Biosorption of heavy metals in industrial wastewater using micro-organisms (*Pseudomonas aeruginosa*)

Olukanni[1], D. O., Agunwamba[2], J. C., Ugwu[1], E. I.

[1]Department of Civil Engineering, Covenant University, P.M.B. 1023, Ota, Ogun State, Nigeria.
[2]Department of Civil Engineering University of Nigeria, Nsukka, Enugu State, Nigeria.
Corresponding Author E-mail: david.olukanni@covenantuniversity.edu.ng; +234-8030726472

ABSTRACT

Heavy metal ions are often present in industrial wastewater, and sometimes there is the need to reduce their concentrations to some certain minimum. Biosorption is one of the methods by which this reduction can be achieved. The purpose of this study is to explore the biosorption technique as an alternative to conventional methods such as reverse osmosis, electro dialysis, ultra filtration, ion-exchange and chemical precipitation in the removal of heavy metal ions from industrial wastewater. Biosorption of the heavy metals namely cadmium, zinc and silicate were conducted using Pseudomonas Aeruginosa. Samples of wastewater from an industrial source were collected in a 2L container and poured into six containers. Measured quantities of the biomass were then introduced into the samples. The concentrations of heavy metals in the samples were determined with an atomic absorption spectrometer. The results yielded a significant reduction in the heavy metal ion concentrations in the samples, with even the highest initial concentration greatly reduced. Biosorption technique can be a very good alternative to conventional methods in terms of availability of materials, cost-effectiveness and absence of precipitates and slurry.

Keywords: Biosorption, Industrial Wastewater, Pseudomonas Aeruginosa, Heavy Metals, Aquatic Environment, Cost-effectiveness.

INTRODUCTION

The occurrence of heavy metals in industrial wastewater is known to cause serious damage to aquatic life, beside the fact that these metals kill microorganisms during biological treatment of wastewater with a resultant delay in the course of water purification (Wierzba, 2010). 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**.** Heavy metals are defined as metals with a specific weight usually more than $5.0g/cm^3$, which is five times higher than the specific weight of water. The toxicity of heavy metals occurs even in low concentrations of about 1.0-10mg/l while some strong toxic metals ions such as Mercury (Hg), Chromium (Cr), Lead (Pb), Zinc (Zn), Copper (Cu), Nickel (Ni), Cadmium (Cd), Arsenic (As), Cobalt (Co), Tin (Sn), etc., are very toxic even in lower concentration of 0.001-0.1 mg/l

(Kapoor *et al.*, 1999; Wang and Chen., 2009). A remarkable increase in the use of heavy metals by industries over the past few decades has inevitably resulted in an increased flux of metallic substances in the aquatic environment. It has been well established by various researchers that modern industry is to a large degree, responsible for contamination of the environment (Olukanni and Ugwu, 2013; Rosa *et al.*, 2009).

Heavy metal pollution occurs directly by effluent outfalls from industries such as refineries, fertilizer and pesticide, metallurgy, iron and steel, leatherworking, photography, electric appliance manufacturing, metal surface treating, and wastewater treatment plants. It also indirectly occurs through the contaminants that enter the water supply from soil/ground water systems and from the atmosphere through rain water (Wang and Chen, 2006; Vijayaraghavan and Yun, 2008; Wang and Chen, 2009; Olukanni and Ugwu, 2013). Lakes, rivers and oceans are being overwhelmed with many

toxic contaminants. Jefferies and Firestone (1984), Olukanni and Adeoye (2012), Olukanni and Ugwu (2013) expressed that metals may accumulate to toxic levels causing ecological damage under certain environmental conditions.

