CADMIUM (Cd) AND LEAD (Pb) UPTAKE POTENTIAL AND SURFACE PROPERTIES OF *Aeromonas* spp. ISOLATED FROM SOIL OF LOCAL MINING SITE

ABSTRACT

Aim: In this study, *Aeromonas* spp. isolated from soil of local mining sites were assessed for their tolerance to some heavy metals and their capability for its uptake.

Material and Methods: The bacteria were isolated from soil of local mining site in Bagega District of Zamfara State in Nigeria. Maximum Tolerable Concentration (MTC) was employed to screen for metal tolerance of the bacteria to Cadmium (Cd) and Lead (Pb) via Agar Plate Method technique. DNA Extraction and 16sRNA Gene Sequence Analysis was carried out to identify the isolates and sequences were compared to those in NCBI database using Basic Local Alignment Search Tool (BLAST).

Results: Similarly, isolates identified were further characterized and validated by subjecting them to 16sRNA gene sequence analysis. Similarity search indicated a close genetic relatedness to *Aeromonas* spp. in NCBI database. The *Aeromonas* spp. Identified showed maximum tolerance to Pb at 27ppm and Cadmium at 24ppm. Bioaccumulation assay carried out revealed *Aeromonas* sp. BDL2 to take up Pb by as high as 70%. Biosorption capacity of their respective dried biomass assayed showed higher Pb uptake at 95.9% for *Aeromonas* sp. BDL2. Statistical analysis revealed a significant difference ($P \leq 0.05$) between the isolates and their respective biomass in the uptake Pb and Cd. Surface examination different bands indicated the presence of carboxyl, amine, amides, sulfates and other aromatic groups and significant shifts of surface molecules.

Conclusion: *Aeromonas* sp. BDL2 identified in this study exhibited higher tolerance to Pb. Surface molecules and their positional properties revealed the presence of hydroxyl, carboxyl, amine and amide group ligands with significant shifts after accumulation and biosorption experiment suggesting interaction between these surface molecules and heavy metal ions.

Keywords: Biosorption, Bioaccumulation, Uptake, Soil

INTRODUCTION

As our societies had gradually grown wealthier, it has created more diverse pollutants with no effective remediation measure in place. Population growth, urbanization and industrialization will in no distant future outperform the reduction strategies in place in the control of hazardous wastes being generated by human activities. Reference [1] reported heavy metals and metalloids to be widespread and of great concern, as they have both direct and indirect consequences on both human health, soil habitat and microbiota. Microorganisms' uptake heavy metals actively (bioaccumulation) and/or passively (adsorption). As maintained by [2] that microbial cell walls, which mainly consist of polysaccharides, lipids and proteins, offer many functional groups that can bind heavy metal ions, and these include carboxylate, hydroxyl, amino and phosphate groups. Among various microbe-mediated methods, the biosorption process seems to be more

feasible for large scale application compared to the bioaccumulation process, because microbes will require addition of nutrients for their active uptake of heavy metals, which increases the biological oxygen demand or chemical oxygen demand in the waste.

Microbes are adapted to thrive in 'adverse conditions' of high acidity / alkalinity / toxicity and high temperature. They can develop 'biological resistance' against any toxic substance in the environment due to special 'jumping genes' [3]. Hence while a number of them may be killed due to high toxicity, some resistant microbes survive and are cultured for further use. Under favorable conditions of growth e.g. pH, temperature and moisture and adequate supply of nutrients like vitamins, magnesium, manganese, copper, sulfur, potassium, phosphorus and nitrogen, microbes can biodegrade / biotransform the complex hazardous organic chemicals into simpler and harmless ones. After the use of 'super bug' in cleaning up oil spills, there has been several successful stories of microbial technique in clean-up of contaminated lands and soils [4]. Whilst microbial remediation (bioremediation) is a well established technology for the removal of organic soil contaminants, the use of microorganisms to transform inorganic contaminants like heavy metals is still being investigated. Environmentalists are viewing microbes as an 'eco-friendly nanofactories' for metal remediation through biotechnological applications employing microbes, such as yeast, bacteria, algae, diatoms and actinomycetes, also there are bacteria which can also ingest the most toxic 'cyanide' from water [4].

MATERIAL AND METHODS

Isolation of Bacteria

Bacteria employed in this study were isolated from soils of local mining sites in Bagega District (11.8648°N, 6.0024°E) of Anka Local Local Government Area of Zamfara State, Nigeria. A sterilized Soil Core Sampler was used to obtain a representative sample from depth of 0-10cm of the soil in those locations. Samples collected were moved to laboratory immediately in tightly sealed container in order to preserve the soil biological properties. Soil samples were sieved with 0.5mm Sieve employing standard microbiological procedures. Serial dilution of the soil was carried out and spread plating was employed using a Nutrient Agar medium. All analysis carried out were in triplicates.

Preparation of Stock Solution

Stock solution of Lead acetate ($Pb(C_2H_3O_2)_2$)(1.6g) and Cadmium (II) Chloride ($CdCl_2$) (1.63g) were dispensed in 1000ml of distilled water to obtain 1000ppm. Working solutions of 0, 1, 2, 4, 8, 16, 32, 64ppm prepared and used in further assays. The pH of the solutions was adjusted using 0.1M H_2SO_4 and 0.1M NaOH. Relevant measures, such as use of standard, positive and negative

controls where necessary, strict cleaning and sterilization of glassware's were absolutely observed [5][6].

Bacterial Biomass Production

Biomass of each of the tolerant species was harvested after growing the cultures in a nutrient broth (NB) medium. For each of the isolates, biomass was obtained from a 72h culture by centrifugation using Selecta Centromix Centrifuge Model 220 at 10000rpm for 15 minutes. Pellets were washed twice with deionized water and dried at 80ºC for 15 minutes [16].

Screening for Cd and Pb Tolerance by the Bacteria

To identify the suitability of the isolates, Maximum Tolerable Concentration (MTC) which is defined as the highest concentration of heavy metal which bacteria can tolerate, was employed to screen for metal tolerance of the bacteria to Cd and Pb. This was achieved via Agar Plate Method in which varying concentrations of the heavy metals (0, 1, 2, 4, 8, 16, 32 and 64ppm) were prepared and incorporated into the Nutrient Agar Medium as described by [7] and adopted by [8], [9] and [10]. Prepared inoculum (0.1ml) of the isolate standardized using McFarland standard was spread plated into each of the varying heavy metal supplemented Nutrient Agar media and incubated at 30°C for 48 hours.

