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LEAD AND CADMIUM DETOXIFICATION BY HALOPHILIC BACTERIA ISOLATED FROM SOLAR SALTERNS IN LEBANON

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LEAD AND CADMIUM DETOXIFICATION BY HALOPHILIC BACTERIA ISOLATED FROM SOLAR SALTERNS IN LEBANON

Abstract

Water contamination by heavy metals has gained considerable attention globally. These inorganic pollutants can enter the aquatic environments *via* different routes thereby threatening biodiversity and human health. Lead and cadmium are hazardous pollutants where their removal by traditional techniques is identified to be costly and ineffective. However, bioremediation by extremophilic microorganisms is considered to be a promising technique as they have considerable potentials to grow in harsh environmental conditions. The present study deals with the isolation of halophilic bacterial isolates from solar salterns in North Lebanon. The isolate H1S9 was identified as *Halomonas venusta* H9 after showing tolerance to 500 mg L^{-1} of lead and cadmium. Conductivity and Fourier Transform Infrared spectroscopy showed that Pb^{2+} and Cd^{2+} were removed by *Halomonas venusta* H9. Transmission Electron Microscopy demonstrated the ability of *Halomonas venusta* H9 biomass to bioaccumulate Pb^{2+} and Cd^{2+} from aqueous solution into the cells. The factors affecting the bioaccumulation process were investigated.

Keywords

Lead, Cadmium, Bioaccumulation, Halophilic bacteria, Halomonas sp.

1. INTRODUCTION

Heavy metals levels have increased substantially mainly because of the industrial activities and the improved lifestyle in modern societies. They have been classified as one of the major inorganic toxicants affecting ecosystem and human health (Feng *et al.* 2018). Cadmium and lead metal ions are considered among these toxicants occupying the second and seventh position, respectively in the "Priority List of Hazardous Substances" published by ATSDR (2015). Moreover, cadmium and its compounds are categorized as a Group 1 and lead compounds as Group 2B carcinogens according to the International Agency of Research on Cancer (Sowmya and Abdulla 2017).

Cadmium is known for its toxicity to living organisms even at 0.001–0.1 mg/L concentrations (Alkorta *et al.* 2004; Tang *et al.* 2006). Exposure to the metal ion can lead to complications in the respiratory and renal systems (Abbas *et al.* 2017). In addition to its ability to disturb enzyme activities, inhibit the DNA-mediated transformation in microorganisms, interfere in the symbiosis between microbes and plants, as well as to increase plant predisposition to fungal invasion (Chellaiah 2018). Lead is also considered poisonous even at low concentrations. The USA Environmental Protection Agency regulations set the permissible limit of lead in drinking water to 0.015 mg/L (EPA 2008). Adverse effects of lead include prevention of cell division, accumulation mainly in bones, brain, kidney and muscles and the increase in lead concentration may cause many serious disorders like anemia, kidney and liver diseases, gastrointestinal damage, nervous disorders and sickness even death (El-Naggar *et al.* 2018). Therefore, it is important to detoxify cadmium and lead ions and to treat wastewaters containing these pollutants before being discharged into the environment.

Several conventional methods have been used for the removal of heavy metals from wastewaters such as chemical precipitation, membrane filtration, chemical oxidation or reduction, ion exchange, electrochemical treatment and evaporation. These methods nowadays are not the most favorable ones due to the drawbacks at the cost level and effectiveness. Bioremediation using biomass to sequester heavy metals using different approaches such as biosorption and bioaccumulation offers a relatively efficient, cheap and environment friendly alternative to remove heavy metals from industrial wastewaters (Timková *et al.* 2018). Bacteria (Pagnanelli *et al.* 2003; Oves *et al.* 2013; Al-Homaidan *et al.* 2015; Muñoz *et al.* 2015), fungi (Iram *et al.* 2015; Kariuki *et al.* 2017) and algae (Abdel –Aty *et al.* 2013; Ghoneim *et al.* 2014) have been used as biosorbents for the removal of lead and cadmium ions from different environments.

Several studies have reported the ability of moderate halophiles to tolerate heavy metals (Garcia *et al.* 1987; Nieto *et al.* 1989; Amoozegar *et al.* 2005; Amoozegar *et al.* 2007; Amoozegar *et al.* 2012; Xu *et al.* 2013; Sowmya *et al.* 2014) but very few studies investigated the removal of heavy metals by moderate halophilic microorganisms (Massadeh *et al.* 2005; Al-Momani *et al.* 2007; Amoozegar *et al.* 2012). Thus, the present study investigated the ability of halophilic bacterial strains to tolerate and remove lead and cadmium from aqueous solutions and evaluated the effects of different parameters on the removal process.

2. MATERIALS AND METHODS

2.1. Sampling and Samples Analysis

Seven water samples were collected from solar salterns in Anfeh, North Lebanon and transferred to labeled sterilized bottles, stored in a thermo box immediately after collection. The sampling area was characterized by having concrete ponds filled with seawater that is left for evaporation to produce salt. The collected samples were preserved at $4 \pm 2\ ^{0}C$ before analysis and during experiments. Electrical conductivity and pH of water samples were measured using conductivity meter (Mi 170 Bench Meter) and pH meter (Ohaus starter 3100), respectively.

2.2. Isolation of the Bacterial Strains

Isolation of halophilic bacterial strains from the samples was made using enrichment culture technique by using marine medium (10% salt concentration) consisting of (gL-1) NaCl, 81; MgCl2, 7; MgSO4.7H2O, 9.6; CaCl2, 0.36; KCl, 2; NaHCO3, 0.06; NaBr, 0.026; yeast extract, 10; protease peptone, 5; glucose, 1 and agar, 20 (Atlas 2005). Marine broth was prepared, divided into aliquots of 50 ml and transferred to Erlenmeyer flasks of 250 ml capacity. One milliliter of each sample was inoculated into each flask and incubated in the shaker incubator (ZHWY-2102C) at 150 rpm and 30 ± 2 0C for 48 hours. Following the incubation period, 0.1 ml was transferred from each flask and spread on marine agar plates, incubation was at 30 ± 2 0C for 48 hours. Thereafter, bacterial strains were further purified by streaking on marine agar plates to obtain individual pure colonies of each strain. Smears from each colony were examined microscopically with Gram staining. Some of the biochemical characteristics were studied according to the methods of Mata et al. (2002), such as catalase, oxidase, citrate utilization, Voges-Proskauer, H2S and indole production. In addition, the production of amylase, lipase, cellulose, gelatinase and pectinase was detected (Barrow and Felthman 1993).

