removal of $Pb^{2+}$by the bacterial isolate. In order to understand better the kind of bacterial functional groups involved in the Pb^{2+} removal process, FT-IR analysis of *Halomonas alkalicola* Ext biomass samples was carried out. The FTIR spectra of unloaded and Pb^{2+} loaded biomass are presented in Fig. 4. The assignment of the bands was referred to the data available for the relevant functional groups. In the infrared spectrum of Pb^{2+} free cells, a broad band including O-H or N-H appeared at 3440 cm^{-1}, C-H stretchinghad a broad

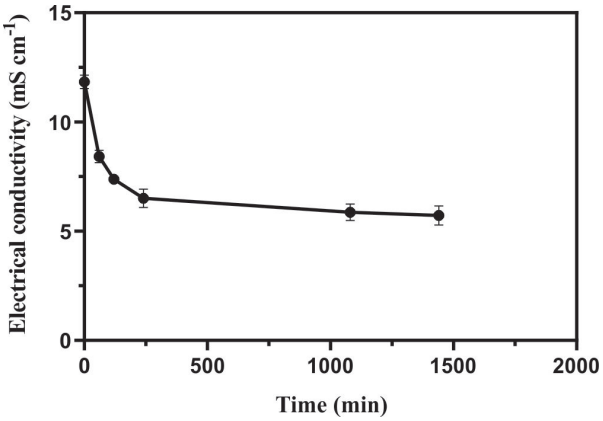


Fig. 3. Electrical conductivity curve of Pb^{2+} solution before and after being interacted with *Halomonas alkalicola* Ext at 30 ± 2 °C (Error bar represents means±SD).

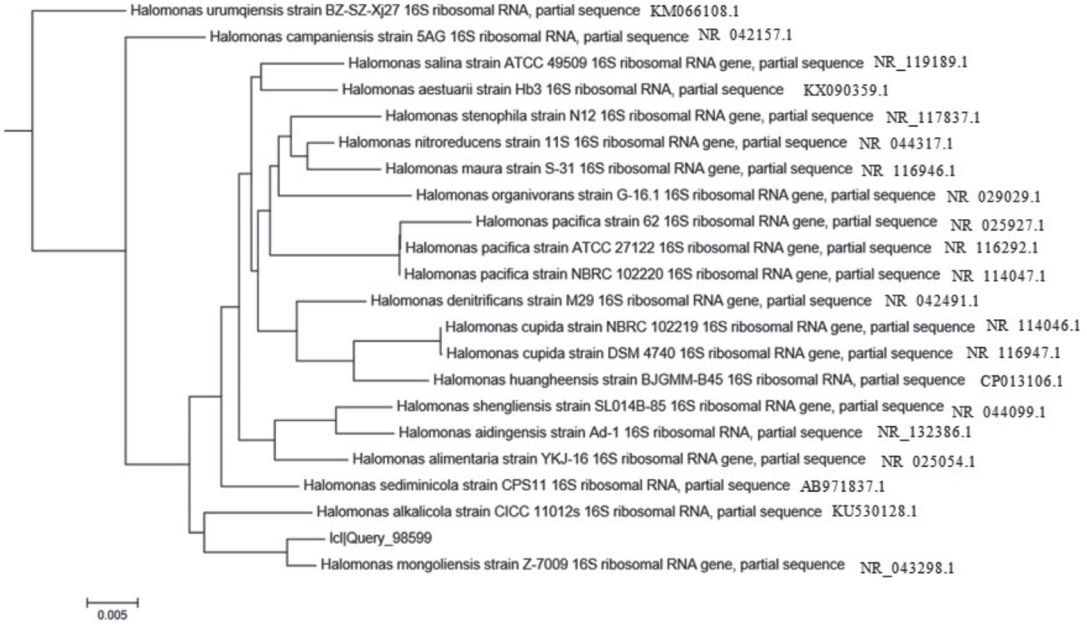


Fig. 2. Phylogenitic tree showing the isolate *Halomonas alkalicola* Ext position and related organisms of *Halomonas* genus based on 16S rRNA gene sequence.

