http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (9): 1496-1499, 2000
© Copyright by the Capricorn Publications, 2000

Selective Bioaccumulation of Mixed Heavy Metals by a Range of Brackish Water Bacteria

E. El-Bestawy
Department of Environmental Studies, Institute of Graduate Studies and Research, University of Alexandria, Alexandria, Egypt

Abstract: Heavy metals represent a significant source of pollution for the aquatic environment. The response of brackish water bacteria to increase concentrations of heavy metals were determined in seven bacterial species originally isolated from Lake Mariut, Alexandria, a heavily polluted lake. They were evaluated for their selectivity to accumulate a range of heavy metals including Fe, Zn, Cu, Pb, Ni and Co. Bacterial isolates identified as *Pseudomonas cepacia, Enterobacter agglomerans, E. coli, Staphylococcus aureus* and three *Bacillus* spp. *B. licheniformis, B. alvei* and *B. cereus.* They were gradually subjected to elevating levels of metals to evaluate their ability for metal bioaccumulation. The effect of heavy metals on bacterial growth was determined as % inhibition of the total viable count (TVC). The amount of accumulated metals (mg metal/g bacterial mass) was determined for each species every 24 hrs for 6 successive days. The selective uptake of metals depending on bacterial species as well as metal type where each species showed high affinity for at least two metals. This property could be efficiently used and manipulated in treatment processes for removing heavy metals. However, the tested bacteria showed more or less similar order of magnitude for metals uptake as Zn > Fe > Cr > Cu > Pb > Co > Ni which explained the toxicity of these metals with some exceptions.

Key words: Metals, selective, accumulation, bacteria, resistant

Introduction

Microorganisms mediate many important processes in the aquatic environment including self-purification from (different pollutants including heavy metals) and nutrient recycling. The development of heavy metals tolerance by the microbial community would allow the maintenance of these important processes despite the heavy metals inputs to the environment. Development of heavy metals tolerance in microorganisms can be done by different means, one of which is to subject the microorganisms to a gradual increase in metal concentration over a period of time (Sakamoto *et al*., 1989; Kai *et al*., 1995).

The removal and recovery of heavy metals from contaminated aqueous systems by microorganisms have several advantages: (1) microbial cells have a high metals adsorbing ability (2) they can absorb metals selectively (3) they produce microbial biomass inexpensively (4) both their growing and immobilized cells have the ability to accumulate metals (5) they pose no disposal problems and (6) they can accumulate metals very rapidly (Nakajima and Sakaguchi, 1986).

Microorganisms posses highly specific metal transport systems, thus they can selectively accumulate certain metals (Macaskie and Dean, 1989; Beveridge, 1989; Kim *et al*., 1995). Some microorganisms Survive the presence of elevating levels of heavy metals by employing detoxification mechanisms and developing tolerance (Timoney, 1989; Rouch *et al*., 1989; Schreiber *et al*., 1990). Examples of positive and negative effects of some of the investigated heavy metals on metabolic activities of microbial cells were discussed including Fe (Abeck *et al*., 1990; Nakajima and Sakaguchi, 1986; Paerl *et al*., 1994), Cu (Erardi *et al*., 1987; Harwood-Sears and Gordon, 1990; Collins *et al*., 1991), Zn and Ni (Fang and Hui, 1994) and Pb (Leborans *et al*., 1998).

The present study is a continuation of a series of studies on the bioremediation of heavy metals from contaminated effluents using naturally occurring bacteria isolated from brackish water. It was started by an investigation on the role of bacteria and phytoplankton in the cycling of pollutant metals in L. Mariut, Alexandria (El-Bestawy, 1993). This was followed by isolation and identification of bacteria with high ability for metals accumulation (Amer, 1997). In the present study, seven of the most promising bacterial species in metal uptake were selected. The main objective is to investigate their selectivity for metals uptake and to enhance their bioaccumulation capabilities by exposing them to a gradual increase in metal levels.

