RESEARCH PAPER:

Fungal biosorption of cadmium and zinc from industrial effluent

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SUMMARY

As a part of the systematic study of bioremediation of heavy metals from soil and aquatic environments, biosorption of Cd and Zn on living, dead and immobilized biomass of native isolate from local industrial effluent and artificial metal solutions were studied. Maximum biosorption (99%) in the order of Zn> Cd was achieved at pH 6.0 within 30 minutes using 1.0g of biosorbent. Immobilization increased stability of living biosorbent. Batch process was found superior over continuous column biosorption. Further, proteins profiles reflected metal toxicity mediated essential metabolic adjustments for high stable efficiency of isolate.

Key words : Fungal Biosorption, Cd, Zn, Industrial effluent

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Heavy metal contamination in soil and water is currently one of the most troublesome environmental problems faced by mankind. A sudden boost in industrial activities has contributed quantitatively to the alarming increase in the discharge of metal pollutants into environmental sink, especially the aqueous environment. Dispersion of the metal ions in the water bodies leads to their biomagnifications through the food chain and results in increased toxicity. This fact renders the removal of heavy metals from aqueous solutions indispensable. Metals discharged into water bodies are not biodegraded but undergoes chemical or microbial transformations, creating large impact on the environment and public health (Nowrot *et al.*, 2006; Green-Ruiz *et al.*, 2008). Metals and their free radicals are highly reactive in terms of attacking other cellular structures. The ability of metals to disrupt the function of essential biological molecules, such as protein, enzyme and DNA is the major cause of their toxicity (Volesky, 1992; Frausto da Silva and Williams, 1993).

Bioremediation technology is economically feasible, easy to apply to contaminated sites and causes little secondary pollution as compared to other known techniques. Since microorganisms have a genetic character for survival strategies in heavy metal polluted habitats, their specific microbial detoxifying mechanisms such as bioaugmentation, biomineralisation and biosorption can be applied eighter "*ex situ*" or "*in situ*" to the design of economical bioremediation processes. Microbial biomass can passively bind large amounts of metal (s), a phenomenon commonly referred to as biosorption(Malik, 2004; Umrania, 2006; Ahluwalia and Goyal, 2007).

Algae, bacteria, fungi and yeasts have proved to be potential metal biosorbents. Among micro organisms, fungal biomass offers the advantage of having a high percentage of cell wall material which shows excellent metal-binding properties. Fungi are known to have good metal uptake systems (Gadd, 1986) with metabolism-independent bio-sorption being the most efficient. The specific mechanisms of uptake differ quantitatively and qualitatively according to the species, the origin of the biomass and its processing .The hyphal wall was found to be a primary site of metal ion accumulation (Tobin *et al.*, 1984). In addition, living biomass may subject to toxic effect of heavy metals at elevated concentration. To overcome the disadvantages; non-viable or dead biomass is preferred (Butter *et al.*, 1998). Potential of filamentous fungi in bioremediation of heavy metal containing industrial effluents and wastewaters has been increasingly reported from different parts of the world. However, filamentous fungi of heavy metals polluted habitat in India are not largely screened and exploited for their bioremediation potential. Therefore, in this study, a native industrial effluent contaminated soil isolate of fungus was used for the removal of Cd and Zn from a local industrial effluent.

MATERIALS AND METHODS

Industrial effluent contaminated soil was used to isolate heavy metal resistant fungi by spread plate technique. Briefly, molasses medium containing molasses-40g/l, agar- 2%, sucrose-20g/l, yeast extract-3g/l, ammonium sulphate-4.50g/l, KH_2PO_4-3g/l, $MgSO_4.7H_2O$-1g/l, citric acid-0.25g/l, $CaCl_2.2H_2O$-0.05g/l conc. was sterilized at 15 lb/inch^2 for 15 min. and was poured into Petriplates. 0.1gm of aseptically collected composite soil was serially diluted in sterile double distilled water. 0.1ml of various dilutions were spread on molasses medium plates in duplicate and incubated at 29^{0}C for 5 days. The fungal growth was observed in 10^{-5} and 10^{-6} dilution plates. Subsequently, the fungus was sub cultured on the same medium. The colony formed by fungus was green velvety in colour from upper side and orange yellow in colour from reverse side of the Petri plate and thus identified as *Asperillus flavus.* Its metal tollerancy was tested by growing the fungus in liquid YPG medium which contained (in g/l): (yeast, 3; peptone, 10; glucose, 20) at various conc. of Cadmium and Zinc (1mg/l, 10mg/l, 50mg/l, 100 mg/l).

