Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.10, 2015
www.iiste.org

Biosorption of some Heavy Metals by Metal Resistant *Bacillus thuringiensis* Isolated from Soil in Basra Governorate- Iraq

Raghad Jaafar[1] Amin Al-Sulami[1] Asaad Al-Taee[2*] Faris Aldoghachi[3]
1. College of Education for Pure Science, Basra University, Basra-Iraq
2. Marine Science Center, Basra University, Basra-Iraq
3. College of Science, Basra University, Basra-Iraq
*E. Mail of the Corresponding Auther: amraltaee@yahoo.com

Abstract
In present study heavy metal resistant bacteria were isolated from soil collected from Fao district in Basra governorate South of Iraq. On the basis of morphological, biochemical, 16S rRNA gene sequencing and phylogeny analysis revealed that, the isolates were authentically identified as *Bacillus thuringiensis*. The minimal inhibitory concentration (MIC) of isolates against cadmium (Cd) and lead (Pb) was determined on solid medium. *B. thuringiensis* showed significant resistance to high concentrations of Pb (1800 mg/l) and Cd (50 mg/l). The biosorption capabilities of *B. thuringiensis* for Cd and Pb were monitored at different ion concentrations and contact times. The functional groups of bacterial surface were determined using Fourier transform infrared, and X-ray powder diffraction analysis.
Key Words: *Bacillus thuringiensis*, Minimal Inhibitory Concentration, Biosorption, Fourier transform infrared, X-ray powder diffraction

Introduction

Heavy metals play an important role in the metabolic processes of the biota, some of them are essential for organisms as micronutrients such as (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, has 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 US-EPA and as a human carcinogen by the International Agency for Research on Cancer (IARC, 1994). Thus, cadmium pollution is 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; Kadirvelu *et al*., 2002) have used for removing heavy metals, but are economically expensive and have disadvantages.

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 metal either actively (bioaccumulation) and/or passively (biosorption) (Shumate and Strandberg, 1985; Anders and Hubert, 1992; Hussein *et al*., 2003). Biosorption exploits various certain natural materials of biological origin including bacteria, fungi, yeast, algae, etc. It can effectively sequester dissolved metal ions out of dilute complex solutions with high efficiency and quickly, therefore it is a suitable candidate for the treatment of high volume and low concentration complex heavy metal wastes (Wang and Chen, 2006). The present study, aims to isolating *Bacillus thuringiensis* from Basra south of Iraq, and evaluating metals biosorption ability, and also studying the effect of metals initial concentration, contact times, and determined the functional groups of bacterial surface using Fourier transform infrared, and X-ray powder diffraction analysis.

Materials and methods

Isolation of bacteria

Three soil samples (30 gm each) were collected from Fao district, 90 Km south of Basra city- Iraq during January 2013. The samples were collected using a sterile plastic bag and transferred within 2h to laboratory for

analysis. One gram of air dried soil sample was serially diluted using 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 reaction, citrate utilization, indole production, methyl red, nitrate reduction, Voges Proskauer, catalase, dextrose, mannitol and sucroseutilization, starch hydrolysis, and gelatin liquefaction tests were determined according to Sneath *et al*. (1986).

16S rRNA based identification

The isolates were identified by sequencing of the 16S rRNA gene. To determine the identity of bacterial isolates, the amplified 16S rRNA gene PCR products obtained from total genomic DNA using primer set 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′), (Lane *et al*., 1985) were sequenced commercially. DNA sequences obtained were compared to sequences available online in a Gen Bank 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 Cd and Pb

The MIC of Cd and Pb of bacteria were determined by disc diffusion method (Wistreich and Lechtman, 1980). The concentrations of Cd and Pb were between 40 - 2500 mgl^{-1}. Filter paper disks were saturated with heavy metals for 30 min, and then added to nutrient agar plates which incubated for 24h at 30ºC. $CdCl_2$ and Pb $(NO_3)_2$ 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).