Metals discharged into water bodies are not biodegraded but undergoes chemical or microbial transformations, creating large impact on the environment and public health (Volesky, 1993). Of the important metals, Hg, Pb, Cd, As and Cr (VI) are regarded as toxic while Lead, Platinum, Argon, Aurum etc, are referred to as precious metals. Uranium and Titanium are known as radionuclide (Wang and Chen, 2009). However, their extensive usage and increasing levels in the environment are of serious concern (Brown and Absanullah, 1971; More, 1990; Volesky, 1990). Various techniques have been employed for the treatment of heavy metal-bearing industrial effluents, which usually include precipitation, adsorption, ion exchange, membrane and electrochemical technologies (Wierzba, 2010). However, these techniques are expensive, not environmentally friendly and usually dependent on the concentration of the waste which is ineffective in much diluted solutions (Volesky, 2001; Volesky and Naja, 2007). The search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated (Olukanni and Ducoste, 2011; Olukanni and Kokumo, 2013). Of the different biological methods, biosorption has been identified and demonstrated to possess good potential to replace conventional methods such as reverse osmosis, electro dialysis, ultra filtration, ion-exchange and chemical precipitation for the removal of heavy metals (Volesky and Holan, 1995; Malik, 2004).

Biosorption refers to the accumulation of metal ions from solution by microbial or plant material. The process utilizes inexpensive dead biomass for selective sequestering of toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents. Some studies have demonstrated microorganism`s ability to remove heavy metals from wastewater with better performance and lower cost (Roane *et al.*, 2005; Violante *et al.*, 2008; Gadd, 2009, 2010). Many types of yeast, fungi, algae, bacteria and some aquatic plants have been reported to have the capacity to concentrate metals from dilute aqueous solutions and to accumulate them inside the cell structure. (Kapoor and Viraghavan, 1995; Volesky and Holan, 1995; Modak and Natarajan, 1996). The early reports from literature described how abundant biological materials could be used to remove at very low cost, even small amounts of toxic heavy metals from industrial effluents (Liu *et al.*, 2004; Moon *et al.*, 2006). The principal advantages of biological technologies for the removal of these pollutants are that; they can be out in-situ at the contaminated site, usually environmentally benign (no secondary pollution) and they are not so expensive (Kapoor *et al.*, 1999; Shankar *et al.*, 2007).

Microbial biomass was used as an adsorbing agent for the removal and recovery of uranium present in industrial effluents and mine wastewater (Nakajima and Sukaguchi, 1986). Biosorption by fungi as an alternative treatment option for wastewater containing heavy metal has been reviewed by Kapoor and Viraghavan (1995) and Modak and Natarajan (1996). Many microbial species such as bacteria, fungi, yeast and algae are known to be capable of adsorbing heavy metals on their surface and/or accumulating within their structure (Campbell and Martin, 1990; Luef, 1991; Mitani and Misic, 1991; Vinita and Radhanath, 1992). Therefore, the aim of this study was to further investigate the toxicity of these metals (cadmium, zinc and silicate) from solution and to evaluate the applicability of pseudomonas aeruginosa for removal of these heavy metals from industrial wastewater.

MATERIALS AND METHOD

The following materials were used for the laboratory analysis: a) Atomic spectrophotometer; b) 2 liter can; c) 21 Cans of 150 ml; d) Measuring cylinder; e) Two bottles of pseudomonas aeruginosa; f) Magnetic stirrer; g) pH meter.

Procedure for analysis of the sample: 0.5 ml of the pseudomonas aeruginosa biomass was added into the sample of various quantities, and 40 ml of the mixture was collected twice from the containers at 20 minutes intervals and metal determinations were carried out. A pH electrode connected to pH meter was used to monitor the pH of the solution. Mixing was achieved with the use of magnetic stirrer. The research work involved the use of atomic spectrophotometer to determine the metals present. The following heavy metals were tested: cadmium, silicon, zinc, lead, iron and copper. The presence of these metals was confirmed by the Atomic Absorption Spectrophotometer (AAS).

