Research Journal of Environmental and Earth Sciences 2(2): 112-117, 2010
ISSN: 2041-0492

Submitted Date: December 23, 2009 Accepted Date: January 10, 2010 Published Date: April 20, 2010

High Chromium Tolerant Bacterial Strains from Palar River Basin: Impact of Tannery Pollution

K. Sundar, R. Vidya, Amitava Mukherjee and N. Chandrasekaran
Nanobio-Medicine Laboratory, School of Biosciences and Technology,
VIT-University, Vellore 632014, India

Abstract: The basic survey study on tanneries and its pollution in the Palar river basin of Vellore District showed that it has been contaminated with heavy metals especially chromium and salts. This study is to improve our understanding to find the Cr contamination level and the ecology of heavy metal tolerance of the native bacterial flora of our study area. Chromium tolerant strains were isolated from contaminated sediments, water and effluents of various tanneries. The minimum and maximum concentration of chromium sediments was in the range of 47.4 and 682.4 mgL^{-1}, with an average of 306.285 mgL^{-1} in the study area. Sixty-eight chromium resistant bacterial strains were isolated and Maximum Tolerance Concentration (MTC) studies have indicated that the tolerance concentrations of the isolates were in the range of 100-3300 mgL^{-1}. These bacterial isolates were also checked for their resistance to other heavy metals like Ni, Pb, Zn, Fe and Cd. Eighty percent of the isolates showed resistance to Ni, Pb, Zn, Fe at 100 ppm level and 45% had shown resistance to Cd. The isolates also had shown tolerance to salt (NaCl) up to 9%. Significant note was found in the concentration of chromium and in the chromium tolerance ability of the bacteria in the study area and these chromium tolerance bacteria can be used as the indicator for the Cr contamination.

Key wards: Cr tolerant bacteria, Palar river, tanneries, Vellore district

INTRODUCTION

Palar is a southern India river, originated from Nandidurg hills of Karnataka state and flows through Karnataka (93 km), Andhra Pradesh (33 km), Tamil Nadu (222 km) and finally convergence into the Bay of Bengal at Vayalur, Tamilnadu, India. The Palar is one of the major rivers flowing through Vellore District (120 km length with 4710 area of river basin). Elevated chromium concentration in the effluents from tanneries poses a serious environmental concern in Vellore district, home of innumerable small and large-scale tanneries.

Palar River is the source of drinking water for 30 towns and 50 villages on its banks and also used for the cultivation purpose. The tanneries present on the banks allowed effluents in the Palar River, which has become more polluted recently and no longer useful for drinking or agricultural purposes. Due to pollution, peoples around the river are suffering from a number of diseases such as asthma, skin disease and stomach ailment and thousands acres of fertile land have become wasteland and no more used for cultivation (Vellore environmental profile). The Ranipet, an industrial area present in the bank of Palar river was reported as the most polluted places of world by Blacksmith Institute in 2006, (World's Worst Polluted Places-the Top Ten, September 2006 http://www.blacksmithinstitute.org/top10/10worst2.pdf) which are heavily contaminated with salts and heavy metals especially Chromium.

Metals contamination is one of the major environmental problems in many countries and these contaminants generally come from various industries like leather, agricultural, textile industries etc (Ganguli and Tripathi, 2002). The most commonly occurring metals at these sites are lead, chromium, arsenic, zinc, cadmium, copper, and mercury. Presence of these metals in groundwater and soils may cause a significant threat to human health and ecological systems (Evanko and Dzombak, 1997).