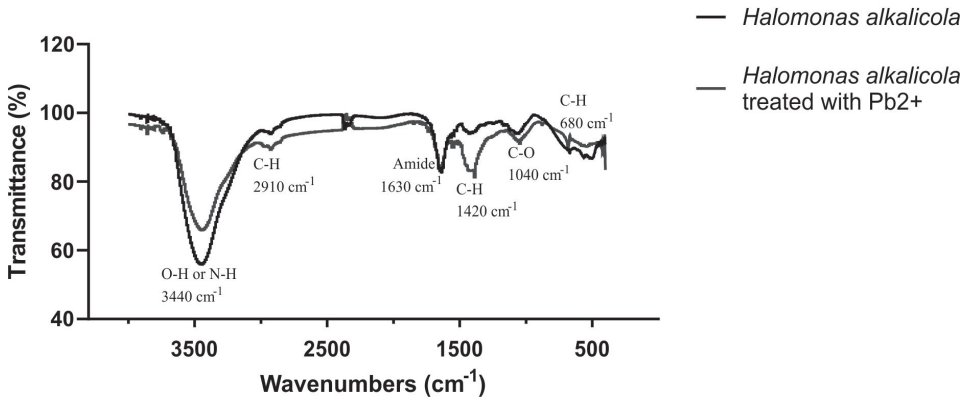
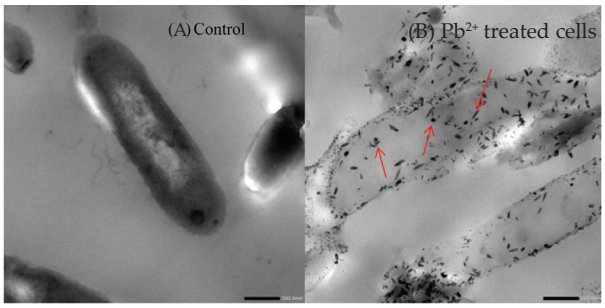


Fig. 4. FT-IR spectra of *Halomonas Alkalicola* cells and Pb^{2+}treated cells displaying the most important bands on the bacterial surface

band at 2910 cm^{-1}, C=O of amide band was assignedat 1630 cm^{-1}. Furthermore, the weak band located at 1040 cm^{-1} represented C-O vibration which is a polysaccharides characteristic peak. The IR spectrum of Pb^{2+} loaded cells revealed a shifting of band at 1040 cm^{-1} which was attributed to the interaction of the metal species with the C-O group. Additionally, there was a slight increase in the transmittance of the band at 3440 cm^{-1} due to the involvement of O-H or N-H groups. It was found that due to the interaction of Pb^{2+}metal ions with the functional groups on the bacterial biomass, the transmittance of the peaks might shift to lower or higher frequencies. These results were in accordance with previously reported studies (Norton *et al.*, 2004; Lodeiro *et al.*, 2006; Tunali *et al.*, 2006; Gabr *et al.*, 2008; Giotta *et al*., 2011). However, at this level we weren't able to determine the removal mechanism of the metal species by the investigated bacterial isolate so further checking of the location of Pb^{2+} in the bacterial cell was done.

Localization of Pb^{2+} within the investigated haloalkaliphilic bacterium using transmission electron microscope

The location of Pb^{2+} within the bacterial cell was evaluated by transmission electron microscope before and after lead loading (Fig. 5A and B). The control cells before the exposure to Pb^{2+}displayed a relatively regular cell shape with a smooth cell wall. After the exposure, the lead treated cells showed an uneven and irregular cell surface containing black dots that represent the precipitated metal. Furthermore, the metal was present in the cytoplasm of *Halomonas Alkalicola* Ext cells indicating its bioaccumulation (arrow). Several studies reported the intracellular uptake of $Pb^{2+}$by



(A) Control (B) Pb^{2+} treated cells

Fig. 5. Transmission electron micrographs of *Halomonas alkalicola*, Ext (A) control untreated cells and (B) cells treated with 0.1 mmol $L^{-1}Pb^{2+}$. Red arrows show the intracellular accumulation of Pb^{2+} in the bacterial cells.

bacterial cells (Ezzouhri *et al.*, 2010; Muñoz *et al.*, 2015; Chen *et al.*, 2015).

