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BIOSORPTION OF LEAD BY INDIGENOUS FUNGAL STRAINS

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Abstract

Industrial effluent is a major environmental threat in Pakistan due to contaminant loads, especially of heavy metals. Bioremediation is a process that is in use to remediate effluents and is ecologically sound. In the present study, fungal strains isolated from effluent and adjacent contaminated soil of Koh-i-noor Textile Mills, Rawalpindi, Pakistan, were explored for the potential to remove lead from aqueous solution. *A. niger* RH 17 and *A. niger* RH 18 strains were tested for metal resistance on Pb-amended plates, which showed maximum resistance up to 6000 and 7000 mg/L, respectively. In media containing 1000 mg/L Pb, maximum lead removal exhibited by *A. niger* RH 17 was 92.04% and that by *A. niger* RH 18 was 93.09%, after three days incubation. The optimum pH for Pb detoxification was 9.0 and 9.5 for *A. niger* RH 17 and *A. niger* RH 18 respectively, with respective removal percentage being recorded as 93.8% and 94.2%. Pb biosorption was also assessed at different temperatures, in media having 1000 mg/L Pb at pH 9.0 and 9.5, for both strains. Maximum removal for both strains was seen at 28˚C. *A. niger* RH18 biosorbed 209.33 mg Pb per gram of the fungal biomass at pH 9.5. These newly isolated fungal strains offer the potential of being used as an effective biosorbent of Pb and bringing about its removal from industrial wastewaters.

Introduction

With rapid industrial development, problems related to pollution are becoming severe. Unfavourable alterations in the environment result in change of energy flow in the universe, and also in its chemical and physical composition. It adversely affects human life through water resources, agriculture and biological products (Naidu *et al*., 1996). In Pakistan, due attention has not been given to the control and management of the industrial wastes and other pollutants. Certain types of industrial development have left an international legacy of soil and water pollution, making the problem even greater. Short-term interests have put a great strain on environment caring capacity (Din *et al.*, 2001).

Lead (Pb) is one of the most important heavy metals, since it poses a great danger for humans, if accumulated in larger amounts. Petrol combustion globally contributes an estimated 60% of total lead emission. Auto-exhaust lead pollution is the main route to introduce lead in soil and vegetation (Mohammed *et al.,* 1996). Lead is widely used in battery manufacturing, printing, pigments, fuels, photographic materials and paint industry (Martins *et al.,* 2006; Parvathi *et al*., 2007). Khan & Jaffar (2002) recorded 33.3-338.7 $\mu g/m^3$ Pb in air, 0.62-21.2 mg/Kg in soil and from below detectable levels to 170 μg/L in water. Field force of Lahore traffic police is reportedly suffering from haemolytic anaemia due to lead poisoning (Arshad & Shafaat, 1997).

The need for economical, effective and safe methods for removing heavy metals from wastewater has resulted in search for unconventional methods that may be useful in reducing the levels of accumulated heavy metals in the environment. There is potential for employing biotechnology for removal of heavy metals. Metal sequestering properties of certain types of microorganisms offer considerable promise. Fungi can accumulate heavy metals and radionuclides even from dilute external concentrations. Fungal cell walls and their components have a major role in biosorption and also take up suspended metal particles and colloids (Ahmad *et al.,* 2006). Fungi can be grown in substaintial amounts using unsophisticated fermentation techniques, and inexpensive growth media (Preetha & Viruthagiri, 2005). Therefore, bioaccumulation carried out by fungi could serve as economical means of treating metal containing effluent. Çabuk *et al*., (2005) removed Pb using various fungal biomasses of *Aspergillus versicolor, Metarrhizium anisoplia var. anisoplia* and *Penicillium verucosum* and found high biosorption of Pb by live treated biomass of *A. versicolor* upto 30.6 mg/g fungal biomass. Using a strain of *Aspergillus terrus* immobilized in polyurethane foam, after 6 days incubation, 164.5 mg Fe, 96.5 mg Cr and 19.6 mg Ni was biosorbed per gram of fungal biomass, from 100% metallurgical effluents supplemented with 1x of glucose (Dias *et al.,* 2002). The studied strain of *Aspergillus terreus* proved to be ideal for treatment of steel foundry effluent. Non-living biomass of *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07 have been shown to adsorb more than 80% Zn from aqueous solution (Faryal *et al.,* 2006). Bioremediation may soon compete with chemical methods in efficiency and cost-effectiveness.