Calculations:

Equation 1 represents the percentage biosorption of heavy metal for the experiment % Biosorption =

$$\left(\frac{1-F}{I}\right)\times100 \quad (1)$$ where: = initial metal concentration

F = final metal concentration

RESULTS AND DISCUSSION

The need to remove heavy metals in industrial wastewater is important before disposal to a nearby river. The results obtained in this work demonstrate the potential removal of heavy metals in wastewater before disposal. Tables 3.1 and 3.2 show the initial concentration of metals in initial and prepared samples while Table 3.3 shows the results of biosorption.

Table 3.1 Initial concentration of metals of sample 1

Sample No.	Mg/l Cd^{++}	Mg/l Pb^{2+}	Mg/l Fe^{++}	Mg/l Ni	Mg/l Cu^{2+}	Mg/l Mg^{++}	Mg/l Na^+	Mg/l Al^{3+}	Mg/l Zn^{2+}	Mg/l Si
1	4.33	Nil	0.01	Nil	0.01	Nil	Nil	Nil	4.89	3.00

Table 2 shows the increase in the temperature, biomass concentration, and the time interval at intervals of 10^0C, 0.5 Mg/ml, and 20 mins, respectively.

Table 3.2 Prepared samples

	Vol.	Initial metal concentration				Temp ^{0}C	Biomass Conc.	Time	pH
Sample unit	100ml	Cd^{++} (mg/l)	Si^{2+} (mg/l)	Zn^{2+} (mg/l)	Total (mg/l)		Mg/ml	Mins	
Sample A	A	5	5	5	15	10	0.5	20	1.2
Sample B	B	10	10	10	30	20	1	40	1.6
Sample C	C	15	15	15	45	30	1.5	60	3.2
Sample D	D	20	20	20	60	40	2	80	5
Sample E	E	25	25	25	75	50	2.5	100	6.1
Sample F	F	30	30	30	90	60	3	120	6.8

Table 3.3 Biosorption results

	Volume	Final metal concentration				% Biosorption of Cd, Si, Zn		
Sample unit	100ml	Cd^{++} (mg/l)	Si^{2+} (mg/l)	Zn^{2+} (mg/l)	Total (mg/l)	Cd	Si	Zn
Sample A	A	2.16	3.77	1.98	7.91	57	25	60
Sample B	B	3.74	6.89	2.99	13.62	63	31	70
Sample C	C	4.08	8.99	2.99	16.06	73	40	98
Sample D	D	1.53	6	0.31	2.71	92	56	80
Sample E	E	13.17	15.04	11.19	39.4	47	70	55
Sample F	F	19.07	21.17	17	57.24	36	49	43
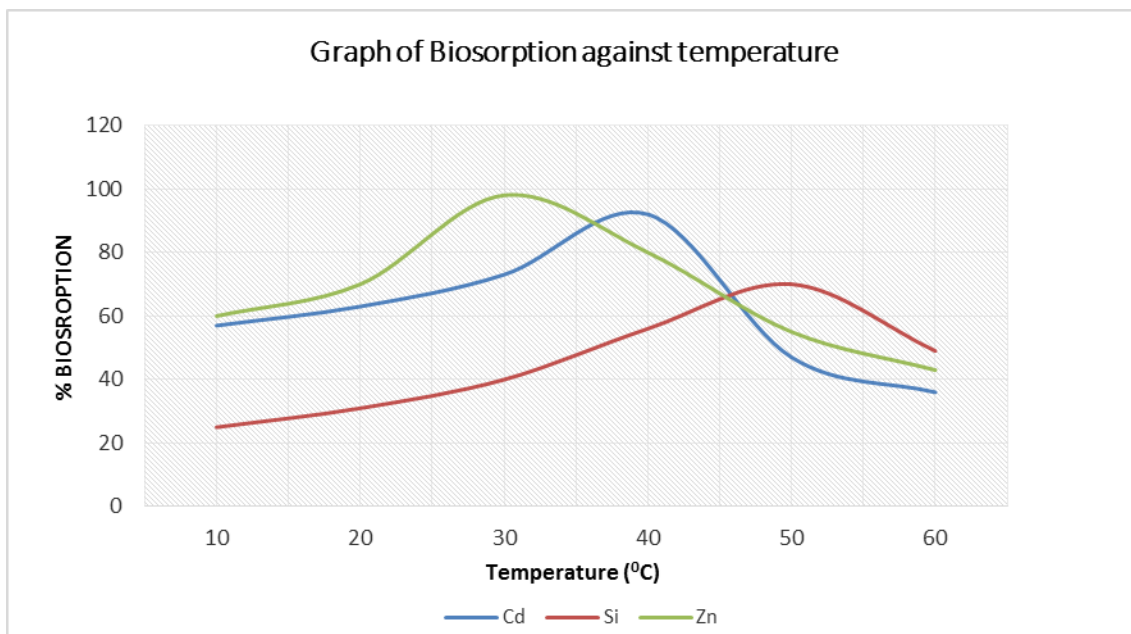
Fig.1 Effect of Temperature on Biosorption of Heavy metals

