academicJournals

Vol. 10(12), pp. 370-375, 28 March, 2016
DOI: 10.5897/AJMR2016.7912
Article Number: E1F962A57733
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Bioaccumulation of cadmium and lead by *Shewanella oneidensis* isolated from soil in Basra governorate, Iraq

Raghad Jaafar[1], Amin Al-Sulami[1] and Asaad Al-Taee[2]*

[1]College of Education for Pure Science, Basra University, Basra, Iraq.
[2]Marine Science Center, Basra University, Basra, Iraq.

Received 9 January, 2016; Accepted 26 February, 2016

In the present study heavy metals resistant bacteria were isolated from soil collected from Al-Zubair district in Basra governorate south of Iraq. On the basis of morphological, biochemical, 16S rRNA gene sequencing and phylogeny analysis, the isolates were authentically identified as *Shewanella oneidensis* in addition to *Bacillus thuringiensis* and *Deinococcus radiodurans*. The minimal inhibitory concentration (MIC) of isolates against cadmium (Cd) and lead (Pb) was determined on solid medium. *S. oneidensis* showed significant resistance to high concentrations of Cd (1000 mgl^{-1}) and Pb (700 mgl^{-1}). The bioaccumilation capabilities of *S. oneidensis* for Cd and Pb were monitored at different ion concentrations and contact times. The transmission electron microscope (TEM) study confirmed the accumulation of Cd and Pb by *S. oneindensis* causing morphological changes.

Key words: *Shewanella oneidensis*, bioaccumulation, minimal inhibitory concentration, heavy metals, transmission electron microscope.

INTRODUCTION

Heavy metals play an important role in the metabolic processes of the biota, some of them are essential for organisms as micronutrients (cobalt, chromium, nickel, iron, manganese and zinc). They are involved in redox processes, to stabilize molecules through electrostatic interactions, as catalysts in enzymatic reactions, and regulating the osmotic balance. On the other hand, cadmium, mercury, lead, have no biological role and are harmful to the organisms even at very low concentration. However, at high levels, both of the essential and non-essential metals become toxic to the organisms (Rathnayake et al., 2010).

Cadmium is widespread and one of the most toxic soil contaminants released by mining and smelting activities, atmospheric deposition from metallurgical industries, incineration of plastics and batteries, land application of sewage sludge, and burning of fossil fuels (Tang et al., 2006). Cadmium is poisonous to plants, animals, and humans (Gupta and Gupta, 1998) and is listed as one of the 126 priority contaminants by the USEPA and as a human carcinogen by the International Agency for Research on Cancer (IARC, 1994). Thus cadmium

*Corresponding author. E-mail: amraltaee@yahoo.com.

pollution is presently attracting more attention from environmentalists worldwide.

Lead (II) is a heavy metal poison which forms complexes with oxo-groups in enzymes to affect nearly all steps in the process of hemoglobin synthesis and porphyrin metabolism. Toxic levels of Pb (II) in man have been associated with encephalopathy appropriations and mental delay (Ademorati, 1996). Conventional physico-chemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis evaporation and sorption (Kadirvelu et al., 2001, 2002) have been used for removing heavy metals but are economically expensive and have disadvantages. Bioremediation is a natural process which depends on bacteria, fungi and plants to altering pollutants as these organisms perform their normal life functions. These organisms have the ability of exploiting chemical contaminants as an energy source in their metabolic processes. Therefore, bioremediation affords alternative tool to destroy or reduce the risky contaminants through biological activity with an effective cost (Salem et al., 2012).

Microbial populations in metal polluted environments become metals resistant (Prasenjit and Sumathi, 2005) so the response of microorganisms towards toxic heavy metals is of importance in view of the interest in the reclamation of polluted sites (Shankar et al., 2007). Microorganisms uptake metals either actively (bioaccumulation) and/or passively (biosorption) (Shumate and Strandberg, 1985; Anders and Hubert, 1992; Hussein et al., 2004). Bioaccumulation is the active method of metal accumulation by living cells. The capacity of living cells to remove metal ions from environment is influenced by environmental growth conditions, as temperature, pH and biomass concentrations (Abd-El-Raheem et al., 2013).

TEM is a useful technique that can help to localize and to identify metals deposited within or around microbial cells. Identification of the site of accumulation is important as it can give clues to the biochemical mechanisms driving metal accumulation. Biological materials which are largely composed of light elements such as C, N, H, O, P, and S, do not deflect the electron beam to the same degree. Thus, it is possible to visualize metals against the faint image of a bacterial cell (Lloyd and Macaskie, 2002).

