Myco and Phyto Remediation of Heavy Metals from Aqueous Solution

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Abstract: Biosorption technique was applied to remove the Ni(II), Cu(II), Cr(III) and Cr(VI) from single metal solution. Seven fungal species viz., *Aspergillus niger, A. terreus, A. flavus, Trichoderma harzianum, Alternaria alternata*, *Rhizopus arrhizus* & *Cunninghamella echinulata*, three agricultural materials viz., *Oryzae sativa* straw (rice straw), *Cicer arietinum* dried seed (gram husk) & *luffa cylindrical* dried fruit (luffa sponge), leaves of five trees i.e., Neem (*Azadaricta indic*a), Dareek (*Melia azedarach*), Bohar (*Ficus benglensis*), Peepal (*Ficus relgiosa*), sunflower (*Helianthus annus*) and charcoal were chosen as adsorbent material. Laboratory biosorption experiments were performed with different concentrations of each of four metals. Results showed highly significant sequestering capacity of all selected biosorbents for both Cr(III & VI) in comparison to Cu(II) and Ni(II) ions. Removal efficiency of candidate biosorbents reached up to 80%, 58% and 52% for Cr(III & VI), Cu(II) and Ni(II) ions, respectively.

Key words: Biosorption, Metals, Fungi, Agricultural waste, Tree leaves

Introduction

Industrial wastewater is considered as most notorious source of heavy metal pollution in the surrounding environment (Sun et al., 2010). In Pakistan industrial wastewater pollution from the electroplating processing has become most serious issue. Volume of the wastewater produced by such processing units is comparatively much smaller but highly toxic in nature because of the high concentration of copper (Cu), zinc (Zn), nickel (Ni), chromium (Cr), cadmium (Cd) , lead (Pb), various acids and cyanide compounds as compared to prescribed limits of National Environmental Quality Standards (Javaid et al., 2010).

Now the attention is being focused on reducing reliance upon expensive chemical methods, and finding alternatives directing attention towards biological technique like Biosorption. Biosorption is ability of certain biological material to sequester contaminates like heavy metals from adjoining environment in economic and eco-friendly way. It occurs through bindings of metal ions with chemical groups present on the biosorbent cell wall surface. Biosorption technique offers several advantages over conventional treatment methods including cost effectiveness, efficiency, minimization of chemical/biological sludge, requirement of additional nutrients, and regeneration of biosorbent with possibility of metal recovery. A diversity of adsorbents like microorganisms (fungi, bacterial, algae and yeast), plant by-products (rice straw and husk, wheat straw and husk, chick pea husk) and waste material (fallen leaves and peels) have been utilized to remove heavy metals from aqueous medium (Javaid et al., 2010). Biomaterials like fungi have been proved efficient and economic for removal of metal ions from aqueous solution due to high percentage of cell wall material, which shows excellent metal binding properties (Das et al., 2008). Among the fungi, *Aspergillus*, *Trichoderma* and *Penicillium* are the most important group that degrades variety of polysaccharides in agricultural waste, soil and feces of cattle and sheep (Sun et al., 2010). So far, Ali et al. (2007) results showed *Trichoderma viride* is successful as biosorbents for the removal of Zn, Pb and Cd from the aqueous media. Rajender et al. (2008) examined tremendous ability of *Aspergillus niger*, *A. sydoni* and *Penicillium janthinellum* to remove Cr(VI) ions @ 91.03, 87.95 and 86.61% from aqueous solution as well as from electroplating effluent. Pal et al. (2010) showed cell surface functional groups of the fungus might act as ligands for metal sequestration and varied their findings with *Aspergillus niger* during Cd and with *R. arrhizus* during Pb biosorption from the aqueous culture media. Findings of Hmambika et al. (2011) indicted more than 60-95% metal ions like Cu, Cd and Pb were removed due to application of *Aspergillus* sp. *Penicillium* and *Cephalosporium* sp**.** from aqueous solution.