Biosorption experiments

The equilibrium, kinetics data of the biosorbent *B. thuringiensis* were obtained by performing batch experiments. The experiments were carried out in 250 ml flasks to which 100 ml solution of either Cd or Pb, and l ml of biomass from exponential phase were added. The mixture was stirred at 180 rpm at 30 ℃ and 15 ml of sample was collected at interval times (2, 4, 6, 24 and 48 h), centrifuged at 3000 rpm for 10 min. The remaining concentration of metals was analyzed by the flame atomic absorbance spectrophotometer (Thermo Scientific ICE 3000 Series AA Spectrometer USA). Each experiment was carried out twice and the mean values were reported. The difference between the initial metal ion concentration and final metal ion concentration was considered as metal bound to the biosorbent (Sethuraman and Kumar, 2011).

Effect of contact time on biosorption

Experiments to determine the equilibrium time required for biosorption was performed using 1 ml of cell biomass from the initial metal concentration (50 mgl^{-1}) of either Cd or Pb in 100 ml of metal solution at pH 6 for Cd and Pb, at 30 °C. and were taken at the desired interval time of 2, 4, 6, 24 and 48 h. and subsequently centrifuged at 3000 rpm for 10 min. The heavy metal concentration in the supernatant was analyzed by flame atomic absorption spectroscopy.

Effect of initial metal concentration

The experiments of the effect of initial concentration of Cd and Pb were performed at different concentrations (5, 10, 25, 50) mgl^{-1} at optimum temperature and pH for each metal, Aliquots of 1ml of cells of *B. thuringiensis* were added to 100 ml solution of either metal at 5, 10, 25 and 50 mgl^{-1} and incubated for 24h on orbital shaking incubator at 180 rpm. Aliquots of 15 ml were collected, centrifuged at 3000 rpm for 10 min and analyzed as mentioned in biosorption exp.

FTIR analysis

The Fourier transform infrared (FT-IR) analysis was done with PerkinElmer spectrometer model 100 series (sample preparation UATR) (UPM-Malaysia).

X-ray powder diffraction analysis (XRD)

The powder X-ray diffraction analysis was performed using a Shimadzu diffractometer model XRD 6000 (UPM-Malaysia). The diffractometer employed Cu-Kα radiation to generate diffraction patterns from powder crystalline samples at ambient temperature. The Cu-Kα radiation was generated by Philips glass diffraction, X-ray tube broad focus 2.7KW type. The crystallite size D of the samples was calculated using the Debye–Scherrer's relationship. Where D is the crystallite size, λ is the incident X-ray wavelength, β is the Full Width at Half-Maximum (FWHM), and θ is the diffraction angle.

The Scherrer equation can be written as:

$D = K \lambda / \beta \cos \theta$

Results and Discussion

Characterization and molecular identification of isolated bacteria

The selected bacterium was characterized and identified by using standard morphological, physiological and biochemical tests (Table 1). It was presumptively identified as *Bacillus* sp. The sequence of 16S rRNA of this bacterium was submitted to Blastn {database 16S ribosomal RNA sequences (Bacteria and Archaea) Megablast} http://www.ncbi.nlm.nih.gov/blast. It indicated a close genetic relatedness of this bacterium with the rRNA sequence of *Bacillus thuringiensis.* This genus represents a common soil bacteria and have been reported as soil inhabitants (Oves *et al.,* 2013).

Table 1: Morphological and biochemical characteristics of *B. thuringiensis*

Tests employed	Characteristics observed
Morphology	
shape	Rod
pigment	-
Gram reaction	+
Biochemical reaction	
Citrate utilization	+
Indole	+
Methyl red	+
Nitrate reduction	-
Oxidase	-
Voges Proskauer	+
Catalase	+
Carbohydrate utilisation	
Glucose	+
Mannitol	-
Sucrose	+
Hydrolysis	
Starch	+
Gelatin	+

(+) and (-) represent positive and negative reaction respectively

Minimum inhibitory concentration

MIC is the lowest concentration of the heavy metals that completely inhibited bacterial growth (Froidevaux *et al*., 2001). *B thurirngiensis* showed significant resistance to high concentrations of Pb, the MIC was 1800 mgl^{-1}, while to cadmium was 50mgl^{-1}. This result is higher than those of Oves *et al* (2013) who observed that, *B. thuringiensis* strain OSM29 could survive at 1500mgl^{-1} of lead, but less in the case of cadmium. This reflects a strain difference and this result is supported by the fact that cadmium is one of the most powerful biological inhibitors, so the growth of bacteria was inhibited with cadmium, even at low concentrations (Qing *et al*. 2007).

