International Journal of Advanced Multidisciplinary Research (IJAMR)
ISSN: 2393-8870
www.ijarm.com

Research Article

A Study on degradation and characterization of heavy metals in industrial effluents waste using *Pseudomonas* sp. isolated from soil samples

P. Manikandaraja and R. Senthilkumaran

Department of Microbiology, King Nandhivarman College of Arts and Science, Thellar, Tamil Nadu, India
Corresponding Author: *rsenthilsurya@gmail.com*

Keywords

Heavy metal,
Pseudomonas sp ,
plating techniques,
Zinc, copper and Nickel,
biosorption studies

Abstract

The present investigation was carried out to study the removal of heavy metal by *Pseudomonas* sp. Isolated from garden soil samples by employing plating techniques. The isolated bacterial members were identified by Gram's staining, motility, IMViC and sugar fermentation methods. The isolated bacterial members were used as inoculants for heavy metal removal treatments. Biosorption of three heavy metals namely Zinc, copper and Nickel were conducted using individual cultures of *Pseudomonas* sp. The Zinc biosorption studies using individual cultures of *Pseudomonas* sp. resulted 99.3% of sorption at pH 5, temperature 32oC and biomass concentration of 0.5 mg/ml in 50 minutes period of contact time.

Introduction

Metals are extensively used in several industries, including mining, metallurgical, electronic, electroplating and metal finishing. The presence of metal ions in final industrial effluents is extremely undesirable, as they are toxic to both lower and higher organisms. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage (Jefferies and Firestone, 1984). Of the important metals, Mercury, lead, cadmium, Arsenic and Chromium (VI) are regarded as toxic; whereas, others, such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing levels in the environment are of serious concerns (Brown and Absanullah, 1971; Moore, 1990; Volesky, 1990). Various techniques have been employed for the treatment of metal bearing industrial effluents, which usually include precipitation, adsorption, ion exchange, membrane and electrochemical technologies but these techniques are expensive, not environment friendly and usually dependent on the concentration of the waste which are ineffective in very diluted solutions. Therefore, the search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated. It was only in the 1990s that a new scientific area developed that could help to recover heavy metals and it was bioremediation.

Heavy metals are defined as metals with a specific weight usually more than 5.0 G/cm3, which is five times higher than water. The toxicity of heavy metals occurs even in low concentrations of about 1.0-10 mg/L. Of the 90 naturally occurring elements, 21 are non-metals, 16 are light-metals and the remaining 53 (with as included) are heavy metals. Certain metals have been known to be toxic for centuries. For example, Theophrastus of Erebus (370-287 B.C.) and Pliny the Elder (23-79) both described poisonings that resulted from Arsenic and Mercury. Other heavy-metals, such as cadmium were not recognized as poisonous until the early nineteenth century (Young, 2000).

Non living cells of microorganisms has been found to be highly promoting as these can be regenerated and reused for a number of times . Biomasses of bacteria such as *Pseudomonas aeruginosa* PU21 . *Bacillus cereus*. *Enterobacter* spJI, *Pseudomonas putida*. *Geobacillus thermodenitrificans* have been found to show high absorption capacities of lead. The cell walls of the gram-negative bacterial species have high content of potentially active chemisorption sites such as peptidoglycan, lipopolysaccharide and protein. Limited information is available on the lead removal by the two gram-negative bacterial species viz., *Pseudomonas oleovorans* (*P. oleovorans*) and *Brevundimonas vesicularis* (*B. vesicularis*). Only in case of the latter species such study has been undertaken and that too without pretreatment and under different conditions. It has been found by some studies that the pretreatment of microbial biomasses by some chemicals such as NaOH, Na2CO3, HCl enhance the efficiency of

biosorption. Therefore, it was thought to study the biosorption of Pb (II) ions by two bacterial species, *P. oleovorans* and *B. vesicularis* after their pretreatment with 0.1N NaOH, then, to use the determined optimum conditions for the removal of Pb (II) ions from wastewater samples and to propose the mechanism of its biosorption on the basis of 1HNMR studies.

Pseudomonas have very simple nutritional requirement and grow chemo organotrophically at neutral pH (Madigan *et al*., 2009) as also established in this work. They are Gram negative, no gas formed when glucose is fermented and oxidase positive (which help in distinguishing them from enteric bacteria) and finally they are motile with a help of a flagella; single or multiple (Madigan *et al*., 2009). These characteristics agree with the biochemical tests that help distinguish the isolates from each other . The bacteria isolated and identified in this work are *Chryseobacterium indologenes, Klebsiella oxytoca, Pasteuralla pneumotropica, Enterobacter cloacae, Proteus mirabilis, Klebsiella ornothinolytica, Pseudomonas aeruginosa, Chryseobacterium meningosepticum, Chryseomonal luteola, Photobacterium clamsela, Enterobacter sakazakii, Acinotobacter baumannii, Serratia liquefaciens,* and *Citrobacter koseri*. It is clear that the isolates found in this study are metal resistant. Previous research has indicated that heavy metal resistance of *P. aeruginosa* can be used to exploit for cleaning up industrial wastewater and bioremediation of heavy metal contaminated soil (Raja and Selvam, 2009).