Materials and Methods

Microorganisms: Seven bacterial species originally isolated from heavily polluted brackish water of Lake Mariut, Alexandria were selected for this investigation. They were previously identified as *Bacillus cereus, B. alvei, B. licheniformis, Staphylococcus aureus, Pseudomonas cepacia, Enterobacter agglomerans* and *E. coli* (Amer, 1997). The selected bacterial proved to exhibit high ability for metals uptake.

Metals bioaccumulation assays: Metals bioaccumulation by the selected bacteria was determined at three elevating metal-concentrations (Table 1).

Stock solutions of the investigated metals (Fe, Zn, Cu, Co, Pb and Ni) were prepared from chloride salts in de-ionized water and sterilized by filtration through 0.22 millipore membrane filter. Each of the selected species was inoculated in 100 nutrient broth medium (NB), incubated at 37°C and agitated at 120°C rpm for 3 hrs to get into the early log phase of growth before the addition of metals. Metals were then added to bacterial cultures and incubated at the same growth condition. After 6-hr exposure, 10 ml samples of each bacterial cultures at the different metals levels in addition to control cultures were plated under aseptic conditions.

Bacterial cells were harvested by centrifugation at 8000 rpm, weighed and digested with concentrated nitric acid (AR grade) according to the Standard Methods for the examination of water and wastewater (Greenberg *et al*., 1995). Metals were then determined using atomic absorption spectrophotometer. The same procedure w as repeated after 18 hrs, then every 24 hrs for 5 successive days. For bacterial count samples including a control sample were taken, serially diluted and then

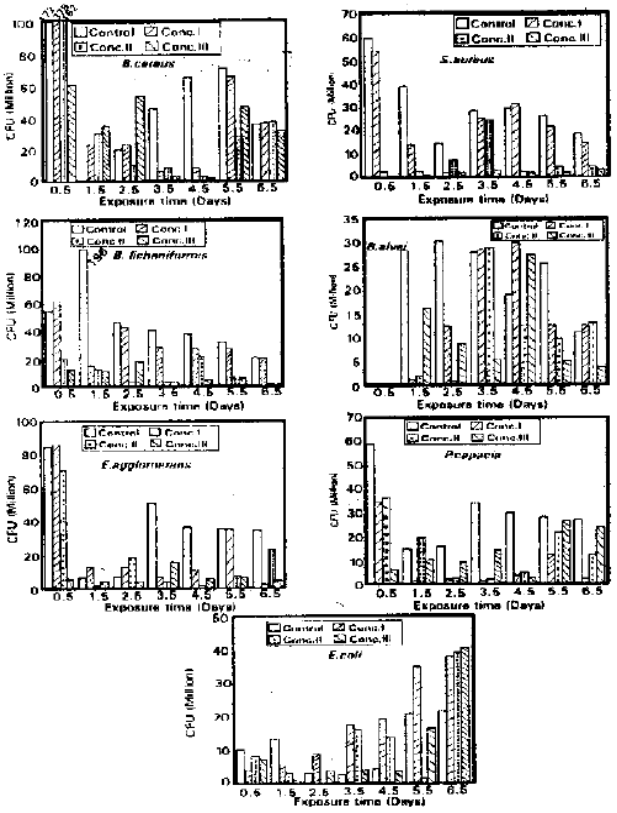

Fig. 1: Effect of mixed heavy metals on the growth of different bacterial species