Chromium is used to call as metal with two faces, that it can be either beneficial or toxic to animals and humans depending on its oxidation state and concentrations (Zayed *et al.*, 1998). Cr(III) is considered to be a trace element essential for the proper functioning of living organisms (Wang *et al.*, 2009). Nutritionally, at lower concentrations, Cr(III) is an essential component of a balanced human and animal diet for preventing adverse effects in the metabolism of glucose and lipids, e.g., impaired glucose tolerance, increased fasting insulin, increased cholesterol and triglycerides, and hypoglycemic symptoms (Zayed and Terry, 2003). Cr(III) at increased concentrations can interfere with several metabolic

Corresponding Author: N. Chandrasekaran, Nanobio-Medicine Laboratory, School of Biosciences and Technology, VIT-University, Vellore 632014, India

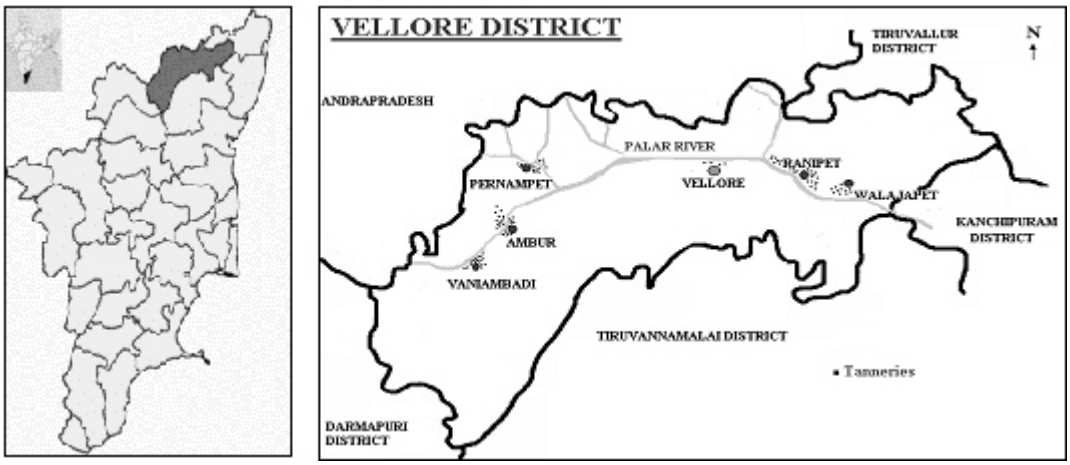

Fig. 1: Palar river basin showing study locations and tanneries

processes because of its high capability to coordinate various organic compounds resulting in inhibition of some metalloenzyme systems (Zayed *et al*., 1998).

Chromium is extensively used in industries, like electroplating, paint and pigment manufacturing, textile, fertilizer and leather tanning (Ganguli and Tripathi, 2002). These industries discharges trivalent and hexavalent chromium with waste effluent to the soil and surface water. Chromium, which is generated by various industries, occurs in different oxidation states but Cr (III) and Cr(VI) are the most significant. Hexavalent chromium is acutely toxic, mutagenic, and carcinogenic (Lee *et al*., 2008). In addition, Cr (VI) is highly mobile in most environments, mainly due to its soluble nature (Fukai, 1967). Despite the fact that heavy metals are acutely toxic to most microbes, there are metal-tolerant bacteria. Long-term exposure to metals imposes a selection pressure that favors the proliferation of microbes that are tolerant / resistant to this stress. This has been investigated by assaying habitats exposed to anthropogenic or natural metal contamination over an extended period of time (Hutchinson and Symington, 1997). This present study is an attempt made to isolate chromium tolerant bacteria from the Palar river basin, which is polluted by the metals contamination.

Reports on the occurrence of Cr tolerance strains from naturally occurring chromium-percolated ecosystem such as tannery-contaminated soil are not common. Vaniambadi, Ambur and Ranipet of Vellore District, Tamilnadu, India are reported to be rich in Cr but no microbiological studies have been made so far on these environment. In this study, indigenous bacteria which showed tolerance to Cr were isolated from Cr-contaminated sites of Palar river basin.

MATERIALS AND METHODS

Study Area: The study locations are tannery accumulated places of Palar river basin of Vellore District, which include Vaniambadi, Ambur, Pernambut, Ranipet and Walajapet. This study was done during the year 2008-2009.