Effect of Contact Time

Fig. (1) shows the effect of contact time on Cd and Pb uptake by this bacterium. As the rate of metal ion biosorption of Cd and Pb ions per unit mass of sorbent increased sharply up to 2h and then slightly decreased in case of Pb gradually, and equilibrium reached after 24h for both of metals.

Contact time is one of the important factors of biosorption process, and the explanation of the highest rate of metal ion biosorption in the beginning is due to the high affinity of free metal ion binding sites on absorbent to

the active sites on the surface of cell become saturated by metal ion within 2h. The order of biosorption rate was Pb > Cd. These indicate the equilibrium time at which an equilibrium metal ion concentration is presumed to have been attained and it seems that the affinity for Cd binding is greater than Pb. In this context, Zoubolis *et al* (2004)) and Volesky (1990) observed that, the initial shortest time period of sorption process is important for a high rate of metal sorption. Similar results have also determined by Gabr *et al* (2008) for Ni and Pb biosorption. Marandi (2011) showed biosorption of Mn and Cu by *B. thuringiensis* where increased sharply up to 30 minutes and then slowed gradually, as a result of the availability of active metal binding sites at the beginning of the experiment. After 50 minutes metal uptake became very slow for both metal ions and equilibrium reached after 120 and 150 minutes for Mn and Cu respectively. Giri (2012) mentioned that, the percentage removal of living cells of *Bacillus cereus* biomass was found to increase from 50.11% to 90%, 45.33% to 85.32% and 43.16% to 80.11% for 5 min to 60 min of contact time, for initial chromium (VI) concentration of 1 mgl^{-1}, 5 mgl^{-1} and 10 mgl^{-1} respectively. The change in the rate of removal might be due to the fact that initially all sorbent sites are vacant and also the solute concentration gradient was high. .

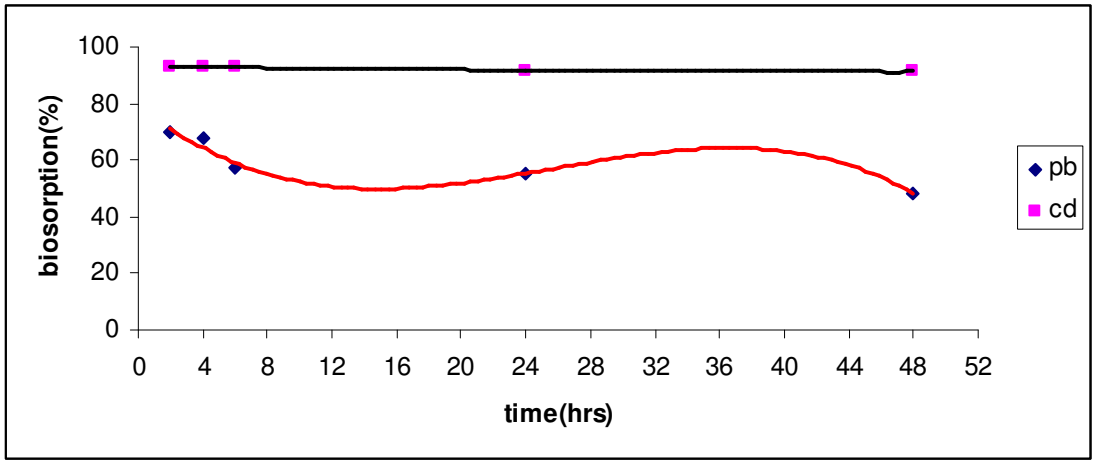

Figure 1: Effect of contact time on the absorption of Lead and Cadmium (II) ions with initial concentration 50 mg/l.

