Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (3):** 296-303 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

ResearchArticle

OPEN ACCESS

Cadmium Bioremediation by Metal - Resistant Mutated Bacteria Isolated from Industrial Effluent

Pattanayak B.*, Mittra B. and Dhal N.K.

Dept. of Biotechnology, F.M.University, Balasore; Department of E& S,CSIR-IMMT, Bhubaneswar, Odisha, India

*Corresponding Author E-mail: pattanayak32@gmail.com

ABSTRACT

Bioremediation of metal pollutants from industrial wastewater using metal resistant bacteria is a very important aspect of environmental biotechnology. In this study, three strains of Pseudomonas aeruginosa were isolated from Industrial effluent of Nalco,district of Anugul, Odisha. The bacterial identity was determined by various biochemical tests. Among them, isolate number one could grow on Muller-Hinton agar medium containing 6mM cadmium ion (Cd^{2+}) and was therefore selected for further study. The isolates were subjected to mutation by two mutagenic agents (Acridine Orange and Acriflavine) using gradient plate and SIC techniques. The Minimum Inhibitory Concentration of Cd^{2+} for the isolate one after mutation was increased to 7mM. Removal of Cd^{2+} using mutated and wild type strains of this bacterium was carried out at different time intervals (10-300 minutes). It was observed that within 60 minutes, 94.7% of cadmium was removed in 30mg/L of Cd^{2+} solution. However, with 60mg/L Cd^{2+} solution, only 53.58% and 38.68% Cd^{2+} removed were achieved by mutated and wild type bacteria, respectively. The equilibrium data was fitted by Langmuir isotherm equation and the related parameters for Cd^{+2} were derived. Based on the data obtained in this study, it can be concluded that biomass of this bacterium can be used for bioremediation of cadmium from industrial waste processing plants with high efficiency.

Key words: *Bioremediation, Cadmium, Mutation, Pseudomonas aeruginosa, Metal resistant bacteria.*

INTRODUCTION

Along with industrial progress, environmental pollutants like toxic heavy metals are widely spreading throughout the world. This is especially true for developing countries like China and India[20]. The uncontrolled discharges of large quantity of heavy metal-containing wastes create huge economical and health care burden particularly for the people living near that area (since the effluents of the industries excreted into the environment and through food chain, affect humans and animals from various anthropogenic sources such as industrial wastes, automobile emissions, mining activity and agricultural practices as well). The important toxic metal pollutants like cadmium, nickel and lead enter to the water bodies through industrial wastewater treatment plants[1,6]. Cadmium is the most dangerous metal ion characterized by high stability and toxicity. It is not degradable in nature and will thus, once released to the environment, stay in circulation. Cadmium is known to bind with essential respiratory enzymes[15] causing oxidative stress and cancer[2].

Cadmium contamination has been also reported particularly in soils containing waste materials from zinc mines and in sludge amended soils fertilized with cadmium rich phosphate fertilizers[21]. The current low world market price of cadmium motivates the development of new applications that by time may develop

into new sources of emissions to the environment not covered by existing regulation. Therefore, decontamination of these pollutants through bioremediation process and other biotechnological means are prerequisite for any future decision by the governments.

The potential use of metal-resistant microorganisms in the treatment of heavy metal contaminated wastewater plants has become more important[24]. Different biomass types, such as bacteria, fungi and algae, have been screened and studied extensively by many authors over the past decades with the aim of identifying highly efficient metal removal biological systems[10,11,26,27].

Many efforts have been devoted to the isolation of heavy metal-resistant bacterial strains during the past years. *Staphylococcus aureus*[16] *Escherichia coli*[14] were found to exclude cadmium ion (Cd^{2+}) from cell surface. Katarina *et al.*,[12] studied cadmium resistant bacterial community isolated from Industrial effluents contaminated by cadmium ions. Among bacteria from bacterial community short cadmium resistant gram-negative rods were predominated. Biochemical tests assigned the eight isolates to six bacterial species, *Alcaligenes xylosoxidans, Comamonas testosteroni, Klebsiella planticola, Pseudomonas putida, Pseudomonas fluorescens,* and *Serratia liquefaciens*. Cadmium-resistant bacterial isolates were able to remove cadmium from solution and the efficiency of cadmium removal correlated with the amount of additionally synthesized proteins in the cell fractions.