Sample Collection and Chromium Analysis: Sediment samples were collected from the tannery accumulated sites like Vaniambadi, Ambur, Pernambut, Ranipet and Walajapet of Vellore district, Tamilnadu, India (Fig. 1). For chromium analysis, the water samples were collected in linear polyethylene containers with polyethylene cap. The samples were preserved immediately by acidifying with concentrated to pH<2 (1.5 ml conc. HNO_3/L sample) and stored at 4ºC for further analysis (APHA, 2005).

The microwave digestion system MARS from CEM Corporation was used for the digestion of soil samples. The digested samples were analyzed with Perkin Elmer precisely Optical Emission Spectrometer (ICP-OES) Optima 5300 DV and Atomic Absorption Spectrophotometer (AAS) VARIAN SPECTRAA.

Sample collection for microbiological analysis: For microbiological analysis the samples were collected in the same locations in sterile containers and brought to the laboratory in ice boxes. The samples were collected every 30 days intervals for one year (APHA, 2005).

Enrichment isolation of chromium-resistant bacteria: Chromium-resistant bacteria were isolated from sediment samples collected in sterile glass containers from Vaniambadi, Ambur, Pernambut, Walajapet and Ranipet of Vellore District, Tamilnadu, India. These samples are rich in salt and chromium concentration. Soil samples were serially diluted and plated on Nutrient agar plates amended with 100 mgL^{-1} of chromium in the form of $K_2Cr_2O_7$ for chromium (VI) and $Cr(NO_3)_3 \cdot 9H_2O$ for chromium (III). The medium amended with trivalent chromium was maintained at pH 3.5 to avoid the precipitation and all the plates were incubated at 37ºC for 2-4 days. Chromium-tolerant strains representing different

Table 1: Concentration of chromium in sediments of study locations in Palar river basin

Chromium conc.(mg l^{-1})	Vaniambadi	Ambur	Pernambut	Ranipet	Walajapet
Minimum	241.7	65.6	47.4	197.2	238.5
Maximum	589.3	396.6	289.2	523.2	682.4
Mean	366.5	282.6	184.2	351.5	346.2

Table 2: MTC (Maximum Tolerance Concentration) of selected isolates to Cr (III), salt and resistance to other metals

			Resistance to other metals at 100 mg l^{-1}				
Isolates	MTC for Cr III (mg l^{-1})	MTC for Salt (%)	Cd	Ni	Pb	Fe	Zn
Cr(III) 01	1500	9	R	R	R	R	R
Cr(III) 02	1200	8	S	R	S	R	R
Cr(III) 03	1100	6	R	R	R	R	S
Cr(III) 04	800	4	S	S	S	S	R
Cr(III) 05	700	4	S	R	S	R	R

colony morphologies were purified on the same agar medium by repeated culturing and the representative strains of hexavalent and trivalent tolerant were maintained and stored on Nutrient agar slants at -20ºC.

Evaluation of chromium-tolerance: The Maximum Tolerable Concentration (MTC) of all the Cr resistant isolates were determined by well diffusion method and broth dilution method (Calomoris *et al.*, 1984) in PYG medium with Cr(III) and Cr(VI) concentrations ranging from 100 to 40000 mgL^{-1}. The maximum concentration of metal in the medium which support the growth was taken as the maximum tolerable concentration (MTC).

Evaluation of Tolerance to Other Metals and Salt: The isolated Cr tolerant bacterial strains were tested for tolerance to other metals like Zn, Fe, Pb, Cd and Ni at 100 mgL^{-1} by broth dilution method (Calomoris *et al.*, 1984) in PYG medium supplemented with above-mentioned metals. The salt tolerance was checked with NaCl in nutrient broth-by-broth dilution method.