The sorption percentage increased with temperature for the heavy metals and experienced a significant reduction after the optimum temperature was reached. The maximum biosorption capacity of the biosorbent for zinc was achieved at a temperature of 30^0C. Further increase in temperature gave no effect on sorption percentage. Therefore, the optimum temperature needed for the effective biosorption of the heavy metals in this experiment for the three metals ranges from 30^0C to 50^0C
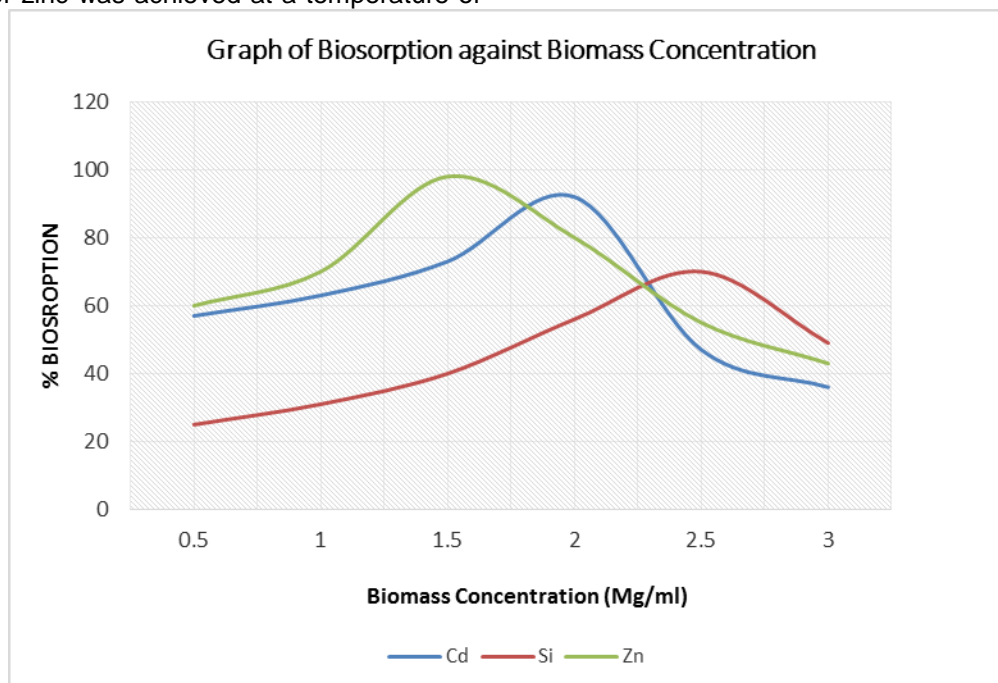
Fig.2 Effect of Biomass concentration on Biosorption of Heavy metals