Apart from fungi, removal of heavy metals by lignicellulosic and plant waste material has been extensively investigated in past decades. The plant and agricultural waste material are good source of cheap, easily and locally available adsorbent with reasonable metal loading capacity. *Cicer arietinum* dried seed (gram husk) showed 99.9% removal of Cr(VI) (Ahalya et al., 2005), *Ficus religiosa* leaves powder was found to be a very good adsorbent for Cr(VI) and Pb (Qaiser et al., 2007) and sunflower (*Helianthus annus*) exhibited 80% removal efficiency for Cr(VI) (Jain et al., 2009). Oboh et al. (2009) found 76.8, 67.5, 58.4 and 41.45 removal efficiency of neem leaves for Cu, Ni, Zn and Pb, respectively. Aslam et al. (2010) showed that *Ficus Religiosa* leaves are the suitable material for Ni(II) biosorption. The potential use of rice straw as an adsorbent for Ni and Cd was suggested El-Syed et al . (2010). Ohbo et al. (2011) stated that *Luffa cylindrica* seeds and sponge mixture is a good alternative biosorbent for Ni, Pb, Cu and Zn ions removal from aqueous solution.

Based on literature survey, present study was conducted to evaluated the Ni(II), Cu(II), Cr(III) and Cr(VI) removal potential of variety of fungal and natural adsorbent from single metal solution at various concentrations.

Methodology

Biosorbents

The pure cultures of fungal species viz., *Aspergillus niger* (FCBP 0074), *A. terreus* (FCBP 0058), *A. flavus* (FCBP 0064), *R. arrhizus* (FCBP 800), *A. alternata* (FCBP 0092), *T. harzianum* (FCBP 0139) and *C. echinulata* (FCBP 0104) were procured from First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences (IAGS), Punjab University. Mycelial biomass of the each fungal species was cultivated in 2% malt extract (ME) broth in 250 mL conical flasks. Inoculated flasks were incubated for 6-7 days under controlled temperature of 25 ± 1° C in stationary phase. Prepared biomass of each candidate fungus was separated from culture broth by filtration and subjected to successive washings with double distilled deionized water followed by drying in oven at 60° C for 24 hours. The dried biomass of each test fungus of 0.5-1 mm was used in biosorption experimentation.

O. sativa straw, *C. arietinum* husk and luffa sponge were obtained from local market. *A. indic*a, *M. azedarach*, *F. benglensis*, *F. relgiosa*, *H. annus* leaves were collected from local environment of University of Punjab Lahore, Pakistan. Each biosorbent material was dried in oven at 100° C for 24 hours and homogenized in a blender to break the cell aggregates into smaller fragments of 0.5-1 mm diameter (mesh size 150 μm). Waste charcoal was acquired from Natural Product laboratory of Herbal Heritage Centre, IAGS, Punjab University and utilized for biosorption experiment after drying at 100 ^{o}C for two hours. Each of the natural biosorbent material was kept in separate airtight bottles for later utilization in biosorption experiments. Table 1 shows list of biosorbens materials selected for current investigation.

Table 1. List of biosorbent materials utilized in present wor**k**

No	Biosorbents	Metal			
	Fungi	**Ni(II)**	**Cu(II)**	**Cr(III)**	**Cr(VI)**
1	*Aspergillus niger*	+	+	+	+
2	*Aspergillus terreus*	+	+	-	-
3	*Aspergillus flavus*	+	+	-	-
4	*Alternaria alternata*	+	+	-	-
5	*Rhizopus arrhizus*	+	+	+	+
6	*Trichodrma harzianum*	+	+	+	+
7	*Cuninnghamalla echinulata*	+	+	-	-
	Agricultural waste				
8	*Oryzae sativa* straw	+	+	+	+
9	Dried seed of *Cicer arietinum*	+	+	+	+
10	Dried fruit of *Luffa cylundrica*	+	+	+	+
	Tree Leaves				
11	*Azadaricta indic*a	+	+	+	+
12	*Melia azedarach*	+	+	+	+
13	*Ficus benglensis*	-	-	+	+
14	*Ficus relgiosa*	-	-	+	+
15	*Helianthus annus*	-	-	+	+
16	Charchol	+	+	+	+