2.3. Preparation of Metal Solutions

Stock solutions of lead and cadmium salts (Pb(NO3)2 and Cd(NO3)2) were prepared by dissolving the known amount of each analytical grade in deionized distilled water to get a concentration of 1000 mgL-1. The desired concentrations were prepared by the dilution of the stock solution with deionized water.

2.4. Determination of Heavy Metal Tolerant Bacterial Strains

Bacterial strains were tested for their ability to tolerate different concentrations of Pb2+ and Cd2+ solutions using broth micro dilution method (CLSI 2012). In one row of the 96 wells plate each heavy metal solution was serially diluted ten times using sterile marine broth. Thereafter, each well was inoculated with bacterial suspension that was diluted to reach 1×106 CFU/ml. The plate was then incubated for 16 to 20 hours at 30 ± 2 0C. The maximum tolerance concentration (MTC) of Pb2+ and Cd2+ for a particular bacterial isolate was determined as the maximum concentration of the metal ion in which bacterial growth was visible. Bacterial isolate that showed highest tolerance to Pb2+ and Cd2+ was selected for further experiments.

2.5. Molecular Identification of the Bacterial Strains

The selected halophilic bacterial isolate was identified using 16S ribosomal RNA (rRNA) gene sequencing. The total genomic DNA was extracted from the bacterial cells using bacterial genomic DNA extraction kit. The amplification reaction was performed with the use of the universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T3') in a thermal cycler (Chakraborty and Das 2014). Polymerase chain reaction was performed and the PCR programme utilized was an initial denaturation at 95 0C for 5 min followed by 30 cycles of 95 0C for 1 min, 55 0C for 1 min and 72 0C for 2 min and a final extension at 72 0C for 10 min. Amplified DNA was purified by PCR purification kit (Sigma-Aldrich, USA). The sequencing of the purified PCR product was done by the GATC Company using ABI 3730xl DNA sequencer and examined for sequence homology 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/). A phylogenetic tree was constructed using the neighbour-joining DNA distance algorithm (Saitou and Nei 1987) using MEGA 3. The partial sequence of 16S rRNA gene of the selected isolate was submitted to NCBI GenBank, and an accession number was assigned.

2.6. Conductivity and FTIR Measurement

After overnight incubation, cells of the organism under investigation were harvested by centrifugation at 6000 rpm for 15 minutes and washed three times with deionized distilled water. Each heavy metal solution was prepared to a concentration of 20 ppm of Pb2+ and 10 ppm of Cd2+ metal ion solution. Then the bacterial pellets were transferred to 10 ml volume of each heavy metal solution and agitated for 5 minutes then incubated overnight at 30 ± 2 0C.

During incubation period, electrical conductivity of Pb2+ and Cd2+ solutions were measured separately at different time intervals (0, 60, 120, 240, 1080 and 1440 minutes) using conductivity meter (Mi 170 Bench Meter).

Dried bacterial pellet samples (metal free and metal loaded) were examined with Fourier Transformed Infrared (FT-IR) Spectroscopy (Thermo Scientific Nicolet iS5 FT-IR) within the wavelength range of 400-4000 cm-1 with 32 scans. FT-IR spectra were used to identify the functional groups present on the bacterial surface and to determine the functional groups involved in the metal binding process.

2.7. Transmission Electron Microscopy

Transmission Electron Microscope was used to examine the localization of Pb2+ and Cd2+ within the investigated microorganism. Bacterial cells, after reaching equilibrium with 20 ppm of Pb2+ and 10 ppm of Cd2+ metal ion solution, were fixed using universal electron microscope fixative. Series dehydration steps were followed using ethyl alcohol and propylene oxide. The samples were then embedded in labeled beam capsules and polymerized. Thin sections of bacterial cells were cut using LKB 2209-180 ultra-microtome and stained with a saturated solution of uranyl acetate for half hour and with lead acetate for 2 min (McDowell and Trump 1976). The same procedure was applied to cells un-treated with Pb2+ and Cd2+. Electron micrographs were taken using a Transmission Electron Microscope (JEM-1400 Plus), at the Electron Microscope Unit, Faculty of Science, Alexandria University, Egypt. The magnification used for the investigated samples was 25000 x, and accelerating voltage 80 Kv.

2.8. Optimization of Pb2+ and Cd2+ Removal by the Selected Bacterial Strain

Bacterial isolate was studied for its ability to detoxify Pb2+ and Cd2+ (nitrate salts) from aqueous solutions. Experiments investigating the effects of biomass dose, pH, initial heavy metal concentrations and time on removal of Pb2+ and Cd2+ in growing bacterial cells were conducted.

2.8.1. Biomass dosage

The effect of biomass dose on the removal of heavy metals (Pb^{2+} and Cd^{2+} metal ions) was studied at different dosages of the bacterial biomass (0.05, 0.1, 0.25, 0.5 and 0.75 grams fresh weight). These bacterial biomasses were prepared through the centrifugation of overnight cultures at 6000 rpm for 15 minutes. Each heavy metal solution (10 ml) (20 ppm of Pb^{2+} and 10 ppm of Cd^{2+}) was added separately to the bacterial pellets. The mixture then was agitated for 5 minutes and incubated overnight at 30 ± 2 ^{0}C. Filtration of the sample was done through a syringe filter (0.45µm). The residual concentrations of Pb^{2+} and Cd^{2+} in the filtrate were determined by atomic absorption spectrophotometry (AAS) (Thermo Scientific iCE 3000 series).

The percentage of Pb^{2+} and Cd^{2+} removal (% R) was calculated using the following equation:

$$\% (R) = (C_i - C_{eq})/ C_i \times 100 \qquad \text{Eq. (1)}$$

C_i is the initial and C_{eq} is the equilibrium concentration of heavy metal (mg.L^{-1}) in water.

2.8.2. Effect of pH

The effect of solution pH on heavy metal ions removal was determined in the pH values of 4, 5, 6, 7, 8 and 9 at a fixed volume and concentration of Pb2+ and Cd2+ (10 ml) (20 ppm of Pb2+ and 10 ppm of Cd2+) and at optimum pellet dose of the bacterial strain. The pH values of the solutions were adjusted using 0.1 M NaOH and 0.1 M H2SO4. All samples were agitated for 5 minutes and then incubated overnight at 30 ± 2 0C. Then the samples were filtered and analyzed for the residual Pb2+ and Cd2+ concentrations as mentioned in the procedure of the previous section. The removal percentage (% R) was calculated using Eq. (1).