The present study, aims at isolating *S. oneindensis* from Basra soil, south of Iraq, and evaluating metals bioaccumulation ability, and also studying the effect of metals initial concentration, contact times, and determine the cellular localization of accumulated metals within this bacterium by using Transmission electron microscope.

MATERIALS AND METHODS

Isolation of bacteria

Three soil samples (30 g each) were collected from AL-Zubair district west of Basra city- Iraq during January 2013. The samples were collected using a sterile plastic bag and transferred within 2 h to laboratory for analysis. One gram of air dried soil sample was serially diluted using sterilized distilled water and spread over nutrient agar. The plates were incubated at 30°C for 24 h.

Bacterial characterization

Properties of the bacteria included gram stain, citrate utilization, indole production, methyl red, nitrate reduction, Voges Proskauer, catalase, dextrose, mannitol and sucrose utilization, starch hydrolysis, and gelatin liquefaction tests were determined according to Sneath et al. (1986).

S16 rRNA gene based identification

The isolates were identified by sequencing of the 16S rRNA gene. To determine the identification of bacterial isolates, the amplified 16S rRNA gene PCR products obtained from total genomic DNA using primer set 27F (5′ AGAGTTTGATCCTGGCTCAG-3′) and 72.1492R (5′ GGTTACCTTGTTACGACTT-3′), (Lane et al., 1985) were sequenced commercially. DNA sequences obtained were compared to sequences available online in a GenBank database (http://www.ncbi.nlm.nih.gov). Homology search was performed using Bioinformatics tools available online BLASTn www.ncbi.nlm.nih.gov/BLA (Altschul et al., 1997).

Determination of minimal inhibitory concentrations (MIC) for Pb and Cd

The minimum inhibitory concentration (MIC) of Cd and Pb of bacteria were determined by disc diffusion method. The concentrations of Cd and Pb were between 40 to 2500 mgl^{-1}. Filter paper discs were saturated with heavy metals for 30 min, and then placed on nutrient agar plates and incubated for 24 h at 30°C. Pb $(NO_3)_2$ and $CdCl_2$ were used to prepare mother solution of these metals in sterile distilled water and were used in various concentrations. The lowest concentrations of Cd and Pb that completely prevented growth of each bacterium were considered as the MIC (Sethuraman and Kumar, 2011).

Bioaccumulation of heavy metals by bacteria

Bacteria were grown in LB broth containing 5, 10, 25 and 50 mgl^{-1} of lead and for cadmium 10, 20, 50 and 100 mgl^{-1} then incubated for 2, 4, 6, 24 and 48 h at 30°C in a shaker incubator at 150 rpm. Three replicates for each concentration have been done, and one as a control. The bacterial cells were harvested by centrifugation at 6000 rpm for 15 min, and suspended in 1 ml of distilled water, oven-dried at 80°C for 1 h and weighted. Metal concentrations were measured by atomic absorption spectrophotometer. Control was represented by the same microbial culture without heavy metals. Each metals concentration is measured with two replicates (Sprocati et al., 2006).

Transmission electron microscope

By centrifuging samples broth culture for 10 min at 3000 rpm, and decanting the supernatant, fixing pellet with 4% gutaraldehyde for 4 h at 4°C and centrifuged again, decanting fixative and adding an appropriate quantity animal serum to submerge sample, and allowing serum to clot. It was washed three times with 0.1 M Cacodylate buffer for 10 min. and Posted fix in 1% Osmium tetrroxide for 2 h at 4°C. Also, it was washed again three times with 0.1 M Cacodylate buffer for 10 min. Dehydrating in series of

Table 1. Biochemical characteristics of *S. oneidensis* isolate from soils.

Tests	Characteristics observed
Oxidase test	+
Catalase test	+
Indole formation	-
Nitrate reduction	-
Production of H_2S	+
Gelatin liquefaction	+
Fermentation of	
Sucrose	+
Fructose	**+**
D-glucose	+

"+"and "-" indicate positive and negative reactions, respectively.

acetone of 35, 50, 75, 95, and 100% for 10, 10,10, 10 and 15 min respectively. Finally, we make infiltration of the specimen with acetone and resin:

Acetone:	Resin	Time
1	: 1	1 h
1	: 3	2 h
100%		Overnight
100%		2 h

Embedding: Specimens were placed into beam capsule filled with resin. Polymerization: polymerized in oven at 60°C for 24 h. Make ultrasectioning, by choosing an area of interest, then cut for ultrathin section, selecting the silver section, picking up a section with a grid, then drying with filter paper. Finally staining with Uranyl acetate for 15 min, and washed double distills water. Lead stained for 10 min, and washed twice in distilled water. This work was done at the Electron Microscope Laboratory Institute of Bioscience, University Putra Malaysia.