Characterization and identification of Isolates

The following tests and analysis were carried out to determine the morphological and biochemical characteristics of the isolates. These are Gram's Staining [11], Spore staining [12], Catalase test [11], Oxidase test [12], Starch hydrolysis test [11], Nitrate reduction test, Triple sugar iron test [12], Urease Production Test [12], Methyl Red Reaction Test [12], Voges-Proskauer test [12], Indole Production Test [12], Citrate utilization and Motility Test [12].

DNA Extraction and 16sRNA Gene Sequence Analysis

The Extraction of the DNA was carried out using the DNeasy blood and tissue kit (Qiagen Ltd., West Sussex, U.K). Amplification of the DNA was performed by PCR using the universal bacterial primers 16srRNA Bact1442-Forward; (5'-AGAGTTGATCCTGGCTCAG-3') and 16srRNA1492-R (5' GGTTACCTTGTTACGACTT-3'). The final PCR mixture (50µl) consisted of 0.4 µM of each primer, 250 µM of each of dNTP, 1U Dynazyme Taq DNA polymerase (Finnzymes Reagents, Thermo Fisher Scientific, Inc. Finland), 1 x PCR Dynazyme buffer with final $MgCl_2$ concentration of 1.5 mM, and 1µl of the template DNA solution. The temperature program consisted of an initial denaturation at 95º C for 1 min, 30 cycles (94°C for 30 s, 60°C for 30 s, 72°C for 1 min) and a final extension step at 72°C for 8 min. PCR products were purified by 0.8 % agarose gel electrophoresis followed by extraction with QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Purified PCR products were sequenced (GATC-Biotech, Konstanz, Germany) using PCR primers and analyzed using BLASTn search [13].

Sequence and Phylogenetic Analysis

The 16S rRNA gene sequences of the isolates were compared to the sequences in the public databases using Basic Local Alignment Search Tool (BLAST). Multiple Sequence Alignment was done using MUSCLE. The 16S rRNA gene sequences with high similarity to those determined in the study were retrieved and added to database and aligned with Mega 7 [14]. Phylogenetic trees were constructed by the Neighbour-Joining method [15] with MEGA 7 package.

Cd and Pb Uptake Assay

A Batch Equilibrium Method (a traditional method of determining metal uptake) as described by [17] was used to determine the uptake of Cd and Pb by the bacteria. A standardized inoculum of 0.5ml was dispensed into a 100ml of Nutrient Broth supplemented with 12ppm of each heavy metal for 72 hours with shaking. Similarly, harvested dried biomass obtained from equal volume of inoculum was dispensed into 100 ml distilled water (with 1% Nitric Acid) supplemented with 12ppm of Cd and Pb and incubate for 72 hours on a rotary shaker at 160 rev/min. Biomass was thereafter separated by centrifugation and the supernatant was analyzed for residual metal concentration by Atomic Absorption Spectrophotometry (AAS). The percentage metal uptake was calculated as follows:

$$\text{Percentage Uptake} = \frac{\text{Heavy metal utilized}}{\text{Heavy metals added}} \times 100$$ [18]

Determination of Surface Characteristics of Biosorbents

Fourier transform infrared spectroscopy (FT-IR) was carried out on the isolates before and after metals uptake experiments to determine surface molecules of the bacteria. Pellets of each of the isolates was obtained and pressed unto a 16mm diameter mould. FT-IR spectra was recorded with a resolution of 1cm-1 in the 4000 to 400cm-1 wave region [9]; [16].

RESULTS

Characterization of Isolates

Morphological and biochemical characterization of the isolates was presented in Table 1. Similarly, isolates identified were further characterized and validated by subjecting them to 16sRNA gene sequence analysis. Similarity search performed using BLASTn indicated a close genetic relatedness to *Aeromonas* spp. in NCBI database as shown in Table 2. Phylogenetic relationship constructed was presented in Figure 1.

Table 1: Morphological and Biochemical Characterization of Isolates

	BDK1	**BDL2**
Gram Reaction	-ve	-ve
Arrangement	Rods	Rods
Motility	+ve	-ve

Spores	+ve	-ve
Starch	+ve	+ve
Catalase	+ve	+ve
Oxidase	+ve	+ve
Indole	+ve	+ve
VP	-ve	-ve
Citrate	+ve	-ve
Urease	+ve	+ve
Gelatin	+ve	-ve
Nitrate Reduction	+ve	+ve
Glucose	+ve	+ve
Lactose	+ve	+ve
Sucrose	+ve	+ve
H_2S	-ve	-ve
Mannitol	+ve	+ve
Gas	-ve	-ve

Table 2: Molecular characterization of isolates indicating closest hits

Isolate Code	Best Hit Match	%Query	%Identity	Accesion No.
BDL2	*Aeromonas jandaei*	95	84.92	LC361001.1
	Aeromonas veronii	95	84.92	MF16714.1
BDK1	*Aeromnas sobria*	65	95.17	LC194875.1
	Aeromonas hydrophila	65	95.05	MN865804.1