Factors affecting Pb^{2+} removal by the bacterial isolate

The results obtained showed that *Halomonas alkalicola* Ext can be used for the removal of Pb^{2+} from aqueous solutions. In addition to its isolation from heavily lead contaminated waste water (26.00 mgL^{-1}) which make this bacteria a good candidate for lead detoxification.

Effect of bacterial biomass on leadremoval process

The amount of biomass is an important parameter affecting lead removal process. As shown in Fig 6, the Pb^{2+}removal percentage increased with the increase in the biomass of *Halomonas alkalicola* Ext. The highest uptake was found at the biomass weight of 0.1 g and beyond this biomass value (0.25 to 0.75 g) the removal percentage remained stable. Thus the optimal biomass that showed highest Pb^{2+} removal

from aqueous solution of 10 mL volume was 0.1 g. This behavior could be explained as the occurrence of competition among the cells that increased as amount of biomass increased leading to decrease in the effectiveness of the surface area having the binding sites. The obtained results were in agreement with previous studies who reported that further increase in biomass concentration has no significant increase on metal removal efficiency (Cho *et al.,* 2004; Masoudzadeha *et al.,* 2011; Abdel – Aty *et al.,* 2013; Chen *et al.,* 2015).

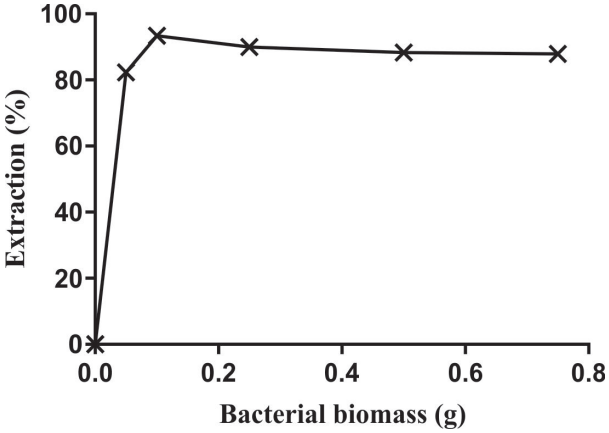


Fig. 6. Bacterial biomass effect on Pb^{2+} extraction by *Halomona salkalicola* Ext (initial concentration 0.1mmol.L^{-1}; pH 10) incubated at 30 ± 2 °C.

Effect of pH

Microorganisms grow in a specific range of pH so the initial pH for growth is one of the major factors influencing heavy metal ions removal from aqueous solutions by bacterial biomass. The Pb^{2+} removal percentage was low at acidic near neutral pH (pH of 6 and 7) and increased when the pH was getting alkaline (Fig. 7). Thus the optimum condition for the removal of Pb^{2+} by *Halomonas alkalicola* was observed at pH 10 (Ma et al 2007 and Mary Mangaiyarkarasi *et al.,* 2011). This result confirm that the bacterial isolate favors the alkaline condition for the extraction of Pb^{2+} compared to neutral or acidic conditions.

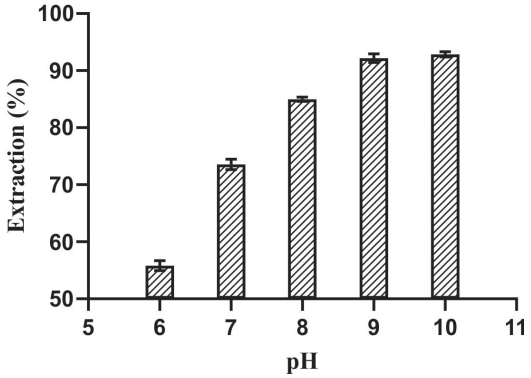


Fig. 7. Effect of pH on the extraction of Pb^{2+} at initial concentration 0.1mmol.L^{-1} by *Halomonas alkalicola* Ext isolate incubated at 30 ± 2 ^{0}C (Error bar represents means±SD).

Lead bioaccumulation capacity by *Halomonas alkalicola*

The data in Fig 9 represents the effect of initial metal concentration on the removal capacity of the bacterial isolate. The uptake capacity of the biomass increased with increasing Pb^{2+} metal ion concentration. However, at higher Pb^{2+} concentrations the uptake capacity remained constant. This could be attributed to the fact that higher heavy metal concentration is toxic to the bacterial cells during the 24 hr incubation period. The obtained result was in agreement with the previous studies reported by Chen *et al.,* 2015 and Shao *et al.,* 2019. Notice that, the bacterial isolate has consumed its need from the metal ion in the studied time interval.