Materials and Methods

Soil Sample Collection

The soil samples were collected from three different sampling stations. They are 1. Garden soil 2. Agriculture soil and 3. Garden soil (Pudhupet**)**

Isolation of bacteria from soil samples by Serial dilution methods

Bacteriological methods

All the soil samples collected in sterile polythene bags and used for isolation of bacteria .

Isolation method of bacteria

Serial dilution

The samples were diluted with different dilution such as 10^{-1}, 10^{-2}, 10^{-3},10^{-4},10^{-5}, 10^{-6}, 10^{-7}

Spread plate technique

0.1 ml of samples were spread on Citrimide agar medium and Nutrient agar and incubated at 37^{o}C for 18 to 24 hours

Colonies

Green color colonies were appeared on Nutrient agar and Citrimide medium. The isolated cultures were then purified by repeated streaking in Nutrient agar and maintained in Nutrient agar slants. The pure cultures were presumptively identified as *Pseudomonas* sp. using Grams staining.

Microbial strains

In the present study four microbial species were used: *Pseudomonas* spp., .The microbes were isolated from the soil and sludge by serial dilution and pour plating method. Strains were maintained in agar plate containing nutrient broth. They were characterized morphologically and on the basis of biochemical reactions .They were transferred weekly to new medium in order to keep metabolic activity and checked for purity by microscopic examination.

Identification of *Pseudomonas* species

Presumptive *Pseudomonas* sp. colonies were then subjected to Gram ‘s staining and a series of biochemical tests such as urease test, indole production, methyl red test, Voges Proskauer test, and cultures which matched typical reaction of standard *Pseudomonas* sp were confirmed as *Pseudomonas* species.

Waste water used

Totally three waste water samples were collected from 3 different areas like Rane NSK, Apollo tyres and Renolds Nisan company.

Removal of heavy metal by Biosorbent method.

Biosorbent preparation

1000 ml of Nutrient medium was prepared with standard composition in a conical flask. The pH for the medium was adjusted accordingly and then the media was sterilized at 15 lb/in2 pressure and 121º C for 30 minutes. Nutrient agar medium Himedia was prepared, autoclaved and allowed to cool. Loop full of bacterial culture was taken and streaked on the agar plate to obtain more colonies. They are later transferred to nutrient broth and grown on specific media (Cetrimide medium-*Pseudomonas* sp.) for subculture. 100 ml of sterilized culture media was transferred to 250 ml Erlenmeyer flask.

The media was allowed to cool and then the 100Yl microbial solution was inoculated into the medium in laminar air flow chamber. The inoculated flasks were incubated in an orbital shaker (Metrex scientific instruments, India) at 250 rpm at 32 0C for 2 days to obtain the biomass. Mixed cultures were

prepared by adding equal amounts of individual cultures. Biomass was harvested from the medium by centrifugation at 9000 rpm for 10 min. The supernatant was discarded and the cells were re-suspended in purified water for washing and again centrifuged as above to make sure that no media remain on the cell surface . This biomass was used for the sorption experiments. Both the biomasses were added in equal amounts for sorption experiments with mixed culture.

Biosorption experiment

Different concentrations of biomass (single cultures) were combined with 100 ml of metal solution in 250 ml Erlenmeyer flask. The flasks were placed on a shaker with a constant speed of 150 rpm and left to equilibrate. Samples were collected at predefined time intervals, centrifuged as above and the amount of metal in the supernatant was determined.

Effect of various parameters on heavy metal removal

Biosorption studies were done using biomass as a function of various parameters such as

a) pH
b) Biomass concentration
c) Temperature
d) Time
e) Initial metal concentration

Effect of pH

The metal sorption monitored for pH range 7.06 NaOH and HCL were used as pH regulators. 1 mg/ml biomass was dispersed in 100 ml of the solution containing 10mg/L of each metal concentration. All flasks were maintained at different pH values 7.06 for about 12 hours. Solutions were centrifuged as above and the supematant was analysed for the residual concentrations of the metal ions. The final pH values have been plotted.