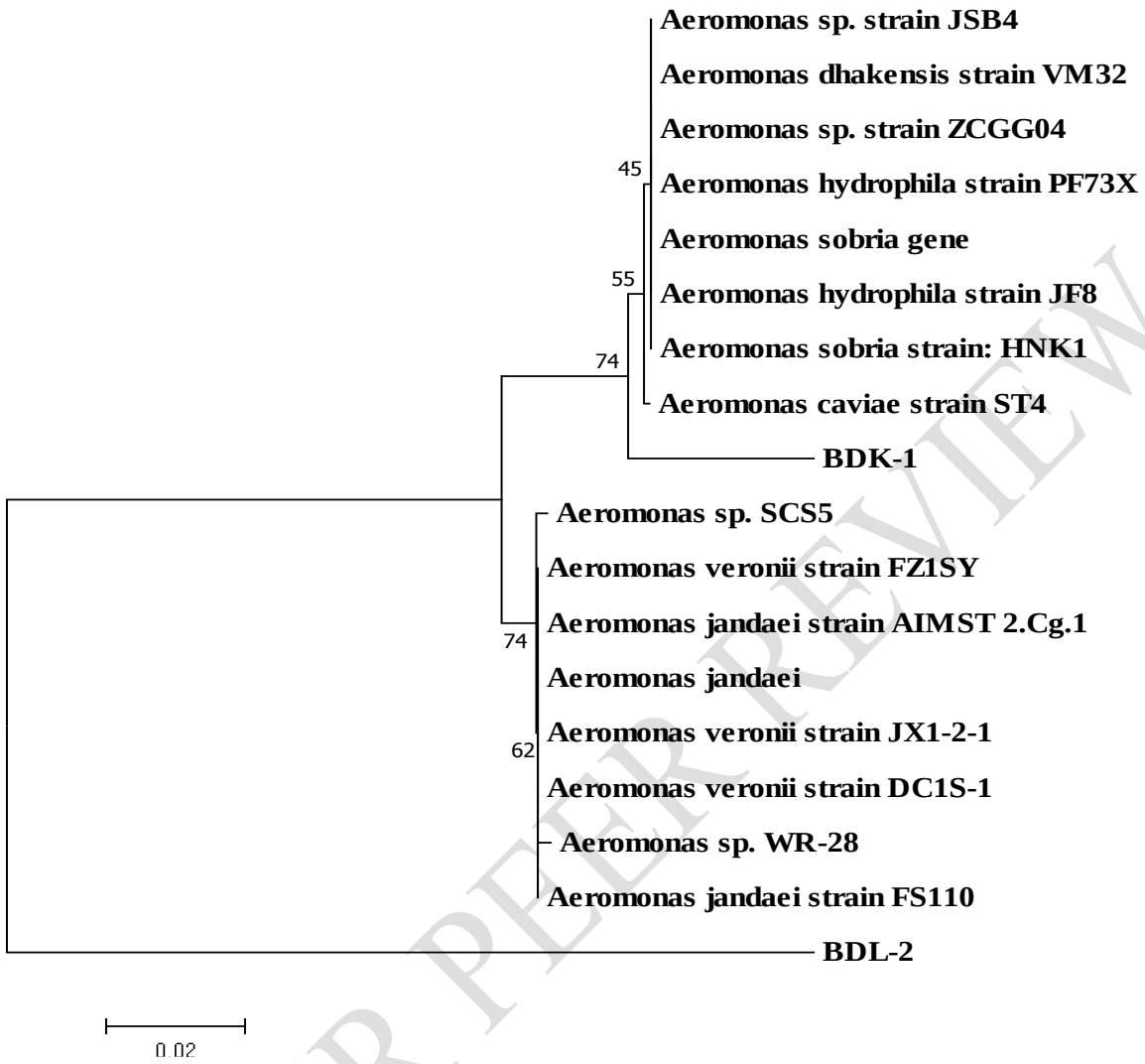

Figure 1: Phylogenetic Tree based on 16SrRNA Sequence Strain BDK1 and BDL2 using Neighbor Joining Method (Bootsrap values were ran at 1000 replications)

Maximum Tolerable Concentration Assay

The *Aeromonas* spp. Identified showed maximum tolerance to Pb at 27ppm and Cadmium at 24ppm as indicated in Figure 2.

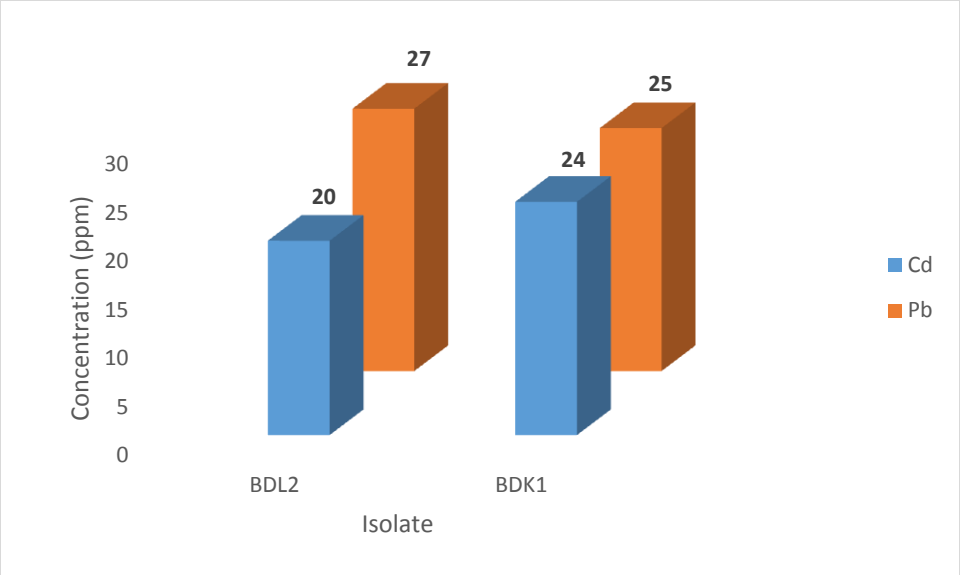

Figure 2: Maximum Tolerable Concentration of Pb and Cd by Isolates

Heavy Metal Uptake Assay

Bioaccumulation assay carried out revealed BDL2 to take up Pb by as high as 70% with BDK1 following closely at 69% as shown in Figure 3 although statistical analysis using Anova revealed no significant difference ($P \geq 0.05$) between the isolates. Biosorption capacity of the respective biomass assayed showed higher percentage of Pb uptake by the bacterial biomass at 94.5 and 95.9% for BDK1 and BDL2 respectively. Statistical analysis employed revealed a significant difference ($P \leq 0.05$) between the isolates and their respective biomass in the uptake Pb and Cd.