The present study was carried out to evaluate potential fungi for use in bioremediation of Pb, a heavy metal present in effluents from various industries. Fungal strains from wastewater and soil contaminated with industrial effluent were first assessed for endurance of Pb by determination of maximum resistance levels of these fungal strains. Optimization of various conditions for removal of Pb was also carried out.

Materials and Methods

In this study, two strains of *Aspergillus niger,* RH 17 and RH 18 were used. These strains were isolated and identified upto species level and maintained on Sabouraud dextrose agar slants at 4°C till further use. During batch culturing in shake flasks, Sabouraud dextrose broth of same composition was used, except that agar was not added.

Selection of lead tolerant strains: In order to determine the maximum tolerance of all the isolated fungi against Pb, the method proposed by Fomina *et al*., (2005) was followed. Sabouraud dextrose agar plates were prepared with the metal salt $Pb(NO_3)_2$ in distilled deionized water, using varying concentrations (1000-9000 mg/L). Inoculation was carried out by using 7 mm diameter agar plug of mycelial growth from the growing edge of the colonies and incubated at 28°C for 7 to 14 days. The size and appearance of the clear zones were recorded every day. *A. niger* RH 17 and *A. niger* RH 18 showed maximum resistance against lead in Pb amended plates, and these strains were selected for further biosorption studies.

Biosorption at various concentrations and pH: Growth conditions of these strains were optimized at various temperatures (28-40°C) and pH (4-12). Both strains were separately inoculated in Sabouraud dextrose broth (100 mL), containing different concentrations of Pb, ranging from 700 to 1300 mg/L, with increments of 100 mg/L. Each flask was inoculated with equal numbers of spores (20 loopsful, corresponding to $1x10^6$ spore/mL), in sterilized conditions, for six days in an orbital shaker (100 rpm) at 28°C. Flasks containing the strains inoculated in Sabouraud dextrose broth (100 mL), without the metal salt were used as control.

Effect of temperature and pH on Pb removal: In order to observe the effect of temperature and pH on metal removal, the experiments were repeated using 1000 mg/L Pb at pH 9.0 and 9.5 for both the strains (the concentration and pH levels which yielded best results in terms of metal removal), and keeping them at three different temperatures (28°, 35° and 40°C) in the orbital shaker.

Analytical methods: The samples (5 mL) were drawn and digested for metal analysis, alongwith biomass which was filtered, dried at 55°C and weighed, at the same time daily from the initiation to the termination of the study, as described earlier (Faryal *et al*., 2006). Residual lead concentrations left in these samples were measured by using air-acetylene flame of Solar Unicam atomic absorption spectrophotometer at 283.3 nm. Determination limit for Pb was 0.05 mg/L, sensitivity 0.05 mg/L and optimum concentration range 1-20 mg/L (Clesceri *et al*., 1989). All biosorption experiments were carried out in duplicate and values used in calculation were arithmetic averages of experimental data. The amount of Pb^{2+} biosorbed per gram of dried biomass was calculated using the following formula:

$$Q = (C_i - C_e) \times V/m$$

where Q = metal ions (mg) biosorbed per gram of biomass, C_i = initial metal ion concentration (mg/L), C_e = final concentration of metal ion (mg/L), m = dry weight of biomass in reaction mixture (g) and V = volume (L) of the reaction mixture (Çabuk *et al.,* 2005; Khani *et al*., 2006).

Statistical analysis: Data are represented as Mean ± Standard Error of Mean. Data were subjected to statistical analysis through Student's 't'-test, as described by Steel & Torrie (1960).