The influence of biomass concentration on the percentage sorption of the heavy metals is shown in Figure 2. The maximum biosorption capacity of the biosorbent for Cadmium was achieved between biomass concentrations of 0.5 to 2 mg/ml. Further increase in biomass concentration beyond 2mg/ml gave no effect on sorption percentage. For Silicon, the optimum bisorption capacity was obtained between the biomass concentrations of 0.5 to 2.5mg/ml. Also for zinc, the biomass concentration was achieved between biomass concentrations of 0.5 to 1.5mg/ml.
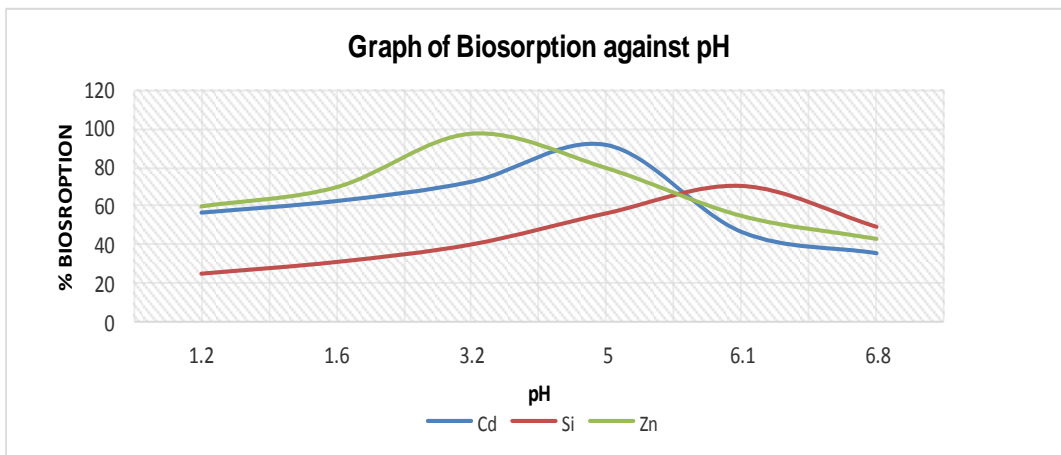

Fig.3 Effect of pH on percentage biosorption of Heavy metals

The effect of pH on the percentage biosorption of heavy metals is depicted in Figure 3. For Cadmium, the sorption increased from 57% at pH 1.2 to 92% at pH 5.0 and later decreased at pH 6.1. For silicon, sorption increased from 25% at pH 1.2 to 70% at pH 6.1 and later decreased with an increase in pH. This implies that an optimum percentage of biosorption was achieved at pH 6.1. Also for Zinc, the sorption increased from 60% at pH 1.2 to 98% at pH 3.2 and significantly decreased with increase of pH. At pH 6.1 and 6.8, it was around 55% and 43%. Figure shows that the maximum percent of biosorption was achieved at pH of 3.2, 5.0 and 6.1 for zinc, cadmium and silicon, respectively.
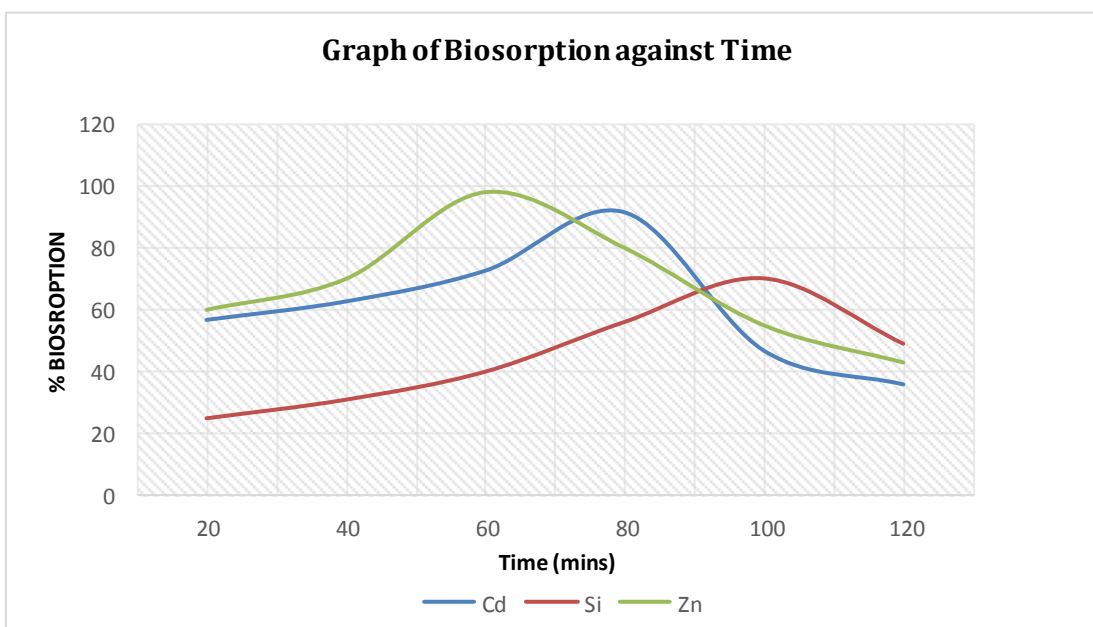