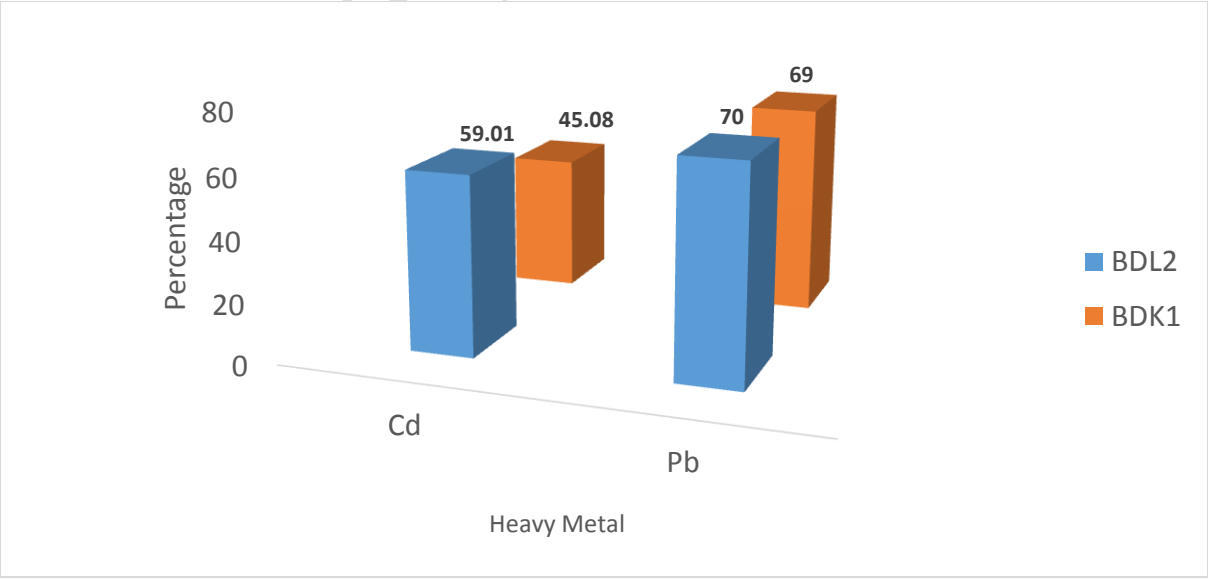

Figure 3: Bioaccumulation of Cadmium and Lead by the Isolates

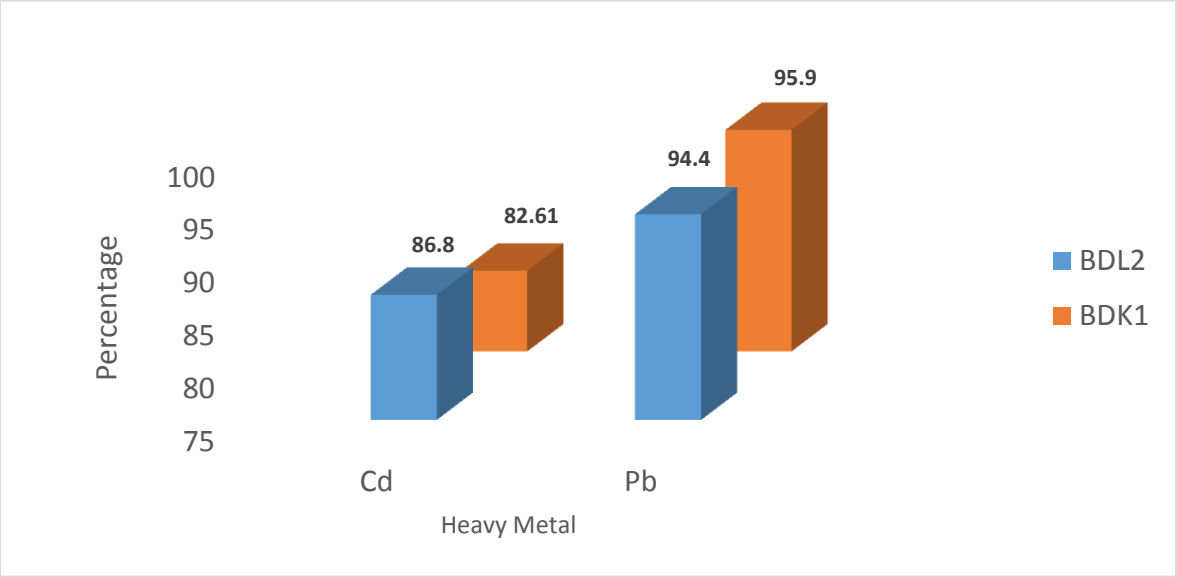

Figure 4: Biosorption of Cadmium and Lead by the Biomass

Determination of Surface Molecules Properties

The surface properties of all the isolate and their respective biomass were carried out using Fourier Transform Infra-Red spectroscopy to ascertain functional groups position prior and after heavy metal accumulation experiments. Wavelengths' at different bands recorded indicated the presence of carboxyl, amine, amides, sulfates and other aromatic groups in the isolates as indicated in Figures 5 to 8 prior to and after Cd and Pb uptake by both the isolate and biomass of the bacteri