2.8.3. Effect of initial metal concentration

The effect of initial metal concentration on the ability of the bacterial strain to remove Pb2+ and Cd2+ from aqueous solution was studied. Ten milliliters of different initial concentration of lead (20, 100, 200 and 500 mg.L-1) and cadmium (10, 100, 200 and 500 mg.L-1) were adjusted to optimum pH value and added to the optimum dose of the bacterial biomass. Mixtures were agitated for 5 minutes then left overnight at 30 ± 2 0C. The mixtures were filtered and residual concentrations of Pb2+ and Cd2+ were determined. The removal percentage (% R) was calculated using Eq. (1).

2.8.4. Effect of contact time

The kinetic studies were performed at different time intervals (20, 30, 60, 120, 240, 360, 1080 and 1440 min) to determine the contact time required for achieving the equilibrium between heavy metals and bacterial biomass. The kinetic study was done using the optimum conditions obtained from the biomass dose, pH and initial metal concentration experiments. The removal percentage (% R) was calculated using Eq. (1).

3. RESULTS AND DISCUSSION

3.1. Analysis of Water Samples

Table 1 represents pH and conductivity analysis of water samples collected from solar salterns, North Lebanon. The results summarized in the table show that the pH is neutral. Conductivity values are high since water samples were collected from areas where salt concentration is high, thus the higher the salinity the greater the conductivity. These results were in accordance with the findings of Zafrilla *et al.* 2010, who analyzed the pH and conductivity values in Redonda and Penalva ponds in Spain and obtained similar results.

Table 1: pH and conductivity analysis of the water samples

Sample / Parameters	1	2	3	4	5	6	7
Temperature (^{0}C)	30	30	31	32	32	30	31
pH	7.80	7.81	7.88	7.43	7.46	7.47	7.38
Conductivity ($mScm^{-1}$)	317	78	38	530	370	362	48

3.2.Screening and Selection of Heavy Metal Tolerant Bacteria

A total of ten bacterial strains were isolated from solar salterns and investigated for their ability to tolerate Pb2+ and Cd2+. Two strains were able to grow in culture medium supplemented with Pb(NO3)2 and Cd(NO3)2 and showed high tolerance against these two toxic metal ions. The bacterial isolate H1S2 was able to tolerate up to 250 ±0.0 mg L-1 of both metal ions, while H1S9 was able to grow in the presence of 500 ± 0.0 mg L-1 of Pb2+ and Cd2+ respectively (Fig. 1). Previous studies of moderate halophiles have reported a lead tolerance range of 100 to 1000 mg L-1 and cadmium tolerance range of 10 to 500 mg L-1 (Nieto et al. 1989; Amoozegar et al. 2012; Sowmya et al. 2014). Thus, H1S9 was then selected for further investigations as it showed resistance to higher Pb2+ and Cd2+ metal ion concentrations than H1S2.

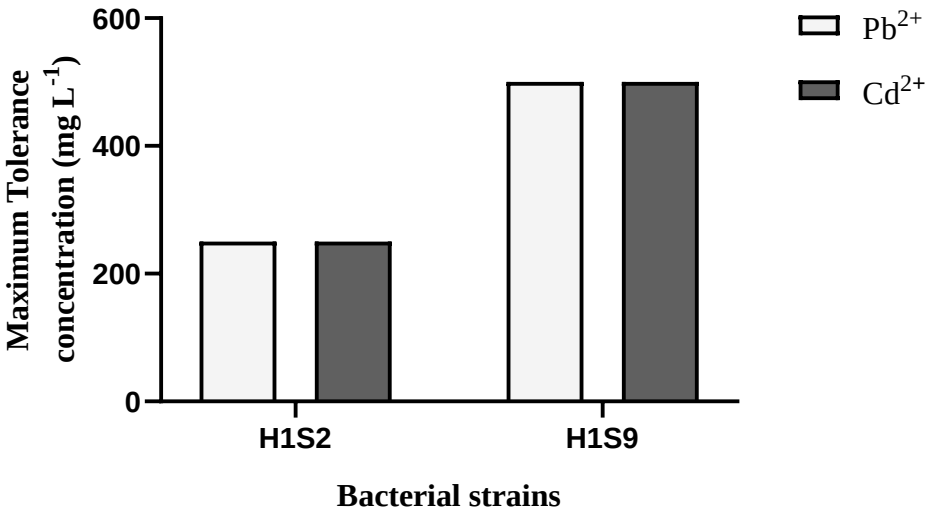


Fig.1: Maximum tolerance concentration of the two bacterial isolates (H1S2 and H1S9) to Pb^{2+} and Cd^{2+} in marine broth medium at 30 ± 2 ^{0}C for 20 hours

3.3.Characterization and Molecular Identification of the Strain H1S9

Findings of the microscopic observation revealed a gram negative, rod shaped and motile H1S9 bacterial strain. The biochemical analyses of the isolate indicated a positive oxidase, catalase, amylase, pectinase and indole. On the other hand, the isolate was found to be negative for lipase, cellulase, gelatinase, Voges-Proskauer, citrate utilization and H_2S production.

In order to further identify this strain, 16S rRNA gene sequence analysis was conducted. The phylogenetic tree based on the16S rRNA sequences showed that strain H1S9 was placed within the genus *Halomonas* and exhibited 99% identity with *Halomonas venusta* strain DSM 4743. The phylogenetic tree presented in Fig. 2 illustrates the maximum similarity of the strain H1S9 with the other 16S rRNA sequences of relevant *Halomonas* species. The Gen-Bank accession number of *Halomonas venusta* H9 was MK357745.

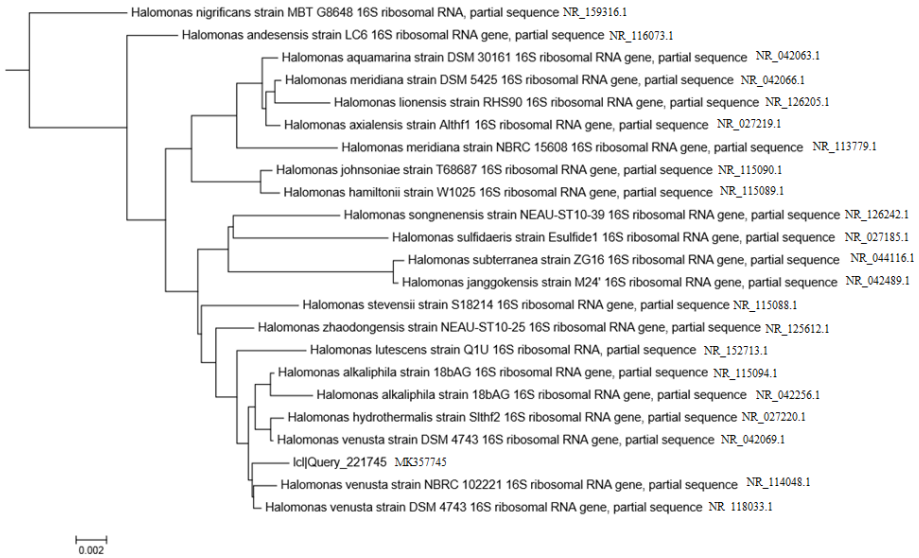


Fig. 2: Phylogenetic tree of the strain *Halomonas venusta* H9 (Gen-Bank accession number MK357745) based on 16S rRNA sequence analysis using MEGA 3.