Results

Selection of lead tolerant strains: Both isolates had optimum pH of 9.0 and temperature of 28°C for growth. By comparison, *A. niger* RH 17 and *A. niger* RH 18 were able to grow up to 6000 and 7000 mg/L of Pb^{2+}, and were selected for further shake flask based detoxification studies. These strains also produced halos around their colonies of various diameters, and their diameter reduced with increase in concentration of the added metal ion.

Biosorption at various concentrations and pH: Maximum Pb removal was obtained at 1000 mg/L. In most cases the maximum biosorption was observed at 3rd day, while maximum Pb biosorption recorded at 1000 mg/L concentration on the 6th day was 92.06% for *A. niger* RH 17 and 92.72% for *A. niger* RH 18 (Fig. 1, A and B). These strains biosorbed 204.57 and 206.04 mg Pb^{+2} per gram of dried biomass, for *A. niger* RH 17 and RH 18, respectively. An increase or decrease in the concentrations of Pb^{+2} resulted in decreased removal of lead per gram of biomass.

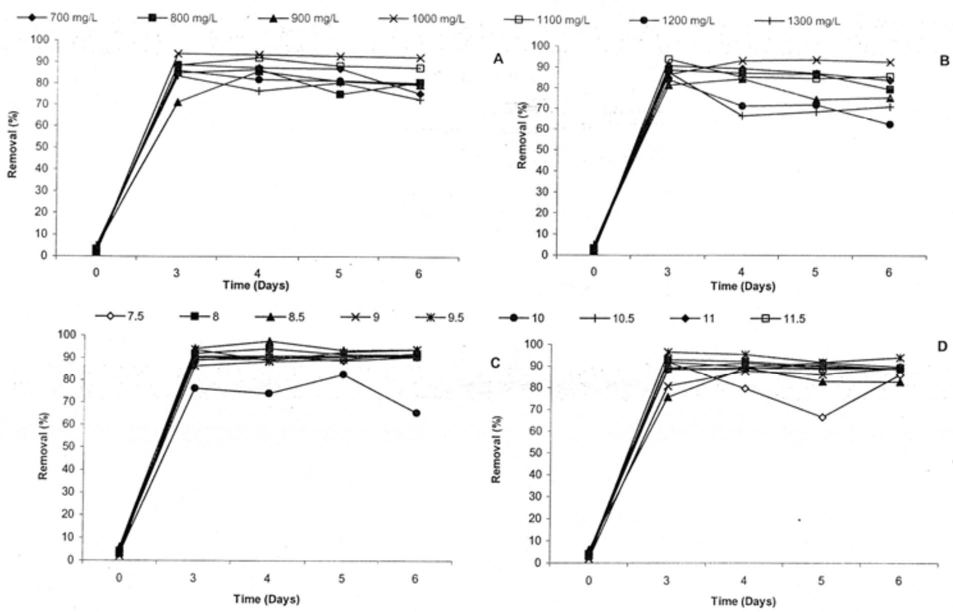

Fig. 1. Percentage removal of lead at different metal concentrations (mg/L), by *Aspergillus niger* strains RH 17 (A) and RH 18 (B), and at various pH by the two strains, respectively (C and D).