RESULTS AND DISCUSSION

Characterization and molecular identification of isolated bacteria

The selected bacterium was characterized and identified by using conventional morphological, physiological and biochemical tests (Table 1). It was presumptively identified as *Shewanella* sp (Holt et al., 2005). The sequence of 16S rRNA gene of this bacterium was submitted to Blastn database 16S ribosomal RNA sequences (Bacteria and Archaea) Megablast http://www.ncbinlm.nih.gov/blast. It indicated a close genetic relatedness of this bacterium with the rDNA sequence of *Shewanella oneidensis* (Holt et al., 2005).

Minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of the heavy metals that completely inhibited bacterial growth (Froidevaux et al., 2001) *S. oneindensis* showed significant resistance to high concentrations of Pb and Cd (700 and 1000 mgl^{-1}) respectively. This may be considered new finding, that the other studies showed different results. Chihomvu et al. (2014) recorded MIC for Pb by *Shewanella* (4 Mm), while MIC was 0 for Cd. Francis and Dodge (1988) and Toes et al. (2008) demonstrated that, the tolerances inhibited growth of different *Shewanella* strains completely at 150 µM Co, 150 - 400 µM Zn, 75 - 150 µM Cd, and 150 µM Cu when cultivated aerobically in 10% LB broth. The effect of the medium on metal toxicity was demonstrated in a study by Toes et al. (2008) where higher tolerances of Cu by *Shewanella* between 75 and 750 µM in more nutrient rich media and the presence of manganese oxides also reduce the toxicity of Cu.

Bioaccumulation

S. oneidensis as sulfate reducing bacteria has the potential to enhance metal retention via extracellular binding, cellular uptake and accumulation of metals, oxidation/reduction processes, and surface mediated mineral precipitation (Burkhardt, 2010). From results of the present study, *S. oneindensis* was able to accumulate Cd than Pb (26.77 and 3.98 mgg^{-1}) at 48 h and at concentrations 50 and 100 mgl^{-1} respectively (Figures 1 and 2). The differences in this accumulation ability for these two metals may be related to different toxicity of these metals to this bacterium. From the results, the accumulation of both metals increases with increasing the time. Varghese et al. (2012) showed that, with increasing time, the biomass of the bacterial strains increased. Likewise, with an increase in biomass, the heavy metals bioaccumulation also increased. The results of the present study showed that the high amount of accumulation occurs with high metals concentration (50 and 100 mgl^{-1}). These results agree with the results reported by Odokuma and Akponah (2010), where they concluded an increasing uptake pattern observed in the respective test isolates as the initial concentration of the various heavy metal salts were increased. These observations suggested that metal uptake may involve diffusion phenomenon, whereby metal ions move from regions of high to low concentrations.

Transmission electron microscope

Cells were evaluated by TEM to observe the locations of precipitate of metals in relation to the *S. oneidensis* cells. In order to differentiate whether extracellular or intracellular reduction occurred, the cells were stained with uranyl acetate. Figure 3 has shown the cells before being exposed to the metals (a). Dark precipitate can be seen around the inside of the cell membrane, indicating

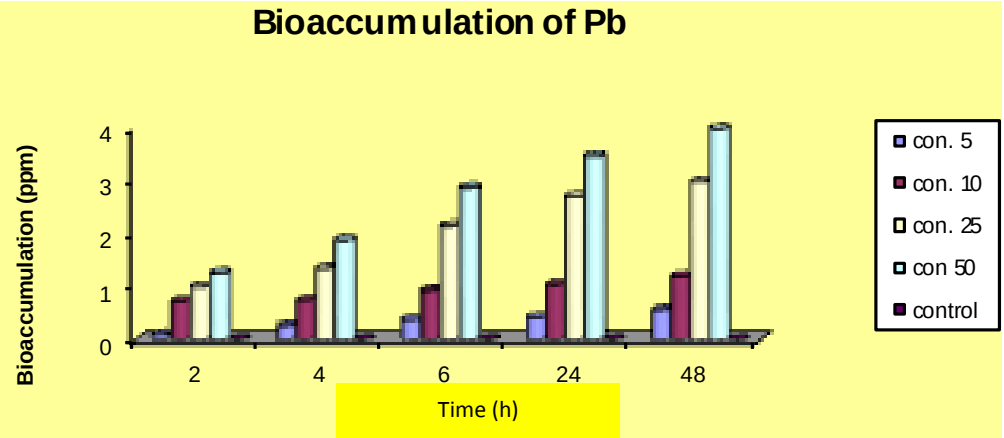

Figure 1. Bioaccumulation of Pb by *S. oneidensis* during different incubation periods and different concentrations.

