BIOREMEDIATION OF HEAVY METALS WITH MICROBIAL ISOLATES

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1 INTRODUCTION

Heavy metal pollution is nowadays one of the most important environmental concerns. Anthropogenic activities like metalliferous mining and smelting, agriculture, waste disposal or industry discharge a variety of metals such as Ag, As, Au, Cd, Co, Cr, Cu, Hg, Ni, Pb, Pd, Pt, Rd, Sn, Th, U and Zn, which can produce harmful effects on human health when they are taken up in amounts that cannot be processed by the organism. Damage may cause adverse reactions in different organs and biological functions, including reproduction and birth defects (Malik, 2004). Fauna and flora are also affected by these detrimental effects.

Physical and chemical methods have been proposed for the removal of these pollutants. Nevertheless, they have some disadvantages, among them cost-effectiveness limitations, generation of hazardous by-products or inefficiency when concentration of polluted materials is below 100 mg l^{-1} (Gavrilescu, 2004; Wang and Chen, 2009). Biological methods solve these drawbacks since they are easy to operate, do not produce secondary pollution and show higher efficiency at low metal concentrations (Chen *et al.*, 2005; De *et al.*, 2008). Microorganisms and plants are usually used for the removal of heavy metals. Mechanisms by which microorganisms act on heavy metals include biosorption (metal sorption to cell surface by physiochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes) intracellular accumulation, and enzyme-catalyzed transformation (redox reactions) (Lloyd, 2002). On the basis of energetic requirements, biosorption seems to be the most common mechanisms (Haferburg and Knothe, 2007). Furthermore, this is the only option when dead cells are applied as bioremediation agent. Nevertheless, systems with living cells allow more effective bioremediation processes as they can self-replenish and remove metals via different mechanisms (Malik et al., 2004). On the other hand, living cells show higher sensitivity to environmental conditions and demand nutritional and energetic sources.

The isolation of heavy metal resistant microorganisms and the understanding of the mechanisms they use in order to remove this kind of pollutants may contribute to the development of improved detoxification processes. The aim of this work is to study the ability of five different compost isolates to remove heavy metals from a multi-polluted solution and to compare efficiency of both living and dead cells.

2 MATERIALS AND METHODS

2.1 Microbial isolates and maintenance

Five multi-resistant fungi (LE48T1, RVT3, RVE48T3, LE48T4 and LT5) were isolated from composting processes made with different starting materials (sewage sludge (L), agricultural waste (RV) and municipal solid waste) at different sampling times according to thermal evolution. Nutrient Broth amended with 50 ppm Cd (II), Cr (VI), Ni (II), Pb (II) and Zn (II) was used as enrichment medium. Resistant strains were isolated after three consecutive cultures at 30 ºC during 48 h using heavy metal enriched Nutrient Agar.

2.2 Living cells study

Heavy metal removal was investigated in Erlenmeyer flasks (250 ml) containing 50 ml of Nutrient Broth supplemented with heavy metal as described above, and inoculated with a 1 cm^2 piece from agar plates in which fungi were grown for 72 h. After incubation (30 ºC for 120 h at 100 rpm), cells were harvested by centrifugation. Both supernatant and biomass (three times washed with NaOH 1N) were digested with HNO_3 67% and H_2O_2 (30% v/v), and metal concentration was determined by atomic absorption spectrometry (ThermoElectron Corporation S Series AASpectrometer, Massachussets, United States).

2.3 Dead cells study

Biomass from five isolates grown in Nutrient Broth with heavy metals were harvested by centrifugation and washed with distilled water three times. The pellet was dried and milled to a final particle size of 1 mm. Aliquots of 0.025 g of dried biomass were added to Erlenmeyer flasks containing 25 ml of heavy metal solution (50 ppm of each Cd^{2+}, Cr^{6+}, Ni^{2+}, Pb^{2+} and Zn^{2+}). Erlenmeyers were shaken (120 rpm) for 90 min at 30 ºC and then suspensions were centrifuged and filtered for biomass removal. Heavy metal concentration in the digested supernatant was measured as previously described.