Fig.4 Effect of Time on Biosorption of Heavy metals

An increase in percentage of biosorption was observed as time increased and later decreased after a longer time as shown in Fig.4. Therefore, at 60 minutes, 80 minutes and 100 minutes for zinc, cadmium and silicon respectively, optimum rate of biosorption was achieved.

CONCLUSION AND RECOMMENDATION

Conclusion: Many aquatic environment face metal concentration that exceed water criteria design to protect the environment, animals and humans. Every essential element is toxic if taken in excess and there is a safe window for essential dose between deficiency and toxicity. Biosorption of heavy metals namely cadmium, Zinc and silicon were conducted using Pseudomonas aeruginosa. The results obtained in this experimental work showed that an increase in pH, Time, Biomass concentration and temperature to a certain level, caused a significant increase in sorption percentage. The cadmium percentage biosorption under optimum condition resulted to 98% at pH 3.2. Also, percentage biosorption of silicon under optimum condition resulted to 70% of sorption at pH 6.1. This implies that Pseudomonas aeruginosa is a very effective biosorbent for removal of Cadmium, Silicon and Zinc in industrial wastewater as evidenced by its biosorptive capacity.

RECOMMENDATION

Based on the results of this work, it is suffice to express that it could be more economical and reliable to use biosorption method to remove heavy metals in wastewater than any other conventional method such as reverse osmosis, electro dialysis, ultra filtration, ion-exchange and chemical precipitation. In addition, this research method is more preferable to conventional method because: (1) It minimizes chemical and biological sludge and (2) There is possibility of metal and biosorbents recovery.

REFERENCES

Brown, B. and Absanullah, M (1971). Effects of heavy metals on mortality and growth, Mar Pollut Bull., 2: 182-187

Campbell, R. and Martin, M. H (1990). Continuous flow fermentation to purify wastewater by the removal cadmium. Water, Air and Soil Pollution, 50: 397-408.

Gadd, G. M (2009). Heavy metal pollutants: environmental and biotechnological aspects. In:

Schaechter M (ed) Encyclopedia of microbiology, 3rd edn. Elsevier, Oxford, pp 321–334.

Gadd, G. M (2010) Metals, minerals and microbes: geomicrobiology and bioremediation.Microbiology 156: 609–643.

Jefferies, D. J and Firestone, P (1984). 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. 1: 17-22.

Kapoor, A and Viraraghavan, T. (1995) Fungal Biosorption-an alternative treatment option for heavy metal bearing wastewater: a review. Bioresour. Technol. 53: 195-206.

Kapoor, A.T., Viraraghavan, T., and Cullimore, D. R (1999). Removal of heavy metals using the fungus spergillus niger, Bioresour. Technol. 70: 95-104.

Luef, E., Prey, T and Kubicek, C. P (1991). Biosorption of Zinc by Fungal mycelial waste. Appl.Microbiol. Biotechnol., 34**:** 688-692.