Although many researchers have studied the bioremediation of cadmium from industrial waste, none has used mutated cadmium resistant bacteria for this purpose. In this investigation, a cadmium resistant bacteria was isolated from Industrial effluent of Nalco, Khullad, situated in the district of Anugul, Odisha. By mutational enhancement technique the bacterial strain was employed for removal of Cd^{2+} using batch bed reactor.

MATERIALS AND METHODS

Effluent sampling and source

100 ml Industrial waste from depth of 0.1 meter of Nalco, Kullad, situated in the district of Anugul, Odisha, were collected in sterile 1 L can for further analysis. The pH of Industrial effluent were measured by pH meter (Metrohm- 691). Concentrations of cadmium in Industrial effluent and biomass were measured by atomic absorption spectrometry (AAS- Shimadzu: AA-6300). Before analysis of cadmium concentration, all samples were filtered through 0.45µm pore size hydrophilic membranes filters (Sartoreious, Millipore, Germany). The cadmium used was in the form of $3CdSO_4.8H_2O$ with 95% purity, purchased from Merck Co. Ltd, (Germany). Muller-Hinton agar and broth were obtained from Hi-Media (Bhubaneswar, India). Mutagenic agents of Acridine Orange and Acriflavine were purchased from Merck Co. Ltd.

Preliminary screening of cadmium resistant bacteria

The preliminary screening of cadmium resistant bacteria was carried out from Industrial effluents by the following two methods. One method was based on serial dilution technique[24] in which 1mL of effluent was added to a tube containing 9mL of sterile 0.75% normal saline (10-1) and mixed well. 1mL supernatant of this dilution was transferred to another 9 mL sterile normal saline (NaOH 0.07%) tube to obtain final volume 10-2. Dilution was repeated till 10-8. 200µL of each dilution was inoculated on to sterile Muller-Hinton agar (MHA) plates containing 0.5 mM $3CdSO_4.8H_2O$ solution, spread thoroughly with sterile glass spreader and incubated aerobically at 35ºC for 24- 96 hours. For control sample, inoculation was done on Muller-Hinton agar medium without cadmium sulphate and incubated along other plates.

In the second method, 1mL of Industrial effluent was inoculated in 10 mL tube with 9 mL Muller-Hinton Broth medium containing 0.5 mM $3CdSO_4.8H_2O$ solution and mixed well by agitation and incubated at 35ºC for 24 hours. All above processes were repeated for one gram soil sample as well. 200µL of soil and sludge suspensions were inoculated onto series of 20 mL sterile Muller-Hinton agar plates and incubated at 35ºC for 24-72 hours. After growth, one loopful of each colony was suspended in 1mL sterile Muller-Hinton broth containing 40% glycerol in 1.5 mL Eppendroff tubes, mixed well and stored at -70ºC[25].

Determination of cadmium sensitivity

After the preliminary isolation of the cadmium resistant bacteria, the minimum inhibitory concentration (MIC) of Cd^{2+} was determined by the agar plate dilution method as described by Malik and Jaiswal[13]. 250 mL of 0.05M stock solutions of the metal salt ($3CdSO_4.8H_2O$) were prepared in sterile DD/W to obtain final and concentrations of 1, 2, 3, 4, 5, 6 and 7 mM $Cd^{2+,}$ respectively. The Petri plates were inoculated with 200 µL log phase liquid culture of isolated bacteria and incubated at 35ºC for 24-96 hours. The MIC was defined as the lowest concentration of the Cd^{2+} that inhibits the visible growth (number of colonies) of the organisms. The Cd^{+2} sensitivity and resistance of the isolates were calculated according to published papers[7,23].

Induction of mutation

The mutagenic compounds of Acridine Orange and Acriflavine have the ability to bind and intercalate with the DNA and cause frame shift mutation[8]. Two methods for induction of mutation were employed in this study. In the first method, Gradient Plate Technique (GPT), stock solutions of mutagenic agents were prepared by addition of 0.1mg of Acridine Orange and Acriflavine into 100 mL sterile double distilled water. Various concentrations of mutagenic agents from stock solution were added to 10 mL sterile melted nutrient agar medium, mixed well and kept as sloping condition. After the medium was solidified, 10 mL melted soft agar was poured into the sterile Petri plates and kept horizontally till it solidified again. The cadmium-resistant bacterial strains that were isolated in pervious stages were inoculated throughout gradient plates and incubated at 35°C for 24-48 hours. The colonies that were grown on the highest concentration of the slop, were selected and used for further study. In the second method, Sub-Inhibitory Concentration (SIC) of each mutagenic agent was determined as previously described[24].