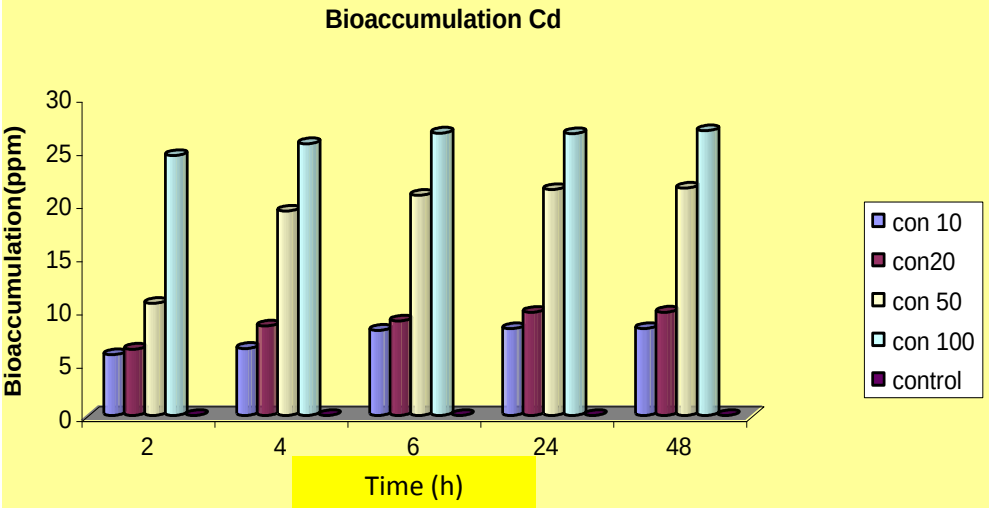

Figure 2. Bioaccumulation of Cd by *S. oneidensis* during different incubation periods and different concentrations.

intracellular Cd and Pb reduction has occurred (b and c). Also, from Figure (3b and c) there were changes in size and shape of cells and some cells have been lysed. These results could add to the toxicity of the substance, and ultimately results in cell death.

The cell surface morphology considerably changed after metals exposure. The cellular localization of the metals bound by the cells of the bacterium was located mainly within the cell membrane. However, some intracellular metal accumulates were also identified in the cytoplasm of the bacterial cells. Merroun et al. (2005) reported that, the cellular localization of the uranium bound by the cells of three types of *Acidithiobacillus ferrooxidans* was studied using TEM. Also, El-Helow et al. (2000) reported that, cell surfaces of cultures treated with cadmium chloride tended to be rough, suggesting that the cell increased its surface to improve the interaction of toxic substances with the cell surface. Also, Singh et al. (2013), reported cell surface morphological changes in *Cryptococcus* sp. after exposure to heavy metals, and which could be observed by the presence of shrunken and distorted cell wall in the presence of Cd and depressions in the presence of Pb and Zn.

Secretion of extracellular polymeric substance by *Desulfovibrio desulfuricans* during biosorption of Zn and Cu was reported to modify its cell surface morphology (Chen et al., 2000). Similarly, El-Meleigy et al. (2013) reported that, high dark dense cytoplasm due to Co^{2+} precipitation is partially emptied with a very thick cell wall; changing in the morphology of vegetative cells of *Bacillus firmus* and *Bacillus subtilis.*