Effect of biomass concentration

Biomass was centrifuged at 9000 rpm and different weights of the biomass range is 0.5.mg/ml were dispersed in solutions containing the 10 mg/L metal concentration. The solutions were adjusted to the optimum pH in which maximum biosorption of the metal ion occurred. Flasks were left for equilibration. The solutions were later centrifuged at 9000 rpm and the metal ion concentrations were determined using the procedures described earlier.

Effect of temperature

Optimum biomass concentration with optimum pH was used to monitor the temperature effect on biosorption. Experiments were carried out at different temperatures is 31.2oC for each culture and kept on rotary shaker at 240 rpm. The samples were allowed to attain equilibrium. The sample collected at regular intervals as above and analyzed for metal concentration.

Effect of time

The cell pellet dispersed in metal solution of 10 mg/L concentration with a working volume of 100 ml. the experiment was carried out at the optimum pH system. Flasks were allowed to attain equilibrium on rotary shaker at 240 rpm and samples were collected at regular time intervals. Centrifugation at 9000 rpm was done and the supernatant was analysed for the residual metal content.

Effect of initial metal concentration

Biosorption experiments were conducted by taking different initial metal concentrations by fixing all the parameters such as biomass concentration, pH, temperature and time. Metal solutions were prepared as stated in section 3.21. With increase in metal concentration (5 mg/L) percentage biosorption was observed.

Results and Discussion

The present study was initiated to assess the removal of heavy metals in industrial effluents by *Pseudomonas* sp. Isolated from soil samples by plating technique.

Biochemical tests for identification of *Pseudomonas* sp.

The isolates from the soil samples showed positive results for indole test, citrate test, catalse test, oxidase test, sugar fermentation tests and TSI tests .It showed negative results to MR -VP test and Nitrate test and coagulase test .It suggests that the isolates are *Pseudomonas* sp.

The results in Triple sugar iron test is the colour change to yellow and yellow in both slant butt region .Sugar fermentation test showed the result from Acid production without gas production .From the positive result of oxidase and catalase test ,it is clearly found that the 3 isolates are *Pseudomonas*. (Table.1).

Bio- sorbent methods – Pseudomans sp. 1

Rane NSK Waste water treated with *Pseudomonas* sp.1

The initial value of Zinc, copper and Nickel were (1.5039, 0.014, 0.010), it was reduced under treatment with bacterial culture (0.4495, 0.008, 0.005) at concentrations of 300 mg than 150 mg of culture pellet samples (Table.2).

Apollo tyres- Waste water treated with *Pseudomonas* sp.1

The initial value of Zinc, copper and Nickel were (0.1216, 0.014, 0.010), it was reduced under treatment with bacterial culture (0.552, 0.010, 0.010) at concentrations of 300 mg than 150 mg of culture pellet samples (Table.2).

Renold Nisan- Waste water treated with *Pseudomonas* sp.1

The initial value of Zinc, copper and Nickel were (0.1769, 0.086, 0.010), it was reduced under treatment with bacterial culture (0.0641, 0.068, 0.005) at concentrations of 300 mg than 150 mg of culture pellet samples (Table.2).

Bio- sorbent methods – Pseudomans sp. 2

Rane NSK Waste water treated with *Pseudomonas* sp.2

The initial value of Zinc, copper and Nickel were (1.5039, 0.014, 0.010), it was reduced under treatment with bacterial culture (0.9034, 0.003, 0.007) at concentrations of 300 mg than 150 mg of culture pellet samples (Table.3).

Apollo tyres- Waste water treated with *Pseudomonas* sp.2

The initial value of Zinc, copper and Nickel were (1.5039, 0.014, 0.010), it was reduced under treatment with bacterial culture (0.0293, 0.008, 0.010) at concentrations of 300 mg than 150 mg of culture pellet samples (Table.3).

Renold Nisan- Waste water treated with *Pseudomonas* sp.2

The initial value of Zinc, copper and Nickel were (1.5039, 0.014, 0.010), it was reduced under treatment with bacterial culture (0.0178, 0.006, 0.007 at concentrations of 300 mg than 150 mg of culture pellet samples (Table.3).

Bio- sorbent methods – Pseudomans sp. 3

Rane NSK Waste water treated with *Pseudomonas* sp.3

The initial value of Zinc, copper and Nickel were (1.5039, 0.014, 0.010), it was reduced under treatment with bacterial culture (0.4071, 0.004, 0.004) at concentrations of 300 mg than 150 mg of culture pellet samples (Table.4).

Apollo tyres- Waste water treated with *Pseudomonas* sp.3

The initial value of Zinc, copper and Nickel were (1.5039, 0.014, 0.010), it was reduced under treatment with bacterial culture (0.0234, 0.008, 0.010) at concentrations of 300 mg than 150 mg of culture pellet samples (Table.4).