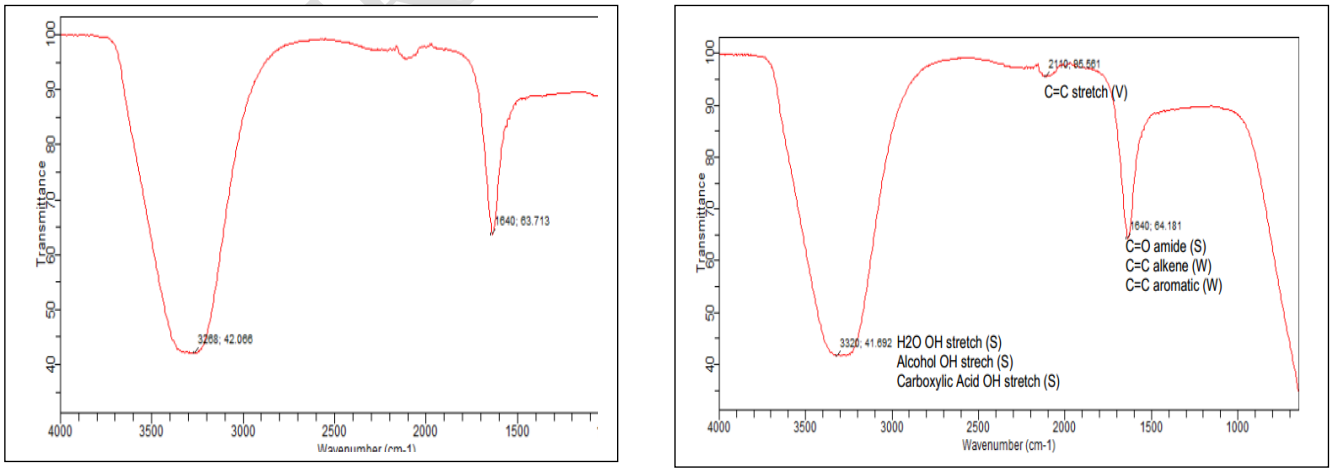

Figure 5: Surface Molecules of *BDL2* before and after accumulation Assay

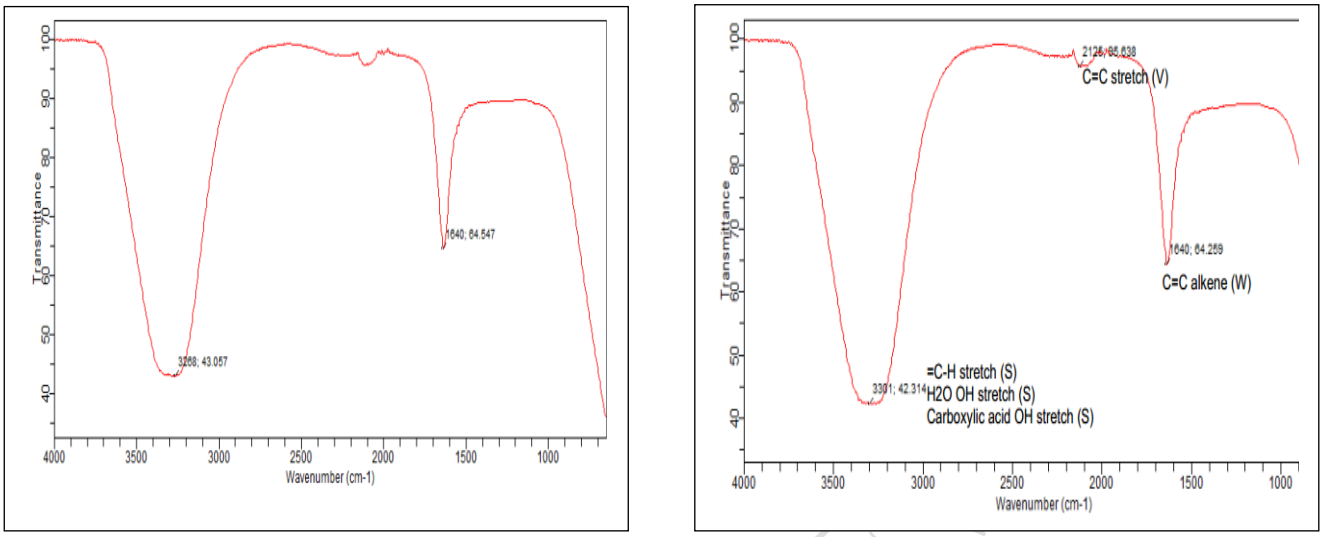

Figure 6: Surface Molecules of *BDK1* before and after accumulation Assay

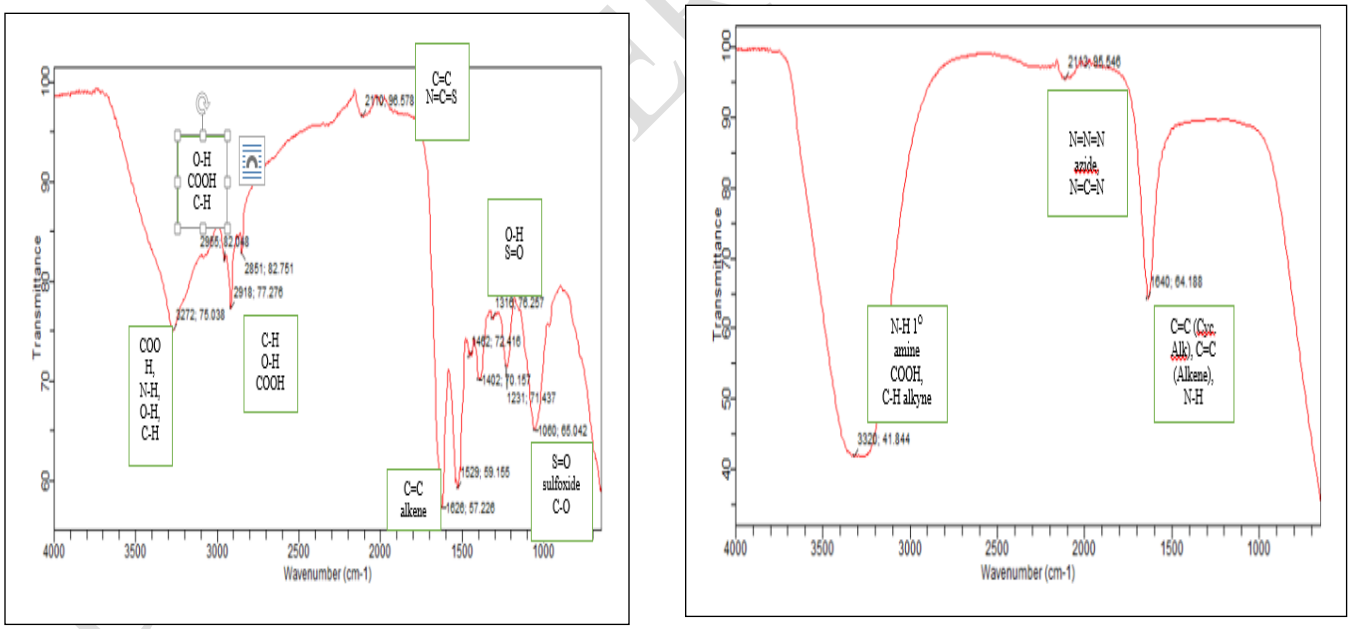

Figure 7: Surface Molecules of *BDL2* biomass before and after biosorption Assay

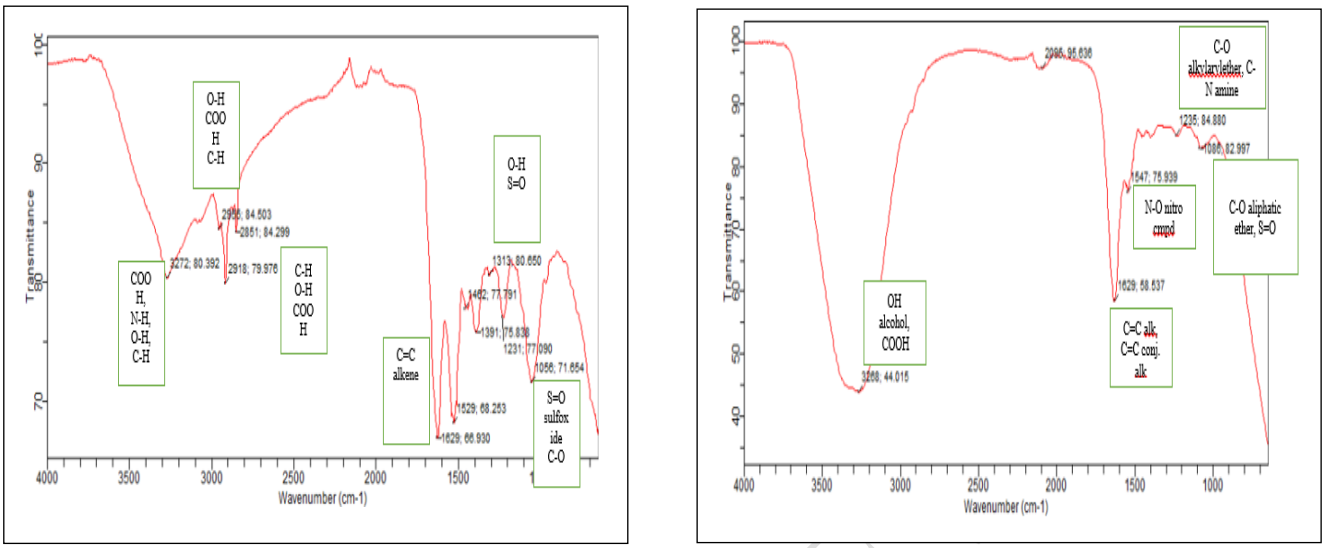

Figure 8: Surface Molecules of BDK1 biomass before and after biosorption Ass

DISCUSSION

In this study, *Aeromonas* spp. identified were capable of tolerating between 20-27ppm of Cd and Lead metals respectively. Metal tolerant qualities that might be inherent in these bacteria and possibly adaptation to their primary environment might have contributed to their adaptation. It was reported how several bacteria species were found to be persistent in metal contaminated soils and other similar environments [19][20][21]. Studies have showed some bacteria can withstand high metal concentrations as reported by [22],[23] and [24] reported that some bacteria to have the capability of tolerating from as low as 2ppm to as high as 100ppm in concentrations of heavy metals. Contrary to the findings in this study, tolerance to individual heavy metals observed by [25] where bacterial isolates were shown to withstand 1900ug/ml of Pb metal which is lower than what was recorded in this study can be attributed to the originating source of the bacteria might have played an important role in the level of tolerance or otherwise as several studies showed those obtained from sludge to have higher tolerant capability to those obtained from soil component [25] [26] although [27] reported the isolation of highly Pb tolerant bacteria from Pb contaminated soil. Similar findings recorded in this study were reported by [28] where 3ppm of Pb was identified as the range of concentration that inhibited bacterial growth. The HTL of *Aeromonas* spp. to Cadmium as observed in this study was 24ppm. Cadmium was a naturally occurring heavy metal and a potential bacterial toxicant having antagonistic effect. Studies by [29] had reported tolerance of some bacterial isolates to a maximum of 0.9ppm of cadmium. Findings in this research are similar to that of [30] who reported similar values in terms of cadmium tolerance by bacterial isolates.

The bacteria identified in this study showed capability of accumulating heavy metals with *Aeromonas* sp. *BDL2* having the uptake capacity of up to 70% for Pb. Affinity of bacteria to metals is widely reported [31][22]. The processes leading to this phenomena can be metabolic and non-metabolic as surface molecules can adsorb various ionic compounds which might be as a result of the opposite charge existing between them. The observation recorded in this study agreed with that of [32] who reported the removal and uptake of heavy metals by bacterial isolates to a near 100% level. To further corroborate the findings in this study, [33] reported about 90.4% uptake of Pb by Gram negative *Pseudomonas* sp. in biosorption of heavy metals studies. But contrary to this study, [34] reported an extremely low uptake of Pb at 0.41% by *Stenotrophomonas* sp. in their study of heavy metal resistance by bacteria and fungal isolates. This variation might have resulted due to differences in genetic make-up and also adaptability of habitats.