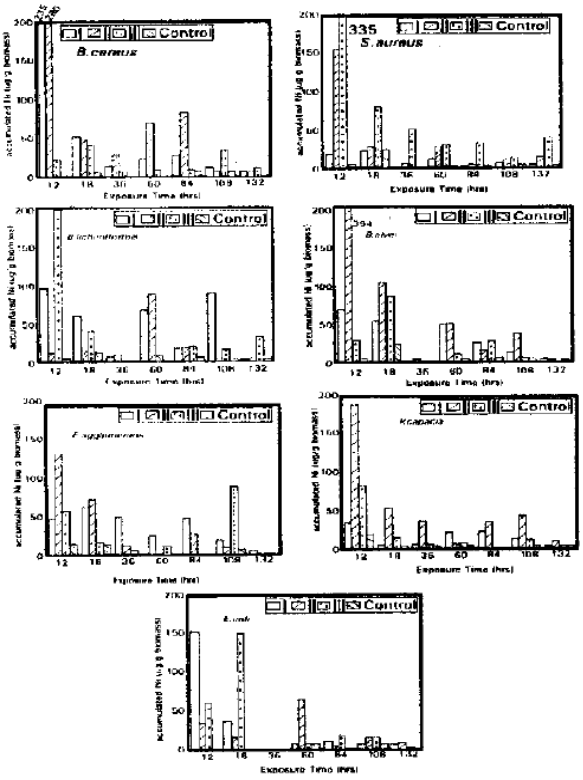

Fig. 2: Levels of Ni accumulated by different bacterial species

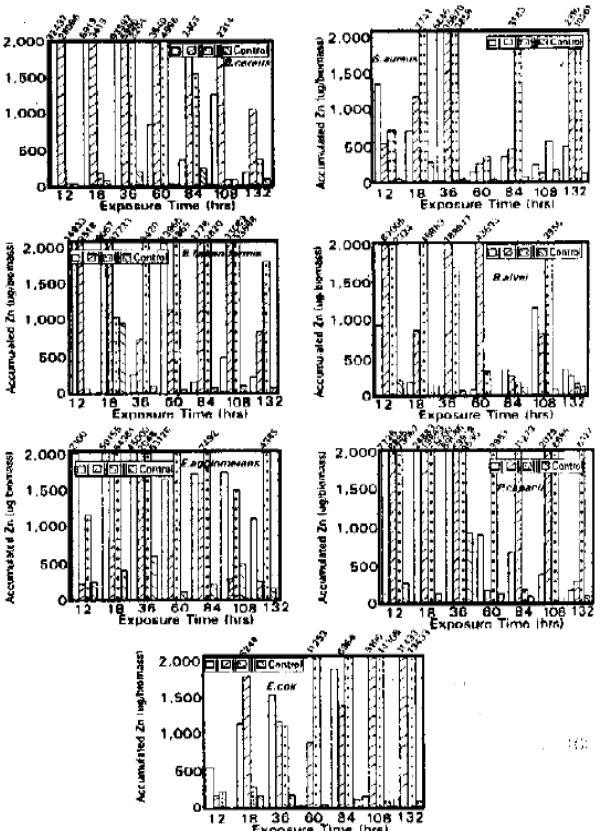

Fig. 3: Levels of Zn accumulated by different bacterial species

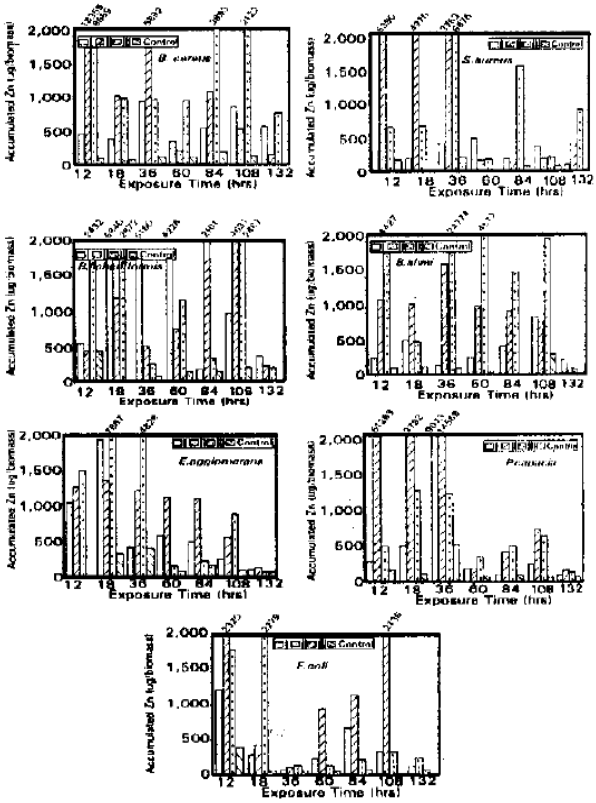

Fig. 4: Levels of Fe accumulated by different bacterial species

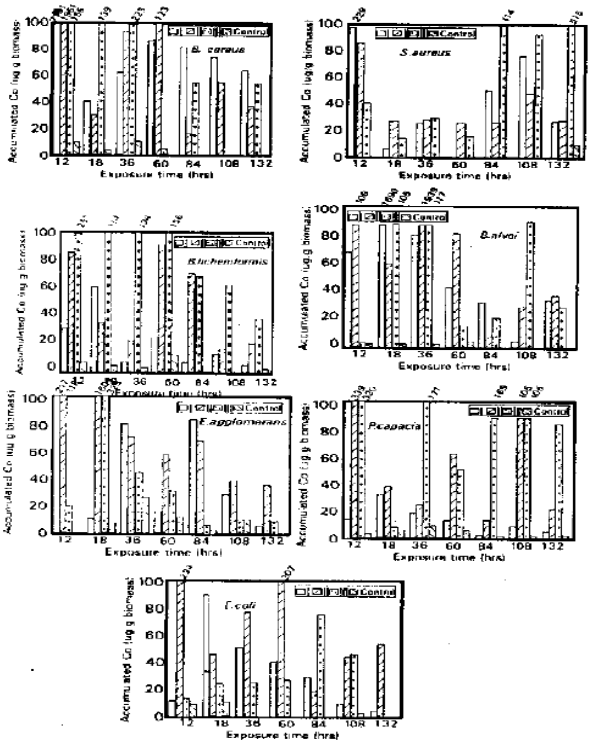

Fig. 5: Levels of Co accumulated by different bacterial species

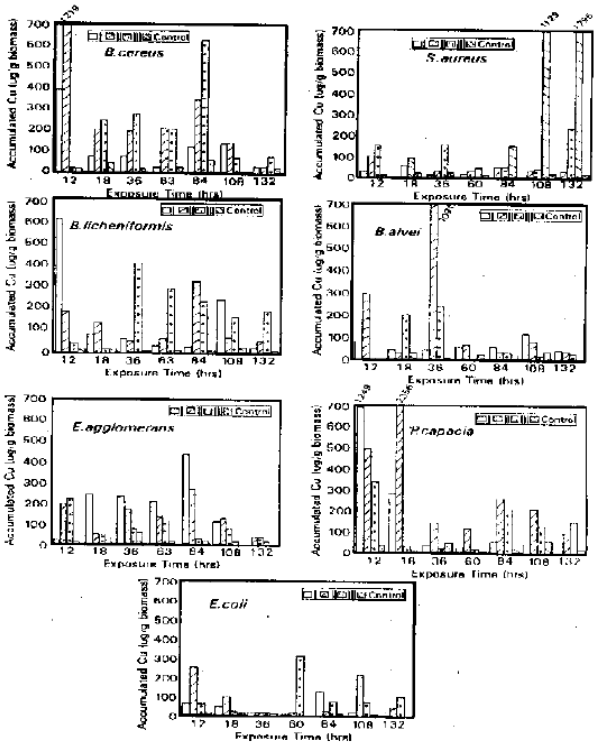

Fig. 7: Levels of Co accumulated by different bacterial species

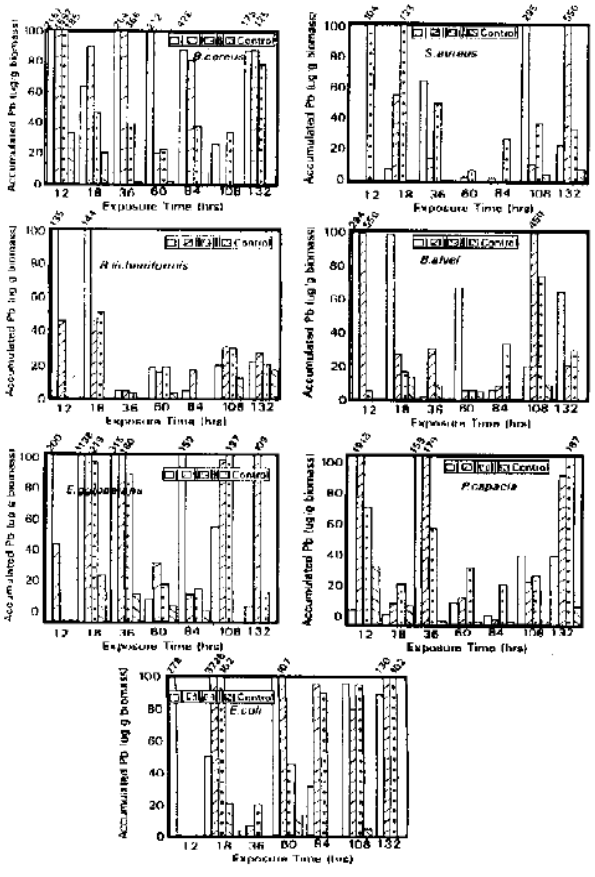

Fig. 6: Levels of Pb accumulated by different bacterial species

Table 1: Concentrations of heavy metals used in the bioaccumulation assays

Metal Conc.	Metals					
(mg/l)	Zn	Co	Fe	Pb	Ni	Cu
I	25.0	10.0	4.0	2.2	2.5	2.5
II	50.0	25.0	8.0	4.4	5.0	5.0
II	100.0	50.0	16.0	8.8	10.0	10.0

inoculated onto nutrient agar plates. Counting bacteria in each sample was carried out after 24 hr incubation at 37°C.

Results and Discussion

Effect of metals concentration on the growth of bacterial species under investigation: The response of bacterial growth to metals varied according to the type of bacteria and to the metals concentration in the growth medium. The