Effluent sample collected from *Hanung Toys and Textile Ltd., Roorkee* was diluted (50 times) analysed for heavy metal content by atomic absorption spectrophotometer (AAS Model- GBC Avanta Ver 1.31) at IIT, Roorkee.

Spores of 6-7 days old culture incubated on molasses medium at 29^{0}C were used for inoculation. The culture was grown at room temp. in YPG medium in a conical flask kept on a orbital shaker agitated at 110 rpm. The fungi grew in a filamentous (mould like) and formed spherical bodies/ pellets. The growth of the fungi was harvested after 3-4 days of growth by filtration using 150ìm sieve. For the treatment of biomass, live harvested biomass (68g) was treated with 0.5N NaOH for 30 min followed by washing with generous amount of distilled water until the pH of the solution reached to neutral range (pH 6.8-7.2) and autoclaved at 15 lb/inch^2 for 20 min. The pretreated biomass was dried at 60°C for 24hrs in hot air oven and powdered into in mortar and pestle.

An initial amount of dried biomass (0.5g) was added to 50ml of effluent solution in four different conical flasks at different pH values of 4, 5, 6 and 7 for the optimization of pH for metal biosorption on a orbital shaker at 110rpm for 24 hours at room temp. and 10ml of samples were withdrawn at regular time intervals (1/2, 1, 2, 4, 8, 12 and 24 hr) for residual amount of Zn and Cd. Then the effect of increased amount of biomass on biosorption was studied by increasing the amount of biomass to 1g, 1.5g and 2g in same conditions as above and for same time period.

Biosorption efficiency by immobilizing the living and dead biomass was studied. The cells of exponentially growing mycelia of the culture were harvested aseptically into a 25ml capacity homogenizer tube. The harvested cells were homogenized and mixed thoroughly. The mixture was then centrifuged at 3000rpm and supernatant was taken and mixed with accurate amount of sodium alginate. The mixture was subsequently pumped through a 5 ml syringe drop wise, into a flask containing sterilized 100 ml of 0.12 M calcium chloride solution .The reaction, which was almost instantaneous, was allowed a retention time of 1 h for complete precipitation that formed spherical beads. The immobilized cells were removed and stored until use at 4^{0}C in 5 mM $CaCl_2$ solution. For dead biomass, the cells of exponentially growing mycelia of the culture were harvested aseptically and autoclaved at 15 lb/inch^2 for 20 min and the same procedure was obtained to immobilize the dead cells as for living cells. Varying amount of dead and living immobilized fungal biomass was added to 100ml effluent and were kept on orbital shaker at 110rpm at room temperature for 24 hours and 10ml of samples were withdrawn at regular time intervals (1, 2, 4 and 24 hr.).

Biosorption by fungus in living mode was also studied. Fungus grown in YPG medium supplemented with various conc. of Cd and Zn (1mg/l, 10mg/l, 50mg/l and 100mg/l) was incubated for 72 hours. Alliquotes were withdrawn at 24hr, 48hr and 72hr for residual amount of Zn and Cd. Continuos coloumn sorption studies was also conducted, effluent solution was run manually from the top of a of 1.7cm diameter and 6cm in length coloumn which was packed with 3g of dried fungal biomass and fraction of eluant were collected at specific intervals of time till the column was saturated and the residual Cd and Zn concentration was analyzed using AAS.

Quntitative and qualitative protein profiles of fungus after undergoing the treatment with different regimes of Cd and Zn were studied following standard protocols of Lowry *et al.*,1951 and by running 12.5% SDS-PAGE. Protein was extracted by urea extraction sample buffer method (1% SDS, 9 M urea, 25 mM tris-HCl pH 6.8, 1 mM EDTA, 0.7 M Beta- mercapto ethanol). Germlings were taken out asceptically, washed once in distilled water and dried on paper towels. Cells were then frozen in liquid nitrogen, and lyophilized overnight. The lyophilized pellets were grinded up in a mortar and pestle weighed at 10 mg dry weight per extraction reaction and an equal volume of glass beads was added. 0.2 ml of urea extraction sample buffer was vigorously mixed with the lyophilized powder, boiled for 2 min, vortexed for 1 min, then boiled for

another minute.