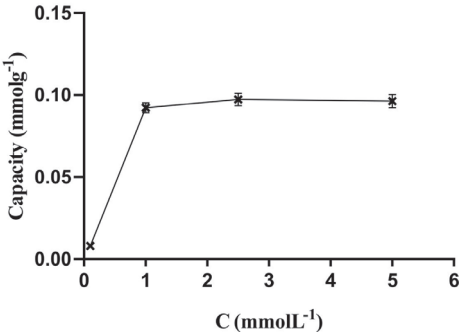


Fig. 8. Pb^{2+} bioaccumulation capacity (mmol g^{-1}) of *Halomonas alkalicola* Ext isolate incubated at 30 ± 2 ^{0}C (Error bar represents means±SD).

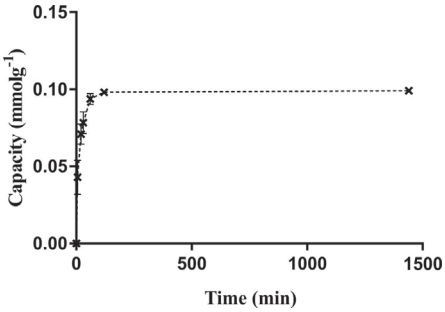


Fig. 9. The uptake of Pb^{2+} by *Halomonas alkalicola* Ext isolate at different contact time at 30 ± 2 ^{0}C incubation temperature (Error bar represents means±SD).

Kinetics of the removal process

The effect of the Pb^{2+} uptake kinetics by *Halomonas alkalicola* Ext was also determined and shown in Fig 9. It was noted that the uptake of Pb^{2+}metal ions occurred in two phases: an initial fast metal uptake followed by slow uptake. This result demonstrates that lead uptake take place by two mechanisms: passive binding to cell surface and intracellular accumulation. Other studies also reported similar results (Wierzba and Latala, 2010, Abdel-Aty *et al.,* 2013, Muñoz *et al.,* 2015; Fadel *et al.,* 2017). These results agreed with the results described in section 3.5.

CONCLUSION

The potential of halo alkaliphilic bacterial isolate to remove lead (II) ions from aqueous solution was investigated in the present study. The bacterial strain isolated from wastewater of tannery industries was able to tolerate up to 1000 mgL^{-1} of Pb^{2+} and to remove the Pb^{2+} ions from aqueous solution by bioaccumulation. The mechanism of the removal process was confirmed using conductivity measurements, FTIR and TEM. Conductivity and FTIR revealed the interaction between Pb^{2+} and the functional groups on the bacterial cell surface. TEM showed the accumulation of the metal ion inside the cell. The optimum operating parameters was proved to be at bacterial biomass of 0.1 g, pH range of 9-10 and incubation temperature of 30 ±2 ^{o}C. Both intracellular accumulation and extracellular absorption may play important roles in the removal of Pb^{2+} at lower concentrations since higher levels of Pb^{2+} could be toxic for the living bacterial cell. Based on the results, this halo alkaliphilic bacterial isolate could be used as a promising, cheap and eco-friendly biosorbent for bioremediation of Pb^{2+} polluted wastewater with alkaline pH. Bioremediation using extremophiles should be further explored since they have the ability to adapt to changes in their environmental surroundings.

REFERENCES

Abdel-Aty, A.M., Ammar, N.S., Abdel Ghafar, H.H. and Ali, R.K. 2013. Biosorption of cadmium and lead from aqueous solution by fresh water alga *Anabaena sphaerica* biomass. *Journal of Advanced Research*.4 : 367-374.

Amoozegar, M.A., Ghazanfari, N. and Didari, M. 2012. Lead and Cadmium Bioremoval by *Halomonas* sp., an Exopolysaccharide-Producing Halophilic Bacterium. *Progress in Biological Science*. 2 : 1-11.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (eds.) 2003. *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc.

Ayangbenro, A.S. and Babalola, O.O. 2017. A New Strategy for Heavy Metal Polluted Environments: A Review of Microbial Biosorbents. *International Journal of Environmental Research and Public Health*. 14 : 94.