3 RESULTS AND DISCUSSION

3.1 Living cells study

Heavy metals were efficiently removed by the growing cells of all the tested isolates, with nickel the most recalcitrant pollutant and lead the most affected by the microbial action. Other authors have described nickel as one of the more resistant heavy metal to bioremediation (Filipović-Kovačević *et al*., 2000; Malik, 2004; Moore *el al*., 2008), both regarding biosorption and intracellular accumulations. Steric hindrances caused by the characteristics of the cation have been postulated as one of the reasons for explaining this low biosorptive capacity (Wong *et al*., 2000), while bioaccumulation might be hampered by the lack of some carrier proteins (Pervez *et al*., 2009). On the contrary, efficiency levels close to 100% have been reported for lead detoxification (Kim *et al*., 2007). These results are mostly associated to multi-metal studies, like this one, in which the presence of other inorganic pollutants seems to decrease the toxicity of Pb^{2+} to fungi (Pan *et al*., 2009) and increase the removal capacity of fungi in comparison to single studies. This effect was also described by Yan and Viraraghavan (2003) when they analyzed the biosorption capacity of *Mucor rouxii* for different heavy metals in binary and ternary solutions.

Cd^{2+}, Cr^{6+} and Zn^{2+} removal activity ranged between 85% and 60%, with intracellular accumulation as predominant mechanisms in most of the cases. Pan *et al*. (2009) observed similar results using *Penicillium* and *Fusarium* as bioremediation agents, as well as Ting and Choong (2009) in their comparison between the ability of a *Trichoderma* isolate to bioaccumulate and biosorb. According to Zucconi *et al*. (2003) and Chojnacka (2007), bioaccumulation involves a first biosorption step which rapidly reaches equilibrium and gives way to intracellular uptake. Thus, bioaccumulation tends to be more important as contact time between heavy metals and growing cells increases.

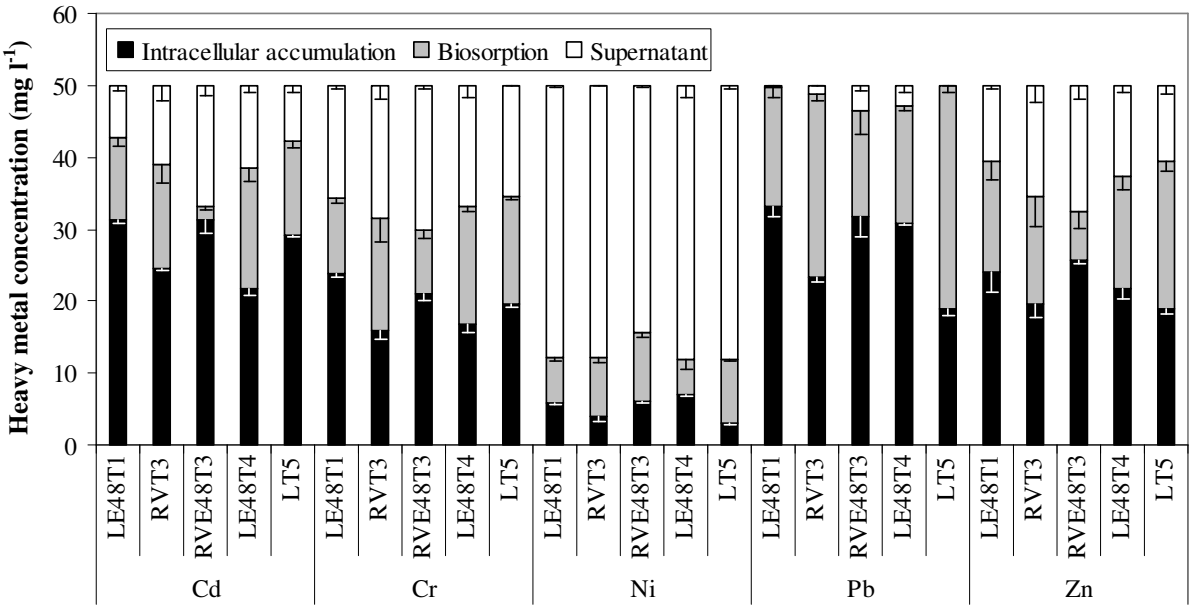

FIGURE 1 **Heavy metal removal by living cells of microbial isolates**

3.2 Dead cells study

Dead cells were less efficient at biosorbing heavy metals than living cells (Fig. 2). The removal capacity ranged between 6% and 34%, with the exception of Pb^{2+}. This element, as occurs when living cells were used, was biosorbed in higher percentages that those observed for the rest of isolates, above 65% in most cases. This result is in contrast to that reported by Chatterjee *et al.* (2010), who found Pb^{2+} was the heavy metal removed in lower proportion by *Geobacillus thermodenitrificans* in a multi-polluted solution, but it is similar to that described by Amini and Younesi (2009) and Chen and Wang (2010). According to these authors, Pb^{2+} removal efficiency was higher than that obtained for the other metals presented in samples.