The Cd and Zn residual concentration in the sorption medium was determined by AAS and the residual concentration R (%) will be calculated as: R = 100 x (Ci – Cf)/Ci

where,

Ci is the initial concentration of metal in the solution (mg/l)

Cf is the final concentration of metal in the solution (mg/l)

RESULTS AND DISCUSSION

Analysis of the effluent sample was done for initial concentration of Cadmium and Zinc. The concentration of Cd and Zn in the sample was found 12.85 mg/L and 14.3 mg/L, respectively (Fig. 1).

pH was optimized for the maximal rate of Cd and Zn biosorption on the fungal surface (0.5g) from definite amount of effluents (50ml). Biosorption was monitored at different time intervals in batch process and data were recorded in the form of Table 1. Data indicate maximal biosorption of both metals (Cd and Zn) in 30 minutes at pH- 6 in the order of Zn (99%)>> Cd (98%). The rate of biosorption was least and steady for next 30 minutes and thereafter. It might be due to the saturation of metal binding

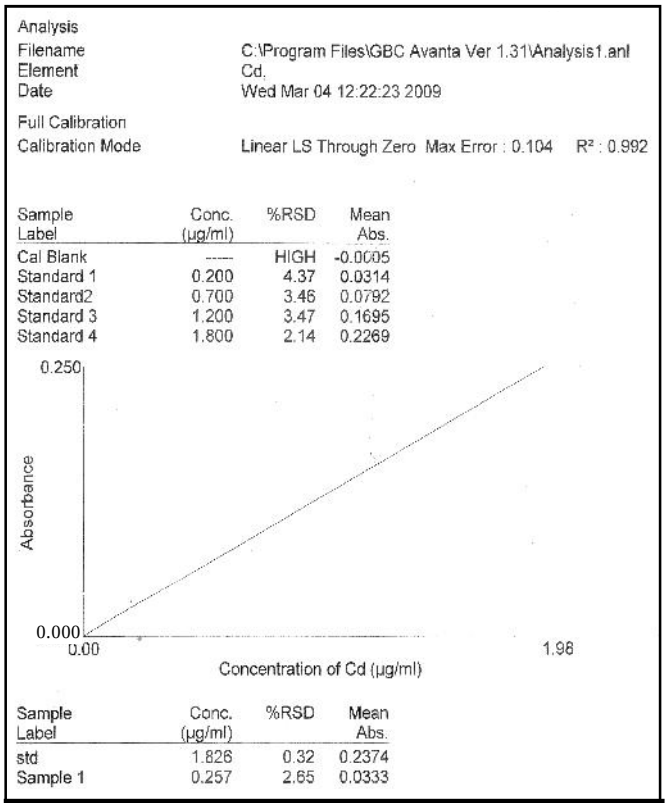

Fig. 1 a : Cd analysis

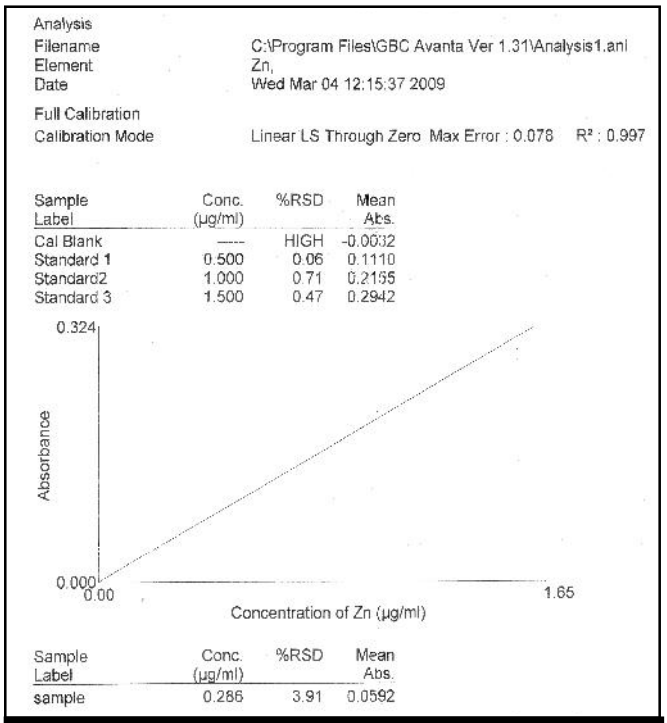

Fig. 1 b : Zn analysis

groups at fungal surface (cell wall) within 30 minutes of time (Crist *et al.*, 1981; Yan and Viraraghvan, 2000).