Balouiri, M., Sadiki, M. and Ibnsouda, S.K. 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. 6 : 71-79.

Barka, N., Abdennouri, M., El Makhfouk, M. and Qourzal, S. 2013. Biosorption characteristics of cadmium and lead onto eco-friendly dried cactus (*Opuntiacus indica*) cladodes. *Journal of Environmental Chemical Engineering*. 1 : 144-149.

Bessbousse, H., Rhlalou, T., Verchère, J.F. and Lebrun, L. 2008. Removal of heavy metal ions from aqueous solutions by ltration with a novel complexing membrane containing poly (ethyleneimine) in a poly(vinyl alcohol) matrix. *Journal of Membrane Science*. 307 : 249-259.

Chen, Z., Pan, X., Chen, H., Lin, Z. and Guan, X. 2015. Investigation of lead(II) uptake by *Bacillus thuringiensis 016*. *World Journal of Microbiology and Biotechnology*. 31 : 1729–1736.

Cho, D.H., Yoo, M.H. and Kim, E.Y. 2004. Biosorption of lead (Pb2+) from aqueous solution by Rhodotorula aurantiaca. *Journal ofMicrobiology and Biotechnology.* 14 : 250-255.

CLSI, 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Kalita, D. and Joshi, S.R. 2017. Study on bioremediation of Lead by exopolysaccharide producing metallophilic bacterium isolated from extreme habitat. *Biotechnology Reports*. 16 : 48-57.

El-Naggar, N.E., Hamouda, R.A., Mousa, I.E., Abdel-Hamid, M.S. and Rabei, N.H. 2018. Biosorption optimization, characterization, immobilization and application of *Gelidiuma mansii* biomass for complete Pb2+ removal from aqueous solutions. *Scientific Reports*. 8 : 13456.

Ezzouhri, L., Ruiz, E., Castro, E., Moya, M., Espínola, F., Cherrat, L., Er-Raioui, H. and Lairini, K. 2010. Mechanisms of lead uptake by fungal biomass isolated from heavy metals habitats. *Afinidad*. 67 : 39-44.

Fadel, M., Hassanein, N.M., Elshafei, M.M., Mostafa, A.H., Ahmed, M.A. and Khater, H.M. 2017. Biosorption of manganese from groundwater by biomass of *Saccharomyces cerevisiae*. *HBRC Journal*. 13 :106-113.

Feng, C.-l., Li, J., Li, X., Li, K.-l., Luo, K., Liao, X.S. and Liu, T. 2018. Characterization and mechanismof lead and zinc biosorption by growing *Verticillium insectorum* J3. *PLoS ONE*. 13(12) : e0203859.

Gabr, R.M., Hassan, S.H.A. and Shoreit, A.A.M. 2008. Biosorption of lead and nickel by living and non living cells of *Pseudomonas aeruginosa* ASU 6a. *International Biodeterioration and Biodegradation.* 62 : 195-203.

Giotta, L., Mastrogiacomo, D., Italiano, F., Milano, F., Agostiano, A., Nagy, K., Valli, L. and Trotta, M. 2011. Reversible binding of metal ions onto bacterial layers revealed by protonation-induced ATR-FTIR difference spectroscopy. *Langmuir*. 27 : 3762-3773.

Guo, H., Luo, S., Chen, L., Xiao, X., Xi, Q., Wei, W., Zeng, G., Liu, C., Wan, Y., Chen, J. and He, Y. 2010. Bioremediation of heavy metals by growing hyper accumulaorendophytic bacterium *Bacillus* sp. L14. *Bioresource Technology*. 101 : 8599-8605.

Gupta, K., Chatterjee, C. and Gupta, B. 2012. Isolation and characterization of heavy metal tolerant Gram-positive bacteria with bioremedial properties from municipal waste rich soil of Kestopur canal (Kolkata), West Bengal, India. *Biologia.* 67 : 827- 836.

Haloi, N.,Sarma, H.P. and Chakravarty, P. 2013. Biosorption of lead (II) from water using heartwood charcoal of Areca catechu: equilibrium and kinetics studies. *Applied Water Science*. 3 : 559-565

Horikoshi, K. 1999. Alkaliphiles: Some Applications of their Products for Biotechnology. *Microbiology and Molecular Biology Reviews.* 63 : 735-750.