Renold Nisan- Waste water treated with *Pseudomonas* sp.3

The initial value of Zinc, copper and Nickel were (1.5039, 0.014, 0.010), it was reduced under treatment with bacterial culture (0.0474, 0.0012, 0.004) at concentrations of 300 mg than 150 mg of culture pellet samples (Table.4).

Cell walls of bacteria are principally composed of peptidoglycans which consist of linear chains of the disaccharide N-acetylglucosamine-β 1,4-Nacetylmuramic acid withpeptide chains. Cell walls of gram negative bacteria are somewhat thinner than the gram positive ones and are also not heavily cross-linked. They have an outer membrane which is composed of an outer layer of lipopolysaccharide (LPS), phospholipids and proteins (Remacle 1990). Biosorption mainly involves a) cell surface complexation, b) ionexchange or affinity and c) micro precipitation. Different microbes have been found to vary in their affinity for different heavy metal(s) and hence differ in their metal binding capacities. Some biomass (es) exhibit preference for certain heavy metal(s) whereas others do not show any specific binding and are broad range (Gupta, et al., 2000).

Among the mechanisms ion affinity dominated due to higher charge density of Chromium (VI). When it comes to Zinc the affinity was towards gram negative Pseudomonas aeruginosa. Here the major role is played by cell surface complexation mechanism than ion exchange or affinity. This may be due to the lower charge density of Mercury when compared to Chromium. Gram positve bacteria normally show low levels of surface complexation due to heavily cross linked peptidoglycan layer (Gupta, *et al.*, 2000). Where as in gram negaitve bacteria most of their lipopolysaccharide (LPS), phospholipids and proteins are exposed on the cell surface and involves in cell surface complexation.

The lower percentage in sorption is due to negative charge (Čerňansky et al., 2007). It is showing its affinity towards gram negative Pseudomonas because the surface of was containing higher levels of carboxylic groups which repel this anion.

Effect of various parameters on heavy metal removal

Biosorption studies using attenuated cells of *Pseudomonas* sp. In the investigation carried out so far, the attenuated cells of *Pseudomonas* sp. were used for the biosorption of Zinc. The parameters influencing the biosorption of Mercury using this single culture of *Pseudomonas* sp. are studied. Futher more the effects of these parameters are discussed below:

Effect of pH

The most important single parameter influencing the sorption capacity is the pH of the adsorption medium. (Goyal *et al.*, 2003). The sorption increasedto 98% at 5 and significantly decreased with increase of pH (Table.5). From this study we can conclude that at pH 5 for *Pseudomonas* sp. maximum percent of biosorption occured. The fluctuation beyond this optimum pH 5 was due to decrease of low availability of

Table: 1 Biochemical test for identification of *Pseudomonas* sp.

S.No	Staining /Bio-chemical tests	*Pseudomonas* sp.1	*Pseudomonas* sp.2	*Pseudomonas* sp.3
1	Gram staining	G (-)	G(-)	G(-)
2	Motility	-	-	-
3	Indole test	+	+	+
4	Methyl red test	-	-	-
5	VP test	-	-	-
6	Citrate test	+	+	+
7	TSI test	+	+	+
8	Oxidase test	+	+	+
9	Catalase test	+	+	+
10	Sugar fermentation tests	+	+	+
11	Nitrate test	-	-	-

Table:2. Heavy metal removal by *Pseudomonas* sp. 1 in all the three collected effluent samples

Rane NSK effluent sample

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	1.5039	0.5544	0.4495
2	Cu	0.014	0.010	0.008
3	Ni	0.010	0.006	0.005

Apollo tyres effluent samples

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	0.1216	0.262	0.552
2	Cu	0.014	0.012	0.010
3	Ni	0.010	BDL	BDL

Renold Nisan effluent samples

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	0.1769	0.0652	0.0641
2	Cu	0.186	0.126	0.089
3	Ni	0.224	0.187	0.089

Table:3. Heavy metal removal by Pseudomonas sp.2 in all the three collected effluent samples

Rane NSK effluent sample

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	1.5039	0.8324	0.9034
2	Cu	0.014	0.008	0.003
3	Ni	0.010	0.008	0.007

Apollo tyres effluent samples

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	0.1216	0.0349	0.0293
2	Cu	0.014	0.010	0.008
3	Ni	0.010	BDL	BDL

Renold Nisan effluent samples

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	0.1769	0.0676	0.0178
2	Cu	0.186	0.096	0.089
3	Ni	0.224	0.184	0.134

Table: 4. Heavy metal removal by *Pseudomonas* sp. 3 in all the three collected effluent samples

Rane NSK effluent sample

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	1.5039	0.4097	0.4271
2	Cu	0.014		
3	Ni	0.010	0.069	0.114

Apollo tyres effluent samples

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	0.1216	0.0339	0.0234
2	Cu	0.014		
3	Ni	0.010	BDL	BDL

Renold Nisan effluent samples

S.No	Name of the metals	Initial value	150 mg of Culture pellet	300 mg of culture pellet
1	Zn	0.1769	0.2347	0.2474
2	Cu	0.186	0.2	0.18
3	Ni	0.224	0.178	0.155

Table.5. Effect of pH on heavy metal removal by Pseudomonas sp.

