

Bioremediation of toxic metal ions from polluted lake waters and industrial effluents by fungal biosorbent

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Alkali-extracted mycelial biomass (biosorbent) from *Aspergillus niger* was effective in sequestering metal ions, especially Zn^{2+} and Cd^{2+} from lake waters from very low concentrations ($\mu g/l$) when compared to Dowex-50. Zinc ions (9 g/l) from an effluent of a battery manufacturing unit could be removed efficiently (>75%) by recycling the biosorbent through five cycles. Similarly, chromium ions from effluents of electroplating industry (420 mg/l) could also be removed by recycling the biomass. Here we report the potential use of this fungal biosorbent in removing toxic metal ions from heterogeneously polluted lake waters and industrial effluents.

DEVELOPING countries are increasingly concerned with pollution due to toxic heavy metals in the environment. Unlike most organic pollutants which can be destroyed, toxic metal ions released into the environment often persist indefinitely, circulating and eventually accumulating throughout the food chain, thus posing a serious threat to mankind. Large proportions of heavy metals are being released into the environment from acid mine drains and industrial waste waters due to inadequacies of technology in processing of metals or through other routes^{1,2}. A reasonable procedure to diminish the escape of metals from industrial effluents is the adoption of low waste-generating technologies coupled with effective effluent treatment.

The use of biological materials for heavy metal removal or recovery has gained importance in recent years due to their good performance and low cost³⁻⁷. Among the various sources, both live and inactivated biomass of microorganisms exhibit interesting metal-binding capacities^{8,9}. Their complex cell walls contain high content of functional groups like amino, amide, hydroxyl, carboxyl, sulphhydryl and phosphate which have been implicated in metal binding¹⁰⁻¹⁴.

Conventional methods for removing metals from industrial effluents before they are disposed off are becoming inadequate, while effective methods are becoming uneconomical¹⁵. An alternative method, based on the adsorptive capacity of microbial biomass, referred to as biosorption, is emerging as a promising 'biosorption technology' for metal removal and recovery^{3,4,16}. Studies from our laboratory had earlier used a nickel-resistant, hyperaccumulating mutant of *Neu-*

rospora crassa to remove toxic Ni^{2+} from aqueous media¹⁷. In later studies, alkali-extracted mycelial biomass of *N. crassa*, *Fusarium oxysporum* and particularly *A. niger* have been demonstrated to have an excellent ability to concentrate silver ions¹⁰. Further, the mechanism of biosorption was demonstrated to be by a direct exchange of toxic ions with resident Ca^{2+}/Mg^{2+} of the biosorbent; carboxyl groups have been shown to be the major binding groups herein¹¹. The objective of the present study is to explore the potential use of a biosorbent from *A. niger* in removing toxic metal ions from polluted lake waters and industrial effluents.

The biomass of *A. niger* was processed as described earlier¹⁰, referred to as 'biosorbent' was used in all experiments. For metal-removal experiments, Hussain Sagar, an eutropically polluted lake in the Hyderabad city, India, was chosen and the metal ion concentrations (Cd^{2+} , Cu^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+}) were determined¹⁸. The biosorbent (25 g fresh weight = 5 g dry weight) was taken in a cheese cloth bag and dipped into the lake waters (2-3 feet deep) for 60 min. The biosorbent was then removed, washed with distilled water and the bound metals were eluted with 0.1 N HCl. The eluate was concentrated and the amount of metals bound was analysed by Atomic Absorption Spectrophotometry (AAS, Perkin-Elmer Model No. 2380). Simultaneously for comparison of efficiency of the biosorbent, 5 g of Dowex-50 (Na^+ form) was taken in a cheese cloth bag and dipped into the lake waters and the experiment was carried out as described above for biosorbent.

For the removal of metals from industrial effluents, biosorbent (2 g) was suspended in 20 ml of the effluent and incubated on a rotary shaker (100 rpm) at $30 \pm 1^\circ C$ for 1 h and the amount of metal remaining was analysed. The biosorbent was reused for 5 cycles after regenerating with Ca^{2+} and Mg^{2+} (0.1 M each) as described earlier¹⁰.