+ indicates material utilized in biosorption experiments for aforementioned metal; indicates material not utilized in biosorption experiment for above mentioned metal

Metals

The stock solutions of Ni(II), Cu(III), Cr(III) and Cr(VI) ions were prepared from respective salts, included Ni $(NO_3)_2$ $6H_2O$, $Cu(NO_3)_2$. $3H_2O$, $Cr(NO_3)_2$. $9H_2O$ and $K_2Cr_2O_7$ by dissolving the exact quantity of salt in double distilled deionized water. Stock solution measuring 1000 mg L^{-1} of each metal ion was further diluted for composing various concentration regimes. On the basis of literature available, four concentrations 50, 100, 300 and 500 mg L^{-1} were prepared from standard solution of Cu(II) and Ni(II) five levels of 5, 15, 25, 35 & 45 mg L^{-1} were made from stock solution of Cr(III) & Cr(VI)

Experiment

Biosorption experiments were performed by suspending 0.1g of fungal and 0.5g of natural biosorbent material in 100 mL of metal solution in 250 mL flask stirred at 150 rpm at pH 4.5 (0.5M NaOH and 0.5M HCl was used to adjust pH in each flask) for 3 hours. The change in working volume due to addition of NaOH and HCl was negligible. These chemicals were added to reaction mixture before the addition of biomass to avoid change in pH value. Different sets of experiments were carried out to appraise the maximum metal accumulating capacity of the biosorbents at different initial concentration of metal ions ranging between 50, 100,

300 & 500 mg L^{-1} for Cu(II) and & Ni(II), and 5, 15, 25, 35 & 45 500 mg L^{-1} in case of Cr(III &VI). After desired contact time, the mixture was filtered through Whatman filter paper No.1 and the residual metal ion concentrations were determined using Atomic absorption spectrophotometer (AAS).

Biosorption data evaluation

The efficiency of the biosorbent or its removal capability (E) was calculated using following equation:

$$q = \left(\frac{C_i - C_f}{m} \right) V \; ; E = \left(\frac{C_i - C_f}{C_i} \right) * 100$$

Where, C_i = initial concentration of the metallic ion (mg L^{-1}); C_f = final concentration of metallic ion (mg L^{-1}); m = dried mass of the biosorbent in the reaction mixture (g) and V = volume of reaction mixture (mL).

Results and Discussion

Comparative analysis of data acquired, in general, exhibited significantly higher sequestering capacity of all selected biosorbents for both Cr(III & VI) in comparison to Cu(II) and Ni(II) ions. Removal efficiency of candidate biosorbents reached up to 80%, 58% and 52% for Cr(III & VI), Cu(II) and Ni(II) ions, respectively (Table 2, 3, 4 & 5). This may also be related to differential electrode potential of various metal ions, resulting in different biosorption affinities (White et al., 1979). Similar concept of stronger chemical and physical affinity for metal ion at greater electronegative bonds and ionic radii has been suggested in other studies (Tsezos &Volesky, 1981, Weast, 1988).

Data acquired on influence of initial concentration of metal ions revealed strong impact of this factor on uptake potential by the biosorbents, the effect being more conspicuous at higher concentrations. Accordingly, adsorption efficiency reduce up to 5-20% for Cu(II) and Ni(II) at 300-500 mg L^{-1} and 2-35% in case of Cr(III &VI) at 35-45 mg L^{-1} by the biosorbents. Over and above, this trend in metal uptake reduction was dominant in case of Cr(III & VI), as 10 amongst the twelve elected biosorbents exhibited this decline in efficiency. Whereas, in case of the Cu(II) and Ni(II) the reduction rate was recorded in half of the biosorbents (6). These results are similar to the observations made by Malkoc et al. (2006), Dubey and Krishna (2007) and Zvinowanda et al. (2010) with different biomaterials. However, the sorption characteristic represented that surface saturation was dependent on the initial metal ion concentrations. At low concentrations adsorption sites took up the available metal more quickly. However, at higher concentrations, more metal ions are left un-adsorbed in solution due to the saturation of binding sites (Lokeshwari & Joshi, 2009).