	Water Sample 1			Water Sample 2			Water sample3		
	Initial Value	150mg	300mg	Initial Value	150mg	300mg	Initial Value	150mg	300mg
Culture Sample 1	1.5039	1.232	0.856	0.1216	0.1098	0.834	0.1769	0.998	0.875
Culture Sample 2	1.5039	0.956	0.657	0.1216	0.0987	0.9234	0.1769	0.1345	0.1209
Culture Sample 3	1.5039	0.9082	0.7768	0.1216	0.1056	0.8965	0.1769	0.1345	0.1098

Table .6. Effect of Temperature on heavy metal removal by *Pseudomonas* sp.

	Water Sample 1			Water Sample 2			Water sample 3		
	Initial Value	**150mg**	**300mg**	**Initial Value**	**150mg**	**300mg**	**Initial Value**	**150mg**	**300mg**
Culture Sample 1	1.5039	1.209	1.002	0.1216	0.1096	0.1011	0.1769	0.1021	0.9045
Culture Sample 2	1.5039	1.1956	0.992	0.1216	0.0923	0.875	0.1769	0.1024	0.934
Culture Sample 3	1.5039	0.9987	0.845	0.1216	0.1043	0.923	0.1769	0.990	0.456

Table.7 . Effect of biomass concentrations on heavy metal removal by *Pseudomonas* sp.

	Water Sample 1			Water Sample 2			Water sample 3		
	Initial Value	**150mg**	**300mg**	**Initial Value**	**150mg**	**300mg**	**Initial Value**	**150mg**	**300mg**
Culture Sample 1	1.5039	1.109	0.908	0.1216	0.1023	0.985	0.1769	0.1067	0.9034
Culture Sample 2	1.5039	1.1045	0.834	0.1216	0.1092	0.875	0.1769	0.1024	0.1011
Culture Sample 3	1.5039	0.934	0.568	0.1216	0.9012	0.576	0.1769	0.908	0.903

Table .8. Effect of Time on heavy metal removal by *Pseudomonas* sp.

[

	Water Sample 1			Water Sample 2			Water sample 3		
	Initial Value	**150mg**	**300mg**	**Initial Value**	**150mg**	**300mg**	**Initial Value**	**150mg**	**300mg**
Culture Sample 1	1.5039	1.302	0.9012	0.1216	0.1023	0.9021	0.1769	0.1236	0.1021
Culture Sample 2	1.5039	1.0923	0.892	0.1216	0.1011	0.1021	0.1769	0.1045	0.0940
Culture Sample 3	1.5039	1.0234	0.9023	0.1216	0.1021	0.1019	0.1769	0.1024	0.0912

Table .9 . Effect of initial metal concentrations on heavy metal removal by *Pseudomonas* sp.

	Water Sample 1			Water Sample 2			Water sample 3		
	Initial Value	**150mg**	**300mg**	**Initial Value**	**150mg**	**300mg**	**Initial Value**	**150mg**	**300mg**
Culture Sample 1	1.5039	1.2098	0.809	0.1216	0.1123	0.1012	0.1769	0.1056	0.1012
Culture Sample 2	1.5039	1.0987	1.0456	0.1216	0.1043	0.1011	0.1769	0.1089	0.0934
Culture Sample 3	1.5039	1.102	1.034	0.1216	0.1011	0.998	0.1769	0.1087	0.0897

surface for sorption at low pH and formation of metal hydroxide and other metal-ligand complexes significantly reduce the amount of metal ions sorbed at high pH (Vijayaraghavan and Yun, 2008).

Effect of biomass concentration

To achieve the maximum biosorption capacity of the biosorbent for Zinc, the biomass concentration was 0.5 mg/ml and it was found that a concentration of 0.5 mg/ml was adequate for maximum percentage of Zinc (Table.7). This may be due to the unavailability of binding sites to the metal and also due to the blockage of binding sites with excess biomass. In this study it was observed that at 0.5 mg/ml concentration showed highest sorption percentages (Vijayaraghavan and Yun, 2008).