3.4. Conductivity and FTIR Measurements

After the incubation of the bacterial strain Halomonas venusta H9 with each heavy metal solution separately (Pb2+ and Cd2+ metal ions), the conductivity of the solution decreased with time (Fig. 3). This proposes that the bacterial strain was able to remove Pb2+ and Cd2+ from aqueous solutions. This finding was further corroborated with FT-IR analysis of the bacterial pellets.

The FTIR spectra of H. venusta H9 cells and metal ions loaded H. venusta H9 cells were analyzed to detect any differences due to the interaction between the functional group on the bacterial surface and the heavy metals (Pb2+ and Cd2+ metal ions) (Fig. 4). The infrared spectrum of heavy metal free cells presents a broad band at 3450 cm-1 which was assigned to O-H or N-H stretching vibrations. Another band appeared at 2960 cm-1 was corresponded to C-H stretching. The band at 1640 cm-1 was attributed to the C=O of amide groups. The weak band appeared at 1090 cm-1 was corresponded to C-O vibration which is a polysaccharide characteristic peak. The IR spectrum of Pb2+ and Cd2+ treated cells revealed a shifting of band at 1090 cm-1 which was attributed to the interaction of the metal species with the C-O group. But there was a slight increase in the transmittance of the band at 3450 cm-1 when the cells were treated with Cd2+ due to the involvement of O-H or N-H groups. These changes in the transmittance of the bands were due to the interaction of metal ions with the functional groups on the H. venusta H9 biomass. These results were in accordance with previously reported studies (Lodeiro et al. 2006; Sari and Tuzen 2008; Giotta et al. 2011; Oves et al. 2013; Muñoz et al. 2015). To further understand the mechanism of metal species removal by the investigated bacterial strain a morphological observation was needed to localize Pb2+ and Cd2+ in the bacterial cell.

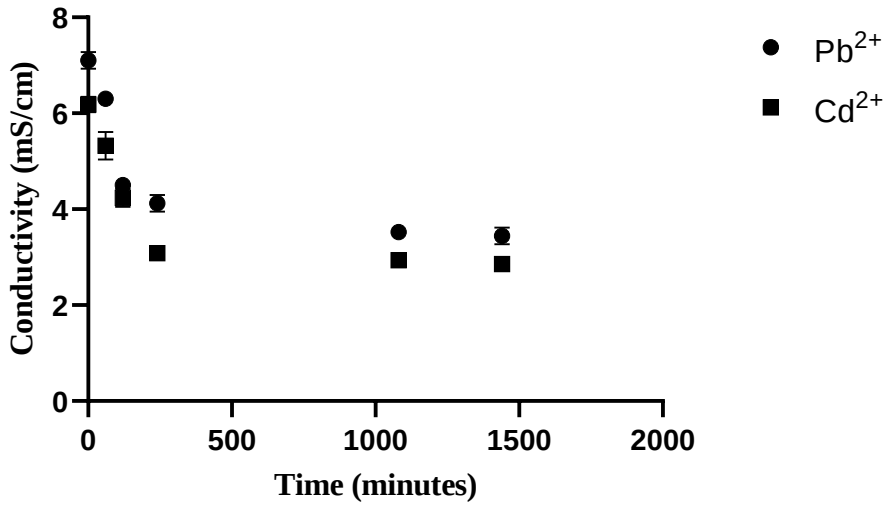


Fig.3: Electrical conductivity measurement of Pb^{2+} and Cd^{2+} (20 ppm of Pb^{2+} and 10 ppm of Cd^{2+}) solution before and after being interacted with *H. venusta* H9 at different time intervals at 30 ± 2 ^{0}C.

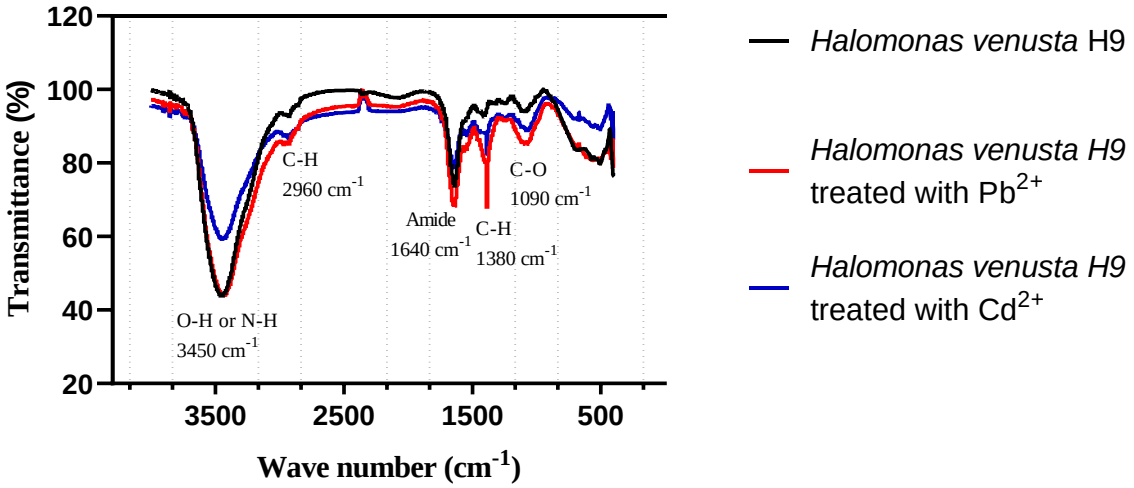
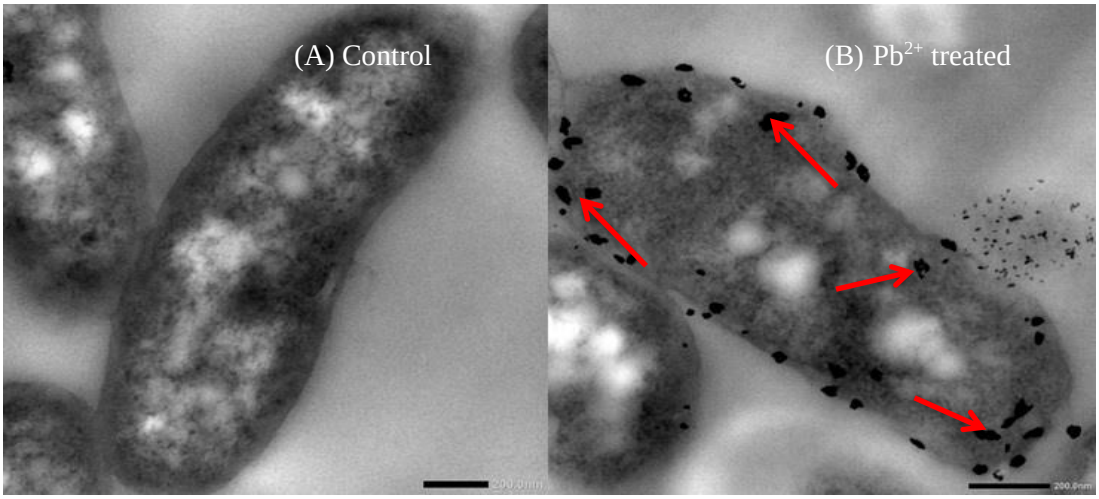