Effect of Initial Metal Ion Concentration

From Fig (2) the absorption capacity of this bacterium for Cd increases by increasing initial metals concentration, while there's fluctuating for sorption of (Pb) with the increasing of metal ion concentration, it was slow when the concentration increased from 5 to 10 mgl^{-1}, but it increased with concentration 25 mgl^{-1} and then reduced with concentration 50 mgl^{-1}. The differences between these two results of removing ions by the same bacterium may be ascribed to variance in a viability of the active site suitable for both metal ions on the surface of the cell. Initial concentration of metal ions, an important factor to be measured for more effective absorption. Higher amounts of metal ions increased the contact probability between the ion and active binding sites on the surface of the biosorbent and subsequently enhanced the metal removal, and thus explain the increase of removal of Cd by bacteria with concentration increase (Marandi, 2012). On the other hand, in case of Pb, the greater uptake of metal by the absorbent materials at the lowest concentration could probably be due to a rapid metal absorbing ability of the bacterial biomass. In contrast, at higher metal concentrations metal ion diffuses into the biomass surface by intraparticle diffusion and therefore, the hydrolyzed ions are likely to diffuse very slowly (Horsfall and Spiff, 2005).

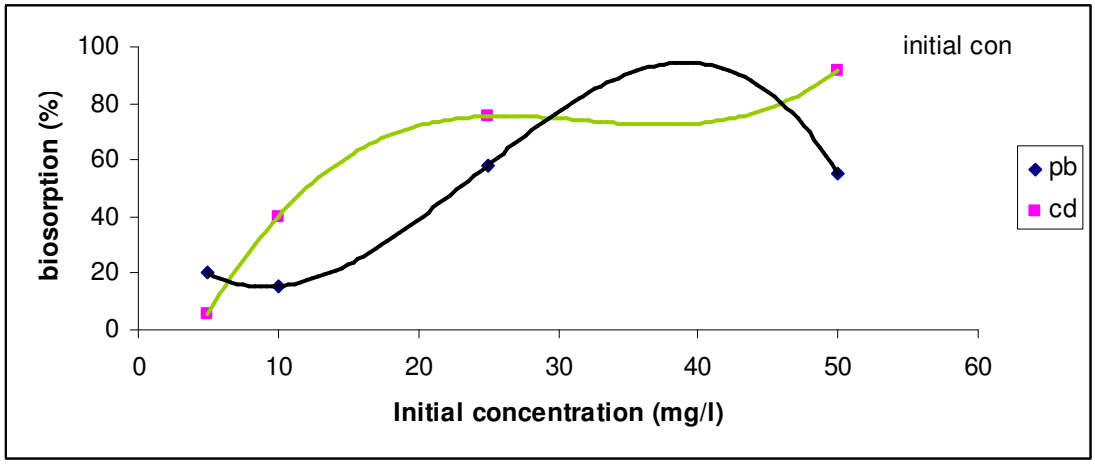

Figure 2: Effect of initial concentration on the biosorption of Lead and Cadmium (II) ions after 24h.

FT-IR spectral analysis

One of the important characteristics of a biosorbent is the presence of its surface functional groups, which are largely characterized by the FTIR spectroscopy method. This technique can only provide a qualitative description. The FT-IR spectra for *Bacillus thuringensis* is given in Fig. (3), they are done in order to characterize the biosorbent. In order to discover which functional groups are responsible for the biosorption process, The FTIR spectra of Pb (II) and Cd (II) loaded and unloaded biosorbent in a range of 280–4000 Cm^{-1} were analyzed. FT-IR spectra for biosorbent, showed the difference between the loaded and unloaded Pb (II) and Cd (II) metal ion (Fig. 3), in all biosorbent. It has an intense absorption band around 3500–3100 Cm^{-1}, which represents the stretching vibrations of amino (N-H) and hydroxyl (O-H) groups in table (3) which clearly states the vibration peak. The spectra of biomass also display absorption peaks at 2925 Cm^{-1} corresponding to stretching of the C-H bonds in the methyl group present in the cell wall structure. (Sethuraman and Kumar, 2011). The absorption band characterization, including C-H in CHO group peak was assigned at 2850 Cm^{-1}, whereas, carbonyl group (C=O) of amide groups at 1646 Cm^{-1}, (COO^{-1}) of the carboxylate groups appeared at 1544 Cm^{-1} (stretching), the band located at 1238 and 1398 Cm^{-1} represent (C-N) and (C-O) respectively. Furthermore, the peak located at 1080 Cm^{-1} was indicative of organic phosphate group P–O of the ($C–PO_4^{-3}$).