Identification of Hexavalent Chromium Resistant Bacterium: Taxonomic studies of the selected Cr(III) resistant bacterium were carried out according to Buchanan and Gibbons (1994). The isolated bacterium was identified by the following taxonomic studies:

- Morphological characteristics (shape and size, gram reaction, motility)
- Cultural characteristics (nutrient agar colonies, slant culture, stab culture)
- Biochemical characteristics (gelatin liquification, milk clotting, indol, H_2S, NH_3 production, Voges-Proskauer reaction, Methyl red test, Starch hydrolysis, Catalase and Urease).
- Molecular Characterization by 16S r DNA sequencing

Molecular Characterization: The DNA was isolated by HiPurA bacterial DNA isolation and purification kit (Himedia, India) and amplified by PCR using a master mix kit, Medoxmix (Medox, India) as per user manual. The bp fragment of the 16S rRNA gene of the strain was sequenced in both the sense and antisense directions. The 16S rRNA sequence was analyzed for the similarity and homology with the existing sequences available in the data bank of National Center for Biotechnology Information (NCBI) using BLAST search. The DNA sequences were aligned and phylogenetic tree was constructed by neighbour joining method using ClustalW software.

RESULTS

Chromium concentration in the Palar River Basin: The chromium concentration in the sediment samples was 47.4 mgL^{-1} as the minimum and 682.4 mgL^{-1} as the maximum with the average of 306.285 mgL^{-1} (Table 1) in the study location. The highest chromium contamination was found in Vaniambadi in the upstream of Palar River, which is having the more number of tanneries among the study area.

Isolation and prevalence of chromium-tolerant bacteria: In search for chromium-resistant microorganisms, a total of 68 Cr-resistant (both Cr(III) and Cr(VI)) bacteria were isolated from soil and water samples following dilution and plating on media amended with 100 mgL^{-1} Cr in the form of $K_2Cr_2O_7$ for chromium (VI) and $Cr(NO_3)_3 \cdot 9H_2O$ for chromium (III) (Red colour line: Not needed , previously already mentioned). The majority of these isolates showed an MTC value of 100 to500 mgL^{-1} Cr(VI), and the maximum was 3300 mgL^{-1} Cr(VI). The prevalence of chromium tolerance bacteria was 31, 23, 21, 13 and 12% in Vaniambadi, Ambur, Walajapet, Ranipet and Pernambut respectively (Fig. 2).

MTC (Maximum Tolerable Concentration) of Isolates to Chromium: Resistance to Cr(VI) by the isolates were tested based on their growth with various levels of Cr(VI) ranging from 100 to 4000 mgL^{-1}. The majority of these isolates showed an MTC value range of 100 to 500 mgL^{-1} Cr(VI), and the maximum was 3300 mgL^{-1} Cr(VI). For Cr(III) the MTC was 1500 mgL^{-1} in the acidic medium (Table 2,3). The percentage of tolerance range is shown in the Fig. 3.

Table 3: MTC (Maximum Tolerance Concentration) of selected isolates to Cr (VI), Salt and resistance to other metals

			Resistance to other metals at 100 mg l^{-1}				
Isolates	MTC for Cr III (mg l^{-1})	MTC for Salt (%)	Cd	Ni	Pb	Fe	Zn
Cr(VI) 01	3300	11	R	R	R	R	R
Cr(VI) 02	3000	6	R	S	S	R	R
Cr(VI) 03	2200	8	S	R	R	S	R
Cr(VI) 04	1600	4	S	S	S	R	R
Cr(VI) 05	1500	6	S	S	R	S	S

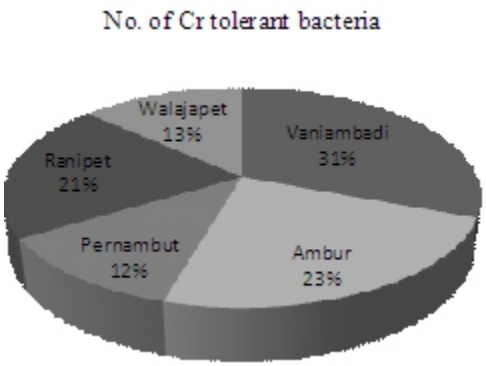

Fig. 2: Distribution of Chromium resistant bacteria in the study area of Vellore District