Microorganisms have applications in selected wastewater metal removal/treatment systems. However, toxicity of metallic contaminants and the highly variable conditions prevalent in many wastes and processed waters preclude the use of living organisms and necessitate the utilization of non-living systems for metal removal. Metal sorption has been well demonstrated with nonmetabolizing and nonliving, microbial biomass^{16,19,20}. Several characteristics of inactive biomass make it an ideal choice for treatment of metal containing waste waters and process streams^{21,22}. Inactive biomass, in general, is not selective in the metal sorbed; rather, it simultaneously removes several different toxic and heavy metals like Cd, Cr, Cu, Hg, Ni, Pb, U, Zn from solution regardless of their concentrations. In addition, the biomass has greater avidity to and removes metals that are relatively more hazardous, due to its low affinity for alkaline earth metals (Ca, Na, K, Mg) (ref. 23).

Most studies described above have been conducted using metal ions in pure solutions and very few have

Table 1. Sequestration of toxic metal ions from lake waters.

Metal ion	M ²⁺ in lake water µg/l*	Amount of metal bound µg/5 g		Litre** equivalents
		Biosorbent	Dowex-50	
Cd ²⁺	8.63 ± 6.5	333.8 ± 155.5	8.69 ± 4.97	38.7
Cu ²⁺	18.1 ± 14.5	85.1 ± 41.2	21.94 ± 9.3	4.7
Zn ²⁺	62.3 ± 21	4562.5 ± 1174.3	111.9 ± 67.3	73.2
Ni ²⁺	17.98 ± 7	90.13 ± 52.48	6.35 ± 3.37	5.3
Co ²⁺	10.65 ± 1.1	84.69 ± 36.75	7.75 ± 2.95	7.9

*Five samples were collected from the same site of the lake over a period of one year and pH varied between 7.9 and 8.5.

**Obtained by dividing biosorbent-bound metal ion by the lake water metal concentration per litre.

Biosorbent or Dowex-50 (5 g) placed in a cheese cloth bag and immersed into lake water (2–3 feet deep) for 60 min. The bound metal ions were desorbed and estimated by AAS (see text).