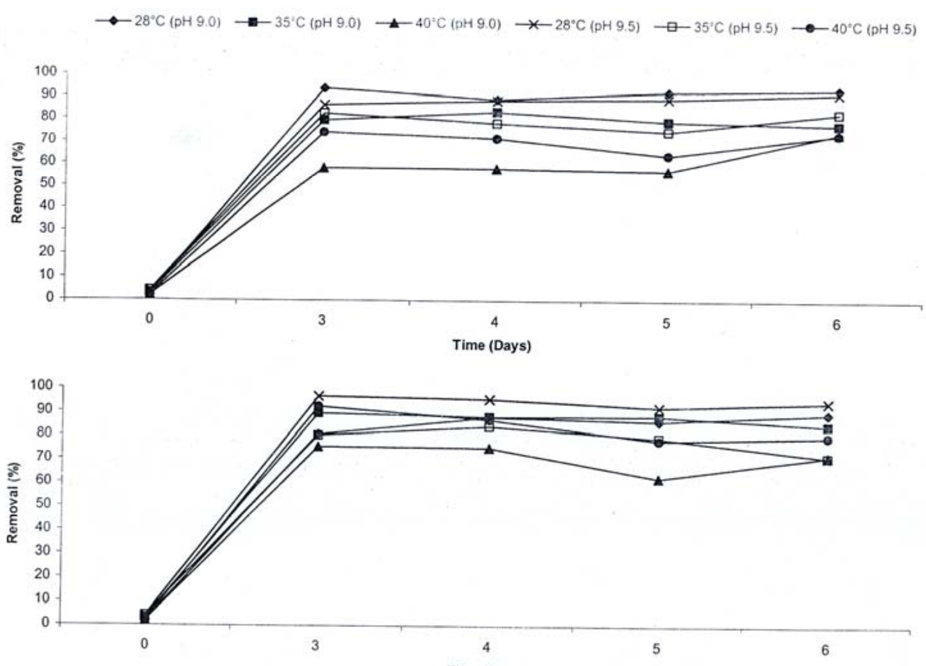

Fig. 2. Percentage removal of lead by *Aspergillus niger* strains RH 17 (A) and RH 18 (B), at different combinations of temperature and pH, from aqueous solution containing 1000 mg/L lead.

At pH 9.0 and 9.5 the two strains showed the best results for Pb removal (Fig. 1, C and D), however, at pH 9.5, maximum lead removal was carried out by *A. niger* 18. *A. niger* RH 17 biosorbed 93.8% Pb (187.06 mg/g) at pH 9.0, and 92% (184 mg/g) at pH 9.5, while *A. niger* RH18 biosorbed 89.4% Pb at pH 9.0 and 94.2% at pH 9.5. At pH 9.5, *A. niger* RH18 biosorbed 209.33 mg Pb per gram of the fungal biomass.

Effect of temperature and pH on Pb removal: While comparing the effect of temperature, best results were obtained at 28°C for both strains. *A. niger* RH17 showed highest detoxification of Pb (93.8%) at pH 9.0, and for *A. niger* RH18, the maximum Pb removal percentage was 94.2% (pH 9.5). At 40°C *A. niger* RH17 biosorbed 74.14% (pH 9.0) and *A. niger* RH18 biosorbed 79.90% (pH 9.5), biosorption per gram of fungal biomass being reduced to 82.37 and 88.77 mg/g. The percentage removal of Pb at 35°C was 88.84% (pH 9.5) and 86.64% (pH 9.0) for *A. niger* RH17 and *A. niger* RH18, respectively.

Discussion

Microbial bioremediation is a promising method of environmental cleanup (Konopka *et al.,* 1999). Amongst the microbial flora present in the effluent of the KTM, fungi were selected for the present study, due to the ease they offer for removal from liquid substrates. The endurance of Pb of each fungal strain was recorded by inoculating them separately in lead amended plates. *A. niger* RH 17 and *A. niger* RH 18 showed the highest resistance, being 6000 and 7000 mg/L, respectively, warranting them to be successful candidates for metal detoxification. The colony diameter of *A. niger* RH 17 after 7 days was 48.4 mm, while in another study (Price *et al.,* 2001), different fungi were able to grow on 5 mM zinc, but *A. niger* grew best with a colony diameter of 84.5mm after 7 days. Both the *A. niger* strains used in the current study (RH17 and RH18), may be involved in solublizing lead, because clear zones or halos were observed in Pb amended agar plates, under and around the colonies, which is an indicator of metal solublization (Sayer *et al*., 1999). Sayer *et al*., (1995), observed that *A. niger* could solubilize Zn when grown on malt extract with 4% (w/v) $Zn_3(PO_4)_2$. Fomina *et al*., (2005) reported lead mineral solubilization as well as accumulation of lead in fungal mycelium, by excretion of various organic acids such as oxalic acid, acetic acid and lactic acid, even at alkaline pH.