The ability of harvested *Aeromonas* spp. biomass to biosorb Cd and Pb assessed showed Aeromonas sp. BDK1 exhibiting highest biosorption capacity of 95.5%. There was significant difference ($P \leq 0.05$) in uptake between isolates and their resultant biomass. The high uptake recorded in the biomass might have been an indication of increase in surface to volume ratio of the biomass to its isolates. Similarly, it is indicative of preservation of cell wall related ligand and other active sites even after harvesting. Similar studies by [35] [36] and [37] have reported higher biosorption capacity in bacterial biomass in comparison to immobilized or living bacterial cells by a difference of up to 50% capacity. In agreement to the findings recorded in this research, [38] reported biomass of *Bacillus* sp. to biosorb higher percentage of heavy metals than living isolates.

Functional groups properties on the surface of each of the isolates and biomass before and after heavy metal accumulation and biosorption experiment were assessed. The Fourier Transform Infra Red Spectroscopy (FTIR) was taken from the range of 4,000 to 1000cm^3 wavelenght. Hydroxyl, Carboxylic, Amide, Aromatic, and other ketone linkages were evident in all the isolates. Findings from this research revealed that peaks observed at between 3200 to 3400 is indicative of the presence of hydroxyl, alcoholic and carboxylic stretches. Presence of these group of surface molecules were reported by [9]. Similarly, findings by [39] and [40] corroborated this study by reporting that hydroxyl and carboxyl groups as well as nitrogen based bioligands including amide and sulphonamides were involved in the binding of Pb. Further studies by [41] reported the presence of these groups in their study of bio-immobilization of lead by *Bacillus* biomass characteristics of *Bacillus* spp. obtained from contaminated soil samples. Furthermore, peaks measured at between 2000 and 2200 wavelenght indicates a variable stretch of alkene presence in the respective isolates whilst that of 1640 measure the presence of amide, aromatic and alkene functional groups. [42] reported the presence of these surface molecules to be indicative of proteins structures. It should be noted that an observable shift in the wavelength occurred after heavy metal experiment at between 3200-3400 for all the isolates. This shift in wavelength might be as result of bonding and complexation due to metal interaction in the isolates. Studies that are similar to this research reported by [9] and [43] reported similar findings

at which peak transmittance actually reduced as a result of metal interaction occurring on the surface. This finding agreed with that of [44][45][46] and [47] who all reported similar observations on heavy metal uptake and surface structure property of different bacterial isolates.