general trend of bacterial growth regardless metal concentration agrees with the normal bacterial growth curve (Dean-Ross and Mills, 1989), with few exceptions regarding *Bacillus* species. However, *S. aureus* was affected by metals concentration in the medium, where its growth was considerably suppressed by increasing metal concentration, which is an indication of the toxic effect of metals in the mixture against *S. aureus.* Although, the same trend was recorded for *P. capacia* and *E. agglomorans,* it was noticed that *P. capacia* retained part of its capacity to grow in the presence of high metal concentration. This could be due to their the depletion of metals in the medium (Hughes and Poole, 1989), or to the acclimatization of the bacterial cells during the growth phases under a high concentration level of metals by different mechanisms such as, reducing metal uptake or a change in the intracellular distribution of ion within the cell (Joho *et al*., 1990; Podlesek *et al*., 1993).

However, *E. coli, B. alvei* and *B. cereus* showed tolerance to

the metals used in this study even at higher concentrations. This indicates that these bacterial strains are metal resistant species and they can consider to be used in removing metals from growth media containing such metals (Fig. 1).

Accumulation of metals by the tested bacteria: Results of Ni accumulation (Fig. 2) indicated that bacterial cells in their early growth stages can accumulate Ni to a certain level, after which Ni accumulation decreased by increasing the incubation time. This could be due to a selective property (Nakajima and Sakaguchi, 1986), or due to a certain resistant mechanism that allow the bacterial cells to alter Ni accumulation (Stoppel and Schlegel, 1995). The previous trend was true for most of the tested bacteria except *B. alvei* and *B. cereu.*

The results of Zn accumulation (Fig. 3), indicated that Zn is an essential element for bacterial growth at it is accumulated at higher levels compared to other elements. This is in agreement with other results obtained by several authors (Hughes and Poole, 1989; Nies, 1992; Podlesek *et al*., 1993). Similar to Zn, most of the tested bacterial strains accumulated Fe as Zn at higher concentration levels (Fig. 4). This is attributed to the well-known role of both Zn and Fe in the metabolic activity of the bacterial cells (Abeck *et al*., 1990) especially as cofactors in the enzymatic activities (Lim, 1989).