Other authors have described this higher efficiency by living fungi cells. Al-Garni *et al.* (2009) report a decrease between 15% and 44% when they compared the results obtained by living and dead cells of *Aspergillus fumigatus* and *Penicillium chrysogenum*. Zucconi *et al.*, (2003) found that living cells of *Paecilomyces lilacinus* showed a higher capacity than dead cells (30% *vs.* 23%). On the contrary, other authors describe opposite results (Gabr *et al.*, 2008; Kaushik *et al.*, 2008), probably as a consequence of the method used to prepare the dead biomass, which affects the efficiency of the biosorbent (Bishnoi and Garima, 2005).

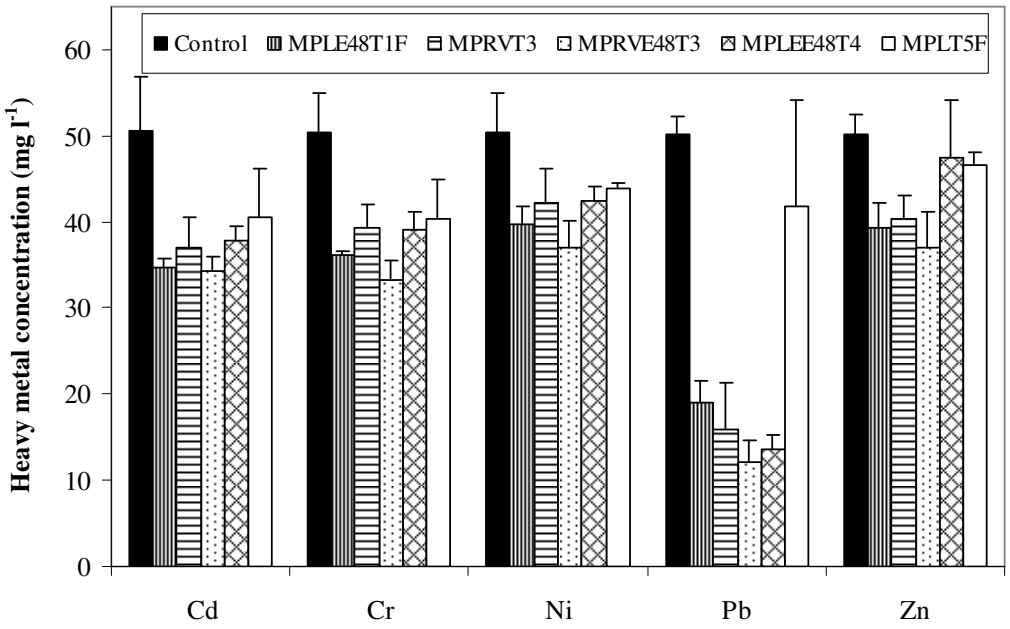

FIGURE 2 **Heavy metal removal by dead cells of microbial isolates**

4 CONCLUSIONS

Fungi isolated from compost have proven to be efficient as detoxification agents in multi-polluted heavy metals aqueous solutions, especially LE48T1 and RVE48T3. In all the cases, living cells showed higher activity than non-living cells and intracellular accumulation seems to be the main mechanism for removing. Although further studies are needed, these results are very promising as a starting point for a potential application of these microorganisms in bioremediation, including those scenarios affecting agricultural soils, which can be polluted with heavy metals as a consequence of the incorporation of amendments such as sewage sludge or municipal solid wastes.

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REFERENCES

Al-Garni S, Ghanem K M, Bahobail A S 2009. Biosorption characteristics of *Aspergillus fumigatus* in removal of cadmium from an aqueous solution. African Journal of Biotechnology 8, 4163-4172.

Amini M, Younesi H 2009. Biosorption of Cd(II), Ni(II) and Pb(II) from aqueous solution by dried biomass of Aspergillus niger: Application of response surface methodology to the optimization of process parameters. Clean - Soil, Air, Water 37, 776-786.