Conclusion

From the findings of this research, it can be concluded that *Aeromonas* spp. BDL2 identified in this study exhibited higher tolerance to Pb with attendant higher uptake of both metals assayed, even though biosorption technique was shown to be efficient process in metal uptake. Surface molecules and their positional properties revealed the presence of hydroxyl, carboxyl, amine and amide group ligands with significant shifts after accumulation and biosorption experiment suggesting interaction between these surface molecules and heavy metal ions.

REFERENCES

[1] Hogan, C.M. (2012). Heavy Metal: in Encyclopedia of Earthe, Eds Cutler J. Cleveland. Washington DC Environmental Information Coalition, National Council of Science and Environment

[2] Dixit R, Malaviya D, Pandiyan K et al (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7(2):2189–2212

[3] Chap, K., Bhupendra, Taak, Pooja (2018) Soil Remediation Through Microbes 101-128 DOI - 10.1007/978-981-13-2420-8_6

[4] US Geological Survey (2006). Bioremediation: Nature's way to a cleaner environment. . http:// water.usgs. gov/wid/html. Yucheng

[5] Adesola, N.A.B., Oyinsiku, O.O., Oyebamiji, J.B.N. and Janet, O.O. (2008). Isothermal and thermodynamic studies of biosorption of zinc (ii) by *Calymperes erosum*. *Journal of Applied Science Resource*, 4(6):716–721.

[6] Kiran, M., Maria, Y., Zobia, N., Abdul, H., Fauzia, Y.H. and Ran, F. (2010). Isotherm studies for determination of removal capacity of bi–metal (Ni and Cr) ions by *Aspergillus niger*. *Pakistan Journal of Botany* 593– 604.

[7] Schmidt T, Schlegel HG (1994) Combined nickel-cobalt-cadmiumresistance encoded by thencclocus of Alcaligenes xylosoxidans31A. J Bacteriol 176: 7045±7054

[8] Sevgi, Ersoy, Coral, Gökhan, Gizir, Ahmet, Sangun, Mustafa (2010) Investigation of heavy metal resistance in some bacterial strains isolated from industrial soils, Turkish Journal of Biology 423- 431 DOI - 10.3906/biy-0901-23

[9] Oves, M., Khan, M.S. and Zaidi, A. (2012). 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

[11] Prescott, L.M., Harley J.P. and Klein, D.A. (2002). *Microbiology*, 5th Edition, McGrawHill, New York. USA

[12] Ochei, J. and Kolhatkar, A. (2000). *Medical Laboratory Science, Theory and Practice*. Tata McGraw Hill Publishers, New Delhi, India.

[14] Tamura K, Stecher, G, Peterson, D., Filipski, A., Kumar, S. (2013) Mega 7: molecular evolutionary genetics analysis version 7.0 Mol Biol Evol 30: 2725– 2729.

[15] Saitou, N., Nei, M. (1987). The neighbor-joining method—a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4: 406– 425

[16] François, F., Carine, L., Jean-Michel, G., Paul, S. Florence, B., Grégory, M., Manon, V., Daniel, G., Anne-Laure, M., David P., Jean, P. *et al.* (2011) Isolation and Characterization of Environmental Bacteria Capable of Extracellular Biosorption of Mercury, *Applied and Environmental Microbiology*, 1097-1106

[17] Khodaverdiloo, H. and Samadi, A. (2011). Batch equilibrium study on sorption, desorption and immobilization of cadmium in some Semi-Arid Zone soils as affected by soil properties. *Soil Resource Journal*, 49: 444-454

[18] Garcia, R., Juan, C., Julio, A., Elena, C. and Buitron, G. (2016). Biosorption of cadmium, Mn, Cr, and Pb from auous solutions by Bacillus sp. *TIP Revista Especilalizada Biologicas* 19(1):5-14

[19]Adekanmbi AO, Falodun OI. Physicochemical, Microbiological and heavy metal studies on water samples and bacteria obtained from Dandaru River in Ibadan. South-Western Nigeria. Afr J Microbiology Res. 2015;9:1357–1365. doi: 10.5897/AJMR2015.7388 [Crossref], [Google Scholar]

[20] Oriomah C, Adelowo OO, Adekanmbi AO. Bacteria from spent engine-oil-contaminated soils possess dual tolerance to hydrocarbon and heavy metals, and degrade spent oil in the presence of copper, lead, zinc and combinations thereof. Ann Microbiol. 2015;65:207–215. doi: 10.1007/s13213-014-0851-x [Crossref], [Web of Science ®], [Google Scholar]

[21]Adekanmbi, A.O., Olawale O. Adelowo, Anthony I. Okoh & Obasola E. Fagade (2019) Metal-resistance encoding gene-fingerprints in some bacteria isolated from wastewaters of selected printeries in Ibadan, South-western Nigeria, Journal of Taibah University
for Science, 13:1, 266-273, DOI: 10.1080/16583655.2018.1561968

[22] Ahemad, M. and Malik, A. (2012). Bioaccumulation of Heavy Metals by Zinc Resistant Bacteria Isolated From Agricultural Soils, *Bacteriology Journal* 2(1): 12-21

[23] Yusuf, I. Mohd, Y. S., Phang Lai Yee, Mohd Arif Syed and Siti Aqlima Ahmad (2014). Biodegradation of Chicken Feather Wastes in Submerged Fermentation Containing High Concentrations of Heavy Metals by Bacillus sp. khayat, *Journal Environmental Bioremediation and Toxicology,* 2014, Vol 2, No 2, 38 -41

[24] Yusuf, I., Ahmad, S.A., Phang, L.Y.,Arif, M.S., Nor, A.S., Khalilah, A., Farrah, A.D., and Mohd, Y. S. (2016). Keratinase production and biodegradation of polluted secondary chicken feather wastes by a newly isolated multi heavy metal tolerant bacterium-Alcaligenes sp. AQ05-001, *Journal of Environmental Management,* 1-14