The tested bacteria also showed tolerant property against Co (Fig. 5). The high accumulation level of Co within the bacterial biomass, at almost all tested concentrations indicated this. This could be attributed to the ability of such bacteria to utilize the toxic Co and/or to a resistant mechanism enabled the bacterial cells to tolerate such high concentration levels of Co (Klerks and Levinton, 1993; Dean-Ross and Mills, 1989).

Accumulation of lead (Pb) was almost similar to Co, except with *B. licheniformis* at the late stages of bacterial growth and partially with *B. alvei* and *S. aereus* at the highest Pb concentration. This could be due to the release of the bound Pb from the sensitive cells, but on the other hand Pb will remain attached to or within resistant cells (Levinson *et al*., 1996).

The trend of Cu accumulation in all tested bacterial cells was very pronounced. The level of Cu. Accumulation was very low, except for *S. aereus* at the late growth stages of the cells at the highest metal concentration. This could be due to the adaptation of this strain when grain under high concentration level of Cu, then a turnover in the accumulation expression allowed the cells to accumulate Cu in a considerable amount that exceeded the levels accumulated in the early growth stages by more than 6-fold. However, the low accumulation of Cu in bacterial cells is expected, where the accumulation of such metal could lead to the mortality of the exposed cells due to the known toxic effect of copper metal to biota generally and in specific to certain microorganisms (Cooksey, 1990; 1993) (Fig. 6).

The previous results indicated that the selected bacterial strains can be used as a good biosorbent for a range of metals in a mixed medium. This could be due to either its tolerant to such metals or to a certain resistant mechanism expressed itself when the bacteria grew under high metal stress. By the way, the growing cells exhibited properties that allowed them to accumulate metals at high rate including the toxic elements. This is proved by the level of bacterial populations (upto 10^6 cfu/ml) that were recorded at when the bacterial cells grew at different elevating levels of metals concentration. On the other hand, the trend of metal accumulation could be due to certain selective property gained by those bacteria to accumulate specific metal without being antagonized regardless the toxicity of some of the tested metals (Fig. 7).

References

Abeck, D., A.P. Johnson, H.C. Korting, W. Stolz, E. Struck-Sauer and O. Braun-Falco, 1990. Effect of iron limitation on protein composition and ultrastructure of *Haemophilus ducreyi*. FEMS Microbiol. Lett., 68: 41-44.

Amer, A.A., 1997. Biotechnological study on mineral btoleaching from polluted aquatic media. M.Sc. Thesis, Alexandria University, Egypt.

Beveridge, T.J., 1989. Role of cellular design in bacterial metal accumulation and mineralization. Ann. Rev. Microbial., 43: 147-171.

Collins, M.L.P., L.A. Buchholz and C.C. Remsen, 1991. Effect of copper on *Methylomonas albus* BG8. Applied Environ. Microbiol., 57: 1261-1264.

Cooksey, D.A., 1990. Genetics of bactericide resistance in plant pathogenic bacteria. Ann. Rev. Phytopathol., 28: 201-219.

Cooksey, D.A., 1993. Copper uptake and resistance in bacteria. Mol. Microbiol., 7: 1-5.

Dean-Ross, D. and A.L. Mills, 1989. Bacterial community structure and function along a heavy metal gradient. Applied Environ. Microbiol., 55: 2002-2009.

El-Bestawy, E., 1993. Studies on the occurrence and distribution of pollutant metals in fresh water phytoplankton and bacteria m lake Mariut, Alexandria, Egypt. Ph.D. Thesis, Manchester University, UK.

Erardi, F.X., M.L. Failla and J.O. Falkinham, 1987. Plasmid-encoded copper resistance and precipitation by *Mycobacterium scrofulaceum*. Applied Environ. Microbiol., 53: 1951-1954.

Fang, H.H. and H.H. Hui, 1994. Effect of heavy metals on the methanogenic activity of starch-degrading granules. Biotechnol. Lett., 16: 1091-1096.

Greenberg, A.E., L.S. Clesceri and A.D. Eaton, 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edn., American Public Health Association, Washington, DC., USA.