Fig.4: FT-IR spectra of *H. venusta* H9 cells, Pb^{2+} treated cells and Cd^{2+} treated cells showing the peak–functional group assignment.

3.5. Localization of Metal Species Within *Halomonas venusta* H9 Cells by Transmission Electron Microscope

In order to understand the interaction between *Halomonas venusta* H9 with Pb^{2+} and Cd^{2+} metal ions, transmission electron microscope imaging was performed. Fig 5 shows images of *H. venusta* H9 unloaded (A and C) and loaded with Pb^{2+} (B) and Cd^{2+} (D). The control cells (untreated) showed a rod shaped bacterial cell with a sharp cell wall. *H. venusta* H9 cells exposed to lead (II) and cadmium (II) metal ions show the presence of the metals both in the cell wall and accumulated in the interior cytoplasm of the cells. Microscopic observation of Pb and Cd precipitates in bacterial cells was reported by several studies (Ezzouhri *et al.* 2010; Hrynkiewicz *et al.* 2015; Muñoz *et al.* 2015; Chen *et al.* 2015) that all suggested an intracellular uptake of Pb^{2+} and Cd^{2+} and this demonstrates the effectiveness of such bacteria in the detoxification of heavy metal from the environment



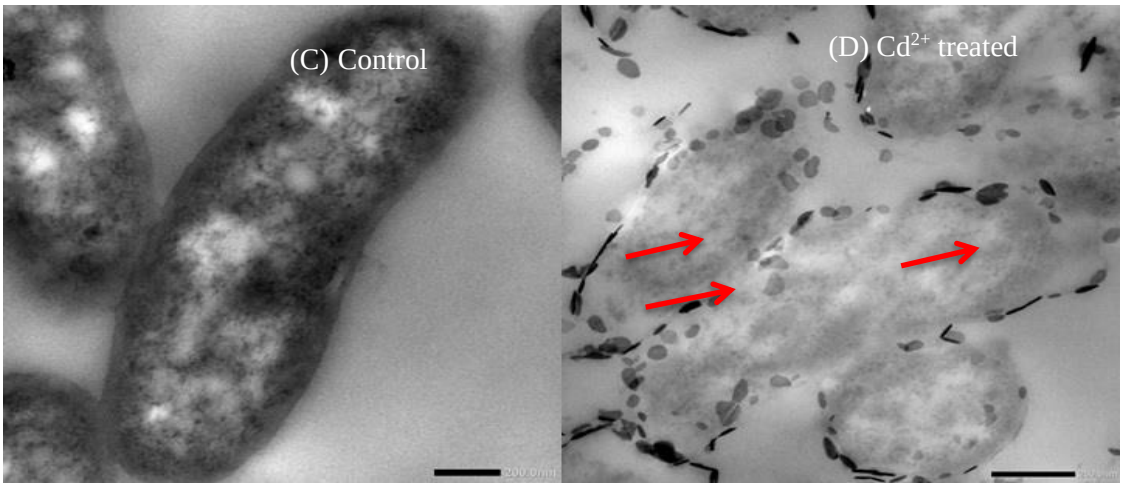


Fig.5: Transmission electron micrographs of *H. venusta* H9 (200 nm magnification), (A and C) control untreated cells, (B) cells treated with 20 ppm of Pb^{2+} and (D) cells treated with 10 ppm of Cd^{2+}. Red arrows show the intracellular accumulation of Pb^{2+} and Cd^{2+} in the bacterial cells.

3.6.Effect of Bacterial Biomass Dose on Removal of Pb^{2+} and Cd^{2+}

The amount of *Halomonas venusta* H9 biomass in the solution is an important parameter affecting metal removal. Pb^{2+} and Cd^{2+} removal percentage increased with the increase in the amount of biomass dose from 0.05 g to 0.1 g wet biomass (Fig. 6) but from 0.25g to 0.75g there was no significant difference of the metal ions removal. This can be due to the removal of all available metal ions in the solution. This result was in accordance with previous studies (Suriya *et al.* 2013; Abdel –Aty *et al.* 2013; Chen *et al.* 2015; Cai *et al.* 2018).

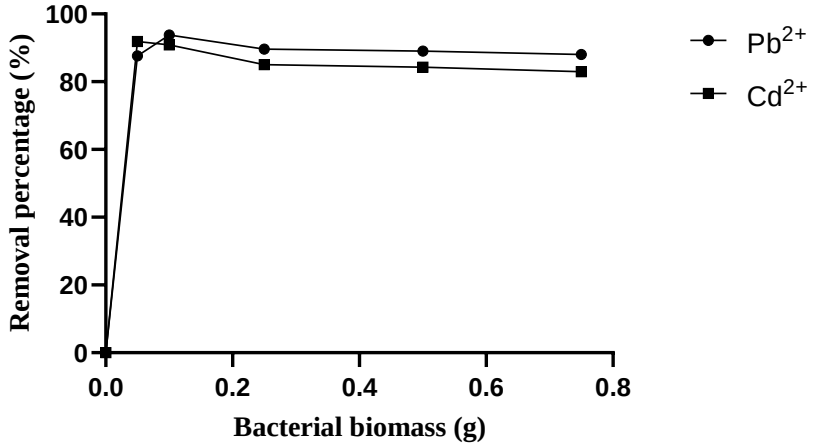


Fig.6: Effect of bacterial biomass on the removal of Pb^{2+} and Cd^{2+} (initial metal concentration 20 ppm of Pb^{2+} and 10 ppm of Cd^{2+}) by *H. venusta* H9 incubated at 30 ± 2 ^{0}C.