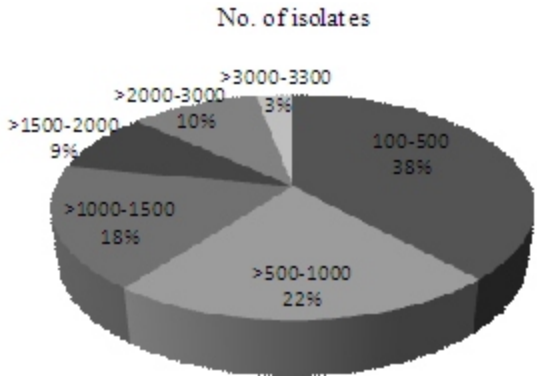

Fig. 3: Range of Chromium tolerance of isolates to Cr(III) and Cr(VI)

Resistance to other metals: The isolated bacterial strains were also checked for the tolerance to other heavy metals like Zn, Fe, Pb, Cd and Ni at 100 mgl^{-1}. among the isolates 38 were showed resistance to Zn, 42 to Fe, 36 to Pb, 41 to Ni and 24 to Cd at 100mgl^{-1} range (Table 2).

Salt tolerance capacity: The salt tolerance capability of the isolates was determined using NaCl. The isolates, which were showing maximum tolerance to Cr, were more tolerant to salt (NaCl). They showed tolerance to salt up to 4 to 9% (Table 2).

Characterization of the selected isolates: The molecular characterization was done for the isolate which had shown maximum tolerance to chromium and found to be *Bacillus cereus* strain VITSCCr02 since it showed 99% similarity in BLAST search which was showing tolerance up to 3300 mgL^{-1} for Cr(VI). The bacteria had shown maximum toleranct to Cr(III) is found ti be *Bacillus* sp. by morphological and biochemical characterization and it is yet to be characterized by molecular methods.

DISCUSSION

This study highlights the chromium contamination in Vellore District and shows the prevalent occurrence of chromate tolerant microbial population in the Palar river basin of Vellore District. These microbes were also observed to be tolerant to other heavy metals like Zn, Fe, Pb, Cd and Ni at 100 mg l^{-1} concentration and salt in the form of NaCl.

The bacterial isolates tolerated a wide range of Cr(III) and Cr(VI) concentrations (100-3300 mgL^{-1}). Most of the reports were on the bacterial tolerance to Cr(VI) and there was not much report on Cr(III) tolerant bacterial populations. Previous reports suggested that, most Cr(VI)-resistant microorganisms are tolerant up to 10 to 1500 mg L_1 of Cr(VI) (McLean and Beveridge, 2001). Chromium (VI) bacterial resistance above 2500 mgL^{-1} has only been reported by Shakoori *et al.* (1999), were isolated from the tannery effluent.

In our study *Bacillus cereus* strain VITSCCr02 showing tolerance to Cr(VI) up to 3300 mgL^{-1} was isolated from the soil and it is also novel in the case of environmental sample. Chromium-resistant bacteria cap able of reducing chromate have been reported from chromium polluted environments and they were showing resistance in less concentration (Mclean and Beveridge, 2001; Arundhati and Paul, 2004).

Isolation of bacteria from polluted environments would represent an appropriate practice to select metal resistant strains that could be used for heavy metal removal and bioremediation purposes (Malik, 2004).

Association between resistance to antibiotics and heavy metals has been reported by several workers (Novick and Roth, 1968; Schottel *et al.*, 1974; Dhakephalkar and Chopade, 1994; Ramteke, 1997). The prevalence of such metal tolerant microorganisms is ecologically important, particularly if they are also antibiotic resistant. The metal resistance with salt tolerance in this study showed the pollution due to

tanneries as these tanneries contributes to salt contamination in the environment.