100µL of log phase bacteria (8 hours grown cell suspension) that was grown on gradient plates of Acridine Orange and Acriflavine were inoculated onto sterile Muller-Hinton Broth (MHB) medium containing different amounts of the above mutagenic agents (400, 800, 1600, 3200 and 6400 µg/mL). All the tubes were incubated at 35°C for 24-48 hours. 100µL from each tube which showed visible growth, was streaked on Muller-Hinton Agar (MHA) medium and incubated at 35°C for 24 hours. Individual colony grown on the highest concentrations of the mutagenic compounds were inoculated on MHA medium containing various concentrations of $3CdSO_4.8H_2O$ (1-8mM) and incubated for 24-48 hours at 35ºC. The bacterial strains that were grown on the highest concentration of $3CdSO_4.8H_2O$ containing plates, were then isolated and stored for Cd^{+2} removal study.

Identification of bacterial isolates

Both, Cd^{+2} resistant gram-negative non-fermentative strains (GNNFR) and gram-positive cocci were isolated. The isolates were tested and characterized by several microbiological key conventional tests for basic differentiation of gram-negative and gram-positive bacteria as previously described in Bergeys Manual of Determinative Bacteriology. Further, the isolates were identified on the basis of biochemical tests of commercial identification systems as shown in **Table 1.**

Mass balance experiment

To determine how much of the Cd^{2+} was precipitated by the cell in an insoluble form, 1.5 mL of the samples were treated with and without cadmium and centrifuged at 10.000 rpm for 15 minutes at room temperature. The supernatant and pellet were analysed for Cd^{2+} content by atomic absorption spectrometry.

Removal of cadmium by Cd resistant biomass

100 mL Erlenmeyer flasks containing 60 mL of 30, 45 and 60 mg/L cadmium solutions were prepared. In one set, 0.5g biomass (wet weight) of mutated bacteria and in the other set, 0.5g biomass from wild type were carefully weighted and separately added to each flask. The pH (8.0) and temperature (24ºC) of the solution were initially measured before adding the biomass. The flasks were placed on stirrer (100 rpm) and the samples were taken and centrifuged at 10.000 rpm every 5 minutes for the first 20 min, and interval of 30, till 300 minutes. The supernatants and biomasses (biomasses were washed with normal saline (0.75%) and again centrifuged) fractions from each specific time were analyzed for the remaining Cd^{2+} by atomic absorption spectrometry For determination of Cd^{+2} concentrations in the biomasses,

bacterial cell residues were dissolved in 1 mL 95% nitric acid (Merck Co.), mixed well by vortexing and diluted to 10 mL with sterile DD/W. Blanks were treated in the same way and analyzed as described above. Simultaneously, total viable count of the organism was determined each time to see any decrease in the colony count.

RESULTS

pH of Industrial effluent was 8.0 and atmospheric temperature was 25°C, while ambient temperature was 20°C. Several mesophilic gram-negative and cadmium resistant bacteria were isolated. The enrichment was better as compared to direct culture method for the isolation of Cd resistant bacteria in shorter time. Also, the isolates in primary enrichment method could grow on 6 mM concentration of Cd^{2+} containing medium. Majority of the bacterial isolates were belonging to gram-negative non-fermentive *Pseudomonas* (4 isolates). One gram-negative coccus was also capable to grow on 2 mM concentrations of $Cd^{2+;}$ but on subsequent inoculation, the strain lost its ability to grow on more than 2 mM Cd^{2+} and was eliminated from the study. Those strains isolated from soil, exhibited MIC=3.5 mM and from Industrial effluent as 6mM for $3CdSO_4.8H_2O$, respectively **(Table 2).**

One *Xanthomonas oryzae* isolated from soil near factory could grow only on 2mM cadmium containing medium; therefore, it was not used for bioremediation study because of low MIC value. Those *P.aeruginosa* isolates grown on the 3.5-6 mM concentration of Cd^{2+} containing

Containing were exposed to 6400mg/L Acriflavine and 9600 the supernatants and remained constant during 3 hours. This indicates that as concentration of Cd^{+2} was increased, the total viable count of the organism decreased and was directly related to the amount of Cd^{+2} removed by the biomass. The Fig. 2 (a & b) shows the Cd^{2+} removal isotherm for mutated bacteria as well as wild type which follow Langmuir model. Table 4 shows the parameters of Langmuir isotherm for Cd^{2+} derived in this investigation.

