academicJournals

Vol. 8(22), pp. 2229-2237, 28 May, 2014
DOI: 10.5897/AJMR2014.6837
Article Number: BEAC6FD45116
ISSN 1996-0808
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Application of bacterial biomass as a potential heavy metal bio-removal agent

Said Mohamed Daboor[1,2]

[1]Head of the Biomedical Sciences Department, Al-Farabi College of Dentistry and Nursing, Riyadh, KSA.
[2]National Institute of Oceanography and Fisheries, Cairo, Egypt.

Received 19 April, 2014; Accepted 12 May, 2014

Water has been the most important element for saving life; the major global health problem has been water pollution that may be due to the wastewater discharge into the water bodies. Several techniques have been used for water treatment that is, physical, chemical and biological methods. Recently, the third method was the most effective one for the wastewater treatment. In this work twenty bacterial isolates were isolated from River Nile, Egypt to study their capability to remove some heavy metals from its solution. Agar plates amended with different concentrations of some heavy metals were used for screening the bacterial capability for removing the tested heavy metals. According to the identification procedures based on the BIOLOG system the bacterial isolate MSNIOF11 showed a similarity of 97% to *Bacillus subtilis* var. *globigii*, so it was given the name as *Bacillus subtilis* var. *globigii* MSNIOF11. The heavy metal removal process was pH and temperature dependent, where the maximum growth and heavy metal removal was recorded at 30°C with neutral pH (7.0). In the first 24 h there was an increase of the metal removal and there was no significant change after 30 h.

Key words: Bioremoval, heavy metals, *Bacillus subtilis*, biomass, biolog, MicroPlats.

INTRODUCTION

Water pollution is an acute problem in the River Nile. In the rise of the increasing urbanization and industrialize-tion, the pollution potential of the River is gaining momen-tum day by day. Dumping wastewater and toxic wastes into the main channel of the River has caused serve pollution in the River to the extent that its water is posing a threat to the survival of aquatic flora and fauna (Dalman et al., 2006).

Water quality has been decreased during this century due to discharge of wastewater into water channels as well as environmental pollutants. This is considered as one of the major global health problems, and cross adap-tation of microbial population to structurally related che-micals may play an important role in the practical deve-lopment and application of bioremediation techniques (Liu and Jones, 1995; Monachese et al., 2012). Pollution of the natural environment by heavy metals is a world-wide problem because these metals are indestructible

E-mail: saiddaboor@yahoo.ca. Tel: 00966-537123317.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

and most of them have toxic effects on living organisms when they exceed a certain concentration (MacFarlane and Burchett, 2000; Ekeanyanwu et al., 2010; Raphael et al., 2011; Aikpokpodion et al., 2012; Ahmed et al., 2013). Heavy metals are of high ecological significance since they are not removed from water as a result of self purification, but accumulate and enter the food chain which inevitably affects the human health resulting in extremely disrupted biological processes (Loska and Wiechula, 2003; Igwe and Abia, 2006; Akpor and Muchie, 2010; Young et al., 2012).

The bio-removal/ processes could offer the possibility to destroy or render various contaminants using natural biological activity. As such, it uses relatively low-cost, low technology techniques, generally have a high public acceptance and can often be carried out on site using a microbial source which has received much attention recently due to the awareness of environmental problems (Saithong and Poonsuk, 2002; Rani et al., 2010; Abioye, 2011). Microorganisms such as bacteria (Daboor and Sabae, 2007; Nanda et al., 2011; Lin and Harichund, 2011; Samarth et al., 2012), algae (El-Sherif et al., 2008; Tamilselvan et al., 2011; Mane and Bhosle, 2012; Kumar and Oommen, 2012), fungi (Selvam et al., 2002; Joshi et al., 2011; Simonescu and Ferdes, 2012; Hemambika et al., 2011) and yeast (Abdul Rehman et al., 2008; Machado et al., 2009, 2010) persist a variety of mechanisms exist for the removal of heavy metals from aqueous solution. Regarding the same process Zhou et al. (2007) used *Bacillus* cells as a factor to remove the chromium ions from the aqueous solution. Jarosławiecka and Piotrowska-Seget (2014) reported that the bacterial extracellular polysaccharides are involved with the lead adsorption.

The main objective of the present study was to isolate and screen heavy metal tolerance of bacterial isolates and evaluate their competence to remove heavy metals from its solution and detect the suitable conditions for the maximum activities under laboratory conditions.

MATERIALS AND METHODS

Sampling and strain isolation

Water samples were collected from five stations at Demitta Branch of RiverNile. These stations are highly polluted with high concentrations of heavy metals due to discharge of industrial effluent. Twenty bacterial isolates (12 as Gram positive and eight as Gram negative) were isolated after seeding the samples on Glu-cose Mineral Salt (GMS) agar plates, (Daboor and Sabae, 2007).

Heavy metal

Heavy metals solutions of zinc sulfate ($ZnSO_4$), lead chloride ($PbCl_2$) and cadmium chloride ($CdCl_2$) were prepared with a final concentration 100 mg/L and kept sterilized for further use.

Screening for bacterial isolates resistant to metal ions

The bacterial isolates were separately streaked on GMS having 100 mg/L of each of the metal solution. After two days of incubation at 30°C the plates were checked for bacterial growth (Daboor and Sabae, 2007).