This study also deals about the chromium tolerance with other metal resistance. There was no obvious pattern between the presence and level of resistance to Cr and the other metals. Multiple metal resistances has been found in other bacteria and the resistance mechanisms and genes involved are typically not common (Nies and Silver, 1990; Trajanovska *et al*., 1997). Multiple heavy metal resistance determinants, namely the Cd, Co, Zn genes (czc), the Co, Ni, Cr genes (cnr, chr) and the Hg (mer) have been isolated from plasmids (Verma *et al*., 2001). The combined resistance to Ni, Cr and Zn was reported by Margesin and Schinner (2001). These reports supports that the metal resistances of the bacteria were interrelated each other.

Incidences of skin and urine infections are very common among tannery workers in India (Das *et al*., 1989). As these workers are in constant contact with tannery effluents and wastes, the causes of most of these infections are microorganisms present in the effluents and wastes. The spread of multiple antibiotic resistant bacteria is one of the most serious threats to the successful treatment of disease (Bhattacherjee *et al*., 1988; Ramteke, 1997). Large numbers of chromate tolerant microorganisms, especially coliforms, were capable of multiple antibiotics resistant and which indeed leads to consequences in the tannery workers.

The introduction of heavy metals, in various forms, in the environment, can produce considerable modifications of the microbial communities and their activities (Hassen *et al*., 2001). It was also confirmed in our study.

The tolerance of the bacterial isolates to both Cr(III) and Cr(VI) showed that, depending on the environmental conditions, the dynamic interconversion between Cr(III) and Cr(VI) in soils, sediments, and aqueous environments is active, with the reduction of Cr(III) to Cr(VI) generally being favored in most situations (Kimbrough *et al*., 1999).

CONCLUSION

The tolerance of soil bacteria to heavy metals has been proposed as an indicator of potential toxicity of metals to other forms of life this is proved in this study that these high chromium tolerant bacteria confirmed the chromium contamination in the study locations of Vellore District.

Microorganisms with the ability to tolerate and reduce Chromium can be used for detoxification of environments contaminated with Chromium so this chromium bacterial strains can be used to remediate the places of Vellore District contaminated with chromium as these are indigenous to this environment.

ACKNOWLEDGMENT

Authors thank Department of Science and Technology (DST), Govt. of India and the Management, VIT University for their support and financial assistance for this project.

REFERENCES

American Public Health Association (APHA), 2005. Standard Methods for the Examination of Water and Wastewater, 20th Edn., Clesceri, L.S., Greenberg, A.E., A.D. Eaton and M.A.H. Franson (Eds.), Washington DC, USA.

Arundhati, P. and A.K. Paul, 2004. Aerobic chromate reduction by chromium-resistant bacteria isolated from serpentine soil. Microbiol. Res., 159: 347-354.

Bhattacherjee, J.W., S.P. Pathak and A. Gaur, 1988. Antibiotic resistance and metal tolerance of coliform bacteria isolated from Gomti river water at Lucknow city. J. Gen. Appl. Microbiol., 34: 391-399.

Buchanan, R.E. and N.E. Gibbons, 1994. Bergy's Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins Co., Baltimore, pp: 1268.

Calomoris, J.J., T.L. Armstrong and R.J. Seidler, 1984. Association of metal-tolerance with multiple antibiotic resistance of bacteria isolates from drinking water. Appl. Environ. Microbiol., 47: 1238-1242.

Das, M., M.P. Misra, A. Kumar, P. Kumar, S. Ahmad and S.V. Chandra, 1989. Chronic chromium poisoning with dermatitis in the leather industry. Contact Dermatitis, 20: 221-222.

Dhakephalkar, P.K. and B.A. Chopade, 1994. High levels of multiple metal resistance and its correlation to antibiotic resistance in environmental isolates of Acinetobacter. Biometals, 7: 67-74.

Environmental Profile of Vellore District. By Operations Research Group, Chennai. Retrieved from: http://www.tnenvis.nic.in/DtProfiles/vellore.pdf.