For each metal different adsorbent were noticed that hold maximum metal adsorption efficiency. For **Cu(II) ions**, *C. arietinum* husk showed significantly greater biosorption efficiency 44-50% within concentration range of 50-500 mg L^{-1}, respectively in comparison to rest of the 12 biosorbents. However, removal rate declined only up 20% in case of *R. arrhizus*, *A. niger*, *A. indic*a, *M. azedarach*. Among rest of adsorbents, *O. sativa* straw, *L. cylindrica* dried fruit, *A. terreus*, *A. flavus, T. harzianum* and *A. alternata* showed up to half time reduction and *C. echinulata* and charcoal exhibited 75% decline in biosorption efficiency as compared to maximum recorded in *C. arietinum* husk (Table 2). *L. cylindrica* dried fruit showed the highest removal efficiency of 44-50% for **Ni(II)** ions followed by *T. harzianum, R. arrhizus*, *O. sativa* straw and leaves of *A. indic*a, *M. azedarach* within concentration range of 50-500 mg L^{-1}. Amongst remaining 6 biosorbents, efficiency was further reduced up to 50-60% in *C. arietinum* husk, charcoal, *A. niger*, *A. terreus*, *A. flavus, A. alternata* and *C. echinulata* in comparison to the highly efficient (44-50%) biosorbent (Table 3). In case of **Cr(III),** four biosorbents viz. *O. sativa* straw, leaves of *F. bengalensis*, *F. religiosa* and *H. annus* hold the greatest biosorption efficiency (80%) at applied concentrations in comparison to rest of adsorbents (Table 4). On the other hand, *F. religiosa* was proved to be most efficient biosorbents (80%) for adsorption of **Cr(VI)** (Table 5).

Disparity in biosorption capacity of different adsorbents may be ascribed to the intrinsic ability of organism, its chemical composition of cell wall leading various types of interaction of metals with adsorbents (Gadd, 1993). This indicates adsorbent variability in metal ions binding affinities for the same or different functional groups (amino, carboxylate, phosphate, sulphahydral, phosphate and thiol) on cell walls. Since in solution all the metal ions are in competition for the available binding sites, a metal that has a higher affinity for particular functional group would bind in greater concentration (Bayramoglu et al., 2003).

Generally we observed that agro-waste and plant leaves exhibited greater adsorption efficiency than fungi. Among the fungi, *T. harzianum* and *R. arrhizus* were found to be good adsorbents of metal ions. Difference among the different fungal species could be owing to marked variations in the wall composition between different fungal taxonomic groups (Siegel et al., 1990; Fourest and Roux, 1992). Generally, major constituents of fungal cell wall are carbohydrates, chitin, chitosan, polyuronide and polyphosphates and proteins that probably participated in metal binding. It has been stated that difference in the high chitin and chitosan content of the cell walls attributed differential metal uptake efficiencies in the fungal biomass (Tsezos &Volesky, 1981).

In case of agro-waste, *O. sativa* straw, *C. arietinum* husk & *L. cylindrica* are ligno-cellulose based. Most of the plant tissues are composed of structural carbohydrates as cellulose, hemicellulose, pectin, lignin, proteins, subreins, mineral salt and waxes (Rowell et al., 2002; Mazali and Alves, 2005). Carbohydrates of lingo-cellulosic mainly contributed in metal binding. Variation in adsorption efficiency of agro-waste materials could be due to the variation in number of fissures and holes. Presence of some fissures and holes indicated the existence of the macroporous structure. Previous findings reported that major contribution of the metal ions uptake is due to micro- and mesoporous structures (Oboh et al., 2009).