Heavy metal removal efficiency detection

The bacterial isolates that showed a positive growth on the agar plates (having heavy metals solutions) were sub cultured again onto modified T-medium (Duxbury, 1981) amended with mixture of heavy metal ions Cd^{2+}, Pb^{2+} and Zn^{2+} (50 mg/L of each one). The plates were swabbed and the growth was measured by the periodic determination of culture density absorbance at wave length of 600 nm using a spectrophotometer (Ultrospec 1100-pro, Amersham Pharmacia Biotech) based on McFarland's scale (Sutton, 2011). The most resistant isolates were inoculated (0.2 g) into 250 mL conical flask containing 50 mL T- medium which has metal ions with 50 mg/L final concentration of each metal ion and incubated in shake condition (50 rpm) for 24 h at 30°C.

Identification and characterization of the metal resistant isolates

Bacterial colonies from the plates showed highly intensiveness growth were picked and streaked on Tryptic Soy Agar (TSA) medium, after overnight incubation at 30°C pure colonies were stained with Gram stain, hence figure out which Biolog Micro Plate could be used.

According to the manufacturer's directions of MicroLog System (Biolog, Hayward, CA, USA) pure bacterial isolates were transferred into Biolog Universal Growth (BUG) medium (Biolog, Hayward, CA, USA) and incubated overnight at 30°C. Bacterial growth were collected and suspended in 0.15 M NaCl using cotton swabs. (Tanase et al., 2011).

Biolog MicroPlates preparation and identification

The isolate was tested to utilize 95 different carbon sources in Gram-positive (GP) MicroPlate (Biolog, Hayward, CA, USA) as recommended by the manufacturer's manual. A plastic disposable loop was used to collect colonies carefully so that there would be minimum carryover of nutrients from the agar when the growth was suspended in 0.15 M NaCl. The turbidimeter (which measured the turbidity at wave length at 590 nm) was blanked with a tube of uninoculated saline. The suspension was then adjusted to fall within the low-limit and high-limit GP MicroPlate turbidity standards supplied by manufacturer. The inoculum, which was always used within 10 min. of preparation, was poured into a disposable plastic reservoir just prior to use. MicroPlates (GP) were inoculated with an eight-channel multi-pipette, with 150 µl of the inoculum being dispensed per well; plates were then generally incubated at 30°C. The carbon source utilization patterns were read with a MicroPlate reader and analyzed for the differentiation of bacterial strains by a cluster analysis program using Biolog database and software (Biolog, Hayward, Calif), with the MicroLog GP data base colour formation in the individual cells of the microtitre plates was measured at 590 nm (Miller and Rhoden, 1991; Holmes et al., 1994).

Biolog system provides identifications if the similarity index of the genus or species was 0.750 or greater after four hours incubation. When a lower similarity value is obtained, the user is prompted to

continue the incubation for 24 h. In this study, all MicroPlates were read at both four hours and confirmed after 24 h even when identification was reported at four hours, a similarity index of less than 0.50 results in an instrument report of not identified (NI). Similarity indices of 0.50 result in a computer report of identification to either the genus or the species level (Miller and Rhoden, 1991; Holmes et al., 1994).

Effect of temperatures on bacterial cells and heavy metals removal

The effect of temperature degree was investigated by using the isolate *B. subtilis var. globigii* MSNIOF11. Heavy metal removal was conducted in 250 mL conical flasks containing 50 mL of GMS broth having heavy metals with final concentration 150 mg/L (50 mg/L of each metal). The flasks (three replicates) were inoculated with 0.2 g bacterial cells and incubated for 48 h at 20, 25, 30, 35 and 40°C. Samples were taken and centrifuged at 10.000 rpm for half hour. The supernatant was analyzed to state the heavy metal remaining in the solution (Daboor and Sabae, 2007) and heavy metal removal was calculated based on its initial concentration according to the equation of Kuycak and Volesky (1988).

$$Q = (C_I - C_F)*V/V_1$$

Q: metal removal; C_I: initial metal concentration; C_F: final metal concentration; V: volume of reaction and V_1: total volume

Effect of pH on bacterial cells and heavy metals removal

The effect of different pH values (5.0, 6.0, 7.0, 8.0 and 9.0) was investigated. Adjusting the pH of the medium using 0.1 N HCl and 0.1 N NaOH, and incubating for 24 h at 30°C, remaining heavy metals in the solution were calculated as described previously based on the equation of Kuycak and Volesky (1988).

Effect of incubation time on bacterial cells and heavy metals removal

To study the effect of different incubation periods on both bacterial growth and heavy metals removal, the pH was adjusted to pH 7.0. After several intervals of time 12, 18, 24, 30 and 36 h of incubation at 30°C, heavy metals residue in the solution were calculated following the method reported by Kuycak and Volesky (1988).

Statistical analysis

The arithmetic means of the three replicates estimations were tabulated and the least significant difference (L.S.D.) at 0.05% confidence limit was calculated according to Pielou (1966).

RESULTS

Biolog MicroLog identification

Only one of the twenty bacterial isolates showed a very good growth in the presence of heavy metals high concentration (data not shown). This isolate was rood shaped positive to Gram staining. After four hours and 24 h the color change within each well in the MicroPlate was red by the automated reader. The tested strain showed 97% similarity with *Bacillus subtilis* var. *globigii* and the data also revealed a very low similarity (2.8%) with *B. pumilus.* The relationship between *Bacillus* strains by carbon sources utilization pattern was shown in Figure 1. The analyzed data by Biolog software illustrated the arrangement and distances of the *Bacillus* species, clarified that both B3 (the selected strain) and B2 have the same unit of taxonomic distances, hence B3 is *Bacillus subtilis* var. *globigii*, for differentiation between the selected strain and others it given the name *Bacillus subtilis* var. *globigii* MSNIOF11.

Effect of different temperatures

Effect of incubation temperatures on the growth of *B.. subtilis* var. *globigii* MSNIOF11 and heavy metal removal were represented in Figure 2. The temperature effect represented significant differences ($P>0.05$) between the percentage of metal ion removal, where maximum activity of the heavy metal removal was recorded at 30°C with the values of 72.30, 68.30 and 70.00% of Cd, Pb and Zn ions.

Effect of different hydrogen ion concentrations

pH values play an important role on heavy metal removal by *B. subtilis* var. *globigii* MSNIOF11,as shown in Figure 3. Hydrogen ion concentrations - pH values- affected both the growth and heavy metal removal by the bacterial isolate. At pH levels of 5.0 and 9.0 no obvious growth was seen and metal removal was indemonstrable, nevertheless, the maximum growth and the maximum metal removal were obtained at pH of 7.0, the logarithmic numbers of viable cells /ml were 7.70, 8.39 and 9.20 for Cd^{2+}, Pb^{3+} and Zn^{2+} at 30°C, respectively and at the same time metal removal percentage was in the order of Cd^{2+}>Pb^{2+}> Fe^{2+} with 69.30, 68.70 and 67.00%,, respec-tively. It was also clear that, there was a significant drop in both growth and metal uptake when the pH was shifted towards both acidic and alkaline media (pH of 6.0 and 8.0).

The percentages of metal uptake were 36.30, 56.70 and 48.70% at pH 6.0, while it was 53.30, 43.30 and 46.6% at pH 8.00 for Cd^{+2}, Pb^{+2} and Zn^{+2}, respectively. The maximum values for Cd, Pb and Zn ions were recorded at pH 7.0, where the removal percentage was in the order Cd^{+2}> Zn^{+2}> Pb^{+2} .

Regarding to the effect of the incubation periods the data represented in Figure 4 showed that after 12 h incubation the microbial growth gradually increased. The metal removal was increased by time until 24 h at which

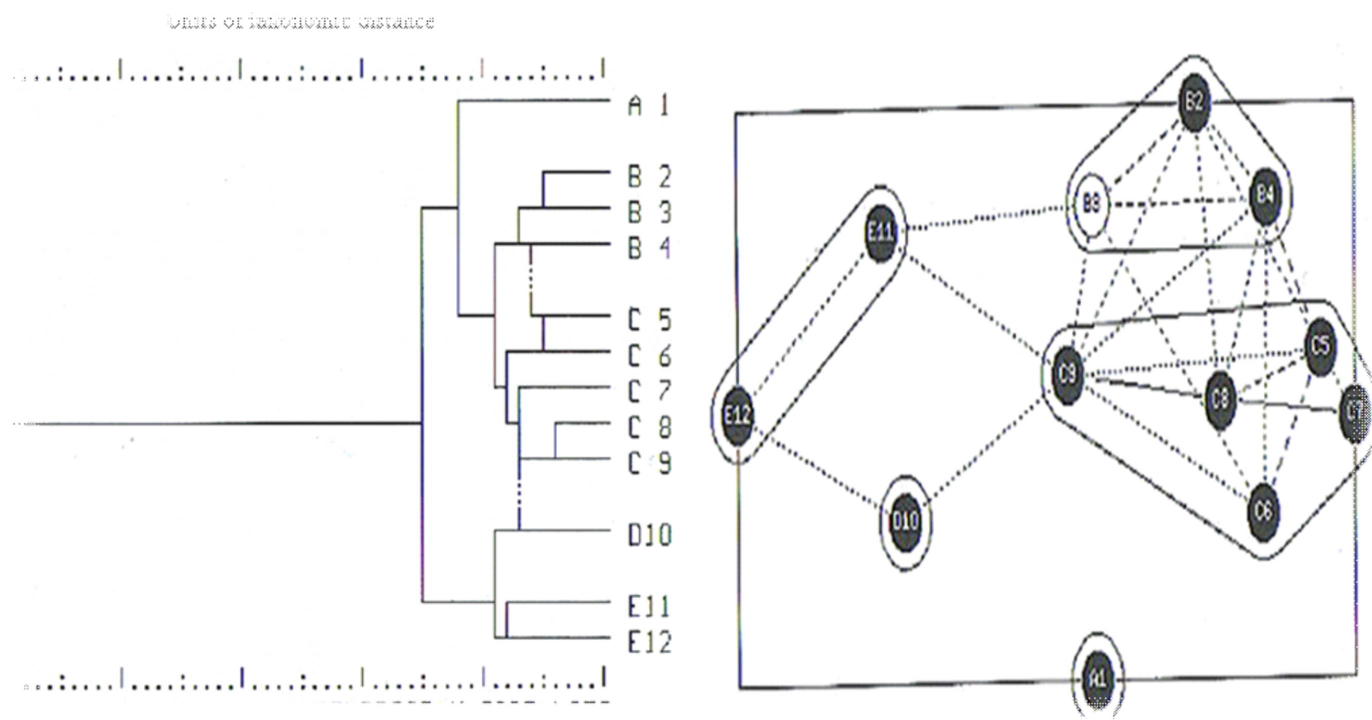

Figure 1. Dendrograms distance generated by Biolog MicroLog identification system; A1: *Bacillus maroccanus,* B2: *B. subtilis* var. *globigii,* B3*: Bacillus* sp.$_{\text{MSNIOF11}}$*, B4*: *B. pumilus,* C5: *B. subtilis,* C6: *B. amyloliquefaciens,* C7: *B. alcalophilusss halodurans,* C8: *Bacillus coagulans***,** C9: *B. licheniformis,* D10: *B. circulans,* E11: *B. azotoformans*and E12: *B. coagulans.*

the highest values of Cd^{2+}, Pb^{2+} and Zn^{2+} removal were detected, but there was no significant effect with time increasing up to 30 h.

DISCUSSION

The results here illustrated the ability of some bacterial isolates to resist the heavy metals toxicity. Only one bacterial isolate showed resistant to the toxicity of all the tested heavy metals, this may be due to biochemical and structural properties, physiological and/or genetic adaptation of microorganisms and environmental modification of metal specification control the surviving of microorganisms in solutions having toxic metals **(**Cooksey, 1993; Blackwell et al., 1995). Hence this bacterial isolate was selected for the heavy metal bioremoval study and identification. It is not possible to isolate and culture microorganisms from their natural habitat and stay behind identification. This will lead to shortage of microbial community composition and function information (Wagner et al., 1993).

In this study, different carbon source profiles were generated by inoculating Biolog GP microtitre plates. The colour development in each well of the GP Biolog microtitre plates reflected the ability of the bacterial community to utilize that specific carbon source. The data indicated that metabolic diversity (substrate utilization), as determined with the Biolog system, could be used to learn more about functional diversity (number of different substrates utilized) and evenness (distribution of species abundance within the community) in natural habitats (Miller and Rhoden, 1991, Heerden et al., 2002; De Paolis and Lippi, 2008).

Some of the *B. subtilis* could survive in the presence of Cd^{+2}, Pb^{+2} and Zn^{+2} at concentration 100 mg/L, while others cannot. Bioaccumulation of heavy metals such as copper, zinc, cadmium, and nickel were reported by several *Bacillus* species **(**Mayer and Beveridge, 1989, Samarth et al., 2012; Odokuma and Akponah, 2012). Heavy metal bioremoval includes the formation of stable complexes between heavy metals and nuclides of microbial biomass and these complexes are generally the result of electrostatic interactions between the metal ligands and negatively charged cellular biopolymers which were produced in both Gram negative and Gram positive bacteria (Ledin and Pedersen, 1996).

The metal bioremoval capacity of living cells from aqueous solutions was influenced by environmental growth conditions, such as temperature, pH value, biomass concentration, and incubation time. It was clear from the present data that, the temperature degree had an important effect on the bioremoval percentage of heavy metal by *B. subtilis* var. *globigii* MSNIOF11 where

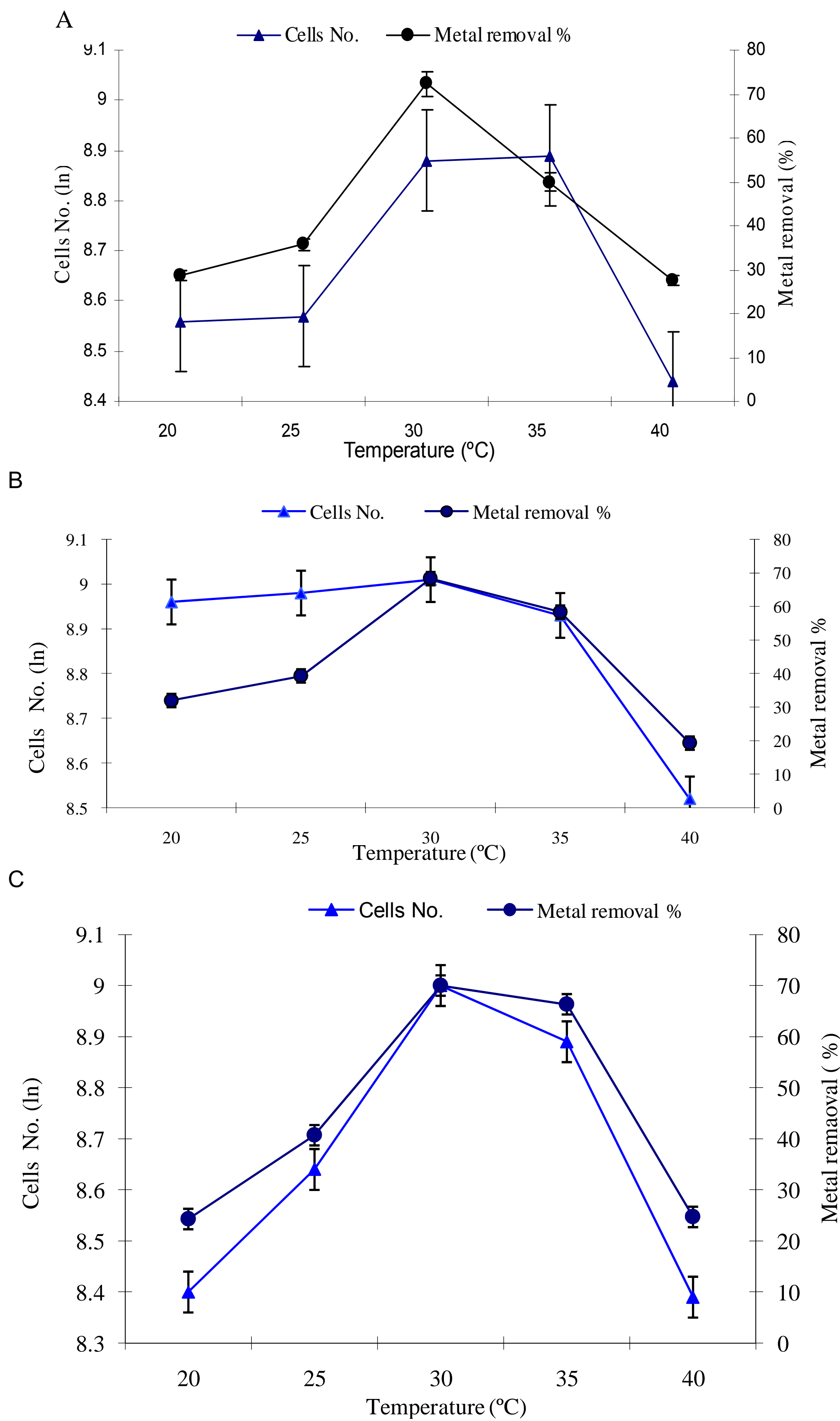

Figure 2. Heavy metal removal and bacterial cell number affected by various temperatures, **(A)** Cadmium, **(B)** Lead and **(C)** Zinc ions.

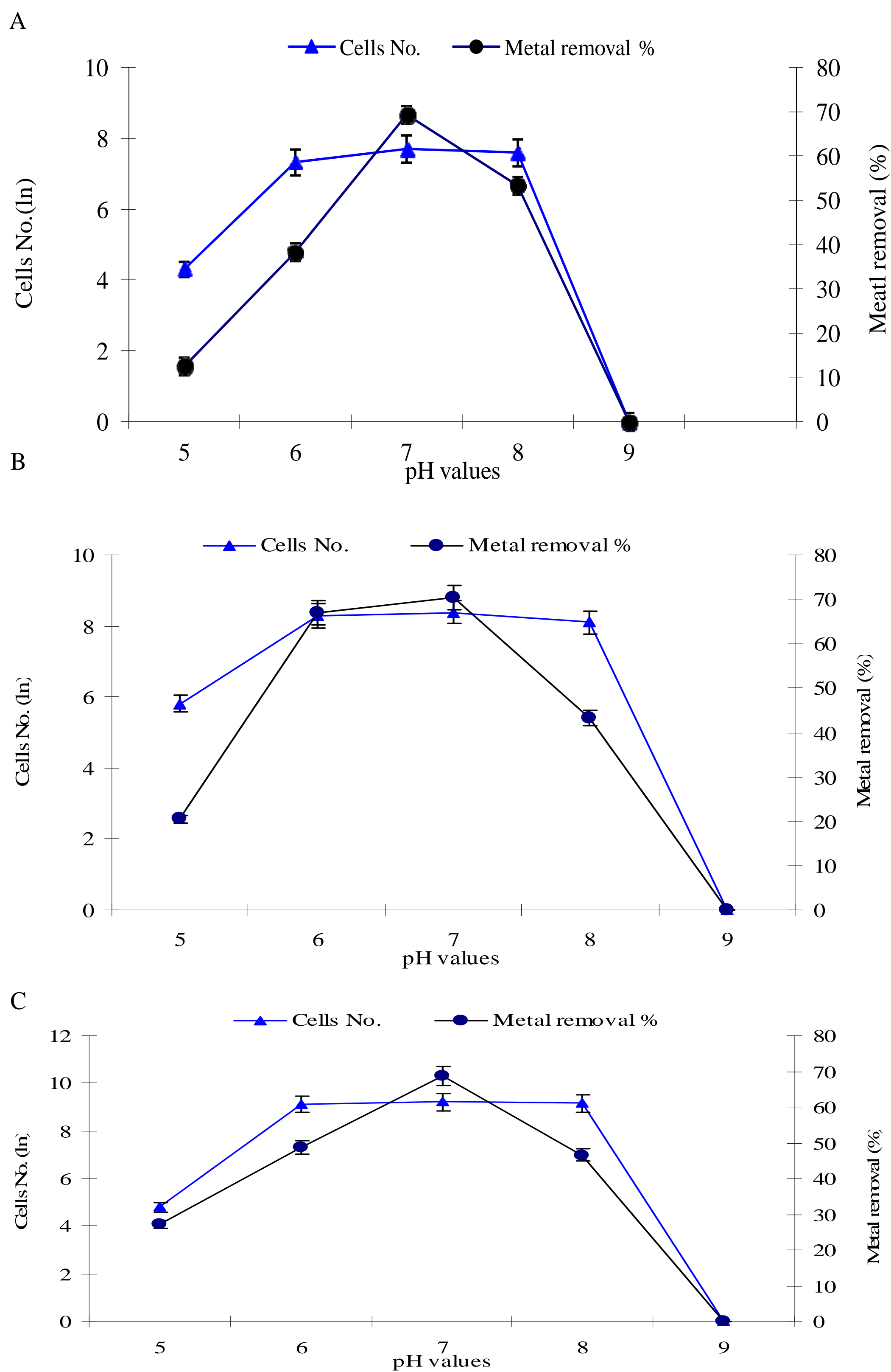

Figure 3. Heavy metal removal and bacterial cell number affected by various pH values, (A) Cadmium, (B) Lead and (C) Zinc ions.

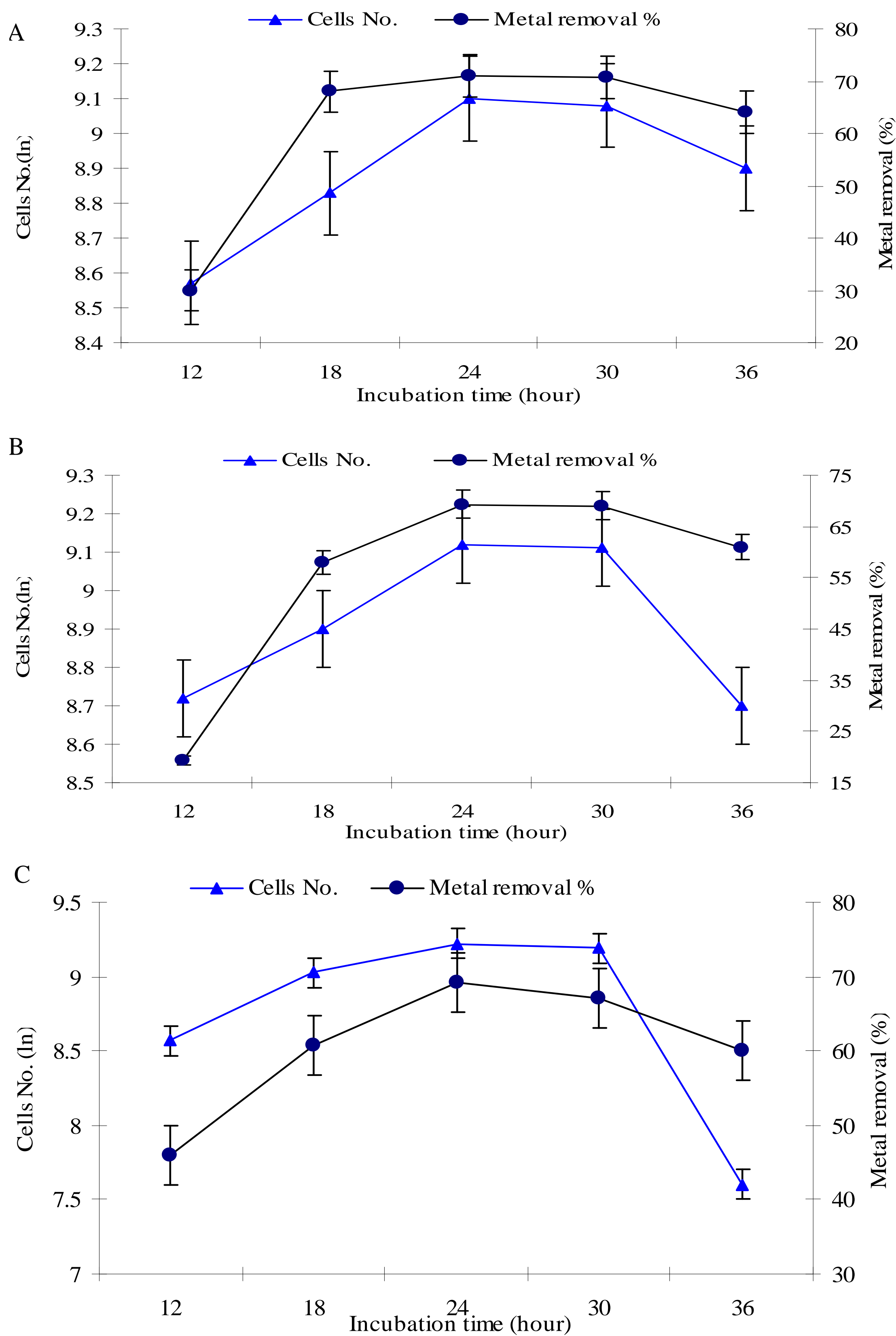

Figure 4. Heavy metal removal and bacterial cell number after various incubation periods, **(A)** Cadmium, **(B)** Lead and **(C)** Zinc.

the uptake of the tested metals increased gradually by increasing temperature from 20 to 30°C and decreased at the higher temperature 35 and 40°C. The maximum metals (Cd^{+2}, Pb^{+2} and Zn^{+2}) bioremoval were recorded at 30°C, which may depend on cell metabolism that are most likely to be inhibited by low temperature, meanwhile the higher temperature also affect the integrity of the cell membrane, these present results are in line with those reported by Brady and Duncan (1994 a,b).

The metal binding to bacterial cell was influenced by many factors including pH, buffer type, ionic strength and incubation time. The present results showed the maximum removal percentage of Cd^{2+}, Pb^{2+} and Zn^{2+} ions by *B. subtilis* var. *globigii* MSNIOF11 at pH7.0. The change of pH values from five to seven may result in an increase in the bacterial cell wall negative charge which favored electrochemical attraction and adsorption of metal (Gourdon et al., 1990). The results here were in accordance with those of Lo et al. (2001), they reported that, the biosorption increased by increasing pH from two to seven, and as the pH value was increased the solubility of the metal decreased which enhanced metal sorption (Tsezos and Volesky, 1982**)**. On the other hand at low pH most of nitrogen containing groups at the bacterial cell wall would be neutral and so preclude the metal sorption (Elliot et al., 1986; Korenevskii et al., 1999) and the high concentrate of hydrogen ion compete with the cations of sorption sites (Mclean and Beveridge, 1990). The results also revealed that, both growth and metal bioremoval were not observed at pH five. In this respect Mera et al. (1992) suggested a delicate competition between H^+ and metal ions for binding into the cells in the presence of competitive cations (Cd^{2+}, Pb^{2+} and Zn^{2+}) would alter energy states of these cells. Also, metal removal was completely inhibited at pH nine that may be due to the formation of insoluble oxides, hydroxides and carbonates at pH above neutrality which reduced the free metal ions (Brady and Duncan, 1994 a, b).

The present results showed that the metal removal also varied with incubation time. When cell suspension of *B. subtilis* var. *globigii* MSNIOF11was exposed to heavy metal in their solutions, the utmost removal was recorded after 24 h the increase of incubation time had a little effect on the metal removal, the stage of the life cycle and cultural conditions affected metal accumulation, the structural features of the cell wall as affected by cell age provide a mechanism to immobilization metals and prevent their entry into the cell (Remacle, 1990; Delgado et al., 1996).

In conclusion the selected bacterial isolate *B. subtilis* var. *globigii* MSNIOF11 had the capability of accumulation of the test metals. The bioremoval process of the tested heavy metals from its solutions was pH and temperature dependant. *B. subtilis* var. *globigii* MSNIOF11 recorded maximum growth and heavy metal removal at 30°C with neutral pH (7.0) during the first 24 h. By passing time that is, after 30 h there was no significant change. For that reason the major directions in the bioremediation technology research contains, studying the microbial communities from contaminated sites with special emphasis on those strains that play major functional roles in pollutant removal. In this respect, characterization of the catabolic potential, as well as accurate taxonomical identification of such types of bacteria is very important. In this study, we identified phenotypic and metabolic traits.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Abdul Rehman RD, Farooq H, Hasnain S (2008). Biosorption of copper by yeast *Loddermyceselongisporus*, isolated from industrial effluents: Its potential use in wastewater treatment. J. Basic Microbiol.48:195-201.

Abioye OP (2011). Biological remediation of hydrocarbon and heavy metals contaminated soil. In: Soil Contamination, MSc Simone Pascucci (Eds), pp. 127-142.

Ahmed MJ,Uddin MN, Islam MN, Islam MS, Islam MF (2013). Physicochemical assessment of soil pollutants due to the ship breaking activities and its impact on the coastal zone of Chittagong , Bangladesh. Eur. Chem. Bull. 2:975-980.

Aikpokpodion PE, Lajide L, Aiyesanmi AF (2012). Assessment of heavy metals mobility in selected contaminated cocoa soils in Ondo State, Nigeria. Global J. Environ. Res. 6:30-35.

Akpor OB, Muchie M (2010). Remediation of heavy metals in drinking water and wastewater treatment systems: Processes and applications. Int. J. Phys. Sci. 5:1807-1817.

Blackwell KJ, Singleton I, Tobin JM (1995). Metal cation uptake by yeast: a review. Appl. Microbiol. Biotechnol. 43:571-589.

Brady D, DuncanJR (1994). a: Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 41:149-154.

Brady D, Duncan JR (1994). b:Cation loss during accumulation of heavy metal cation by *Saccharomyces cerevisiae*. Biotechnol. Lett. 10:543-548.

Cooksey DA (1993). Copper uptake and resistance in bacteria. Mol. Microbiol. 7:1-5.

Daboor SM, Sabae SZ (2007). Bioremoval of certain heavy metal ions by bacterial isolates from the River Nile, Egypt. Afr. J. Biol. Sci. 3:51-59.

Dalman O, Demirak A, Balci A (2006). Determination of heavy metals (Cd, Pb) and traceelement (Cu, Zn) in sediments and fish of the Southeastern Aegean sea (Turkey) by atomic absorption spectrometry. Food Chem. 95:157-162.

Delgado A, Barreiro AM, Novais MJ (1996). Heavy metal biosorption by the mycelium of *Fusarium flocciferum.* Inter. Biodeteri. Biodegrad. 37:241-244.

De Paolis MR, Lippi D (2008). Use of metabolic and molecular methods for the identification of a Bacillus strain isolated from paper affected by foxing. Microbiol. Res. 163:121-131

Duxbury T (1981). Toxicity of heavy metals to soils bacteria. FEMS, Microbiol. Lett**.** 11:217-20.

Ekeanyanwu CR,Ogbuinyi CA, Etienajirhevwe OF (2010). Trace Metals Distribution in Fish Tissues, Bottom Sediments and Water from Okumeshi River in Delta State, Nigeria. Ethiopian J. Environ. Studies Manage. 3(3):12-17

Elliot HA, Liberati, Huang CP (1986). Competitive adsorption of heavy metals by soils. J. Environ. Qual. 15:214-219.

El-Sherif IY, Ashmamy A, Badr S (2008). Biosorption of cadmium and nickel by Nile water algae. J. Appl. Sci. Res. 4: 391-396.

Gourdon R, Bhender S, Rus E, Sofer SS (1990). Comparison of cadmium biosorption by Gram-positive and Gram-negative bacteria from activated sludge. Biotech. Lett. 12:839-42**.**

Heerden J, Korf C, Ehlers MM, Cloete TE (2002). Biolog for the determination of diversity in microbial communities. Water SA .1:29-35.

Hemambika B, Rani MJ, Kannan VR (2011). Biosorption of heavy metals by immobilized and dead fungal cells: A comparative assessment. J. Ecol. Nat. Environ. 3:168-175.

Holmes B, Costas M, Ganner M, On SLW, Stevens M (1994). Evaluation of Biolog System for identification of some Gram-Negative bacteria of clinical importance. J. Clin. Microbiol. 32:1970-1975.

Igwe JC, Abia AA (2006). A bioseparation process for removing heavy metals from wastewater using biosorbents. Afr. J. Biotech. 5:1167-1179.

Jarosławiecka A, Piotrowska-Seget Z (2014). Lead resistance in micro-organisms. Microbiol. 160:12-25.

Joshi PK, Swarup A, Maheshwari S, Kumar R, Singh N (2011). Bioremediation of heavy metals in liquid media through fungi isolated from contaminated sources. Indian. J. Microbiol. 51:482-487.

Korenevskii AA, Khamidova KH, Avakyan ZA, Karavik G (1999).Silver biosorption by Micromycetes. Microbiol. 68:139-145.

Kumar NJ, Oommen C (2012). Removal of heavy metals by biosorption using freshwater alga *Spirogyra hyaline.* J. Environ. Biol. 33:27-31.

Kuycak N, Volesky R (1988). Biosorption for recovery of metals from industrial solutions, Biotech. Lett. 10:137-142.

Ledin M, Pedersen K (1996).The environmental impact of mine wastes-roles of microorganisms and their significance in treatment of mine wastes. Earth Sci. Rev. 41:67-108.

Lin J, Harichund C (2011).Isolation and characterization of heavy metal removing bacterial bioflocculants. Afr. J. Microbiol. Res. 5:599-607.

Liu SM, Jones WJ (1995). Biotransformation of dichloro- aromatic compounds in non adapted and adapted fresh water sediment sturries. Appl. Microbiol. Biotech. 43:725-32.

Lo W, Leung WC, Chua H (2001).Biosorption of heavy metal by bacteria isolated from activated sludge. Appl. Biochem. Biotech. 91:171- 84.

Loska K, Wiechula D (2003). Application of principal component analysis for the estimation of source heavy metal contamination in surface sediments from Rybnik Reservoir. Chemosphere. 51:723-733.

MacFarlane GR, Burchett MD (2000). Cellular distribution of Cu, Pb and Zn in the Grey Mangrove Avicennia marina (Forsk.).Vierh. Aquat. Bot. 68:45-59.

Machado MD, Janssens S, Soares HM, Soares EV (2009). Removal of heavy metals using a brewer's yeast strain of *Saccharomyces cerevisiae*: advantages of using dead biomass. Appl. Microbiol. 106:1792-1804.

Machado MD, Soares EV, Soares HM (2010). Removal of heavy metals using a brewer's yeast strain of *Saccharomyces cerevisiae*: Chemical speciation as a tool in the prediction and improving of treatment efficiency of real electroplating effluents. J. Hazard. Mater. 18:1-3.

Mane PC, Bhosle AB (2012). Bioremoval of some metals by living algae *Spirogyra* sp. and *Spirulina* sp. from aqueous solution. Int. J. Environ. Res. 6:571-576.

Mayer SIT, Beveridge TJ (1989). The sorption of metals to *Bacillus subtilis* walls from dilute solution and simulated Hamilton Harbour water. Can. J. Microbiol. 35:764-770.

Mclean RJC, Beveridge TJ (1990). Metal binding capacity of bacterial surface and their ability to form mineralized aggregates. In: "Microbial Mineral Recovery", H.L.; Ehrlich and C.L.; Brierly, (ed.) McGraw-Hill publishing Co. New York. pp. 185-222.

Mera MU, Kemper M, Doyle R, Beveridge TJ (1992). The membrane induced proton motive force influence the metal binding ability of *Bacillus subtilis* cell walls. Appl. Environ. Microbiol. 58:3837-3844.

Miller JM, Rhoden DL (1991). Preliminary evaluation of Biolog, a carbon source utilization method for bacterial identification. J. Clin. Microbiol. 29:1143-1147.

Monachese M, Jeremy PB, Gregor R (2012). Bioremediation and tolerance of humans to heavy metals through microbial processes: a potential role for probiotics? Appl. Environ. Microbiol. 78:6397-6404.

Nanda M, Sharma D, Kumar A (2011). Removal of heavy metals from industrial effluent using bacteria. Int J. Environ. Sci. 2:781-787.

Pielou EC (1966). The measurement of diversity in different types of biological collection. J. Theor. Biol. 13:131-144.

Odokuma LO, Akponah E (2012). Effect of concentration and contact time on heavy metal uptake by three bacterial isolates. J. Environ. Ch. Ecotoxico. 2(6):84-97.

Rani MJ, Hemambika B, Hemapriya J, Kannan VR (2010). Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: A biosorption approach. Afr. J. Environ. Sci. Technol. 4:77-83.

Raphael CE, Ogbuinyi CA, Etienajirhevwe OF (2011). Trace metals distribution in fish tissues, bottom sediments and water from Okumeshi River in Delta State, Niger. Environ. Res. J. 5:6-10

Remacle J (1990). "The cell wall and heavy metals" In: Biosorption of heavy metals. B;Volesky (ed.).CRC press. Boca Roton, Florida. pp. 83-92.

Saithong K, Poonsuk P (2002). Biosorption of heavy metal by thermo tolerant polymer producing bacterial cells and bioflocculants. Songklanakarin. J. Sci. Technol. 24:421-430.

Samarth DP, Chandekar CJ, Bhadekar RK (2012).Biosorption of heavy metals from aqueous solution using *Bacillus licheniformis.* Int. J. Pure Appl. Sci. Technol. 10:12-19.

Selvam K, Swaminathan K, Song MH, Chae K (2002). Biological treatment of pulp and paper industry effluent by *Fomeslividus* and *Trametesversicolor*. World J. Microbiol. Biotech. 18:523-526.

Simonescu CM, Ferdes M (2012). Fungal biomass for Cu(II) uptake from aqueous system. Pol J environs stud. 21:1831-1839.

Sutton S (2011). Measurement of microbial cells by optical density. J. Validation Technol. 17:46-49.

Tamilselvan N, Saurav K, Kannabiran K (2011). Biosorption of selected toxic heavy metals using algal species *Acanthophoraspicifera.* Pharmacol. online. 1:518-528.

Tanase A, Nicoara A, Vassu T, Chiciudean I, Ionescu R, Csutak O, Stoica I (2011). Phenotypic and Genotypic Analysis on Bacterial Strains from a Karathane Polluted Soil. Rom. Biotech. Lett. 1:141-148

Tsezos M, Volesky B (1982). The mechanism of uranium biosorption by *Rhizopusarrhijus*. Biotechnol. Bioeng. 24:385-401.

Wagner M, Amann R, Lemmer H, Schleiffer KH (1993). Probing activated sludge with oligonucleotides specific for proteobacteria: Inadequacy of culture-dependent methods for describing microbial community structure. Appl. Environ. Microbiol. 59**:**1520-1525.

Young SM, Ishigaa H, Pitawala A (2012).Geochemical assessment of Upper Mahaweli River and Polgolla Reservoir sediments, Sri Lanka. APCBEE Procedia. 1:53-58.

Zhou M, Yungo L, Guangming Z, Xin L, Weihua X, Ting F (2007). Kinetic and equilibrium studies of Cr (VI) biosorption by dead *Bacillus lichenoformis* biomass. World J. Microbiol. Biotech. 23:43-48.