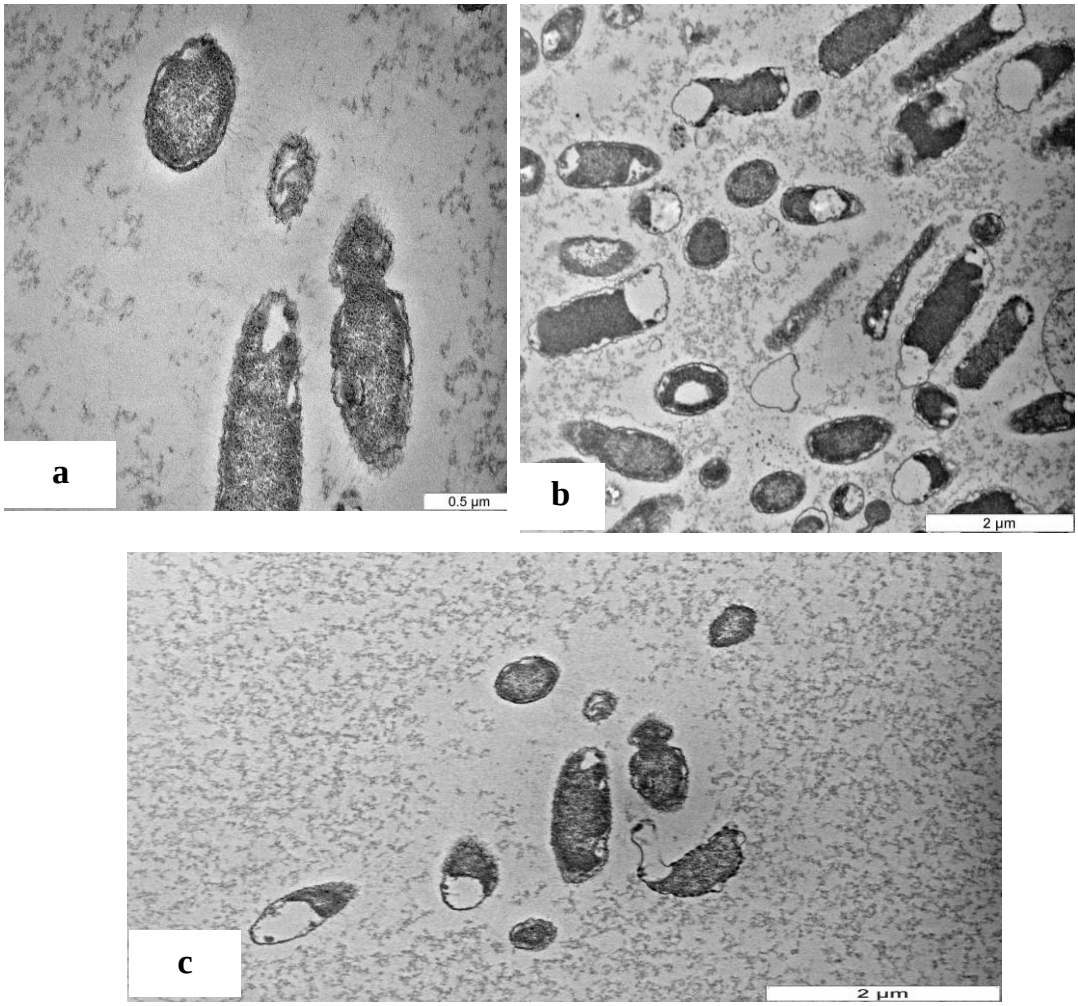

Figure 3. Transmission electron micrographs of *S. oneidensis* a: control, b: treated with 50 mgl^{-1} of Cd for 24 h, c: Treated with 50 mgl^{-1} of Pb for 24 h (Scale of bar 0.5 and 2 µm).

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Ademorati CMA (1996). Environmental Chemistry and Toxicology. Pollution by Heavy metals. Fludex Press Ibadan. pp. 171-172.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucl. Acids Res. 25:3389-3402.

Anders MYJH, Hubert CJ (1992). Bacterial biosorption and retention of thorium and uranyl cations by *Mycobacterium smegmatis*. J. Radioanal. Nucl. Chem. 166:431-440.

Burkhardt EM (2010). Interaction of Fe (III)- reducing bacteria with heavy metals in contaminated soils, Ph.D. Thesis, University of Jena, Germany. P 183.

Chen BY, Utgikar VP, Harmon SM, Tabak HH, Bishop DF, Govind R (2000). Studies on biosorption of zinc (II) and copper (II) on *Desulfovibrio desulfuricans.* Int. Biodeterior. Biodegradation 46:11-18.

Chihomvu P, Stegmann P, Pillay M (2014). Identification and Characterization of Heavy Metal Resistant Bacteria from the Klip River. Int. J. Biol. Vet. Agric. Food Eng. 8(11):1056-1066.

El-Helow ER, Sabry SA, Amer RM (2000). Cadmium biosorption by a cadmium resistant strain of *Bacillus thuringensis*. Regulation and optimization of cell surface affinity for metal cations. Biometals 13:273-280.