In present study leaves were found to be good biosorbents for Cr(III) than rest of metals. This could be due to difference in the metal-attracting groups of the cell walls of these leaves. Leaves of different trees are contained a variety of organic and inorganic compounds. Cellulose, hemicellulose, pectins and lignin present in the cell wall are the most important sorption sites (Volesky, 2003). Leaves have chlorophyll, carotene, anthocyanin and tannin which contribute to metal biosorption. The important feature of these compounds is that they contain hydroxyl, carboxylic, carbonyl, amino and nitro groups which are important sites for metal sorption (Qaiser et al., 2007).

Table 2: Comparative representation of biosorption efficiency of various biosorbents at selected concentrations.
Biosorption conditions: biosorbents concentration, 0.1 g 100 mL^{-1}; pH, 4.5 (the solution pH was not controlled during the experiment); 150 rpm and 25°C for 3 hours.

	#	Biosorbents	Efficiency (%)					#	Biosorbents	Efficiency (%)			
			50 mg/L	100 mg/L	300 mg/L	500 mg/L				50 mg/L	100 mg/L	300 mg/L	500 mg/L
Cu(II)	1	*A. niger*	36	34	35	34	Ni(II)	1	*A. niger*	20	20	16.67	14
	2	*A. terreus*	21	20	20	19.8		2	*A. terreus*	20	21	17	16
	3	*A. flavus*	22	22	23	20		3	*A. flavus*	16	16	15	15
	4	*T. harzianum*	24	24	24	24		4	*T. harzianum*	46	45	43.33	43
	5	*A. alternata*	20	20	19	18		5	*A. alternata*	20	19	15	15
	6	*R. arrhizus*	34	35	36	36		6	*R. arrhizus*	36	40	46.67	46
	7	*C. echinulata*	20	18	16	12		7	*C. echinulata*	20	20	18.67	18
	8	*O. sativa* straw	30	30	30	25		8	*O. sativa* straw	36	38	41	43
	9	*C. arietinum* husk	44	58	54	50		9	*C. arietinum* husk	18	19	30	24
	10	*L. cylundrica* dried fruit	20	25	20	18		10	*L. cylundrica* dried fruit	44	50	51.67	48
	11	*A. indica* leaves	30	29	31	31		11	*A. indica* leaves	32	30	46	45
	12	*M. azedarach* leaves	30	30	33	33		12	*M. azedarach* leaves	36	35	48	46.6
	13	Charcoal	6	7	8	10		13	Charcoal	28	27	26	26

Note: Highlighted rows indicate biosorbent with the maximum biosorption efficiency

Table 3 A&B: Comparative representation of biosorption efficiency of various biosorbents at selected concentrations. Biosorption conditions: biosorbents concentration, 0.1 g 100 mL^{-1}; pH, 4.5 (the solution pH was not controlled during the experiment); 150 rpm and 25°C for 3 hours.

	#	Biosorbents	Efficiency (%)				
			5 mg L	15 mg/L	25 mg/L	35 mg/L	45 mg/L
Cr(III)	1	A. niger	50	50	51.2	51.43	48.89
	2	R. arrhizus	70	70	68	66.86	66.67
	3	T. harzianum	76	73.33	60	54.29	40
	4	O. sativa straw	80	78.67	76	74.29	72.22
	5	C. arietinum husk	80	73.33	64	57.14	48.89
	6	L. cylundrica dried fruit	80	80	80	57.14	51.11
	7	A. indica leaves	60	60	48	48.57	44.44
	8	M. azedarach leaves	66	66.67	58	46.86	44.44
	9	F. benglensis leaves	80	80	80	80	80
	10	F. relgiosa leaves	80	80	80	80	80
	11	H. annus leaves	80	80	80	77.15	75.56
	12	Charcoal	50	46.68	46	46	44.44