3.7. Effects of pH on Pb^{2+} and Cd^{2+} Removal

In order to estimate the role of pH in the removal process, the optimum Halomonas venusta H9 biomass was incubated with each heavy metal solution (20 ppm of Pb2+ and 10 ppm of Cd2+) with varying pH values of 4, 5, 6, 7, 8 and 9. The results (Fig. 7) showed that at low pH (pH= 4) the removal percentage was low and this was due to the presence of excess hydrogen ions in the solution which will compete with Pb2+ and Cd2+ cations for binding on the negatively charged bacterial surface. Then as pH increase to near neutral values (pH 6-7) the removal percentage of Pb2+ and Cd2+ increased and reached its maximum value which was found to be the optimum pH for the growth of H. venusta H9 (Romano et al. 2006). However, at alkaline pH the removal percentage of Pb2+ and Cd2+ decreased. The removal percentage of Pb2+ by the bacterial isolate was higher than that of Cd2+. These results are in accordance with the findings of several studies (Chakravarty and Banerjee 2012; Khan et al. 2016).

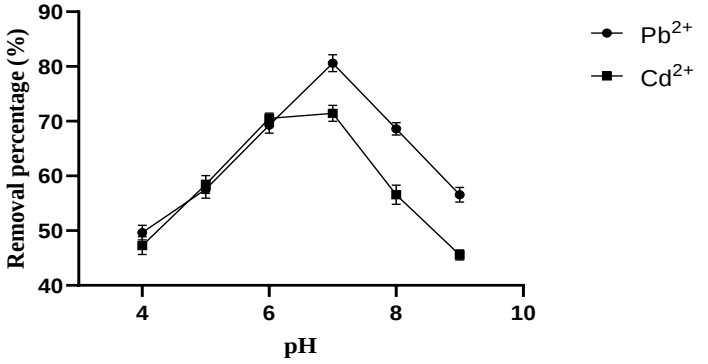


Fig.7: Effect of pH on the removal of Pb^{2+} and Cd^{2+} (initial metal concentration 20 ppm of Pb^{2+} and 10 ppm of Cd^{2+}) by *H. venusta* H9 incubated at 30 ± 2 ^{0}C.

3.8.Effect of Initial Concentration of Heavy Metal on the Removal Process

The removal efficiency of Halomonas venusta H9 (0.1 g wet biomass) was studied at different initial concentrations of each heavy metal (Pb2+: 20, 100, 200 and 500 ppm; Cd2+: 10, 100, 200 and 500 ppm). It was observed that there was an increase in the removal percentage of Pb2+ and Cd2+ as the concentration of heavy metal increased from 20 and 10 ppm of each metal respectively. Then above 100 ppm the removal percentage decreased (Fig. 8). This decrease could be attributed to the interaction of the heavy metals to all active sites reaching saturation of receptors (Suriya et al. 2013; Oves et al. 2013; Arivalagan et al. 2014; Cheng et al. 2017).

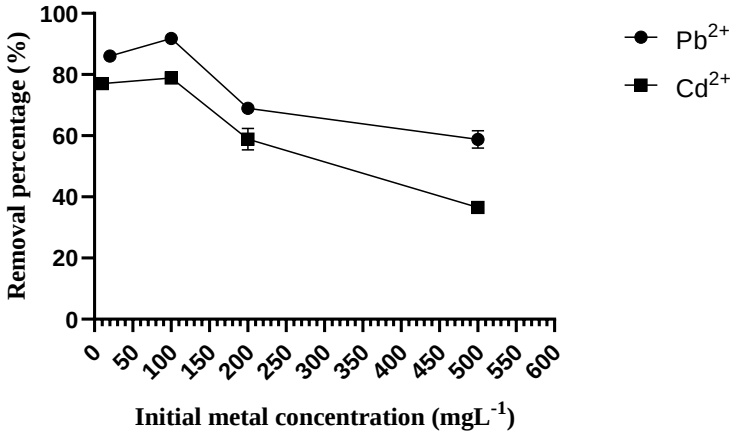


Fig. 8: Removal of Pb^{2+} and Cd^{2+} by *H. venusta* H9 (0.1 g biomass) when incubated with different concentrations of each heavy metal at 30 ± 2 ^{0}C for 24 hours.

3.9. Kinetics of the Removal Process

The kinetics of the removal process was studied to estimate the optimum contact time for the uptake of Pb^{2+} and Cd^{2+} by *Halomonas venusta* H9 (Fig. 9). It was noticed that the removal of Pb^{2+} and Cd^{2+} increase as time increase up to 60 min where the maximum removal was reached then no considerable increase was observed. There was an initial rapid metal uptake followed by a slow uptake. Since a large number of vacant surface sites were available for the biosorption in the initial stage, the biosorption of metal ions was rapid and passive then a slower process occurred which was the bioaccumulation that is active and metabolism dependent process. The uptake of metal ions by microorganisms in batch systems has been shown to occur in two stages: an initial rapid stage (passive uptake), followed by much slower process (active uptake) (Wierzba and Latała 2010; Abdel-Aty *et al.* 2013; Muñoz *et al.* 2015). In addition, the biosorption of Pb^{2+} and Cd^{2+} ions by *H. venusta* H9 was found following the pseudo-second order kinetics. This result is similar to those obtained by other authors with different microorganisms (Febrianto *et al.* 2009; Ezzouhri *et al.* 2010; Muñoz *et al.* 2015).

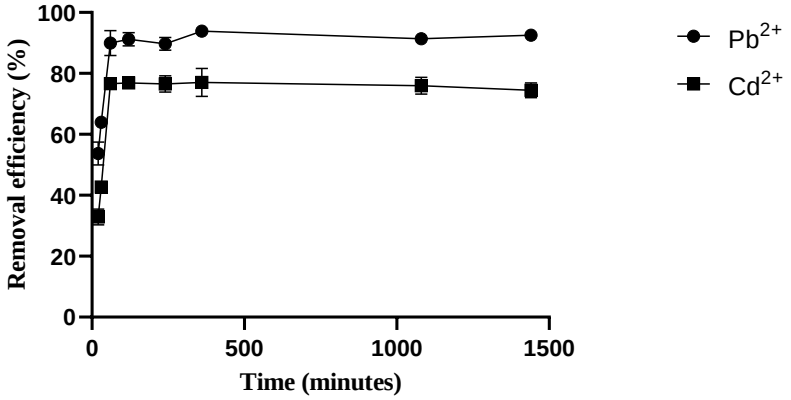


Fig.9: Kinetics for the removal of Pb^{2+} and Cd^{2+} (initial concentration of 100 ppm of each metal) by *H. venusta* H9 (0.1 g biomass) incubated at 30 ± 2 ^{0}C.