Harwood-Sears, V. and A.S. Gordon, 1990. Copper-induced production of copper-binding supernatant proteins by the marine bacterium *Vibrio alginolyticus*. Applied Environ. Microbiol., 56: 1327-1332.

Hughes, M.N. and R.K. Poole, 1989. Metals and Microorganisms. Chapman and Hall, London, ISBN: 9780412244001, Pages: 412.

Joho, M., M. Inouhe, H. Tohoyama and T. Murayama, 1990. A possible role of histidine in a nickel resistant mechanism of *Saccharomyces cerevisiae*. FEMS Microbiol. Lett., 66: 333-338.

Kai, T., M. Nishi and T. Takahashi, 1995. Adaptation of *Thiobacillus ferrooxidans* to nickel ion and bacterial oxidation of nickel sulfide. Biotechnol. Lett., 17: 229-232.

Kim, Y.H., Y.J. Yoo and H.Y. Lee, 1995. Characteristics of lead adsorption by *Undaria pinnatifida*. Biotechnol. Lett., 17: 345-350.

Klerks, P.L. and J.S. Levinton, 1993. Genetic adaptation to heavy metalsun aquatic organisms: A r eview. Environ. Pollut., 45: 173-205.

Leborans, G.F., Y.O. Herrero and A. Novillo, 1998. Toxicity and bioaccumulation of lead in marine protozoa communities. Ecotoxicol. Environ. Safety, 39: 172-178.

Levinson, H.S., I. Mahler, P. Blackwelder and T. Hood, 1996. Lead resistance and sensitivity in *Staphylococcus aureus*. FEMS Microbiol. Lett., 145: 421-425.

Lim, D.V., 1989. Microbiology. West Publishing Co., St. Paul, MN., USA.

Macaskie, L.A. and A.C.R. Dean, 1989. A heavy metal accumulating *Citrobacter* sp. Is unable to accumulate cadmium in the presence of organa-lead. Biotechnol. Lett., 17: 345-350.

Nakajima, A. and T. Sakaguchi, 1986. Selective accumulation of heavy metals by microorganisms. Applied Microbiol. Biotechnol., 24: 59-64.

Nies, D.H., 1992. Resistance to cadmium, cobalt, zinc and nickel in microbes. Plasmid, 27: 17-28.

Paerl, H.W., L.E. Prufert-Bebout and C. Guo, 1994. Iron-stimulated N2 fixation and growth in natural and cultured populations of the planktonic marine cyanobacteria *Trichodesmium* spp. Applied Environ. Microbiol., 60: 1044-1047.

Podlesek, Z., B. Herzog and J. Abroie, 1993. Inducible res1stance to zinc ions m *Bacillus subtitlis*. FEMS Microbial. Lett., 113: 201-204.

Rouch, D.R., B.T. Lee and J. Camakaris, 1989. Genetic and Molecular Bass of Copper Resistance in *Escherichia coli*. In: Metal Ion Homeostasis: Molecular Biology, Hamer, D.H., O.R. Winge and R. Alan (Eds.). Liss Inc., New York, pp: 439-446.

Sakamoto, K., M. Yagasaki, K. Kirimura and S. Usami, 1989. Resistance acquisition of *Thiobacillus thiooxidans* upon cadmium and zinc ion addition and formation of cadmium ion-binding and zinc ion-binding proteins exhibiting metallothionein-like properties. J. Fermentation Bioeng., 67: 266-273.

Schreiber, D.R., F.J. Millero and A.S. Gordon, 1990. Production of an extracellular copper-binding compound by the heterotrophic marine bacterium *Vibrio alginolyticus*. Mar. Chem., 28: 275-284.

Stoppel, R.D. and H.G. Schlegel, 1995. Nickel-resistant bacteria from anthropogenically nickel-polluted and naturally nickel-percolated ecosystems. Applied Environ. Microbiol., 61: 2276-2285.

Timoney, 1998. Survey of metal tolerance in moderately halophilic bacteria. Applied Envirn. Microbiol., 55: 2385-2390.