Iram, S., Shabbir, R., Zafar, H. and Javaid, M. 2015.Biosorption and Bioaccumulation of Copper and Lead by Heavy Metal-Resistant Fungal Isolates. *The Arabian Journal for Science and Engineering*. 40 : 1867-1873.

ISI 2000. Guide for Treatment and Disposal of Effluents of Tanning Industry. Indian Standards Institution, New Delhi, India.

ISW-BDS-ECR 1997. Ministry of Environment and Forest, Inland Surface Water in Bangladesh, Gazette notification 27, Aug.

Jin, Y., Wang, X., Zang, T., Hu, Y., Hu, X., Ren, G., Xu, X. and Qu, J. 2016. Biosorption of Lead(II) by *Arthrobacter* sp. 25: Process Optimization and Mechanism. *Journal of Microbiology and Biotechnology*. 26 : 1428-1438.

Kalita, D. and Joshi, S.R., 2017. Study on bioremediation of Lead by exopolysaccharide producing metallophilic bacterium isolated from extreme habitat. *Biotechnology Reports.* 16 : 48–57.

Kumar, M. and Puri, A. 2012. A review of permissible limits of drinking water. *Indian Journal of Occupational and Environmental Medicine*. 16 : 40-44.

Kumar, S., Tamura, K. and Nei, M. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefingsin Bioinformatics.* 5 : 150-163.

Li, J., Zhou, W.Z., Liu, D.S., Liu, T. and Wang, Z.D. 2017. Biosorption isotherm study of Cd2+, Pb2+ and Zn2+ biosorption onto marine bacterium *Pseudoalteromonas* sp. SCSE709-6 in multiple systems. *Journal of Molecular Liquids*. 247 : 230-237.

Lodeiro, P., Barriada, J.L., Herrero, R. and Sastrede Vicente, M.E. 2006.The marine macroalga Cystoseirabaccata as biosorbent for cadmium (II) and lead (II) removal: kinetic and equilibrium studies. *Environmental Pollution.* 142 : 264-273.

Ma, Z., Zhu, W., Long, H., Chai, L. and Wang, Q. 2007. Chromate reduction by resting cells of *Achromobacter* sp. Ch-1 under aerobic conditions. *Process Biochemistry*. 42 : 1028-1032.

Mary Mangaiyarkarasi, M.S., Vincent, S., Janarthanan, S., Subba Rao, T. and Tata, B.V.R. 2011. Bioreduction of Cr(VI) by alkaliphilic *Bacillus subtilis* and interaction of the membrane groups. *Saudi Journal of Biological Sciences*. 18 : 157-167.

Masoudzadeh, N., Zakeri, F., Lotfabad, T., Sharafi, H., Masoomi, F., Zahiri, H.S., Ahmadian, G. and Noghabi, K.A. 2011. Biosorption of cadmium by *Brevundimonas* sp. ZF12 strain, a novel biosorbent isolated from hotspring waters in high background radiation areas. *Journal of Hazardous Materials*. 197 : 190-8.

McDowell, E.M. and Trump, B.F. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. *Archives of Pathologyand Laboratory Medicine.* 100:405-14.

Muñoz, A.J., Espínola, F., Moya, M. and Ruiz, E. 2015. Biosorption of Pb(II) Ions by *Klebsiella* sp. 3S1 Isolated from a Wastewater Treatment Plant: Kinetics and Mechanisms Studies. *Bio Med Research International.* 2015 : 12.

NEQS 2000. National Environmental Quality Standards for municipal and liquid industrial effluents. Url: http:// www.emc.com.pk/pdf/3-NEQS (New) pdf (Last visited: Sept, 23, 2012). Accessed 23 Sept

Norton, L., Baskaran, K. and McKenzie, T. 2004. Biosorption of zinc from aqueous solution using biosolids. *Advances in Environmental Research.* 8:629-635.

Obuseng, V., Nareetsile, F. and Kwaambwa, H.M. 2012. A study of the removal of heavy metals from aqueous solutions by *Moringa oleifera* seeds and amine-based ligand 1,4-bis[N, N-bis(2picoyl) amino] butane. *Analytica Chimica Acta*. 730 : 87-92

Oves, M., Saghir Khan, M. and Zaidi, A. 2013. Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil. *Saudi Journal of Biological Sciences.* 20 : 121-129.