4. CONCLUSION

Halomonas venusta H9 showed high tolerance (up to 500 ppm) for both heavy metal ions (Pb^{2+} and Cd^{2+}) was further studied for its potential to remove these heavy metals from aqueous solutions. The removal of metal ions was determined by measuring the conductivity, FTIR analysis and TEM. It was found that *H. venusta* H9 was able to uptake these heavy metals through bioaccumulation process. The optimal conditions that showed highest removal percentage of heavy metals by *H. venusta* H9 were at biomass of 0.1 g, pH of 6-7 and initial metal concentration of 100 ppm. Thus, this study supports the use of *H. venusta* H9 as an economically viable and environmentally friendly biomass.

REFERENCES

- Abbas, S.Z., Rafatullah, M., Hossain, K., Ismail, N., Tajarudin, H.A., & Abdul Khalil, H.P.S. (2017). A review on mechanism and future perspectives of cadmium resistant bacteria. *International Journal Environmental Science and Technology*, 15, 243–262.
- Abdel-Aty, A.M., Ammar, N.S., Abdel Ghafar, H.H., & Ali, R.K. (2013). Biosorption of cadmium and lead from aqueous solution by freshwater alga *Anabaena sphaerica* biomass. *Journal of Advanced Research*, 4, 367-374.
- Al-Homaidan, A.A., Alabdullatif, J.A., Al-Hazzani, A.A., Al-Ghanayem, A.A., & Alabbad, A.F. (2015). Adsorptive removal of cadmium ions by *Spirulina platensis* dry biomass. *Saudi Journal of Biological Sciences*, 22, 795–800.

- Alkorta, I., Hernandez-Allica, J., Becerril, J.M., Amezaga, I., Albizu, I., & Garbisu, I. (2004). Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead and arsenic. *Reviews in Environmental Science and Biotechnology*, 3,71–90c
- Al-Momani, F.A., Massadeh, A.M., & Hadad, Y.A. (2007). Uptake of zinc and copper by halophilic bacteria Iiolated from the Dead Sea shore, Jordan. *Biological Trace Element Research*, 115, 291-300.
- Amoozegar, M.A., Hamedi, J., Dadashipour, M., & Shariatpanahi, S. (2005). Effect of salinity on the tolerance to toxic metals and oxyanions in native moderately halophilic spore-forming bacilli. *World Journal of Microbiology and Biotechnology*, 21, 1237–1243.
- Amoozegar, M.A., Ghasemi, A., Razavi, M.R., & Naddaf, S. (2007). Evaluation of hexavalent chromium reduction by chromate resistant moderately halophile, *Nesterenkonia* sp. strain MF2. *Process Biochemistry*, 42, 1475–1479.
- Amoozegar, M.A., Ghazanfari, N., & Didari, M. (2012). Lead and cadmium bioremoval by *Halomonas* sp., an exopolysaccharide-producing halophilic bacterium. *Progress in Biological Sciences*, 2, 1-11.
- Arivalagan, P., Singaraj, D., Haridass, V., & Kaliannan, T. (2014). Removal of cadmium from aqueous solution by batch studies using *Bacillus cereus. Ecological Engineering*, 71, 728–735.
- Atlas, R.M. (2005). Handbook of media for environmental microbiology. CRC Press, New York, pp. 84–256.
- ATSDR—Priority List of Hazardous Substances. (2015). Summary data for 2015 priority list of hazardous substances. http://www. atsdr.cdc.gov/spl/ (accessed December 20, 2015).
- Barrow, G.I., & Feltham, R.K.A. (eds) (1993). Cowan and Steel's Manual for the identification of medical bacteria, 3rd edn. University Press, Cambridge.
- Cai, Y., Li, X., Liu, D., Xu, C., Ai, Y., Sun, X., Zhang, M., Gao, Y., Zhang, Y., Yang, T., Wang, J., Wang, L., Li, X. & Yu, H. (2018). A novel Pb-resistant *Bacillus subtilis* bacterium isolate for co-biosorption of hazardous Sb(III) and Pb(II): Thermodynamics and Application Strategy. *International Journal of Environmental Research and Public Health*, 15, 1-18.
- Chakraborty, J., & Das, S. (2014). Characterization and cadmium-resistant gene expression of biofilm-forming marine bacterium *Pseudomonas aeruginosa* JP-11. *Environmental Science and Pollution Research*, 21, 14188–14201.
- Chakravarty, R., & Banerjee, P.C. (2012). Mechanism of cadmium binding on the cell wall of an acidophilic bacterium. *Bioresource Technology*, 108, 176–183.
- Chellaiah, E.R. (2018). Cadmium (heavy metals) bioremediation by *Pseudomonas aeruginosa*: a minireview. *Applied Water Science*, 8, 154-164.
- Chen, Z., Pan, X., Chen, H., Lin, Z., & Guan, X. (2015). Investigation of lead(II) uptake by *Bacillus thuringiensis* 016. *World Journal of Microbiology and Biotechnology*, 31, 1729–1736.
- Cheng, J., Yin, W., Chang, Z., Lundholm, N., & Jiang, Z. (2017). Biosorption capacity and kinetics of cadmium(II) on live and dead *Chlorella vulgaris. Journal of Applied Phycology*, 29, 211–221.
- 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.
- El-Naggar, N.E., Hamouda, R.A., Mousa, I.E., Abdel-Hamid, M.S., & Rabei, N.H. (2018). Biosorption optimization, characterization, immobilization and application of *Gelidium amansii* 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., & Lairini, K. (2010). Mechanisms of lead uptake by fungal biomass isolated from heavy metals habitats. *Afinidad*, 67, 39-44.
- Febrianto, J., Kosasih, A.N., Sunarso, J., Ju, Y-H., Indraswati, N., & Ismadji, S. (2009). Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies. *Journal of Hazardous Materials*, 162, 616–645.
- Feng, C-l., Li, J., Li, X., Li, K-l., Luo, K., Liao, X-s., & Liu, T. (2018). Characterization and mechanismof lead and zinc biosorption by growing *Verticillium insectorum* J3. *PLoS ONE*, 13, e0203859.