Fig. 1: Removal of Cd2+ by P.aeruginosa one, isolated from active sludge effluent in solution containing (a) 30mg/L and (b) 60mg/L Cadmium

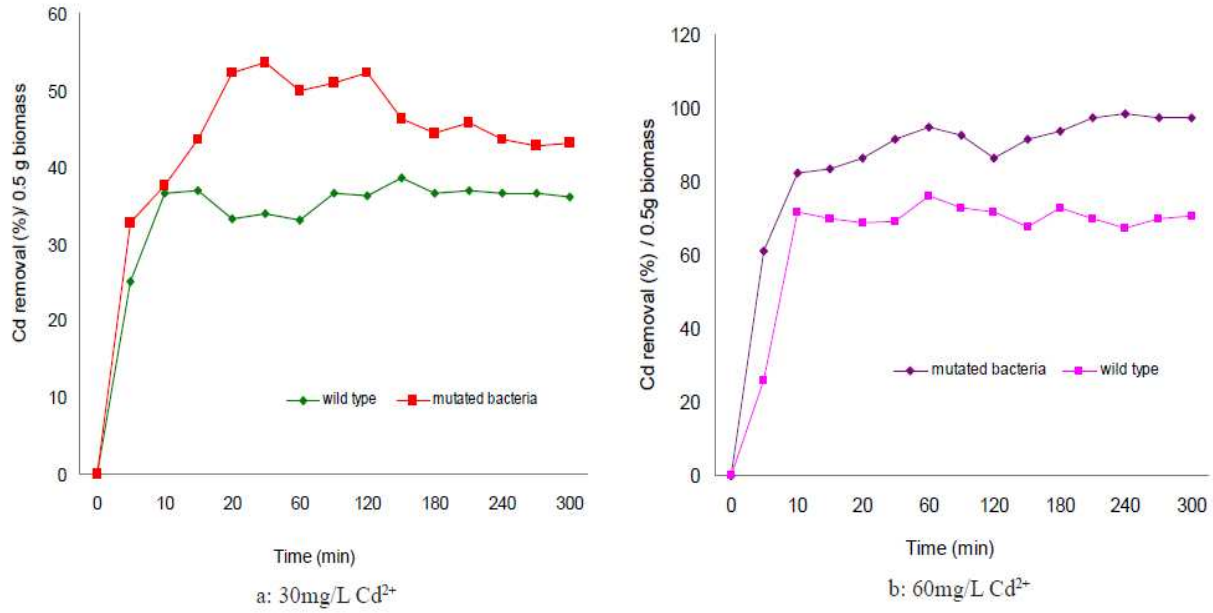

Fig. 2: Langmuir isotherm plot for removal of Cd+2 by (a) wild type and (b) mutated bacteria