Both fungal strains showed maximum Pb removal at 1000 mg/L metal concentration. Similarly, Ilhan *et al*., (2004) reported 27% lead biosorption by *Penicillium lanosa-coeruleum*. However, with any increase or decrease in concentration from 1000 mg/L, the rate of Pb sorption decreased, as was also recorded for Cr and Cd by *Aspergillus* spp. and *Rhizopus* spp., isolated from agricultural fields treated with sewage/industrial effluents, in which at 25°C, *Aspergillus niger* biosorbed highest at 4 mM, as compared to 2 and 6 mM, thus reflecting the same pattern of biosorption dependence on the initial concentration of the metal ions (Ahmad *et al*., 2005). Maximum removal was recorded on the third day, while desorption and adsorption occurred in following days. Maximum removal was observed at 1000 mg/L after 72 hours, while Santos & Lenzi (2000), reported that water hyacinth (*E. crassipes*) with biomass 20g/L was highly efficient in lead absorption since it absorbed 90% of the contaminating solution ($Pb(NO_3)_2$), after 48 hours.

A direct proportionality was observed between pH and lead biosorption capacity up to 9.5. Optimization of pH in shake flask experiments showed that *A. niger* 17 biosorbed 93.7% Pb at pH 8.5, 93.8% at pH 9.0 and 92% at pH 9.5 while *A. niger* 18 biosorbed 82.98% Pb at pH 8.5, 89.4% at pH 9.0 and 94.2% at pH 9.5, Likewise the present study, live biomass of *Mucor rouxii* treated with NaOH has been reported to have Pb^{2+} biosorption capacity of 36.69 mg/g. In our study, a steady Pb removal was obtained at pH 9.0 for *A. niger* RH 17 and pH 9.5 for *A. niger* RH 18, i.e., alkaline pH corresponding to their respective environment (Faryal & Hameed, 2005). These results showed that alkaline pH was best for lead removal in accordance with the pH values of soil and effluent from which strains were isolated. The uptake of Pb^{2+}has been reported to be strongly affected by pH (Yan & Viraraghavan, 2003).

Yin *et al*., (2001) found that Cd^{2+} biosorption from aqueous solution by *Laminaria japonica* was pH dependent and a maximum uptake capacity of about 1.3mmol/g dry weight at pH 6.0. *R. arrhizus, M. meihei, P. chrysogenum* have shown maximum sorption capacity, 200mg/g biomass, for lead at pH 7.0 (Fourest *et al.,* 1994). Kim *et al*., (1995) reported lead uptake capacity per gram dry biomass of *U. pinnatefida* as 350 mg, within a pH range of 3 to 4. pH is a major factor influencing the adsorption (Pan *et al.,* 2000).

Temperature also played an important role in Pb biosorption. The difference of the action of the two selected strains on Pb^{2+} removal, with respect to temperature was also studied. The temperature ranges used were 28°C, 35°C and 40ºC. Best Pb removal was observed at 28ºC, although Pb removal was also remarkable at the other two temperatures, but the Pb biosorption per gram of the biomass was reduced. The biomass produced by both strains increased with the increase in temperature from 28ºC to 40ºC at pH 9.0 but biosorption of lead decreased per gram of biomass. At 40ºC, there was least Pb^{2+} removal as compared to the other temperatures used, where as the biomass produced was highest at this temperature, average being 0.9-0.95g dried biomass/100mL media. *R. arrhizus* adsorptive capacity for Cr^{6+} and Fe^{3+} reportedly increased with increasing temperature with the range of 25-45ºC and 25-35ºC, respectively (Sağ & Kutsal, 1996). *Z. ramigera* and *R. arrhizus* have been shown to exhibit the highest lead adsorptive rate at pH 4.5-5.0, temperature range 25-45ºC and with increasing metal ion concentrations, 150-200mg/L and 200-300gm/L, for *Z. ramigera* and *R. arrhizus* respectively. In our study, pH 9.0 and 9.5, 28°C and 1000 mg/L Pb^{2+} concentration were best for both *A. niger* strains in terms of Pb removal. The two strains only differed at their preferential pH for metal removal, in terms of the environmental conditions.