Cd and Zn biosorption was recorded through batch experiments for optimal pH at different time intervals and different regimes of fungal biomass. Results are presented in the form of Fig. 2.Biosorption rate of Zn was more as compared to Cd. Maximum biosorption was recorded within 30 minutes. Doubling of biomass (0.5g- 1.0g) caused no or least increase in heavy metal biosorption from fixed volume of effluent (50ml). Thereafter, rate of biosorption was inversely related to increase in biomass. This might be due to the low exposure/ electrostatic repulsion in same charge of metal capturing groups of fungal cell wall with the increased concentration of

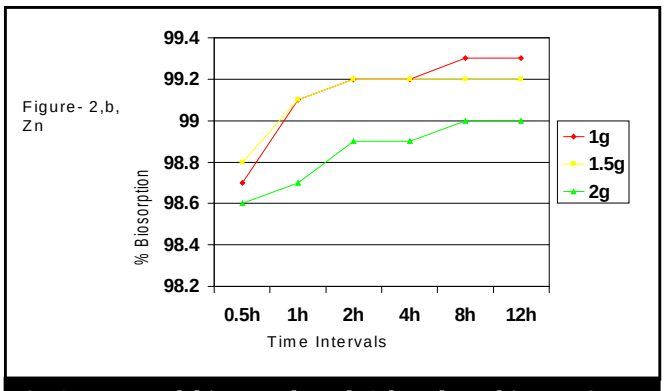

Fig. 2 : Fungal biomass based Cd and Zn biosorption at different time intervals

Table 1: Optimization of pH (room temp, rpm: 110)

Time (hours)	Samples	Conc. of cadmium (mg/L)	% reduction of Cd	Conc. of zinc (mg/L)	% reduction of Zn
0.5	pH 4	0.355	97.3	0.246	98.2
	pH 5	0.379	97.0	0.239	98.3
	pH 6	0.208	98.3	0.180	98.7
	pH 7	0.221	98.2	0.214	98.5
1	pH 4	0.198	98.4	0.179	98.7
	pH 5	0.146	98.8	0.153	98.9
	pH 6	0.120	99.0	0.114	99.2
	pH 7	0.218	98.3	0.146	98.9
2	pH 4	0.192	98.5	0.168	98.8
	pH 5	0.140	98.6	0.150	98.9
	pH 6	0.116	99.0	0.109	99.2
	pH 7	0.194	98.4	0.139	99.0
4	pH 4	0.188	98.5	0.159	98.8
	pH 5	0.136	98.9	0.141	99.0
	pH 6	0.108	99.1	0.105	99.2
	pH 7	0.189	98.5	0.133	99.0
8	pH 4	0.180	98.5	0.159	98.8
	pH 5	0.134	98.9	0.138	99.0
	pH 6	0.108	99.1	0.102	99.2
	pH 7	0.180	98.5	0.130	99.0
12	pH 4	0.178	98.6	0.156	98.9
	pH 5	0.130	98.9	0.134	99.0
	pH 6	0.105	99.1	0.099	99.3
	pH 7	0.180	98.5	0.125	99.1
24	pH 4	0.174	98.6	0.152	98.9
	pH 5	0.126	99.0	0.131	99.0
	pH 6	0.102	99.2	0.097	99.3
	pH 7	0.177	98.6	0.122	99.1

biomass in fixed volume of effluent (50ml) or this may reflect the shortage of metal (below the threshold value) concentration in the solution. Therefore, it is not useful to increase the biomass beyond 1g/50ml.

During biosorption experiment by fungus in living mode, a general increase in rate of biosorption was observed with the increase in metal concentration in media (solution). Rate of biosorption was found maximum at 24 h with sharp decrease thereafter. $\geq$ 90% biosorption was achieved at 50 and 100mg/L concentration of Cd and Zn, respectively (Table 2). These results reflect physiological adaptations of fungus towards the increased concentration of heavy metals in the solution (culture media). Decrease in biosorption at 1mg/L of Zn and 100mg/L Cd reflects the nature of two metals as micronutrient and toxic enviourenmental factor, respectively. Similarly, increase in biosorption at 10mg/L Zn and 100mg/L Zn reflects dual regimes, its supraoptimum and toxicity in order to develop tolerance and adaptation strategies in terms of induction of metal binding proteins: metallothioneins synthesis (Fig. 3).