Effect of temperature

In the studies of biosorption using attenuated cells of *Pseudomonas* sp. it was observed that the temperature range 32º C was found to be favorable than that of the lower or higher temperatures. Maximum sorption of around 98% was seen at 32ºC. In these experiments there was an increase in sorption percentage with increase in the temperature till 32ºC (Table.6). A gradual decrease in sorption percentage was observed after that. This is because of the shrinkage of cells at higher and lower temperatures which reduces the surface area of contact (Vijayaraghavan and Yun, 2008). From this we can conclude that the temperature 34ºC was favorable for biosorption of Mercury using *Pseudomonas* sp.

Effect of contact time

The adsorption experiments of Zinc were carried out for different contact times with a fixed adsorbent dose of 0.5 mg/ml concentration at pH 5 at 32ºC. The results showed that indicate the maximum sorption attained at 60 min for Zinc (Table.8).

Effect of initial metal concentrations

The adsorption experiments of Zinc were carried out for different contact times with a fixed adsorbent dose of 5 mg/ml concentration at pH 5 at 32ºC. The results showed that indicate the maximum sorption attained at 5mg/ml for Zinc (Table.9).

References

Addour, L; Belhocine, D; Boudries, N; Comeau, Y; Pauss, A and Mameri, N. (1999).“Zinc Uptake by *Streptomyces rimosus* Biomass Using A Packed-Bed Column”, J Chem Technol Biotechnol., 1999; 74; 1089-1095.

Aderhold, D; Williams, CJ and Edyvean, RGJ. “The removal of heavy-metal ions by seaweeds and their derivatives”, Bioresource Technology., 1996; 58; 1-6.

Agency for Toxic Substances and Disease Registry (ATSDR), Department of Health and Human Services, 1999.

Ahalya, N; Ramachandra, TV and Kanamadi, RD. “Biosorption of heavy metals”, Res. J. Chem. Environ., 2003; 7; 71-78.

Aksu, Z. “The biosorption of copper (II) by C. vulgaris and Zramigera”, Environ Technol., 1992; 13; 579-586.

Asku, Z; Kutsal, T; Gun, S; Haciosmanoglu, N. and Gholminejad, M. “Investigation of biosorption of Cu(II), Ni(II), and Cr(VI) ions to activated sludge bacteria”,Environmental Technolog., 1991;12; 915-921.

Baes, CF; Mesmer, RE. “The Hydrolysis of Cations”. New York: John Wiley and Sons;1976; 241–310.

Baral, SS; Das, SN and Rath, P. “Hexavalent Chromium removal from aqueous solution by adsorption on treated sawdust”, Biochem.Eng. J., 2006; 13; 216–222.

Beveridge, TJ; Murray, RGE. “Uptake and retention of metals by cell walls of Bacillus subtilis”, J Bacteriol., 1976; 127; 1502–1518.

Boyle, RW and Jonasson, IR. “The geochemistry of Arsenic and its use as an indicator element in geochemical prospecting”, Journal of Geochemical Exploration., 1973; 2; 251–296.

Brown, B and Absanullah, M. “Effects of heavy metals on mortality and growth”, Mar Pollut Bull., 1971; 2; 182–187.

Chang, CY; Tsai, WT; Ing, CH and Chang, CF. “Adsorption of polyethylene glycol (PEG) from aqueous solution onto hydrophobic zeolite”, J. Colloid Interface Sci., 2003; 260; 273-279.

Cossich, ES; Tavares, CRG and Ravagnani, TMK. “Biosorption of Chromium(III) by *Sargassum* sp. Biomass”, Electronic Journal of Biotechnology., 2002; 5;133-141.

Costa, ACA and Leite, SGF. “Metals Biosorption by sodium alginate immobilized *Chlarella homosphaera* cells”, Biotechnol Left., 1991; 13; 55-562.

Doyle, RJ; Matthews, TH; Streips, UN. “Chemical basis for selectivity of metal ions by the *Bacillus subtilis* cell wall”, J Bacteriol., 1980; 143; 471–480.

Ercole, C; Veglio, F; Toro, L; Ficara, G and Lepidi, A. “Immobilisation of microbial cells for metal adsorption and desorption”. In: Mineral Bioprocessing II. Snowboard. Utah 1994.

Florence, TM. “Trace Element Speciation in Biological Systems”. In: Batley, G. E. ed. Trace Element Speciation: Analytical Methods and Problems. Boca Raton, Florida: CRC Press, 1989; 319-338.

Gadd, GM. “Heavy metal and radionuclide by fungi and yeasts”. In: P.R. Norris and D.P. Kelly (Editors), Biohydrometallurgy. A. Rowe, Chippenham, Wilts., U.K., 1988.

Gadd, GM. “Metal and Microorganisms: A Problem of Definition”, FEMS Microbiology Letters., 1992; 100; 197-204.