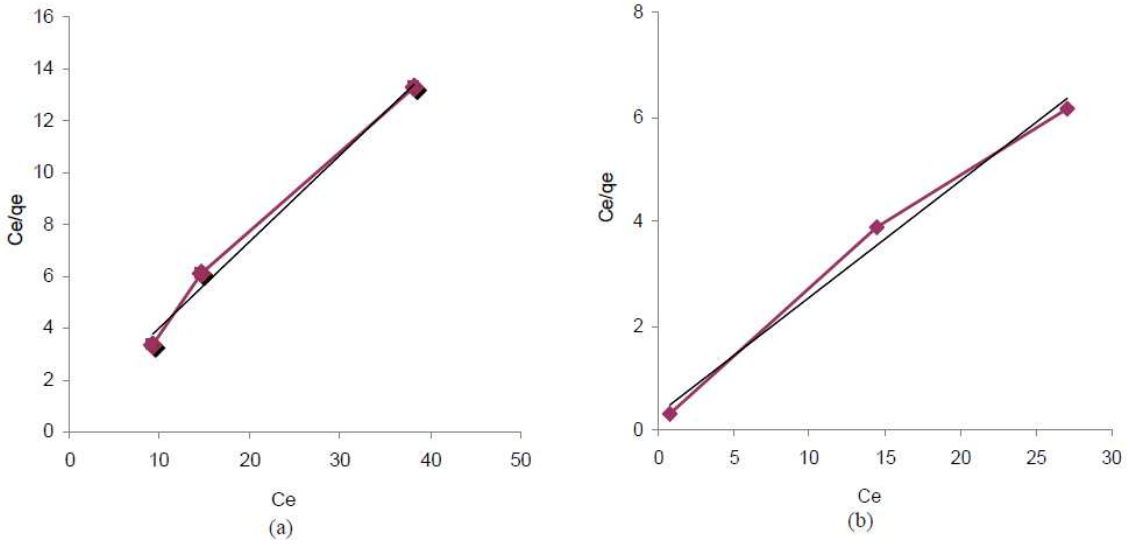

Table 1: Biochemical tests for identification of *P. aeruginosa* isolated from Industrial Effluent of Nalco Smelter Plant

Biochemical test	**Properties**
Morphology	Short Rods
Gram staining	Gram negative
Motility	+
Growth at 42^0C	+
Growth at 4^0C	-
Fluoroscent pigment	+
Odor	Fruity odor
H_2S production in TSI	_
Gas production	_
Growth on Simmon	+
Citrate agar	+
Urea test	+
Oxidase	+
Arginine dehydrolase	+(Weak)
OF	oxidative
Indole	_
Gelatinase	+

Table 2: Minimum Inhibitory Concentration (MIC) of Cd^{+2} for three *Pseudomonas* strains isolated from Industrial effluent before and after mutation

P. aeruginosa isolates	**Source**	**MIC (before Mutation) mM**	**MIC (after Mutation) mM**
1.	Effluent	6	7
2.	Effluent	3.5	4
3.	Effluent	2.5	3

Table 3: Gradient Plate Method (GPM) and Subinhibitory Concentration (SIC) of mutagenic agents Acridine Orange (AO) and Acriflavine (AF) for *P. aeruginosa* strains 1, 2, and 3 isolated from effluent

P. aeruginosa isolates	**Concentration**		
	GMP	**SIC***	
	AO	AF	AO	AF
1.	9600	6400	6400	3200
2.	9600	6400	6400	3200
3.	9600	6400	6400	3200

*SIC was defined as maximum concentration of mutagenic agent that bacteria could grow.

Table 4: Langmuir isotherm coefficients for cadmium

Langumir Model for Mutated Bacteria				Langumir Model for Wild type Bacteria		
	Q_{max}	K_L	R^2	Q_{max}	K_L	R^2
	4.47	1.58	0.9945	3.01	0.47	0.9908

DISCUSSIONS

The data obtained in this study clearly shows that with employment of cadmium resistant mutated biomass, bioaccumulation of Cd^{2++} from Cd-containing solution considerably increased. *P. aeruginosa* isolate 1 could efficiently remove 94.7% in 30 mg/L of Cd^{+2} solution within 60 min. The results were consistent with previously report for strain E1[28]. Strain E1 with resistance to 18 mM/L cadmium isolated medium were exposed to 6400 mg/L Acriflavine and 9600 Fig. 2 (a & b) shows the Cd^{2+} removal isotherm for mutated bacteria as well as wild type which follow Langmuir model. Table 4 shows the parameters of Langmuir isotherm for Cd^{2+} derived in this investigation.

One *Xanthomonas oryzae* isolated from soil near factory could grow only on 2mM cadmium containing medium; therefore, it was not used for bioremediation study because of low MIC value. Those *P. aeruginosa* isolates grown on the 3.5-6 mM concentration of Cd^{2+} containing medium were exposed to 6400mg/L Acriflavine and 9600 mg/L Acridine Orange as shown in Table 3. The isolates could grow well on these concentrations and not any changes in the colony characteristics or bacterial morphology was observed after exposure to these mutagenic agents. Table 2. compares MIC values of Cd^{2+} before and after exposure to these two mutagenic agents. The MIC increased to 7 mM for *P. aeruginosa* isolate1 after exposure to the above agents. Fig. 1 (a and b) shows removal of Cd^{2+} by mutated *P. aeruginosa* isolate 1 and wild type in 30 and 60 mg/L of Cd^{2+} solution, respectively. 94.7% removal was achieved till 60 minutes in 30 mg/L of Cd^{2+} by mutated bacteria. However, in 60 mg/L concentration of cadmium, only 53.58% Cd^{+2} was removed by mutated bacteria and 38.68% by wild type. In all cases the Cd^{2+} concentration rapidly decreased during the first 15 minutes in the supernatants and remained constant during 3 hours. This indicates that as concentration of Cd^{+2} was increased, the total viable count of the organism decreased and was directly related to the amount of Cd^{+2} removed by the biomass.