Fungal strains, isolated from the textile effluent and adjacent contaminated soil have an appreciable capacity of lead biosorption. The most suitable incubation period for metal removal is three days, as maximum Pb removal was recorded on the 3rd day, and after that, in most of the cases, desraption occurred. Both strains showed maximum lead removal at alkaline pH, which was the pH of their isolation site, whereas most of the literature reports that many fungi can remove metals at acidic pH. That is a remarkable capacity of both the strains, and another interesting feature is that these strains showed an excellent lead removal capability over a wide range of temperature. It would suggest that these fungal strains may be used in future for bioremediation of wastewater and heavy metals contaminated soils.

References

Ahmad, I., M.I. Ansari and F. Aqil. 2006. Biosorption of Ni, Cr and Cd by metal tolerant *Aspergillus niger* and *Penicillium* sp., using single and multi-metal solution. *Indian Journal of Experimental Biology,* 44(1): 73-76.

Ahmad, I., S. Zafar and F. Ahmed. 2005. Heavy metal biosorption potential of *Aspergillus* sp. and *Rhizopus* sp., isolated from wastewater treated soil. *Journal of Applied Sciences and Environmental Management,* 9(1): 123-126.

Arshad, M. and Y.K. Shafaat. 1997. Effect of lead poisoning on some haematological parameters of Lahore traffic police. *Biologia,* 43(1): 51-54.

Çabuk, A., S. İlhan, C. Filik and F. Çalişkan. 2005. Pb^2 biosorption by pretreated fungal biomass. *Turkish Journal of Biology,* 29:23-28.

Clesceri, L.S., A.E. Greenberg and R.R. Trussel. 1989. *Standard methods for the Examination of water and waster water*. 17th Edition, APHA/AWWA/WPCF Publications, Washington D. C. Part. 3000.

Dias, M.A., I.C. Lacerda, P.F. Pimentel, H.F. deCastro and C.A. Rosa. 2002. Removal of heavy metals by an *Aspergillus terreus* strain immobilized in polyurethane matrix. *Lettres in Applied Microbiology,* 3 4(1): 46-50.

Din, S., A.M. Salariya, J. Hafeez, M. Yasin and M. Ashraf. 2001. Heavy metals contents of some vegetables collected from fields irrigated with polluted water. *Pakistan Journal of Scientific Research,* 53(3-4): 103-107.

Faryal, R. and A. Hameed 2005. Isolation and characterization of various fungal strains from textile effluent for their use in bioremediation. *Pakistan Journal of Botany,* 37: 1003-1008.

Faryal, R., A. Lodhi and A. Hameed 2006. Isolation, characterization and biosorption of zinc by indigenous fungal strains *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07. *Pakistan Journal of Botany,* 38: 817-831.

Fomina, M., S. Hillier, J.M. Charnock, K. Melville, I.J. Alexander and G.M. Gadd. 2005. Role of oxalic acid over-excretion in toxic metal mineral transformations by *Beauveria caledonica*. *Applied and Environmental Microbiology,* 71(1): 371-381.

Fourest, E., C. Canal and J.C. Roux. 1994. Improvement of heavy metal biosorption by mycelial dead biomasses (*Rhizopus arrhizus, Mucor miehei and Penicillium chysogenum*): pH control and cationic activation. *FEMS Microbiology Reviews,* 14(4): 325-332.

İlhan, S., A. Çabuk, C. Filik and F. Çalişkan. 2004. Effect of pretreatment on biosorption of heavy metals by fungal biomass. *Trakya Üniversitesi Fen Bilimleri Dergisi (Trakya University Journal of Science),* 5(1): 11-17.

Khan, A. and M. Jaffar. 2002. Lead contamination of air, soil and water in the vicinity of Rawal Lake, Islamabad. *Journal of Applied Sciences,* 2(8): 816-819.