The FT-IR spectra of the loaded biomass varied with the metal species Cd and Pb. A stretching of bands appearing at 1070 and 1074 Cm^{-1} was revealed in the FT-IR spectrum, which was attributed to the interaction of sorbed metals Cd and Pb with phosphate groups, respectively. In addition, shifting of bands observed in 1646–1644 Cm^{-1} (Cd and Pb) after biosorption could be due to the involvement of carboxyl groups. Similarly, stretching of bands from 1396 to 1387 Cm^{-1} was due to the involvement of hydrogen bonds as reported by (Sar *et al*., 1999). The bands located between 3484 and 3283 Cm^{-1} however, verified the interaction of hydroxyl and amine groups. The transmittance of the peaks in the loaded biomass was substantially lower than the unloaded bacterial biomass. These changes suggest that bond stretching occurs to a lesser degree due to the presence of metals and therefore, peak transmittance is consequently reduced. In agreement with our findings, numerous workers have also reported similar results (Tunali *et al*., 2006; Lodeiro *et al*., 2006; Gabr *et al*., 2008; Giotta *et al.,* 2011). Conclusively, the formation of varying spectra following adsorption of metal ions on the bacterial biomass validated the contribution of functional groups in metal binding. However, it is difficult to pinpoint the exact mechanism as to how metals are adsorbed onto the microbial biomass due to some unidentified peaks appearing in this experiment.

Table 3: Assignments of Infrared absorption bands

Wave numbers (Cm-1)	**Intensity shape**	**Assignment**
3500-3750	Sharp	O-H stretching
3100-3500	Strong-broad	N-H stretching
2850-2950	Variable	C-H stretching
1400-1660	Variable	N-H bending
1280-1430	Variable	C-H bending
1160-1420	Variable	O-H bending
900-1350	Variable	C-N stretching
900-1380	Variable	C-O stretching