CONCLUSIONS

The findings of this study suggest that Industrial effluent containing high levels of Cd toxic metal may have led to the soil-effluent microbial population developing resistance strategies against metal toxicity. The Langmuir model was derived based on several assumptions (the surface was homogeneous, adsorption on the surface was localized and each site could only accommodate one molecule or atom). In the other study, Cd-resistant bacteria were isolated from Cd-contaminated Industrial waste effluents by Prapagdee and Watcharamusik[19]. One isolate, TAK1, was highly resistant to cadmium toxicity. TAK1 was isolated from effluent contaminated with a high Cd concentration (204.1 mg/kg). The removal of the heavy metal ions by some gram-negative bacterial species such as *E. coli* and *P. syringae* were studied by Cohen *et al.*,[5] and Cabral[3] respectively. *P. aeruginosa* was found to detoxify Cd^{2+} through production of intracellular cadmium-binding proteins[9]. A Cd^{2+-} hyperresistant bacterial strain HQ-1 was isolated from a lead–zinc mine by Qing Hu *et al.*,[17]. Shakibaie *et al.*,[24] isolated *P. aeruginosa* strains that accumulated high concentrations of copper and zinc after exposure to mutagenic compounds. From present study it can be concluded that the strain 1 can be efficiently used for bioremediation.

Acknowledgement

The authors are grateful to the E& S Department for providing instruments and facilities of this research containing waste pollutants with minimum cost and high efficiency.

REFERENCES

1. Ajmal, M. Mohammad, A. Yousuf, R. and Ahmad, A., Adsorption behaviour of cadmium, zinc, nickel and lead from aqueous solution by *Mangifera indica* seed shell. *Indian. J. Environ. Health.* **40(1):** 5-26 (1998)