Liu, H., Ramnarayanan, R and Logan, B. E (2004). Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ. Sci. Technol. 38: 2281–2285.

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

Mitani, T and Misic, D. M (1991). Copper Accumulation by Penicillium sp. Isolated from soil.Soil Sci. Plant Nutr. 1991, 37(2): 347-349.

Modak, J. M., Natarajan, K. A and Saha, B (1996). Biosorption of copper and zinc using wasteAspergillus niger biomass. Miner. Metall. Process., 13(2): 52-57.

Moon, H., Chang, I.S and Kim, B. H (2006). Continuous electricity production from artificialwastewater using a mediator-less microbial fuel cell. Bioresour Technology. 97:621–627.

Nakajima, A and Sakaguchi, T (1986). Selective accumulation of heavy metals by microorganisms. Appl. Microbiol. Biotechnology. 24: 59-64.

Olukanni, D. O and Adeoye, D. O (2012) Heavy Metal Concentrations in Road Side Soils from Selected Locations in the Lagos Metropolis,Nigeria. International Journal of Engineering and Technology. Vol. 2 (10).

Olukanni, D. O. and Kokumo, K. O (2013) Efficiency Assessment of a Constructed Wetland Using Eichhornia Crassipes for Wastewater Treatment. American Journal of Engineering Research (AJER). Vol. 2 (12): 450-454.

Olukanni, D. O and Ugwu, N. C (2013). Mud Rotary Drilling in Southern Nigeria: Potential Adverse Effects of its By-Products on the Environment. International Journal of Water resources and Environmental Engineering IJWREE Vol. 5 (5): 262-271.

Roane, T. M., Pepper, I. L and Miller, R. M (2005). Microbial remediation of metals. In:Crawford RL, Crawford DL (eds) Bioremediation principles and applications. Cambridge University Press, Cambridge, pp 312–340.

Rosa, M. P.S., Arelis, A. R, Jose, M.G.M and Domingo, C. M (2009). Biosorption of chromium, copper, manganese and zinc by Pseudomonas aeruginosa AT18 isolated from a site ontaminated with petroleum. Bioresource Technology, 100(4):1533-1538.

Shankar, C., Sridevi, D., Joonhong, P., Michael, D and Kaliannan T (2007). Biosorption of Chromium and nickel by heavy metal resistant fungal and bacterial isolates. Journal of hazardous materials 147: 270-277.

Vijayaraghavan, K., Yun, Y. S (2008). Bacterial biosorbents and biosorption. Biotechnol. Adv.26: 266–291.

Vinita, V. P., Radhanath, P. D (1992). Biorecovery of zinc from industrial effluent using native microflora. Intern. J. Environmental Studies, 44: 251-257.

Violante, A., Huang, P. M and Gadd, G. M (2008). Biophysicochemical processes of heavy metals and Metalloids in soil environments. Wiley, Chichester.

Volesky, B (1993). Biosorption and biosorbents, in Biosorption of heavy metals, edited by Volesky (CRCPress, Boca Raton, Florida) 1990a, 3-5.

Volesky, B and Holan Z (1995). Biosorption of heavy metals. Biotechnol. Prog.11: 235-250 .

Volesky, B (2001). Detoxification of metal-bearing effluents: Biosorption for the next century. Hydrometallurgy 59: 203–216.

Volesky, B and Naja, G (2007). Biosorption technology: starting up an enterprise. Int. J. Technol. Transf. Commer. 6: 196–211.

Wang, J. L and Chen, C (2006). Biosorption of heavy metals by Saccharomyces cerevisae: a review. Biotechnol Adv 24: 427–451.

Wang, J. L and Chen, C (2009). Biosorbents for heavy metals removal and their future a review.Biotechnol Adv. 27: 195–226.

Wierzba, S (2010). Heavy metals biosorption from aqueous solution by Pseudomonas Sp. G. Proceedings of Biotechnology and Molecular Biology, ECOpole, Opole University, Opole