Khani, M.H., A.R. Keshtkar, B. Meysami, M.F. Zarea and R. Jalali. 2006. Biosorption of uranium from aqueous solutions by non living biomass of marine algae *Cystoseira indica*. *Electronic Journal of Biotechnology (online),* http://www.ejbiotechnology.info/content/vol9/issue2/full/8/index.html.

Kim, Y.H., Y.J. Yoo and H.Y. Lee. 1995. Characteristics of lead biosorption by *Undaria pinnatefida*. *Biotechnology Letters*, 17(3): 345-350.

Konopka, A., T. Zakharova, M. Bischoff, L. Oliver, C. Nakatsu and R.F. Turco. 1999. Microbial biomass and activity in lead-contaminated soil. *Applied and Environmental Microbiology*, 65(5): 2256-2259.

Martins, B.L., C.C.V. Cruz, A.S. Luna and C.A. Henriques. 2006. Sorption and desorption of Pb^{2+} ions by dead *Sargassum* sp. biomass. *Biochemical Engineering Journal,* 27(3): 310-314.

Mohammed, T.I., I. Changyen and I. Bekele. 1996. Lead pollution in East Trinidad resulting from lead recycling and smelting activities. *Environmntal Geochemistry and Health,* 18: 123-128.

Naidu, R., R.S. Kookuna, D.P. Oliver, S. Rogers and M.J. McLaughlin. 1996. Contaminants and the soil environment in the Austrilasia-Pacific Region. *Proceedings of the First Australasia-*

Pacific Conference on Contaminants and Soil Environment in the Australasia-Pacific Region, held in Adelaide, Australia 20-24 February 1996. Kluwer Acedamic Publishers. 629-646.

Pan, J.F., R.G. Lin and L. Ma. 2000. A review of heavy metal adsorption by marine algae. *Chinese Journal of Oceanology and Limnology*, 18(3): 260-264.

Parvathi, K., R. Nagendran and R. Nareshkumar. 2007. Lead biosorption onto waste beer yeast by-product, a means to decontaminate effluent generated from battery manufacturing industry. *Electronic Journal of Biotechnology (online),* http://www.ejbiotechnology.info/content/ vol10/issue1/full/13/index.html.

Preetha, B. and T. Viruthagiri. 2005. Biosorption of Zinc(II) by *Rhizopus arrhizus*: equilibrium and kinetic modelling. *African Journal of Biotechnology,* 4(6): 506-508.

Price, M.S., J.J. Classen and A.G. Payne. 2001. *Aspergillus niger* absorbs copper and zinc from swine wastewater. *Bioresource Technology*, 77: 41-49.

Sağ, Y. and T. Kutsal 1996. Fully competitive biosorption of chromium (VI) and iron (III) ions from binary metal mixtures by *R. arrhizus*: Use of the competitive langmiur model. *Process Biochemistry*, 31(6): 573-585.

Santos, M.C.D. and E. Lenzi. 2000. The use of aquatic macrophytes (*E. crassipes)* as a biological filter in the treatment of lead contaminated effluents. *Environmental Technology*, 21: 615-622.

Sayer, J.A., J.D. Cotter-Howells, C. Watson, S. Hillier and G.M. Gadd. 1999. Lead mineral transformation by fungi. *Current Biology,* 9: 691-694.

Sayer, J.A., S.L. Raggett and G.M. Gadd. 1995. Solubilization of insoluble metal compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. *Mycological Research*, 99: 987-993.

Steel, R.G.D. and J.H. Torrie. 1960. Sampling from a normal distribution. In: *Principles and procedures of statistics with special reference to biological sciences*. McGraw-Hill Book Company Inc., New York, U.S.A., pp. 49-66.

Yan, G. and T. Viraraghavan. 2003. Heavy-metal removal from aqueous solution by fungus *Mucor rouxii. Water Research*, 37(18): 4486-4496.

Yin, P., Q. Yu, Z. Lin and P. Kaewsarn. 2001. Biosorption and desorption of cadmium (II) by biomass of *Laminaria japonica*. *Environmental Technology*, 22(5): 509- 514.

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