El-Meleigy MA, Abed NN, Sari IP (2013). Fate of cobalt and nickel in *B. firmus and B. subtilis*. Nat. Sci. 9(8):175-189.

Francis AJ, Dodge CJ (1988). Anaerobic Microbial Dissolution of Transition and Heavy Metal Oxides. Appl. Environ. Microbiol. 54(4):14.

Holt HM, Hansen BG, Bruun B (2005). *Shewanella algae* and *Shewanella putrefaciens*: Clinical and microbiological characteristics Clin. Microbiol. Infect. 11:347-352.

Hussein H, Ibrahim SF, Kandeel K, Moawad H (2004). Biosorption of heavy metals from waste water using *Pseudomonas* sp. Electron. J. Biotechnol. 7(1):30-37.

IARC (1994). Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry [M]. In: Monographs on the evaluation of carcinogenic risks to humans. Lyon: WHO Press 58:444.

Kadirvelu K, Thamaraiselvi K, Namasivayam C (2001). Adsorption of nickel (II) from aqueous solution onto activated carbon prepared from coir pith. Sep. Purif. Technol. 24: 477-505.

Kadirvelu K, Senthilkumar P, Thamaraiselvi K, Subburam V (2002).

Activated carbon prepared from biomass as adsorbent: elimination of Ni (II) from aqueous solution. Bioresour. Technol. 81:87-90.
Lloyd JR, Macaskie LE (2002). Environmental Microbe-Metal Interactions. American Society for Microbiology Press, Washington, DC. pp 3-30.
Merroun ML, Raff J, Rossberg A, Hennig C, Reich T, Selenska-Pobell S (2005). Complexation of uranium by cells and S-layer sheets of *Bacillus sphaericus* JG-A12. Appl. Environ. Microbiol. 71(9):5532-5543.
Odokuma LO, Akponah E (2010). Effect of concentration and contact time on heavy metal uptake by three bacterial isolates. J. Environ. Chem. Ecotoxicol. 2(6):84-97.
Prasenjit B, Sumathi S (2005). Uptake of chromium by *Aspergillus foetidus*. J. Mater. Cycles Waste Manage. 7:88-92.
Rathnayake IVN, Megharaj M, Bolan N, Naidu R (2010). Tolerance of Heavy Metals by Gram Positive Soil Bacteria. Environ. Eng. 2(4):1185-1190.
Salem IB, Sghaier H, Trifi H, Héni S, Khwaldia K, Saidi M, Landoulsi A (2012). Isolation and characterization of a novel *Micrococcus* strain for bioremediation of strontium in radioactive residues. Afr. J. Microbiol. Res. 64:851-858.
Sethuraman P, Kumar MD (2011). *Bacillus subtilis* on Pb^{2+} ion removal from aqueous solution by Biosorption. Res. J. Pharm. Biol. Chem. Sci. 2(4):247.
Shankar C, Sridevi D, Joonhong P, Dexilin M, Thamaraiselvi K (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J. Hazard. Mater. 146:270-277.
Shumate ES, Strandberg WG (1985). Accumulation of metals by microbial cells. Compr. Biotechnol. 13:235-247.
Singh P, Raghukumar C, Parvatkar RR, Mascarenhas-Pereira MBL (2013). Heavy metal tolerance in the psychrotolerant *Cryptococcus* sp. isolated from deep-sea sediments of the Central Indian Basin. Yeast 30(3):93-101.
Sneath PHA, Mair NS, Sharpe ME, Holt JG (1986). Bergey's manual of systematic bacteriology. Vol. 2. Williams & Wilkins, Baltimore, U.S.A.
Sprocati AR, Alisi C, Segre L, Tasso F, Galletti M, Cremisini C (2006). Investigating heavy metal resistance, bioaccumulation and metabolic profile of a metallophile microbial consortium native to an abandoned mine. Sci. Total Environ. 366:649-658.
Tang XY, Zhu YG, Cui YS, Duan J, Tang L (2006). The effect of ageing on the bioaccessibility and fractionation of cadmium in some typical soils of China. Environ. Int. 32:682-689.
Toes ACM, Geelhoed JS, Kuenen JG, Muyzer G (2008). Characterization of heavy metal resistance of metal-reducing *Shewanella* isolates from marine sediments. Geomicrobiol. J. 25:304-314.
Varghese R, Krishna MP, Babu A, Mohamed HA (2012). Biological removal of lead by *Bacillus* sp. obtained from metal contaminated industrial area. J. Microbiol. Biotechnol. Food Sci. 2(2):756-770.