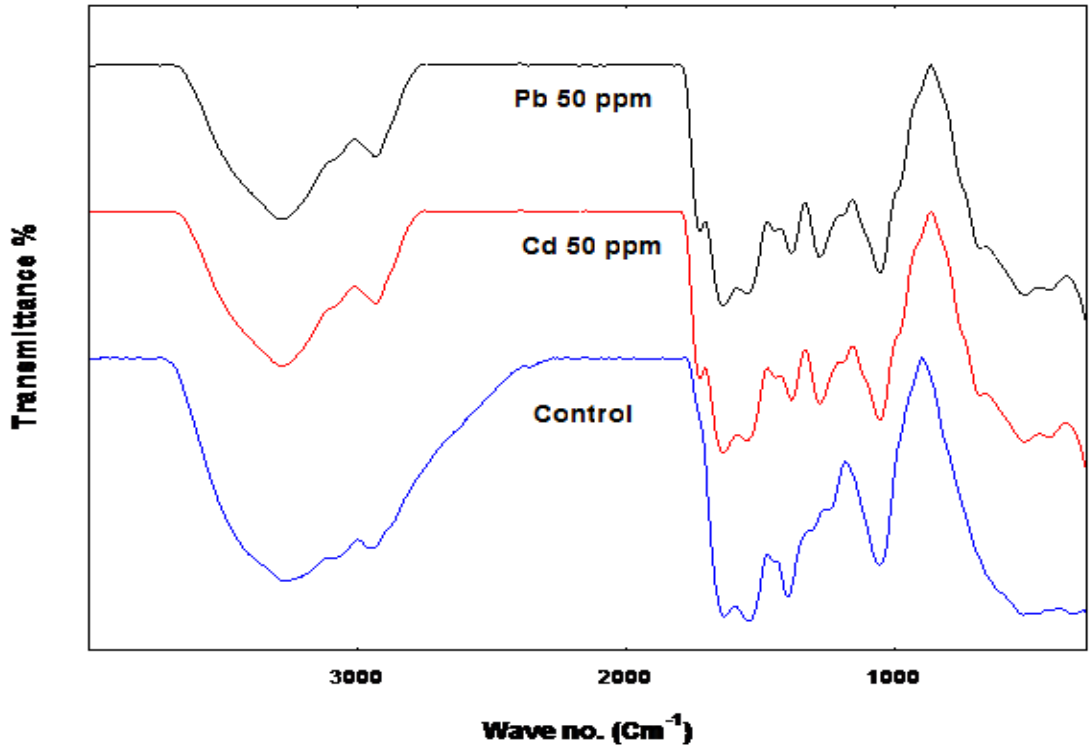

Figure 3: The FTIR Spectra of *B. thuringiensis* in Pb(II), with Cd (II) loaded and without metal loaded.

X-ray powder diffraction analysis (XRD)

The XRD spectra were used to confirm the crystalline nature of the biosorbent (un-loaded *Bacillus thuringiensis)* and loaded with Cd (II) and Pb (II) ions nanoparticles and the pattern is exhibited in Figure (4).The XRD spectrum of Cd (II) and Pb (II) nanoparticales exhibits strong peaks at 2-theta value of 29.9, 37.8, 44.1, 64.4 and 77.8^{o} corresponding to (200), (420), (114), (640) and (822) planes, respectively. The XRD spectrum is compared with the excited spectrums of control that have been published by the Joint Committee on Powder Diffraction Standards (JCPDS file no. 00-002-097). The average crystal size of the un-loaded control, loaded with Cd (II) 50ppm, and Pb (II) 50ppm nanoparticle is estimated from the broadening plane (114) by using the Debye-Scherrer Eq. (1). $D= K \lambda / \beta \cos \theta$.

Where D is the average crystal size, k is the Debye constant (0.9), λ is the X-ray wave length (0.15438nm), β is the full width at half maximum of the peak (FWHM), and θ is the diffraction angle. The average size of the particles was around 54,55,45nm, for Cd (II) 50 mgl^{-1}, Pb (II) 50mgl^{-1} and control respectively.