Golab, Z; Breitenbach, M. “Sites of copper binding in *Streptomyces pilosus*”, Water Air Soil Pollut., 1995; 82; 713–21.

Goldwater, LJ. “Mercury in the environment”, Sci. Am., 1971; 224; 15-21.

Goyal, N; Jain, SC. and Banerjee, UC. “Comparative studies on the microbial adsorption of heavy metals”, Advances in Environmental Research., 2003; 7; 311-319.

Gupta, R; Ahuja, P; Khan, S; Saxena, RK and Mohapatra, H. “Microbial biosorbents: meeting challenges of heavy metal pollution in aqueous solutions”, Current Science., 2000; 78; 967–973.

Hammaini, A. “Simultaneous uptake of metals by activated sludge”, Minerals Eng., 2003; 16; 723-729.

Hoyle, B and Beveridge, T J. “Binding of metallic ions to the outer membrane of *Escherichia coli*”, Appl. Environ. Microbiol., 1983; 46; 749–752.

Huang, CP; Huang, C and Morehart, AL. “The removal of Cu(II) from dilute aqueous solutions by *Saccharomyces cerevisiae*”, Water Research., 1990; 24, 433–439.

Iqbal, M and Saeed, A. “Production of an immobilized hybrid biosorbent for the sorption of Ni (II) from aqueous solution”, Process Biochem., 2007; 42; 148–157.

Jalali, R; Ghafourian, H; Asef, Y; Davarpanah, SJ. and Sepehr, S. “Removal and recovery of lead using non living biomass of marine algae”, Journal of Hazardous Materials., 2002; 92; 253-262.

James, BR; Petura, JC; Vitale, RJ and Mussoline, GR. “Oxidation-reduction chemistry of Chromium: Relevance to the regulation and remediation of chromate-contaminated soils”, J. Soil Contamination., 1997; 6; 569-580.

Jefferies, DJ; Firestone, P. “Chemical analysis of some coarse fish from a Suffolk river carried out as part of the preparation for the first release of captive-bred otters”, J Otter Trust., 1984; 1; 17–22.

Kaewsam, JT; Yu, QP and Ma, W. “Pre- treated biornass of marine macroalgae as low cost high efficiency adsorbent for heavy metal ions. J. Chin. Inst. Chern.Eng., 2000;31; 411-415.

Kang, SY; Lee, JU and Kim, KW. “Biosorption of Cr(III) and Cr(VI) onto the cell surface of *Pseudomonas aeruginosa*”, Biochem Eng J., 2007; 36; 54–58.

Keskinan, O. Heavy metal adsorption characteristics of a submerged aquatic plant (*Myriophyllum spicatum*). Process Biochem., 2003; 1-5.

Kuyucak, N and Vole sky, B. “Biosorbents forrecovery of metals from industrial solutions”, Biotechnol Left., 1988; 10; 137-142.

Lopez, A; Lazaro, N; Priego, JM and Marques, AM. “Effect of pH on the biosorption of nickel and other heavy metals by *Pseudomonas fluorescens* 4F39”, J Ind Microbiol Biotech., 2000; 24; 146–151.

Malik, A. “Metal bioremediation through growing cells”, Environ Int., 2004; 30; 261–78.

Mercury source protocol, Utah department of environmental quality, April-2008.

Moore, JW. “Inorganic Contaminants of Surface Water Residuals and Monitoring Priorities”, New York: Springer-Verlag; 1990; 178–210.

Muraleedharan, TR and Venkobachar, C. “Mechanism of cobalt biosorption”, Biotechnol. Bioeng., 1990; 33; 823-831.

Nies, DH. “Microbial Heavy-metal Resistance”. Appl Microbiol Biotechnol., 1999; 51;730-750.

Norton, L. “Biosorption of zinc from aqueous solutions using biosolids. Adv. in Environ. Res., 2003; 6; 2342-2348.

Nourbakhsh, M; Sag, Y; Ozer, D; Aksu, Z and Caglar, A. “A comparative study of various biosorbents for removal of Chromium (VI) ions from industrial wastewaters”, Process Biochemistry, 1994; 29; 1-5.

Onishi, H and Baur, WH. Arsenic. In: Wedepohl KH (ed) Handbook of geochemistry, 1969; 11-3. Springer, Berlin Heidelberg New York, p 33.

Pagnanelli, F; Toro, L and Veglio, F. “Olive mill solid residues as heavy metal sorbent material: a preliminary study”, Waste Management., 2002; 22; 901-907.

Petanen, T. “Accessment of Bioavailable Concentrations and Toxicity of Arsenite and Mercury in Contaminated Soils and Sediments by Bacterial Biosensors”. University of Helsinki, Academic Dissertation.2001.