[25] Marzan, L.W., Mehjabeen, H., Sohana, A.M., Yasmin, A. Masudul, A.M. and Chowdhury, A. (2017). Isolation and Biochemical Characterization of Heavy Metal Resistant Bacteria from Tannery Effluent in Chittagong City, Bangladesh, *Egyptian Journal of Aquatic Research* (2017) 43, 65–74

[26] Igiri, B.E. ,Stanley I. R. Okoduwa, Grace O. Idoko, Ebere P. Akabuogu, Abraham O. Adeyi, and Ibe K. Ejiogu(2018) Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review, Journal of Toxicology Volume 2018, Article ID 2568038, 16 pages https://doi.org/10.1155/2018/2568038

[27] Jebara, S.H., Souhir, A., Imen, C.F., Manel, C., Omar, S. and Moez, J. (2014). Identification of effective Pb resistant bacteria isolated from *Lens culinaris* growing in lead contaminated soils, *Journal of Basic Microbiology* DOI 10.1002/jobm.201300874

[28] Neethu, C.S., Mujeeb-Rahiman, K.M., Saramma, A.V. and Mohamed, H.A.A. (2015). Heavy-Metal Resistance in Gram-negative Bacteria Isolated from Kongsfjord, *Arctic Canadian Journal of Microbiology* 61: 429-435.

[29] Oaikhena, E.E., Dominic, B.M., Samuel, D.D., Muhammad, M.N. and Ali, A.H. (2016). Bioremediation Potentials of Heavy Metal Tolerant Bacteria Isolated from Petroleum Refinery Effluent, *American Journal of Environmental Protection* 5(2): 29-34 http://www.sciencepublishinggroup.com/j/ajep doi: 10.11648/j.ajep.20160502.12

[30] Smrithi, A. and Usha, K. (2012). Isolation and Characterization of Chromium Removing Bacteria from Tannery Effluent Disposal Site. *International Journal of Advanced Biotechnology and Research* 3(3): 644-652

[31] Rani, J.M., B. Hemambika*, J. Hemapriya and V. Rajesh Kannan (2010) Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: A biosorption approach African Journal of Environmental Science and Technology Vol.

4(2), pp. 077-083, Available online at http://www.academicjournals.org/AJEST ISSN 1991-637X © 2010 Academic Journals

[32] Sofu A. Sayilgan E. Guney G. (2015). Experimental design for removal of Fe(II) and Zn(II) ions by different lactic acid bacteria biomasses. *International Journal of Environmental Research* 9(1), 93–100

[33] Shamim, S. (2017). Biosorption of Heavy Metals, *IntechOpen Publication*.

[34] Congeevaram, S.D., Park, S., Dexilin, M. and Thamaraiselvi, K. (2007). Biosorption of Chromium and Nickel by Heavy Metal Resistant Fungal and Bacterial Isolates. *Journal of Hazardous Materials* 146(1-2):270-277

[35] Srinath, T. T. Verma, P.W. Ramteke, S.K. Garg, F. (2002). Chromium (VI) biosorptionand bioaccumulation by chromate resistant bacteria, *Chemosphere* 48: 427–435.

[36] Tuzen, M. O. Dogan, C. Usta, M. Soylak (2007). Biosorption of copper(II), lead(II),iron(III) and cobalt(II) onBacillus sphaericus-loaded Diaion SP-850 resin, *Analytica Chimica Acta* 581: 241–246

[37] Benmalek, Y. M. and Fardeau, L. (2016). Isolation and characterization of metal-resistant bacterial strain from wastewater and evaluation of its capacity in metal-ions removal using living and dry bacterial cells. *International Journal of Environmental Science and Technology* 13:2153–2162 DOI 10.1007/s13762-016-1048-6

[38] Velasquez, L. and Dussan, J. (2009). Biosorption and bioaccumulation of heavy metals on dead and living biomass of *Bacillus sphaericus*. *Journal of Harzadous Materials*, 167: 713–716

[39] Cabuk, A., Ilhan S., Filik C. and Caliskan F. (2005). Pb^{2+} Biosorption by pretreated fungal biomass. Turkish Journal of Biology 29: 23.

[40] Anna Jarosławiecka and Zofia Piotrowska-Seget Lead resistance in micro-organisms, Microbiology (2014), 160, 12–25 DOI 10.1099/mic.0.070284-0

[41] Qiao, W., Yunhao Zhang, Hao Xia, Yang Luo, Si Liu, Shiyu, W. and Weihan, W. (2019). Bioimmobilization of lead by Bacillus subtilis X3 biomass isolated from lead mine soil under promotion of multiple adsorption mechanisms, *Royal Society of Chemistry Journal* http://dx.doi.org/10.1098/rsos.181701

[42] Faghihzadeh, F., Nelson, M., Anaya, M., Laura, A., Schifman, V. and Oyanede, C. (2016). Fourier transform infrared spectroscopy to assess molecular-level changes in microorganisms exposed to nanoparticles, *Nanotechnology Environmental Engineering* 1:1 DOI10.1007/s41204-016-0001-8

[43] Blessy Baby Mathew and Nideghatta Beeregowda Krishnamurthy, Screening and identification of bacteria isolated from industrial area groundwater to study lead sorption: Kinetics and statistical optimization of biosorption parameters, *Groundwater for Sustainable Development*, https://doi.org/10.1016/j.gsd.2018.07.007

[44] Norton, L., Baskaran, K. and Mckenzie, T. (2004). Biosorption of Zinc from Aqueous Solution Using Biosolids. *Advance Environmental Journal Research* 8:629-635

[45] Lodeiro, P., Barriada, J.L., Herrero, R. and Sastre, V. (2006). The Marine Micro-alga *Cystosreira baccata* as Biosorbent for Cd and Pb Removal, *Environmental Pollution* 142: 264-273

[46] Gabr, R.M., Hassan, S.H.A. and Shoreim, A.A.M. (2008). Biosorption of Pb and Ni by living and non-living cells of *Pseudomonas aeruginosa*. *International Journal of Biodegradation and Biodeterioration*, 62: 195-203

[47] Giotta, L., Mastrogiocomo, D., Italiano, F., Milano, F., Agostiano, F. et al. (2011). Reversible Binding of Metal ions onto bacterial layers revealed by Protonation-induced ATR-FTIR Difference Spectroscopy, *Langmuir* 27: 3762-3773