Comparative Cd and Zn uptake by living and dead immobilized fungal biomass was studied at different time intervals.Maximum (98-99%) biosorption of both metals occurred in 1h by 1g of living as well as dead immobilized biomass (Table 3). A general increase in case of living biomass might be due to stabilized metabolic activities under immobilized conditions. Enhancement in heavy metal biosorption by living biomass after immobilization

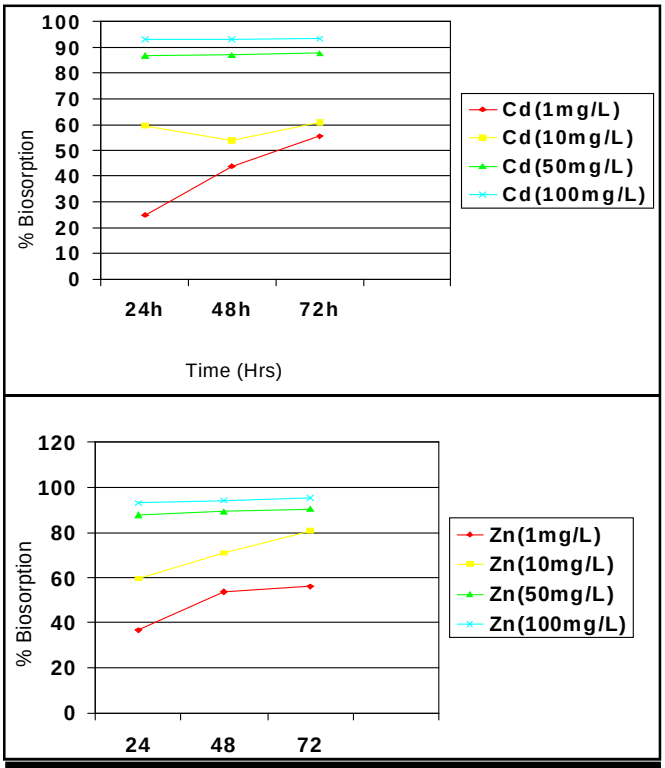

Fig. 3 : Metal enrichment based rate of biosorption at different time intervals of 24 h

Table 2 : Biosorption rates of Cd and Zn by living fungus

Time interval (hours)	Biosorption rates (mg/L/h)							
	Cd (1mg/L)	Zn (1mg/L)	Cd (10mg/L)	Zn (10mg/L)	Cd (50mg/L)	Zn (50mg/L)	Cd (100mg/L)	Zn (100mg/L)
24	.010	.015	.204	.248	1.80	1.82	3.87	3.88
48	.008	.007	.020	.046	.008	.030	.007	.040
72	.004	.008	.028	.041	.019	.016	.004	.048

Table 3 : Comparative metal uptake by living and dead immobized biomass at diiferent time intervals and varying amount of biomass (room temp, 110 rpm)

Time intervals (Hours)	Biomass	Metal uptake by biosorbent							
		Living immobilized				Dead immobilized			
		Cd mg/L	% reduction of Cd	Zn mg/L	% reduction of Zn	Cd mg/L	% reduction of Cd	Zn mg/L	% reduction of Zn
1	1g	0.13	99	0.15	99	0.120	98	0.22	98
	2g	0.14	99	0.15	99	0.14	99	0.17	99
	5g	0.14	99	0.15	99	0.17	99	0.19	99
	10g	0.15	99	0.16	99	0.16	99	0.18	98
2	1g	0.13	99	0.15	99	0.13	99	0.16	99
	2g	0.14	99	0.15	99	0.13	99	0.15	99
	5g	0.14	99	0.15	99	0.17	99	0.12	99
	10g	0.15	99	0.16	99	0.16	99	0.18	99
4	1g	0.13	99	0.14	99	0.11	99	0.12	99
	2g	0.14	99	0.14	99	0.12	99	0.12	99
	5g	0.14	99	0.14	98	0.17	99	0.12	99
	10	0.15	99	0.16	99	0.15	99	0.17	99
12	1g	0.12	99	0.14	99	0.11	99	0.12	99
	2g	0.13	99	0.14	99	0.11	99	0.11	99
	5g	0.14	99	0.15	99	0.17	99	0.19	99
	10g	0.14	99	0.15	99	0.15	99	0.17	99

was seen (Table 3 and Fig. 3 a and b). No significant increase in biosorption was seen due to increase in amount of biomass and time of exposure.

Effluent was run continuously on a column made up of dead fungal biomass as mentioned in methodology. After 4h of metal saturation on column, eluent contained 2.643mg/L Cd and 4.221 mg/L Zn and showed nearly 90% and 85% biosorption, respectively.