	#	Biosorbents	Efficiency (%)				
			5 mg/L	15 mg/L	25 mg/L	35 mg/L	45 mg/L
Cr(VI)	1	A. niger	47.2	47.33	46	45.71	44.44
	2	R. arrhizus	34	33.33	32	31.43	32.22
	3	T. harzianum	70	68	65.2	65.14	65.11
	4	O. sativa straw	57.6	56.67	56	55.71	55.55
	5	C. arietinum husk	72	70	68	67.71	66.67
	6	L. cylundrica dried fruit	30	26.67	24	20	17.78
	7	A. indica leaves	66	56.67	48	45.71	40
	8	M. azedarach leaves	40	33.33	30	28.57	28.89
	9	F. bengalensis leaves	52	42.67	39.6	35.14	33.33
	10	F. relgiosa leaves	80	80	80	80	80
	11	H. annus leaves	60	46.67	46	45.14	44.44
	12	Charcoal	52	66.67	72	72	73.33

Note: Highlighted rows indicate biosorbent with the maximum biosorption efficiency

Conclusion

Perusal of results acquired on metal removal capability of the biosorbnets revealed that among thirteen selected candidates for Cu(II) and Ni(II) half of them proved to be 30-50% efficient with concentration range of 50-500 mg L^{-1}. For removal of Cu(II) from aqueous solution, *C. arietinum* husk, *A. niger* and *R. arrhizus* could be utilized. For Ni(II), six namely *T. harzianum*, *R. arrhizus*, *O. sativa* straw, *L. cylindrical* dried fruit, leaves of *A. indica* and *M. azadarch* were found as efficient adsorbents. Five biosorbents viz. *R. arrhizus*, *O. sativa* straw, leaves of *F. bengalensis*, *F. religiosa* and *H. annus* hold the greater biosorption efficiency 80% for Cr(III). Four biosorbents, *T. harzianum, C. arietinum* husk*, F. religiosa* leaves and charcoal were recorded to be best option (75%± 5) as Cr(VI) sequestering agents at applied concentrations (5-50 mg L^{-1}).

References

Ahalya, N., Kanamadi, R. D., & Ramachandra, T. V. (2005). Biosorption of chromium(VI) from aqueous solutions by the husk of bengal gram (*Cicer arientinum*). *Electronic Journal of Biotechnology*, *8,* 258-264.
Ali. E. H., & Hashem, M. (2007). Removal Efficiency of the Heavy Metals Zn(II), Pb(II) and Cd(II) by Saprolegnia delica and Trichoderma viride at Different pH Values and Temperature Degrees. *Mycobiology*, *35*, 135-144 (
Aslam, M. Z., Ramzan, N., Naveed, S., & Ferooz, N. (2010). Ni(II) removal by biosorption using *Ficus religiosa* (peepal) leaves. *Journal of the Chilean Chemical Society*, *55*, 81-84.
Bayramoglu, G., Bektas, S., & Arica, M. Y. (2003). Biosorption of heavy metals on immobilized white-rot fungus *Trametes versicolor*. *Journal of Hazardous Material*, *101*, 285-300.
Das N., Vimala, R., & Karthika, P. (2008). Biosorption of heavy metals-An Overview. *Indian Journal of Biotechnology*, *17*, 159-169.

Dubey, S. P., & Krishna, G. (2007). Adsorption of chromium (VI) on low cost adsorbents derived from agricultural wastematerial. *Journal of Hazardous Materials, 145*, 465-470.

El-Syed, G.O., Dessouki, H.A., & Ibrahim, S.S. (2010). Biosorption Of Ni (II) And Cd (II) Ions From Aqueous Solutions Onto Rice Straw. *Chemical Sciences Journal*, *2010*, CSJ-9.

Fourest, E., & Roux, J. C. (1992). Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. *Applied Microbiology Biotechnology*, *3*, 399–403.