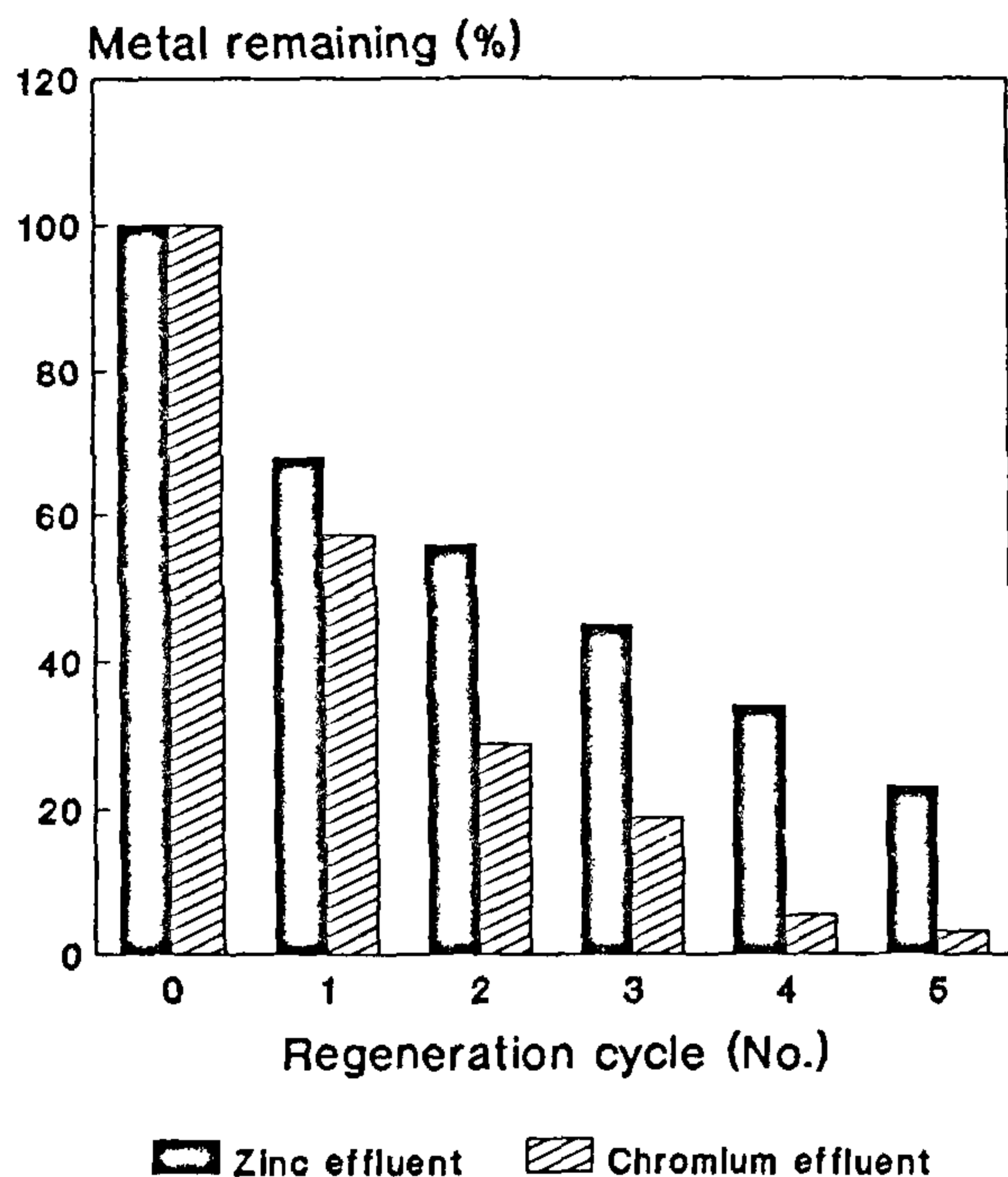


Figure 1. Biosorbent (2 g) was suspended in 20 ml of effluent containing zinc (9 g/l) or chromium (420 mg/l) for 60 min. The metal ion remaining was estimated by AAS. After regeneration, the biosorbent was reused up to 5 cycles (see text).

used industrial effluents to our knowledge^{24,25}. In the present study, we have used the polluted effluents directly without manipulating their composition. The results show that cadmium and zinc were more effectively sequestered from the lake waters at very low concentrations (ppb or µg/l) as compared with other metal ions by the biosorbent. The efficacy of the biosorbent *vis-à-vis* equal weight of Dowex-50 indicated the former's superiority in binding metal ions (Table 1). This is due to Dowex-50 readily binding nonspecifically divalent ions

present in lake waters (like Ca²⁺ and Mg²⁺), thereby attaining saturation with these ions (data not shown) and being there after unable to sequester toxic ions present at very low concentrations. If the binding capacity of the biosorbent is calculated on the basis of litre equivalents, the biosorbent (5 g) is able to remove almost 75 l equivalents of zinc and around 40 l equivalents of cadmium: for copper, cobalt and nickel, equivalence was around 5–10 l (Table 1). The metal ion concentrations noted here are well below the permissible limits suggested by the Indian Council of Medical Research²⁶. It should be noted here that when the same metal ions were present in a mixture at high concentrations (25 mM each), the order of binding by the biosorbent (from pure solutions) was Cu > Zn > Cd > Co = Ni (ref. 11). This shows that other unknown factors influence the order of preference for metal sorption from such heterogeneously polluted waters.

Zinc removal from an industrial effluent (from a battery manufacturing unit) which contains a high concentration of zinc (9 mg/ml) was highly effective with the biosorbent; five cycles of biosorbent treatment resulted in more than 75% metal removal. In another effluent from an electro plating industry containing chromium (420 µg/ml), the biosorbent was able to remove more than 95% of chromium (five cycles). One of the interesting features regarding Zn²⁺ sequestration from effluent of a battery manufacturing unit is that in the first cycle of use (Figure 1) about 30% of Zn²⁺ is removed by the biosorbent (60 mg zinc/2 g fresh wt). On a dry weight basis, this amounts to 15% of biosorbent weight (as dry weight accounts for 20% of fresh weight). In earlier studies¹¹, Zn²⁺ binding was 9–10% from a pure solution containing 50 mM (about 3 mg/ml). The higher binding noted herein is possibly due to a 3-fold higher concentration of Zn²⁺ present in the effluent. The accumulation of toxic metal ions is based on a mole for mole exchange with the resident calcium and magnesium ions of the biosorbent. The low affinity of Ca²⁺ and Mg²⁺ ions for the biosorbent makes them excellent counter ions for the heavy metals that form more stable complexes with the unprotonated binding sites. This factor has been taken as an advantage for the regeneration of the biosorbent and its repeated use¹¹.

Ideal properties for a biosorbent are: i) it should sequester toxic metal ions both from dilute and concentrated solutions, ii) should not face antagonism with essential metal ions, iii) should be reusable, iv) should not by themselves contaminate the environment. Overall, the biosorbent used in the present study has most of these features. Further scale-up studies are required to determine the actual potential of this biosorbent for decontaminating metal ions from industrial effluents.

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