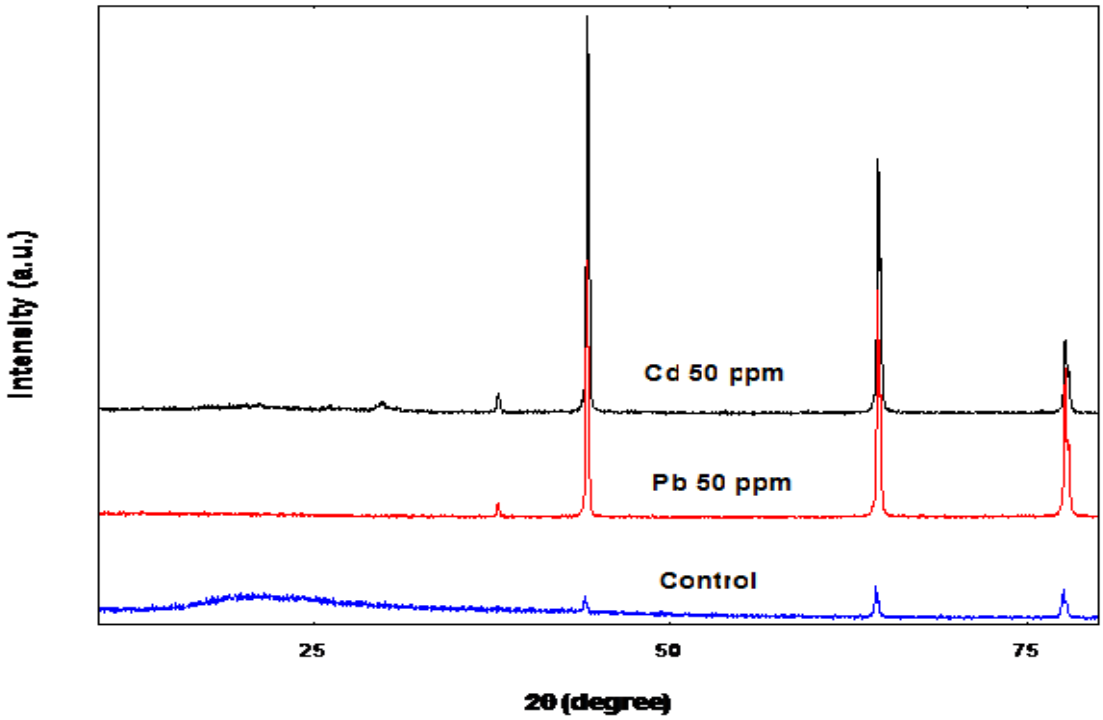

Figure 4: XRD analysis of *B. thuringiensis* biomass before and after Pb and Cd biosorption

References

Ademorati, C. M. A. (1996). Environmental Chemistry and Toxicology. Pollution by Heavy metals. Fludex press Ibadan. 171-172.

Anders, M. Y. J. H; Hubert, C. J. (1992). Bacterial biosorption and retention of thorium and uranyl cations by *Mycobacterium smegmatis*. J. Radio Anal. Nucl. Lett. 166: 431- 440.

Altschul, S. F.; Madden T. L.; Schaffer, A. A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25: 3389–3402 .

Cheng, Z.; Yang, H.; Yu, L.; Cui, Y.; Feng, S. (2006). Preparation and magnetic properties of Y3Fe5O12 nanoparticles doped with the gadolinium oxide, J. Magn. Mater. 302: 259–262.

Fourest, E.; Roux C. J. (1992). Heavy metal biosorption by fungal mycilial by-products: mechanisms and influence of pH. Appl. Microbiol Biotechnol. 37(3), 399-403.

Froidevaux, R.; Krier F.; Nedjar-Arroume N et al. (2001). Antibacterial activity of a pepsin-derived bovine hemoglobin fragment[J]. EBS Lett, 491, 159–163.

Gabr, R.M.; Hassan, S.H.A.; Shoreit, A.A.M. (2008). Biosorption of lead and nickel by living and non living cells of *Pseudomonas aeruginosa* ASU 6a. Int. Biodeterior. Biodegrad. 62,195–203.

Garvie, R.C. (1965). The occurrence of metastable tetragonal zirconia as a crystallite size effect, J. Phys. Chem. 69, 1238–1243.

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.

Giri, A.K. (2012). Removal of arsenic (III) and chromium (VI) from the water using phytoremediation and bioremediation technique. PhD Thesis. Odisha, India.

Gong, R.; Dingh Liu Y.; Chen Q Liu. (2005). Lead biosorption and desorption by intact and pretreated Spirulina maxima biomass Chemosphere. 58, 125-130.

Gupta, U.C.; Gupta, S. C. (1998). Trace element toxicity relationships to crop production and livestock and human health implications for management. J. Commun. Soil Sci. Plan. 29,1491-1522.

Horsfall, M.; Spiff, A.I. (2005). Effect of metal ion concentration on the biosorption of Pb^{2+} and cadmium Cd^{2+} by caladium bicolour (wild cocoyam). Afr. J. Biotechnol. 4, 191–196.

Hussein, H., Farag S., Moawad H. (2003). Isolation and characterisation of *Pseudomonas* resistant to heavy metals contaminants. Arab J Biotechnol., 7,13-22.
Http:// www.ncbi.nlm.nih.gov.

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.

Klug, H.P.; Alexander, L.E. (1974). X-ray diffraction procedures: for polycrystalline and amorphous materials, in: Harold P. Klug, Leroy E. Alexander (Eds.), 2nd edition, Wiley-VCH, ISBN: 0-471-49369-4, May 1 1974, p. 992.

Lane, D. J.; Pace, B.; Olsen, G. J.;Stahl, D. A.; Sogin, M. L.; Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci U S A, 82, 6955–6959.