Fungal isolate grown at different conc. of Cd and Zn was harvested for the extraction and analysis of cellular proteins. No significant change in the proteins content at 1 and 100mg/L Cd treatments might be due to their respective non toxicity and induction of adaptation strategy in the fungal isolate. Increase in protein content at 10 and 50 mg/L Cd treatment might be due to fungal metabolic adjustments under stressed conditions (Table 4).

Table 4 : Fungal protein content at different regimes of Cd and Zn

Metal concentration	Total protein (mg/g fresh weight)
Control (00)	5.6
Cd (1mg/L)	5.9
Cd (10mg/L)	7.1
Cd (50mg/L)	6.8
Cd (100mg/L)	6.0
Zn (1mg/L)	5.0
Zn (10mg/L)	6.2
Zn (50mg/L)	4.2
Zn (100mg/L)	5.4

Fungal proteins were separated into polypeptides through 12.5% SDS-PAGE (Fig. 4).Induction of general polypeptides was due to all Cd regimes and 50mg/L Zn treatment reflects fungal metabolic adaptation under heavy metal toxicity. Normal band intensity of 1mg/L Zn

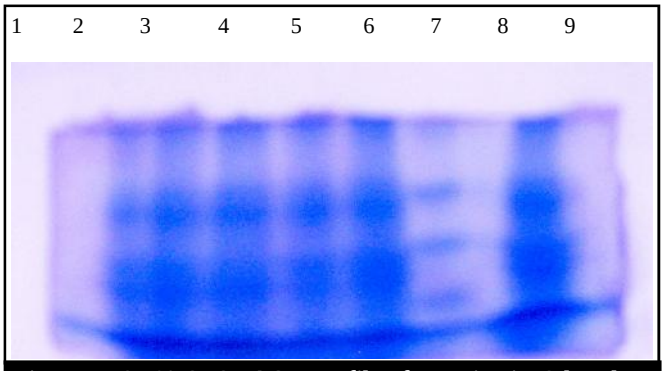

Fig. 4 : 12.5% SDS-PAGE profile of proteins in Cd and Zn treated fungal isolate where

Lane	Treatment (mg/L)	Heavy metal
1	Control (00)	-
2	1.0	Cd
3	10.0	Cd
4	50.0	Cd
5	100.0	Cd
6	1.0	Zn
7	10.0	Zn
8	50.0	Zn
9	100.0	Zn

treatment indicates optimal metabolic activities in fungal isolate.

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REFERENCES

Ahluwalia, S.S. and Goyal, D. (2007). Microbial and plant derived biomass for the removal of heavy metals from waste water. *Bioresour. Technol*.**, 98**; 2243-2257.

Butter, T.J., Evison, L.M., Hancoch, H.F.S., Matis, K.A., Philipson, A., Sheikh, A. J., Zouboulis, A.I. (1998). The removal of cadmium from dilute aqueous solution by biosorption and electrolysis at laboratory scale. *Water Res.,* **32**(2) : 400- 406.

Crist, R.H., Oberholser, K., Shank, K. and Nguyen, M. (1981). Nature of bonding between metallic ions and algal cell walls. *Environ. Sci. Technol*., **15**: 29- 33.

Frausto da Silva, J.J.R., Williams, R.J.P. (1993). *The Biological Chemistry of The Elements*: The inorganic chemistry of life. (London Press, Oxford).

Gadd, G.M. (1986). Fungal responses towards heavy metals. *Microbes in Extreme Environment,* : Academic Press, London, **42**: 83–110.

Lowery, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurements with the following phenol reagents. *J. Biol. Chem.*, **193** : 265-275.

Malik, A. (2004). Metal bioremediation through flowing cells. *Environ. Internat.*, **30** : 261- 278.

Nowrot, T., Plusquia, M., Hogervost, J., Roets, H.A., Colis, H., Thijs, I., Vangronsvald, I., Vanheeke, E. and Staessen, J.A. (2006). Environmental exposure to cadmium and risk of cancer, a perspective population based study. *Lancet Oncol*., **7** : 119-126.

Tobin, J.M., Copper, D.G. and Neufeld, R.J., (1984). Uptake of metal ions by *Rhizopus arrhizus. Appl. Environ. Microbiol.*, **47**: 821–824.

Umarania, V.V. (2006). Bioremediation of toxic heavy metals using acidothermophilic autotrophies. *Bioresour. Technol*., **97**: 1237- 1242.

Volesky, B. (1992). Biosorption of heavy metals *(CRC Press, Bocaraton FL)*, **24**: 13- 14.

Yan, G. and Viraraghavan, T. (2000). Effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii. Water SA* , **26**: 119- 123.