2. Banjerdkji, P. Vattanaviboon P. and Mongkolsuk, S., Exposure to cadmium elevates expression of genes in the oxy R and Ohr R regulons and induces cross-resistance to peroxide killing treatment in *Xanthomona scampestris*. Appl. Environ. Microbiol. **71 (4):** 1843-1849 (2005)
3. Cabral, J.P., Selective binding of metal ions to *Pseudomonas syringae* cells. Microbios. **71:** 47-53 (1992)
4. Chelliah, E.R. Sundaresan, S. and Govindan, S.S., Adaptive and cross resistance to cadmium (II) and zinc (II) by *Pseudomonas aeruginosa* BC15. Biologica. **63(4):** 461-465 (2008)
5. Cohen, I. Bitan, R. and Nitzan, Y., The effect of zinc and cadmium ions on *Escherichia coli.* B. Microbios. **68:** 157-168 (1991)
6. Denise, P. Higham, P. Sadler. J. and Michael, D., Cadmium Resistance in *Pseudumunas putida:* Growth and Uptake of Cadmium. *J. General Microbiol.* **131:** 2539-2544 (1989)
7. El-Helow, E.R. Sabry, S.A. and Amer, R.M., Cadmium biosorption by a cadmium resistant strain of *Bacillus thuringiensis*: regulation and optimization of cell surface affinity for metal cations. *BioMetals*. **13:** 273–280 (2000)
8. E.Glass, R., Gene function; *E.coli* and its heritable elements. Croom Helm Ltd, 2-10 St Johns Road, London SW11., 113-114 (1982)
9. Hassen, A. Saidi, N. Cherif, M. and Boudabous, A., Effects of heavy metals on Pseudomonas aeruginosa and *Bacillus thurengiensis* Biores. Technol. **65:** 73-82 (1998)
10. Herrero, R. Lodeiro, P. Rey-Castro, C. Vilariño, T. and Sastre de Vicente, M.E., Removal of inorganic mercury from aqueous solutions by biomass of the marine macroalga *Cystoseira baccata*. Water. Res. **39:** 3199–3210 (2005)
11. Kapoor, A. and Viraraghavan, T., Fungal biosorption: an alternative treatment option for heavy metal bearing wastewaters. Bioresour. Technol. **53:** 195–206 (1995)
12. Katarina, C. Darina, S. Vladimir, K. Miloslava, P. Jana, H. Andrea Puškrov, B. and Ferianc, P., Identification and characterization of eight cadmium resistant bacterial isolates from a cadmium-contaminated sewage sludge, Biologia. Bratislava. **59:** 817—827 (2004)
13. Malik, A. and Jaiswal, R., Metal resistance in Pseudomonas strains isolated from soil treated with industrial wastewater. *World. J. Microbiol. Biotechnol.* **16:** 177–182 (2000)
14. Mitra, R.S. Gray, R.H. Chin, B. and Berstein, I.A., Molecular mechanisms of accumulation in *Escherichia coli* to toxic levels of cadmium. *Journal of Bacteriology.* **121:** 1180-1188 (1975)
15. Nies, D.H., Efflux mediated heavy metal resistance in prokaryotes. FEMS. *Microbiol. Rev.* **27(2-3):** 313-39 (2003)
16. Novick, R.P. and Roth, C., Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. Bacteriology. **95:** 1335-1342 (1968)
17. Qing, H. Hongyan, Q. Zhihui, B. Minna, D. Jinghai, Z. Fanyuanhang, Z. and Hongxun, Z., Biosorption of cadmium by a Cd^{2+} hyperresistant *Bacillus cereus* strain HQ-1 newly isolated from a lead and zinc mine. *World. J. Microbiol. Biotechnol.* **23:** 971–976 (2007)
18. Paul, V.I. and Jayakumar, R., A comparative analytical study of the cadmium and humic acids contents of two lentic water bodies in Tamil nadu, India. *Iran. J. Environ.Health Sci and Eng.***7(2):** 137-144 (2010)
19. Prapagdee, B. and Watcharamusik, A., Adaptive and cross-protective responses against cadmium and zinc toxicity in cadmium-resistant bacterium isolated from zinc mine. *Braz. J. Microbiol.* **40:** 838-845 (2009)
20. Raja, S. Ravikrishna, R. Yua, X.Y. Leea, T. Chenc, J. Murugesand, K. Xinhua, S. Qingzhongc, Y. Kalliat, T. and Collett, J.L., Fog chemistry in Texas-Louisiana Gulf Coast corridor. *Atmospheric Environment J.* **42(9):** 2048-2061 (2008)
21. Raskin, I. and Ensley, B.D. (2000). Phytoremediation of toxic metals. John Wiley & Sons, New York.
22. Romero, P. (1990). Resistance to antibiotics and heavy metals of *Pseudomonas aeruginosa* isolated from natural water. *Appl. Bacteriol.* **68:** 625-632.
23. Sabry, S. Ghzlam, H.A. and Abou-Zeid, D.M., Metal tolerance and antibiotic resistance patterns of bacterial population isolated from sea water. Appl. Microbiol. **82:** 245-252 (1997)

24. Shakibaie, M.R. Khosravan, A. Farmhand, A. and Zare, S., Application of metal resistant bacteria by mutational enhancement technique for bioremediation of copper and zinc from industrial wastes. *Iran. J. Environ Health. Sci. Eng.* **5:** 251-256 (2008)
25. Shakibaie, M.R. Dhakephalker, P. Kapadnis, B.P. and Chopade, B.A., Silver resistance in *Acinetobacter baumannii* BL54 occurs through binding to an Ag-Binding Protein. *Iranian J Biotechnology.* **1(1):** 41-46 (2003)
26. Vieira, Regine HSF. and Volesky, B., Biosorption: a solution to pollution. International Microbiology. **3(1):** 17–24 (2000)
27. Viraghavan, T., Fungal biosorption-an alternative treatment option for heavy metal bearing wastewaters: a review. *Bioresource Technol.* **53(3):** 195-20 (1995)
28. Zeng, X. Tang, J. Liu, X. and Jiang, P., Isolation, identification and characterization of cadmium-resistant *Pseudomonas aeruginosa* strain E1. *J. Cent. South. Univ. Technol.* **16:** 0416−0421 (2009)