Evanko, C.R. and D.A. Dzombak, 1997. Remediation of Metals-Contaminated Soils and Groundwater. E Series: TE-97-01. Retrieved from: http://www.cluin.org/download/toolkit/metals.pdf.

Fukai, R., 1967. Valency state of chromium in seawater. Nature, 213: 901.

Ganguli, A. and A.K. Tripathi., 2002. Bioremediation of toxic chromium from electroplating effluent by chromate-reducing *Pseudomonas aeruginosa* A2Chr in two bioreactors. Appl. Microbiol. Biotechnol., 58: 416-420.

Hassen, A., Z. Jerboui, M. Chérif, N. Saidi, S. Gharbi and A. Boudabous, 2001. Impact of heavy metals on the selective phenotypical markers of *Pseudomonas aeruginosa.* Microb Ecol., 48(1): 99-107.

Hutchinson, T.C. and M.S. Symington, 1997. Persistence of metal stress in a forested ecosystem near Sudbury, 66 years after closure of the O'Donnell roast bed. J. Geochem. Explor., 58: 323-330.

Kimbrough, D.E., Y. Cohen, A.M. Winer, L. Creelam and C. Mabuni, 1999. A critical assessment of chromium in the environment. Crit. Rev. Environ. Sci. Technol., 29: 1-46.

Lee, S.E., J.U. Lee, H.T. Chon and J.S. Lee, 2008. Microbiological reduction of hexavalent chromium by indigenous chromium-resistant bacteria in sand column experiments. Environ. Geochem. Health, 30: 141-145.

Malik, A., 2004. Metal bioremediation through growing cells. Environ. Int., 30: 261-278

Margesin, R. and F. Schinner, 2001. Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an alpine glacier skiing area. Appl. Environ. Microbiol., 67: 3127-3133.

McLean, J. and T.J. Beveridge, 2001.Chromate reduction by a Pseudomonad isolated from a site contaminated with chromated copper arsenate. Appl. Environ. Microbiol., 67: 1076-1084.

Nies, A., D. Nies and S. Silver, 1990. Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from Alcaligenes eutrophus. J. Biol. Chem., 265: 5648-5653.

Novick, R.P. and C. Roth, 1968. Plasmid linked resistance to inorganic salts in Staphylococcus aureus. J. Bacteriol., 95: 1335-1342.

Ramteke, P.W., 1997. Plasmid mediated co-transfer of antibiotic resistance and heavy metal tolerance in coliforms. Ind. J. Microbiol. 37: 177-181.

Schottel, L., A. Mandal, D. Clark, S. Silver and R.W. Hedges, 1974. Volatilization of mercury and organomercurials determined by F factor system in enteric bacilli. Nature, 251: 335-337.

Shakoori, A.R., S. Tahseen and R.U. Haq, 1999. Chromium-tolerant bacteria isolated from industrial effluents and their use in detoxication of hexavalent chromium. Folia Microbiol., 44(1): 50-54.

Trajanovska, S., M.L. Britz and M. Bhave, 1997. Identification of lead resistant Bacteria from a heavily contaminated sites. Biodegradation, 8: 113-124.

Verma, T., T. Srinath, R.U. Gadpayle, P.W. Ramteke, R.K. Hans and S.K. Garg, 2001. Chromate tolerant bacteria isolated from tannery effluent. Bioresour. Technol., 78: 31-35.

Wang, Y., W. Xu, Y. Luo, L. Ma, Y. Li, S. Yang and K. Huang, 2009. Bioeffects of chromium (III) on the growth of *Spirulina platensis* and its biotransformation. J. Sci. Food Agric., 89(6): 947-952.

Zayed, A., S. Gowthaman and N. Terry, 1998. Phytoaccumulation of toxic trace elements by wetland plants: I. Duckweed (*Lemna minor* L.). J. Environ. Qual., 27: 715-721.

Zayed, A.M. and N. Terry, 2003. Chromium in the environment: factors affecting biological remediation. Plant Soil, 249: 139-156.