Bishnoi N R, Garima A 2005. Fungus - An alternative for bioremediation of heavy metal containing wastewater: A review. Journal of Scientific & Industrial Research 64, 93-100.

Chatterjee S K, Bhattacharjee I , Chandra G 2010. Biosorption of heavy metals from industrial waste water by *Geobacillus thermodenitrificans*. Journal of Hazardous Materials 175, 117-125.

Chen C, Wang J 2010. Removal of heavy metal ions by waste biomass of *Saccharomyces cerevisiae*. Journal of Environmental Engineering 136, 95-102

Chen X C, Wang Y P, Lin Q, Shi J Y, Wu W X, Chen Y X 2005. Biosorption of copper (II) and zinc (II) from aqueous solution by *Pseudomonas putida* CZ1. Colloids and Surfaces B: Biointerfaces 46, 101-107.

Chojnacka K 2007. Bioaccumulation of Cr(III) ions by blue-green alga *Spirulina* sp. Part I. A comparison with biosorption. American Jouranl of Agricultural and Biological Sciences 2, 218-223.

De, J, Ramaiah N, Vardanyan L 2008. Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. Marine Biotechnology 10, 471-477.

Filipović-Kovačević Z, Sipos L, Briški F 2000. Biosorption of chromium, copper, nickel and zinc ions onto fungal pellets of Aspergillus niger 405 from aqueous solutions. Food Technology and Biotechnology 38, 211-216.

Gabr R M, Hassan S H A, Shoreit A A M 2008. Biosorption of lead and nickel by living and non-living cells of Pseudomonas aeruginosa ASU 6a. International Biodeterioration & Biodegradation 62, 195-203.

Gavrilescu M 2004. Removal of heavy metals from the environment by biosorption. Engineering in Life Sciences 4, 219-232.

Haferburg G, Kothe E 2007. Microbes and metals: Interactions in the environment. Journal of Basic Microbiology 47, 453-467.

Kaushik S, Juwarkar A, Malik A, Satya S 2008. Biological removal of Cr (VI) by bacterial isolates obtained from metal contaminated sites. Journal of Environmental Science and Health, Part A 43, 419-423.

Kim S U, Cheong Y H, Seo D C, Hur J S, Heo J S, Cho J S 2007. Characterisation of heavy metal tolerance and biosorption capacity of bacterium strain CPB4 (*Bacillus* spp.). Water Science & Technology 55, 105-111.

Lloyd J R 2002. Bioremediation of metals: The application of microorganisms that make and break minerals. Microbiology Today 29, 67-69.

Malik A 2004. Metal bioremediation through growing cells. Environment International 30, 261-278.

Moore B A, Duncan J R, Burgess J E 2008. Fungal bioaccumulation of copper, nickel, gold and platinum. Minerals Engineering 21, 55-60.

Pan R, Cao L, Zhang R 2009. Combined effects of Cu, Cd, Pb and Zn on the growth and uptake of consortium of Cu-resistant *Penicillium* sp. A1 and Cd-resistant *Fusarium* sp. A19. Journal of Hazardous Materials 171, 761-766.

Pervez A, Shahbaz S, Shah M M, Mahmood Q, Mirza N 2009. Assessing bioaccumulation of heavy metals in sporocarp of *Pleurotus ostreatus*. World Applied Sciences Journal 7, 1498-1503.

Ting A S Y, Choong C C 2009. Bioaccumulation and biosorption efficacy of *Trichoderma* isolate SP2F1 in removing copper (Cu(II)) from aqueous solutions. World Journal of Microbiology & Biotechnology 25: 1431-1437.

Wang J, Chen C 2009. Biosorbents for heavy metals removal and their future. Biotechnology Advances 27, 195-226.

Wong J P K, Wong S Y, Tam N F Y 2000. Nickel biosorption by two chlorella species, *C. vulgaris* (a commercial species) and *C. miniata* (a local isolate). Bioresource Technology 73, 133-137.

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

Zucconi L, Ripa C, Alianiello F, Benedetti A, Onofri S 2003. Lead resistance, sorption and accumulation in a *Paecilomyces lilacinus* strain. Biology and Fertility of Soils 37, 17-22.