- Garcia, M.T., Nieto, J.J., Ventosa, A., & Ruiz Berraquero, F. (1987). The susceptibility of the moderate halophile *Vibrio costicola* to heavy metals. *Journal of Applied Bacteriology*, 63, 63–66.
- Ghoneim, M.M., El-Desoky, H.S., El-Moselhy, K.M., Amer, A., Abou El-Naga, E.H., Mohamedein, L.I., & Al-Prol, A.E. (2014). Removal of cadmium from aqueous solution using marine green algae, Ulva lactuca. *Egyptian Journal of Aquatic Research*, 40, 235–242.
- Giotta, L., Mastrogiacomo, D., Italiano, F., Milano, F., Agostiano, A., Nagy, K., Valli, L., & Trotta, M. (2011). Reversible binding of metal ions onto bacterial layers revealed by protonation-induced ATR-FTIR difference spectroscopy. *Langmuir*, 27, 3762-3773.
- Hrynkiewicz, K., Złoch, M., Kowalkowski, T., Baum, C., Niedojadło, K., & Buszewski, B. (2015). Strain-specific bioaccumulation and intracellular distribution of Cd2+ in bacteria isolated from the rhizosphere, ectomycorrhizae, and fruitbodies of ectomycorrhizal fungi. *Environmental Science and Pollution Research*, 22, 3055–3067.
- Iram, S., Shabbir, R., Zafar, H., & Javaid, M. (2015). Biosorption and bioaccumulation of copper and lead by heavy metal-resistant fungal isolates. *Arabian Journal for Science and Engineering*, 40, 1867–1873.
- Kariuki, Z., Kiptoo, J., & Onyancha, D. (2017). Biosorption studies of lead and copper using rogers mushroom biomass '*Lepiota hystrix*'. *South African Journal of Chemical Engineering*, 23, 62-70.
- Khan, Z., Rehman, A., Hussain, S.Z., Nisar, M.A., Zulfiqar, S., & Shakoori, A.R. (2016). Cadmium resistance and uptake by bacterium, *Salmonella enterica* 43C, isolated from industrial effluent. *AMB Express*, 6, 54-70.
- Lodeiro, P., Barriada, J.L., Herrero, R., & Sastre deVicente, M.E. (2006). The marine macroalga Cystoseira baccata as biosorbent for cadmium (II) and lead (II) removal: kinetic and equilibrium studies. *Environmental Pollution*, 142, 264-273.
- Massadeh, A.M., Al-Momani, F.M., & Haddad, H.I. (2005). Removal of lead and cadmium by halopholic bacteria isolated from the Dead Sea shore, Jordan. *Biological Trace Element Research*, 108, 259–269.
- Mata, J.A., Martinez-Canovas, J., Quesada, E., & Bejar, V. (2002). A detailed phenotypic characterization of the type strains of *Halomonas* species. *Systematic and Applied Microbiology*, 25, 360–375.
- McDowell, E.M., & Trump, B.F. (1976). Histologic fixatives suitable for diagnostic light and electron microscopy. *Archives of Pathology and Laboratory Medicine*, 100, 405-414.
- Muñoz, A.J., Espínola, F., Moya, M., & Ruiz, E. (2015). Biosorption of Pb(II) ions by *Klebsiella* sp. 3S1 isolated from a wastewater treatment plant: Kinetics and mechanisms studies. *BioMed Research International*, 2015, 1-12.
- Nieto, J.J., Fernandez-Castillo, R., Marquez, M., Ventosa, A., Quesada, E., & RuizBerraquero, F. (1989). Survey of metal tolerance in moderately halophilic eubacteria. *Applied and Environmental Microbiology*, 55, 2385-2390.
- Oves, M., Khan, M.S., & 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., Esposito, A., Toro, L., & Vegli"o, F. (2003). Metal speciation and pH effect on Pb, Cu, Zn and Cd biosorption onto *Sphaerotilus natans*: Langmuir-type empirical model. *Water Research*, 37, 627–633.
- Romano, I., Lama, L., Nicolaus, B., Poli, A., Gambacorta, A., & Giordano, A. (2006). Halomonas alkaliphila sp. nov., a novel halotolerant alkaliphilic bacterium isolated from a salt pool in Campania (Italy). *Journal of General and Applied Microbiology*, 52, 339–348.
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425.
- Sari, A., & Tuzen, M. (2008). Biosorption of Pb(II) and Cd(II) from aqueous solution using green alga (*Ulva lactuca*) biomass. *Journal of Hazardous Materials*, 152, 302–308.
- Sowmya, M., Rejula, M.P., Rejith, P.G., Mohan, M., Karuppiah, M., & Hatha, A.A.M. (2014). Heavy metal tolerant halophilic bacteria from Vembanad Lake as possible source for bioremediation of lead and cadmium. *Journal of Environmental Biology*, 35, 655–660.

- Sowmya, R., & Abdulla, M.H. (2017). Handbook of Metal-Microbe Interactions and Bioremediation. In: Das S, Dash H (eds) Cadmium and Lead Tolerance Mechanisms in Bacteria and the Role of Halotolerant and Moderately Halophilic Bacteria in Their Remediation. Boca Raton: CRC Press.
- Suriya, J., Bharathiraja, S., & 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.
- Syed, S., & Chinthala, P. (2015). Heavy metal detoxification by different *Bacillus* species isolated from solar salterns. *Scientifica*, 2015, 1-8.
- Tang, X.Y., Zhu, Y.G., Cui, Y.S., Duan, J., & Tang, L. (2006). The effect of ageing on the bioaccessibility and fractionation of cadmium in some typical soils of China. *Environment International*, 32, 682–689.
- Timková, I., Sedláková-Kaduková, J., & Pristaš, P. (2018). Biosorption and Bioaccumulation Abilities of Actinomycetes/Streptomycetes Isolated from Metal Contaminated Sites. *Separations*, 5, 54-68.
- US Environmental Protection Agency. June (2008). Lead and Copper Rule: A Quick Reference Guide, EPA 816-F-08-018.
- Wierzba, S., & Latała, A. (2010). Biosorption lead(II) and nikel(II) from an aqueous solution by bacterial biomass. *Polish Journal of Chemical Technology, 12*, 72-78.
- Xu, L., Xu, X-W., Meng, F-X., Huo, Y-Y., Oren, A., Yang, J-Y., & Wang, C-S. (2013). *Halomonas zincidurans* sp. nov., a heavy-metal-tolerant bacterium isolated from the deep-sea environment. *International Journal of Systematic and Evolutionary Microbiology*, 63, 4230–4236.
- Zafrilla, B., Martínez-Espinosa, R.M., Alonso, M.A., & Bonete, M.J. (2010). Biodiversity of Archaea and floral of two inland saltern ecosystems in the Alto Vinalopó Valley, Spain. *Saline Systems*, 6, 1-12.
- http://www.ncbi.nlm.nih.gov/BLAST/