Pagnanelli, F., Jbari, N., Trabucco, F., Martý´nezb, M.E., Sa´nchezd, S. and Toroa, L. 2013. Biosorption-mediated reduction of Cr(VI) using heterotrophically-grown *Chlorella vulgaris*: Active sites and ionic strength effect. *Chemical Engineering Journal*. 231 : 94-102.

Peterskova, M., Valderrama, C., Gibert, O. and Cortina, J.L. 2012. Extraction of valuable metal ions (Cs, Rb, Li, U) from reverse osmosis concentrate using selective sorbents. *Desalination*. 286 : 316-323.

Ren, G.M., Jin, Y., Zhang, C.M., Gu, H.D. and Qu, J.J. 2015.

Characteristics of *Bacillus* sp. PZ-1 and its biosorption to Pb (II). *Ecotoxicology and Environmental Safety*.117 : 141-148.

Safdar, M., Mustafa, S., Naeem, A., Mahmood, T., Waseem, M., Tasleem, S., Ahmad, T. and Siddique, M.T. 2011. Effect of sorption on Co(II), Cu(II), Ni(II) and Zn(II) ions precipitation. *Desalination*. 266 : 171-174.

Shao, W., Li, M., Teng, Z., Qiu, B., Huo, Y. and Zhang, K. 2019. Effects of Pb(II) and Cr(VI) Stress on Phosphate-Solubilizing Bacteria (*Bacillus* sp. Strain MRP-3): Oxidative Stress and Bioaccumulation Potential. *International Journal of Environmental Research and Public Health.* 16 : 1-14.

Srijaranai, S., Autsawaputtanakul, W., Santaladchaiyakit, Y., Khameng, T., Siriraks, A. and Deming, R.L. 2011. Use of 1-(2-pyridylazo)-2-naphthol as the post column reagent for ion exchange chromatography of heavy metals in environmental samples. *Microchemical Journal.* 99 : 152-158.

Sulaymon, A.H., Sharif, A.O. and Al-Shalchi, T.K. 2011. Removal of cadmium from simulated wastewaters by electrode position on stainless steel tubes bundle electrode. *Desalination and Water Treatment*. 29 : 218-226.

Suriya, J., Bharathiraja, S. and Rajasekaran, R. 2013. Biosorption of Heavy Metals by Biomass of Enterobacter Cloacae Isolated From Metal-Polluted Soils. *International Journal of ChemTech Research.* 5: 1329-1338.

Tang, X., Zhai, L., Lin, Y., Yao, S., Wang, L., Ge, Y., Liu, Y., Zhang, X., Zhang, T., Zhang, L., Liu, J. and Cheng, C. 2017. *Halomonas alkalicola* sp. nov., isolated from a household product plant. *International Journal of Systematic and Evolutionary Microbiology*. 67 : 1546 - 1550.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J. 2012. Heavy metal toxicity and the environment. *Experientia Supplementum*. 101 : 133-164.

Tiquia-Arashiro, S.M. 2018. Lead absorption mechanisms in bacteria as strategies for lead bioremediation. *Applied Microbiology and Biotechnology.* 102 : 5437-5444.

Tunali, S., Cabuk, A. and Akar, T. 2006. Removal of lead and copper ion from aqueous solutions by bacterial strain isolated from soil. *Chemical Engineering Journal.* 115 : 203-211.

US Environmental Protection Agency. June, 2008. Lead and Copper Rule: A Quick Reference Guide, EPA 816-F-08-018.

WHO. 2004. *Guidelines for Drinking-Water Quality,* Third edition. World Health Organisation, Geneva, Switzerland (2004).

Wierzba, S. and Latala, A. 2010. Biosorptionlead (II) and nikel (II) from an aqueous solution by bacterial biomass. *Polish Journal of Chemical Technology. 12* : 72-78.

Zouboulis, A.I., Loukidou, M.X. and Matis, K.A. 2004. Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. *Process Biochemistry*. 39 : 909 -916.

http://www.ncbi.nlm.nih.gov/BLAST/