Lodeiro, P.; BarriadaJ.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. Environ. Pollut. 142, 264–273.

Marandi, R. (2011). Bioextraction of Cu (II) Ions from Acid Mine Drainage by *Bacillus thuringiensis*.Internat. J. Biol. Eng. 1(1), 11-17.

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 J.Biol. Sciences . 20, 121–129.

Patterson, A. (1939). The Scherrer formula for X-ray particle size determination, Phys. Rev. 56 , 978.

Prasenjit, B.; Sumathi S. (2005). Uptake of chromium by *Aspergillus foetidus*. J. Mater. Cycles Waste Manage. 7, 88-92.

Qing, HU.; Min-na, D.; Hong-yan, QI.; Xiang-ming, XIE; Guo-qiang, Z.; Min, Y. (2007). Detection isolation and identification of cadmium-resistant bacteria based on PCR-DGGE. J. Environ. Sci. 19, 1114–1119.

Rathnayake, V. N.; Megharaj, M.; Bolan, N.; Naidu, R. (2010). Tolerance of Heavy Metals by Gram Positive Soil Bacteria. Intern. J. Civil Environ. Eng. 2, 4.

Roane, T.M.; Pepper, I.L. (2000). Microbial responses to environmentally toxic cadmium. Microbial. Eco. 38: 358-364, R.K. Poole & G.M. Gadd, "Metals: Microbe Interactions, IRL Press Oxford, 1-37.
Sar, P.; Kazy, S.K.; Asthana, R.K.; Singh, S.P. (1999). Metal adsorption and desorption by lypholized Pseudomonas aeroginosa. Int. Biodeterior. Biodegrad. 44, 101–110.

Shankar, C.; Sridevi, D.; Joonhong, P.; Dexilin, M.; Thamaraiselvi, K. (2007). Biosorption of chromium and nickel by heavy metal resistant funga and bacterial isolates. J. Hazard. Mater. 146, 270-277.

Sethuraman, P.; Kumar M.D. (2011). *Bacillus subtilis* on Pb^{2+} ions removal from aqueous solution by Biosorption. Res. J. Pharmaceut. Biol. Chem. Sci. 2 (4), 247.

Shumate, ES.; Strandberg, WG. (1985). Accumulation of metals by microbial cells. Compr. Biotechnol. 13, 235-247.

Sneath, P. H. A.; Mair, N.S.; Sharpe, M. E.; Holt, J.G. (1986). Bergey's manual of systematic bacteriology. Vol.2. Williams & Wilkins, Baltimore, U.S.A

Tang, X. Y.; Zhu, Y. G.; Cui, Y. S. et al. (2006). The effect of ageing on the bioaccessibility and fractionation of cadmium in some typical soils of China. J. Environ. Intern. 32, 682-689.

Tunali, S.; Ahmet C.; Abuk b.; Akar T. (2006). Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. Chem. Eng. J. 115, 203–211.

Volesky, B.(1990). Biosorption and biosorbents: In Biosorption of heavy metals. CRC Press, Florida, 3-44.

Wang, J.; Chen, C. (2006). Biosorption of heavy metals by *Saccharomyces cerevisiae*: Rev. Biotechnol. Adv. 24, 427-451.

Wistreich, MD.; Lechtman, D. (1980). Laboratory exercises in microbiology. 3rd ed. Vol 1, Glencoe puplishing Co.,Inc. p. 200 , 312, 401.

Yilmaz, E.I. (2003)."Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1", Res. Microbiol. 154, 409-415.

Zhang, H.B. ; Yang, M. X. ; Shi, W.; Zheng, Y.; Sha, T.; Zhao, Z.W. (2007). Bacterial diversity in mine tailings compared by cultivation and cultivation independent methods and their resistance to lead and cadmium. 54, 705-712.

Zoubolis, A.I.; Loukidou, M.X.; Matis, K.A. (2004). Biosorption of toxic metals from aqueous solution by bacteria strains isolated from metal polluted soils. Process Biochem. 39, 909–916.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar