International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 12 (2014) pp. 326-335**
http://www.ijcmas.com

Original Research Article

Bioremediation of cadmium by *Bacillus safensis* (JX126862), a marine bacterium isolated from mangrove sediments

Rajesh Priyalaxmi[1]*, Athiappan Murugan[2], Paul Raja[3] and Kasper Diraviya Raj[3]

[1]Suganthi Devadason Marine Research Institute, Tuticorin, Tamil Nadu, India
[2]Department of Microbiology, Periyar University, Salem, Tamil Nadu, India
[3]St.Xavier's College (Autonomous), Tirunelveli, Tamil Nadu, India.

**Corresponding author*

A B S T R A C T

Heavy metal pollution due to an increase in industrialization has become a serious issue in Tuticorin coast. The common metal pollutants are copper (Cu), zinc (Zn), nickel (Ni), lead (Pb), mercury (Hg), chromium (Cr) and cadmium (Cd). Enumeration of total microbial load of mangrove ecosystem states that bacterial load was higher than the fungal and Actinomycetes load and was found that only two bacterial strains (PB-5 and RSA-4) had cadmium resistance. Heavy metal resistance assay against $CdCl_2$, $PbNO_3$, $CuSO_4$, $ZnCl_2$ and $CoCl_2$ at different concentrations (20ppm, 40ppm, 60ppm, 80ppm and 100ppm) revealed that RSA-4 strain was the best. Percentage of cadmium reduction in the medium, absorption by the cells, growth and pigment production was evaluated at various pH (5, 6, 7, 8 and 9) with two different concentrations (40 and 60 ppm) of cadmium and the results showed that the removal of cadmium by reduction and absorption was to a maximum of 83.5, 39% and 98.10, 92% for 40 and 60 ppm of cadmium, respectively at pH 7. The growth of the strain RSA-4 was maximum at pH 7 and the pigment production was decreased with increasing cadmium concentration and its production was maximum at pH 5. The potent strain RSA-4 was identified as *Bacillus safensis* by phylogenetic analysis and received an accession no. JX126862 from gene bank. This investigation has proved that the mangrove ecosystem plays an important role in reduction and biosorption of cadmium as it contains wide microbial diversity.

Keywords

Tuticorin, mangrove, heavy metal, cadmium, *Bacillus safensis*

Introduction

Heavy metals are the most important pollutants in marine environments (Safahieh *et al.*, 2012). The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and thereby inhibition of metabolic processes. According to the World Health Organization (WHO, 2008), the metals of most immediate concern include cadmium, chromium, cobalt, copper, lead, nickel, mercury and zinc, which contaminate the soils, ground water, sediments and surface waters are

extremely toxic to biological and ecological systems (Sundar *et al.,* 2010; Choudhury and Pradhan, 2011) and are responsible for their persistence in the food chain (Tamil Selvi *et al.,* 2012). Each heavy metal has unique biofunctions or biotoxicities (Wei *et al.,* 2009). Among metals, cadmium is a non essential element and highly toxic to organisms even at very low dosages. Cadmium damages cells by strong affinity to glutathione and sulphydryl groups in proteins (Cunningham and Lundie, 1993) and displacement of zinc and iron ions from proteins. Moreover, these metals known to cause detrimental effect in humans such as brain damage, reproductive failures, nervous system failures and tumor formation (Zaki and Farag, 2010). Furthermore, researches indicated that cadmium is related to reactive oxygen species (ROS) and cancer (Zeng *et al.,* 2010). In Japan, Itai-itai disease occurred due to high concentration of cadmium in silver mine waste water, causing skeleton deformation and spontaneous fractures (Faryal, 2003). Some reports have shown that indigenous microbes tolerate high heavy metal concentrations in different ways (Wei *et al.,* 2009).

One such way is bioremediation of heavy metals by which the microbes such as bacteria and fungi to absorb or convert them to non-toxic products (Safahieh *et al.,* 2012) through their different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization or biosorption (Lin and Lin, 2005; Preetha and Viuthagiri, 2005; Wuyep *et al.,* 2007; Kumar *et al.,* 2007). With the characteristics of quick reproducible velocity and easy control, microbe is preferable to use in environmental treatment (Zeng *et al.,* 2010). Increased industrialization in Tuticorin coast has become the major cause marine pollution by receiving heavy amount of effluents containing organic compounds, chlorinated hydrocarbons and heavy metals including mercury from the fertilizer and heated effluents and fly-ash are discharged from the thermal power plant. The present study deals with the enumeration of marine microbes and assessing their heavy metal tolerance from mangrove ecosystem of Tuticorin coast.

Materials and Methods

Isolation of microorganisms

Sediments associated on rhizosphere of mangrove plant, decomposing plastic samples and also free mangrove sediments were collected from different sites of mangrove area near Roach park (N 08˚46' 582 and E 78˚ 09'363), Tuticorin coast. 1 gm of sediment sample was serially diluted and 0.1 ml of aliquots was plated separately on Zobell marine agar, Sabouraud dextrose agar and Starch casein agar medium for enumeration of bacteria, fungi and actinomycetes, respectively. The plates were incubated at room temperature and growth is expressed in CFU/g. Respective media's were supplemented with cycloheximide (50 mg/l), chloramphenicol (50 mg/l) and both to permit the growth of bacteria, fungi and actinomycetes. The microbes of different morphology were streaked on plate and pure colonies were maintained at 4°C.

Isolation and Screening for heavy metal resistant bacteria

The selective isolation of resistant bacteria to some heavy metals was done by streaking the isolates onto marine agar supplemented with 10ppm of different metal salts ($HgSO_4$, $CdCl_2$, $PbNO_3$, $CuSO_4$, $K_2Cr_2O_7$, $ZnCl_2$ and $CoCl_2$). Control plates, without isolate were also maintained (Rajbanshi, 2008). Heavy metal resistance assay was carried out by well plate method for selected potential strains against $CdCl_2$, $PbNO_3$, $CuSO_4$, $ZnCl_2$

and $CoCl_2$ following the method of (Altalhi, 2009).

Effect of pH in bioremediation of Cadmium by the resistant RSA-4 strain

Bioremediation of cadmium was carried out in two sets following the method of (Akujobi *et al.,* 2012). One set involves the reduction of cadmium in medium and second set involves removal of cadmium by cell absorption. Two culture flasks containing 100 ml of SCA medium was supplemented with Cd of two concentrations such as 40ppm and 60 ppm respectively and were maintained at various pH 5, 6, 7, 8 and 9 following inoculation with 5% of culture. Media without Cd was also inoculated with the same bacterium and uninoculated media containing Cd served as controls. All the cultures including controls (in duplicate) were incubated for 8 days at room temperature (30°C). To estimate the Cd reduction and absorption by cells, 6ml culture from each of the above flasks was centrifuged (6000 rpm for 10 min at 10°C) and the supernatant was analyzed for Cd concentration. Harvested cells (biomass) were then washed twice with de-ionized distilled water, autoclaved and dried in an oven at 80^oC for 5 h. The dried powdered biomass was used for extraction process.

Effect of Cadmium on growth and pigment production by RSA-4

The influence of Cadmium on pigment production and growth by RSA-4 strain (5%) was estimated with two concentrations of cadmium (40 ppm and 60 ppm) at different pH such as 5, 6, 7, 8 and 9. To estimate the growth and pigment production, 6ml culture from each of the above flasks was centrifuged (6000 rpm for 10 min at 10°C) and the supernatant was analyzed for pigment production. Harvested cells (biomass) were then washed twice with phosphate buffer (0.1 M NaH2PO4-Na_2HPO_4; pH, 7.1) following the procedure of (Akujobi *et al.,* 2012). The amount of pigment production and growth was monitored by measuring optical density of the cultures spectrophotomettrically at 420 nm and 600 nm, respectively.

Heavy metal analysis

Collected supernatant and harvested cells were digested with 10 ml of a mixture perchloric acid: nitric acid ($HCLO_4$:HNO_3-1:5v/v). Acid digestion was carried out on a hot plate at 70-100^oC until yellow fumes of HNO_3 and white fumes of $HClO_4$ were observed. The digested sample was dissolved in distilled water, filtered through Whatman no.1 filter paper to remove impurities and made up to 25 ml following the method of (Sharma and Fulekar, 2009**)**. The made up samples were transferred to polythene bottles and were analyzed for cadmium content using Atomic Adsorption Spectrometer (AAS ELICO SD194).

Identification of cadmium resistant bacteria (RSA-4) by 16SrRNA sequencing

Bacterial DNA isolation and purification was performed using Bacterial genomic DNA isolation kit. The isolated DNA was then amplified by polymerase chain reaction (PCR). The PCR was carried out using PCR Master Mix Kit. The size and purity of the genomic DNA was analyzed by agarose-gel electrophoresis was carried out. The PCR product was directly sequenced using ABI 3500 XL genetic analyzer. RNA sequences were compared with already submitted sequence in NCBI database using BLAST software and phylogenetic tree was viewed using tree view software to analyze evolutionary relationships among sequences of isolated microorganism and nearest neighbours.

Results and Discussion

Selection of Cadmium resistant strain

35 strains of bacteria isolated from various samples of mangrove ecosystem were selected based on different morphology and assessed for their degradation activity against 7 heavy metals salts ($HgSO_4$, $CdCl_2$, $PbNO_3$, $CuSO_4$, $K_2Cr_2O_7$, $ZnCl_2$ and $CoCl_2$). Among them, two cadmium resistant bacteria (PB-5 and RSA-4) were chosen since it is most toxic heavy metal and they were subjected to heavy metal resistance assay against 6 heavy metals ($CdCl_2$, $PbNO_3$, $K_2Cr_2O_7CuSO_4$, $ZnCl_2$ and $CoCl_2$) at various concentrations (20, 40, 60, 80 and 100 ppm) (Table 1). The strain RSA-4 strain was selected for further studies as it showed resistant to most of the heavy metals tested and cadmium at higher concentration (Fig. 1).

Influence of pH in Cadmium degradation, Growth and Pigment production:

The effect of pH on cadmium degradation on the basis of reduction and absorption, growth and pigment production was assessed at various pH (5, 6, 7, 8 and 9) with two different concentrations (40 and 60 ppm) of cadmium and the results indicated that the reduction and absorption of cadmium was at a maximum of 83.5, 39% and 98.10, 92% for 40 and 60 ppm of cadmium, respectively at pH 7 for RS-4 strain (Fig. 3 and 4). The growth was at a maximum at pH 5 but in the presence cadmium the growth was found to be maximum at pH 7 (Fig. 5) and the pigment production was high without cadmium at pH 5 (Fig. 2) and the level of production gradually decreased with increase in pH and cadmium concentration.

Identification of Cadmium resistant strain

The potent strain was identified as *Bacillus safensis* by comparative analysis of the sequence with the NCBI database which showed that RSA-4 strain (1500 bp) was close to the member of the genus *Bacillus* sp. and showed 98% homology with *Bacillus safensis* and the sequences were deposited at Gene Bank and got a Gene Bank accession number as JX126862.

The bioremediation of heavy metal by marine strains are not new to our coast. The effect of heavy metals copper, mercury, cadmium and nickel and enzymatic activity of the five bacterial strains isolated from mangrove sediments of Tuticorin coast have been reported by (Prabhahar *et al.,* 2012). Selection of bacteria was made since it is the fast grower. 35 bacterial colonies of different morphology were selected and when screened in the preliminary study, PB-5 and RSA-4 strain had the capability to degrade the most toxic heavy metal, cadmium. The quantity of cadmium degradation by these strains was observed favorably in heavy metal resistance assay. Similar methodology was followed by (Kermani *et al.,* 2010) for cadmium resistant *Pseudomonas aeruginosa.* Among the two strains, RSA-4 strain was found to tolerate cadmium up to the concentration of 80 ppm while PB-5 strain only upto 60 ppm. Similar results are obtained by (Abd-Elnaby *et al.,* 2011) with *Vibrio harvei.* (Baskar and Prabakaran, 2011) stated that the bacteria present in the rhizoshere sediment of mangrove ecosystem have the resistance against heavy metals. Similar to their finding the present work revealed that the strain RSA-4 from the rhizoshere sediment of mangrove ecosystem is highly resistant to toxic heavy metal cadmium. The pH of the medium was found to have prominent role in growth and degradation efficiency of heavy metal resistant strain and so this study was done at various pH (5, 6, 7, 8 and 9) in the present work. Based on this same idea,

several workers have carried out studies at various pH. For example, (Congeevaram *et al.,* 2007) determined the optimum pH and temperature conditions for both the growth and heavy metal removal was lower (5–5.2) than that for bacterial isolates (7). The present study showed that the optimal pH for growth of bacteria was pH 7 at a temperature of 30°C which is supported by (Raja *et al.,* 2009) who isolated *Proteus vulgaris* (BC1), *Pseudomonas aeruginosa* (BC2), *Acinetobacter radioresistens* (BC3) and *Pseudomonas aeruginosa* (BC5) based on high level of heavy metal and antibiotic resistances from sewage water sample. (Sinha and Mukherjee, 2009) reported a significant ability to remove more than 75% and 89% of the soluble cadmium during the active growth phase from the growth medium by *Pseudomonas aeruginosa* strain KUCd1. Similar finding was observed in the present work that during 40 ppm of cadmium, the active growth of bacterium reduced 83.5% while in 60 ppm only 39%. In the present study, the inhibition of growth of the bacterium was observed as the concentration of cadmium chloride increases which indicates the concentration based degradation. It can be substantiated by the work of (Vijayalakshmi *et al.,* 2011) that the increasing concentrations of cadmium inhibited the growth of *Bacillus subtilis* and *Corynebacterium rubrum*. The study on cadmium's influence in pigment production by *Bacillus safensis* was carried out similar to (Abdul-sada, 2008) described the role of heavy metals (HgCl, $MgSo_4$, Zn_2O_3, $MgCO_3$, Na_2SO_4, $C_{10}H_{20}O$, EDTA, $NiSO_4$, $CuCl_2$ and $CdCl_2$) to influence the production of bacterial pigments by *Pseudomonas aeruginosa*. Reduction in yellow pigment production was found in RSA-4 strain on exposure to cadmium and thus the present work suggests its application in detection and monitoring of cadmium in the environment. Similarly, (Cheung and Gu, 2002) proved that reduction in blue colour pigment production occurs with *Vogesella indigofera* occurs on exposure of higher concentrations of chromium nearly 150 µg of chromium/ml. The characterization of cadmium resistant bacteria from mangrove ecosystem was analyzed phylogenetically and the active strain was identified as *Bacillus safensis*. Identification of marine strains by molecular characterization is found to be very common nowadays. A very few reports are available on isolation of *B. safensis* viz., (Nath *et al.,* 2012) reported *Bacillus safensis* from sweet meat whey which had the capability to degrade copper, lead and Cadmium. (Khaneja *et al.,* 2010) reported the production of yellow orange and pink pigment in *Bacillus* sp. which was sampled from soil, sea water and the human gastrointestinal tract and phylogenetic profiling using 16S rRNA sequences revealed the presence of *Bacillus safensis, Bacillus marisflavi, Bacillus indicus, Bacillus firmus,* and *Bacillus altitudinus.*

From these observations, it can be concluded that the mangrove environment could be used as a promising source for isolation of heavy metal resistant bacteria. The study suggested that pH plays an important role in the bioremediation of cadmium and stresses the importance of bacteria in eco-friendly method mitigate environmental pollution; the mangrove environment can be used to isolate many microbes with bio-degradation potential. The active bacterium, *Bacillus safensis* (JX126862) observed in this study can potentially be used in cadmium treatment. Further studies on the mechanism of cadmium removal will provide new insight towards future scopes in this area of research.

Table.1 Heavy metal resistance activity of PB-5 and RSA-4 against various heavy metals in different concentrations

Sl.No	Heavy Metals	Concentration (ppm)	PB-5	RSA-4
1	$CdCl_2$	20	+	+
		40	+	+
		60	+	+
		80	-	+
		100	-	-
2	$PbNo_3$	20	+	+
		40	+	+
		60	-	+
		80	-	+
		100	-	+
3	$CuSO_4$	20	-	+
		40	-	-
		60	-	-
		80	-	-
		100	-	-
4	$K_2Cr_2O_7$	20	-	-
		40	-	-
		60	-	-
		80	-	-
		100	-	-
5	$ZnCl_2$	20	-	+
		40	-	+
		60	-	+
		80	-	+
		100	-	+
6	$CoCl_2$	20	-	+
		40	-	+
		60	-	+
		80	-	+
		100	-	+

Fig.1 Cadmium resistance assay of RSA-4

+ Presence of growth **- Absence of growth**

Fig.2 Cadmium reduction in medium by *Bacillus safensis* at various pH

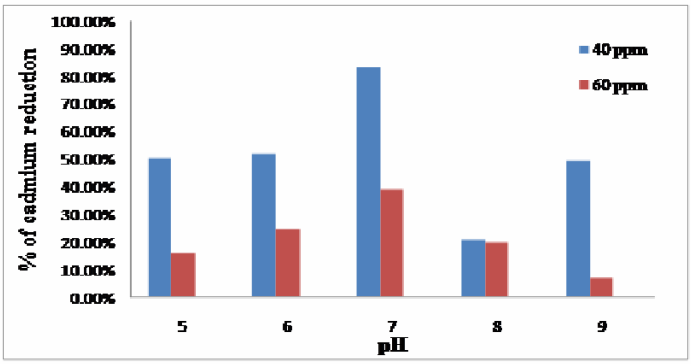


Fig.3 Cadmium absorption by the cells of *Bacillus safensis* at various pH

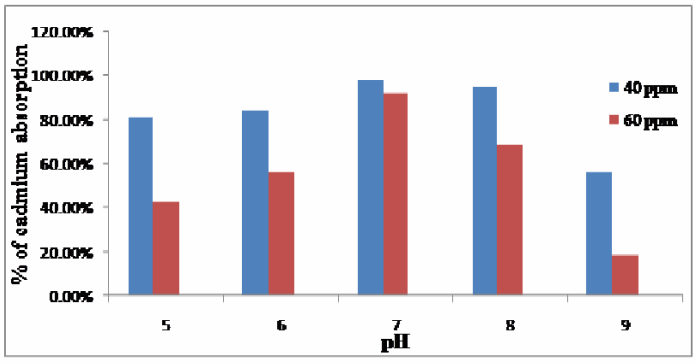


Fig.4 Effect of pH and cadmium on the growth of *Bacillus safensis*

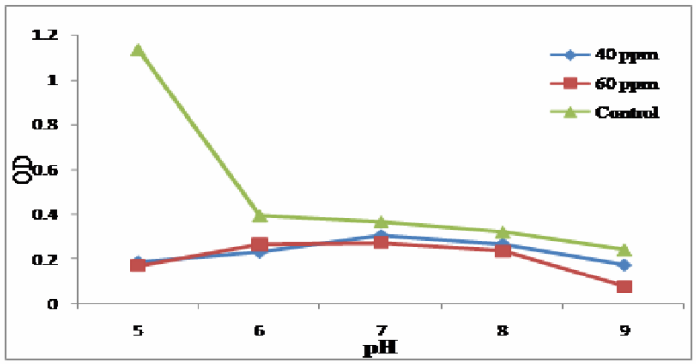
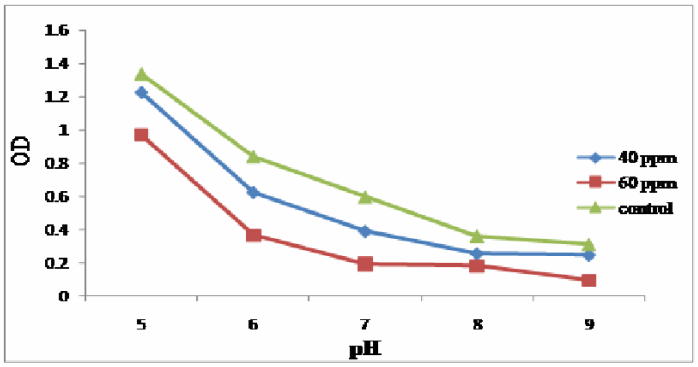


Fig.5 Effect of pH and cadmium on the pigment production by *Bacillus safensis*



Acknowledgement

The author is thankful to the Suganthi Devadason Marine Research Institute for permitting to attend the conference. It is the author's pleasure to put on records his heartfelt gratitude and profound thanks to Dr. S. Ramesh, Sri Paramakalyani College, Alwarkurichi for their recognition and continued support.

References

Abd-Elnaby, H., Abou-Elela, G.M., El-Sersy, N.A. 2011. Cadmium resisting bacteria in Alexandria Eastern harbour (Egypt) and optimization of cadmium bioaccumulation by *Vibrio harveyi*. *Afr. J. Biotechnol*. 10: 3412–3423.

Abdul-Sada, H.K. 2008. A resistance study of *Pseudomonas aeruginosa* to heavy metals. *Med. J. Bobylon*, 5(1): 16–23

Akujobi, C.O., Odu, N.N., Okorondu, S.I. 2012. Bioaccumulation of lead by *Bacillus* species isolated from pig waste. *J. Res. Biol.*, 2: 083–089.

Altalhi, A.D. 2009. Plasmids profiles, antibiotic and heavy metal resistance incidence of endophytic bacteria isolated from grapevine (*Vitis vinifera* L.). *Afr. J. Biotechnol.*, 8: 5873–5882.

Baskar, B., Prabakaran, P. 2011. Isolation and characterization of copper resistant *Exiguobacterium* strains isolated from rhizosphere soil of

Avicennia marina. *Res. J. Pharm. Biol. Chem. Sci.,* 2: 640–646.

Cheung, K.H., Gu, J. 2002. Bacterial color response to hexavalent chromium, Cr^{6+}. *J. Microbiol.* 40: 234–236.

Choudhury, A.R., Pradhan, S. 2011. Role of potentially important microbes in bioremediation. *Sci. Cult.,* 77: 324–330.

Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., Thamaraiselvi, K. 2007. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *J. Hazard. Mater*., 146: 270–277.

Cunningham, D. P., Lundie, L. L. 1993. Precipitation of cadmium by *Clostridium thermoaceticum*. *Appl. Environ. Microbiol.,* 59: 7–14.

Kermani, A.J.N., Ghasemi, M.F., Khosravan, A., Farahmand, A., Shakibaie, M.R. 2010. Cadmium bioremediation by metal-resistant mutated bacteria isolated from active sludge of industrial effluent. *Iran J. Environ. Health Sci. Eng.,* 7: 279–286.

Khaneja, R., Perez-Fons, L., Fakhry, S., Baccigalupi, L., Steiger, S., To, E., Sandmann, G., Dong, T.C., Ricca, E., Fraser, P.D., Cutting, S.M. 2010. Carotenoids found in *Bacillus*. *J. Appl. Microbiol.,* 108: 1889–1902.

Kumar, S., Hatha, A.A.M., Christi, K.S. 2007. Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. *Rev. Biol. Trop.,* 55: 777–786.