Gadd, G. M. (1993). Interaction of fungi with toxic metals. *New Phytology, 124*, 25-60.

Hmambika, B., Rani, M. J., & Kannan, V. R. (2011). Biosorption of heavy metals by immobilized and dead fungal cells: A comparative assessment. *Journal of Ecology and the Natural Environment*, *3*, 168-175.

Jain, M., Garg, V. K., & Kadirvelu, K. (2009). Chromium VI) removal from aqueous system using Helianthus annuus (sunflower) stem waste. *Journal of Hazardous material*, *162*, 365-372.

Javaid, A., Bajwa, R., & Javaid, A. (2010). Biosorption of heavy metals using a Dead Macro Fungus: Evaluation of Equilibrium and Kinetic Models. *Pakistan Journal of Botany, 42*, 2105-2118.

Lokeshwari, N., & Joshi, K. (2009). Biosorption of heavy metal (Chromium) by using biomass. *Global Journal of Environmental Research, 3*, 29-35.

Malkoc, E., Nuhoglu, Y., & Dundar, M. (2006). Adsorption of chromium (VI) on pomace-An olive industry waste: Batch and column studies. *Journal of Hazardous Materials, 138,* 142-151.

Mazali, I. O., & Alves, O. L. (2005) Morphosynthesis: high fidelity inorganic replica of the fibrous network of loofa sponge (*Luffa cylindrica*). *Anais Da Academia Brasileira De Ciências, 77,* 25-31.

Oboh, I., Aluyor, E., & Audu, T. (2009). Biosorption of Heavy Metal Ions from Aqueous Solutions Using a Biomaterial. *Leonardo Journal of Sciences, 14*, 58-65.

Pal, T. K., Bhattacharyya, S., & Basumajumdar, A. (2010). Cellular distribution of bioaccumulated toxic heavy metals in Aspergillus niger and Rhizopus arrhizus. *International Journal Pharmaceutical and Biological Sciences, 1,* 1-6.

Qaiser, S., Saleemi, A. R., & Ahmad, M. M. (2007). Heavy metals uptake by agro based waste material. *Electronic Journal of Biotechnology*, *10*, 410-416.

Rajender, K., Narsi, B., Garma, & Kiran, B. (2008). Biosorption of chromium(VI) from aqueous solution and electroplating wastewater using fungal biomass, *Chemical Engineering Journal*, *135*, 202-208.

Rowell, R. M., Pettersen, R., Han, J. S., Rowell, J. S., & Tshabalala, M. A. (2005), Cell wall chemistry. In: Handbook of wood chemistry and wood composites. Ed. Rowell, R.M., CRC Press, Boca Raton, FL., pp. 35-72.

Siegel, S. M., Gallun, M., Keller, P., & Siegel, B. Z. (1990). Filamentous fungi as metal biosorbents: A review. *Water, Air and Soil Pollution, 53*, 335-344.

Sun, Y-M., Horng, C-Y., Chnag, F-L., & Tiang, W-X. (2010). Biosorption of lead, Mercury and Cadmium by *Aspergillus terreus* immobilized in natural matrix. *Polish Journal of Microbiology*, *59,* 37-44.

Tsezos, M., & Volesky, B. (1981). Biosorption of uranium and thorium. *Biotechnology and Bioengineering*, 23, 583-604.

Volesky, B. (2003). Sorption and Biosorption (ISBN #0-9732983-0-8). BV Sorbex, Montreal-St.Lambert, Canada, 316.

Weast, R. C. (1988). CRC Handbook of Chemistry and Physics. CRC Press, Inc., Boca Raton, Fla.

Zvinowanda, C. M., Okonkwo, J. O., Agyei, N. M., Staden, M. V., Jordaan, W., & Kharebe, B. V. (2010). Recovery of Lead(II) from Aqueous Solutions by *Zea mays* Tassel Biosorption *American Journal of Biochemistry and Biotechnology 6*, 1-10.