Prasad, MNV; Freitas, HMO. “Metal hyperaccumulation in plants—biodiversity prospecting for phytoremediation technology”, Electron J Biotechnol., 2003; 6; 285– 321.

Remacle, J., in Biosorption of Heavy Metals (ed. Volesky, B.), CRC Press, Boca Raton, Florida, 1990, pp. 83–92.

S Čerňansky, S; Urik, M; Ševc, J; Littera, P and Hiller, E. “Biosorption of arsenic and cadmium from aqueous solutions”, African Journal of Biotechnology.,2007; 6; 1932-1934.

Sag, Y and Kutsal, T. “Determination of the biosorption heats of heavy metal ions on *Zoogloea ramigera* and *Rhizopus arrhizus*”, Biochem. Eng. J.,2000; 6; 145–151.

Sanchez, A; Balleste, A; Blazquez, ML; Gonzalez,F; Munoz, J and Hammaini, A. “Biosorption of copper and zinc by Cymodocea nodosa. FEMS Microbiol. Rev., 1999; 23; 527-536.

Selatnia, A; Boukazoula, A; Kechid, N; Bakhti, MZ; Chergui, A and Kerchich, Y. “Biosorption of lead (II) from aqueous solution by a bacterial dead *Streptomyces*

rimosus biomass", Biochem Eng J., 2004b; 19; 127–135.
Singh, S; Rai, BN and Rai, LC. "Ni (II) and Cr (VI) sorption kinetics by *Microcystis* in single and multimetallic system", Proc. Biochem., 2007; 36; 1205–1213.
Snow, ET. "Effects of Chromium on DNA replication in vitro", Environ Health Perspect 102 (Suppl 3); 1994; 41-44.
Thakur, IS. Environmental Biotechnology Basic concepts and Applications.2006.
van der Wal, A; Norde, W; Zehnder, AJB and Lyklema, J. "Determination of the total charge in the cell walls of gram-positive bacteria", Colloids Surf. B Biointerfaces ., 1997; 9; 81–100.
Veglio, F; Beolchini, F. "Removal of metals by biosorption: a review",
Vieira, RHSF and Volesky, B. "Biosorption: a solution to pollution", Int Microbiol., 2000; 3; 17–24.
Vijayaraghavan, K and Yun, YS. "Utilization of fermentation waste (*Corynebacterium glutamicum*) for biosorption of Reactive Black 5 from aqueous solution", J Hazard Mater., 2007b;141; 45–52.
Vijayaraghavan, K; Yun, YS. "Bacterial biosorbents and biosorption", Biotechnology Advances., 2008; 26; 266–291.
Vole sky, B. "Biosorbent Materials", Biotechnoi. Bioeng Symp., 1986; 16: 121-126.
Volesky B; Schiewer, S. "Biosorption of metals". In: Flickinger M, Drew SW, editors. Encyclopedia of Bioprocess Technology. New York:Wiley; 1999; 433–53.
Volesky, B (ed) Biosorption of heavy metals. CRC Press, Boca Raton, FL, 1990.
Volesky, B and Holan, ZR. "Biosorption of heavy metals", Biotechnol Prog., 1995; 11; 235–250.
Volesky, B. "Biosorbents for metal recovery", TIBTECH, 1987; 5; 96–101.
Volesky, B. "Biosorption and biosorbents", In: Volesky B (ed) Biosorption of heavy metals. CRC Press, Boca Raton, FL, 1990; 36.
Woolson, EA. "Emissions, cycling and effects of Arsenic in soil ecosystems". In Biological and Environmental Effects of Arsenic, Elsevier Science Publishers B.V.,Amsterdam. 1983, p. 76.
Wu, FC; Tseng, RL and Juang, RS. "Kinetic modeling of liquid-phase adsorption of reactive dyes and metal ions on chitosan", Water Res.,2001; 35; 613-618.
Xia, Y and Liyuan, C. "Study of gelatinous Supports for Immobilizing Inactivated Cells of *Rhizopus oligosporus* to Prepare Biosorbent for Lead Ions", The International Journal of Environmental Studies., 2002; 5; 1-6.
Xie, JZ; Chang, HL and Kilbane, JJ. "Removal and Recover of Metal Ions from Wastewater Using Biosorbents and Chemically Modified Biosorbents", Bioresource Technology., 1996; 57: 127-136.
Yang, X; Duri, BAl ."Kinetic modeling of liquid-phase adsorption of reactive dyes on activated carbon", J. Colloid Interface Sci., 2005; 287; 25-34.
Young, RV. ed. World of Chemistry. Michigan: Gale Group, 2000.