Lin, C.C., Lin, H.L. 2005. Remediation of soil contaminated with the heavy metal (Cd^{2+}). *J. Hazard. Mater*., 122: 7– 15.

Nath, A., Dutta, S., Chowdhury, R., Bhattacharjee, C. 2012. Micro and macro-kinetics of di-auxic microbial growth in the presence of lactose and glucose-Experimental and modeling. World Congress on Biotechnology, Hyderabad, India, May 4, 2012

Prabhahar, C., Saleshrani, K., Tharmaraj, K., Vellaiyan, M. 2012. Heavy metal pollution in mangrove region: A review. *Int. J. Pharm. Biol. Arch.,* 3: 513–518.

Preetha, B., Viruthagiri, T. 2005. Biosorption of zinc (II) by *Rhizopus arrhizus*: equilibrium and kinetic modeling. *Afr. J. Biotechnol.,* 4: 506–508.

Raja, C.E., Selvam, G.S., Omine, K. 2009. Isolation, identification and characterization of heavy metal resistant bacteria from sewage. Proceedings of International Joint Symposium, Pp. 205–211.

Rajbanshi, A. 2008. Study on heavy metal resistant bacteria in guheswori sewage treatment plant. Our Nature 6(1): 52–57.

Safahieh, A., Abyar, H., Roostan, Z., Zolgharnein, H., Mojoodi, F. 2012. Isolation and characterization of *Pseudomonas* resistant to heavy metals and poly aromatics hydrocarbons (PAHs) from Persian Gulf sediments. *Afr. J. Biotechnol.,* 11: 4418–4423.

Sharma, J., Fulekar, M.H. 2009. Potential of *Citrobacter freundii* for bioaccumulation of heavy metal – copper. *Biol. Med.,* 1: 7–14.

Sinha, S., Mukherjee, S.K. 2009. *Pseudomonas aeruginosa* KUCD1, a possible candidate for cadmium bioremediation. *Braz. J. Microbiol.,* 40: 655–662.

Sundar, K., Vidya, R., Mukherjee, A., Chandrasekaran, N. 2010. High chromium tolerant bacterial strains from Palar River Basin: Impact of Tannery Pollution. *Res. J. Environ. Earth Sci.* 2: 112–117.

Tamil Selvi, A., Anjugam, E., Archana Devi, R., Madhan, B., Kannappan, S., Chandrasekaran, B. 2012. Isolation and characterization of bacteria from tannery effluent treatment plant and their tolerance to heavy metals and antibiotics. *Asian J. Exp. Biol. Sci.*, 3: 34–41.

Vijayalakshmi, S., Usha, V., Ahmad Rather, R. 2011. Invitro responses of various bacteria to cadmium chloride: growth and biochemical analysis. *Asian J. Exp. Biol. Sci.*, 2: 12–17.

Wei, G., Fan, L., Zhu, W., Fu, Y., Yu, J., Tang. M. 2009. Isolation and characterization of the heavy metal resistant bacteria CCNWRS33-2 isolated from root nodule of *Lespedeza cuneata* in gold mine tailings in China. *J. Hazard. Mater.*, 162: 50–56.

WHO. 2008. Guideline for drinking water quality recommendations, Vol. 1, World Health Organization, Geneva.

Wuyep, P.A., Chuma, A.G., Awodi, S., Nok, A.J. 2007. Biosorption of Cr, Mn, Fe, Ni, Cu and Pb metals from petroleum refinery effluent by calcium alginate immobilized mycelia of *Polyporus squamosus*. *Sci. Res. Essays.*, 2: 217–221.

Zaki, S., Farag, S. 2010. Isolation and molecular characterization of some copper biosorped strains. *Int. J. Environ. Sci. Tech.*, 7: 553–560.

Zeng, X., Tang, J., Jiang, P., Liu, H., Dai, Z., Liu, X. 2010. Isolation, characterization and extraction of mer gene of Hg^{2+} resisting strain D_2. *Trans. Nonferrous Met. Soc. China*, 20: 507−512.