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Original Research Article

# Biosorption of chromium by fungal strains isolated from polluted soil

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**Abstract**

Various human activities are responsible directly or indirectly for discharge of toxic heavy metals in the environment. Bioremediation is an emerging technology for removing the heavy metals from the contaminated environment. In the present study, soil samples from nearby region of Union Carbide India Ltd. (UCIL) which is responsible for disastrous Bhopal gas tragedy and dispersion of various toxic metals in the soil, air and water of that area. A high concentration of chromium was observed in soil samples. A total of 12 isolates were recovered from the soil samples. Among all isolates studied, the most tolerant isolate belonged to the genus *Aspergillus*, with a MIC of 400 to 850 mg/l. Out of which, the ability of *Aspergillus niger* was highest to accumulate or uptake Cr (64 percent) at initial concentration of 500 ppm Cr. Hence, from the present study it can be concluded that indigenous fungi can be a novel tool for bioremediation. Soil fungi seem to be well adapted to metals and could effectively be used as a metal biosorbent. Metal tolerance appears to be an added advantage when using live cells for metal removal.

**Key Words:** Bioremediation; heavy metals; indigenous fungal isolates; industrial effluents; biosorption.

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## INTRODUCTION

The soil and water contaminations are frequently occurred by toxic heavy metals and organic pollutants as a consequence of human activities become a key concern in environmental and health problem. Several toxic metals (Cd, Cu, Cr, Hg, Pb, Mn, As, Ni, Zn, etc.) from industrial wastewater and other human activities are directly or indirectly released into the environment. Soils are the major sink for heavy metals released into the environment by aforementioned anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation[1], and their total concentration in soils persists for a long time after their introduction[2]. Changes in their chemical forms (speciation) and bioavailability are, however, possible. Heavy metal contamination of soil may pose risks and hazards to humans and the ecosystem through: direct ingestion or contact with contaminated soil, the food chain (soil-plant-human or soil-plant-animal human), drinking of contaminated ground water, reduction in food quality (safety and marketability) via phytotoxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems[3-5]. Physico-chemical methods such as reverse osmosis, solvent extraction, lime coagulation ion exchange and chemical precipitation[6] for removal of heavy metals from wastewater are very expensive and these do not remove heavy metals from wastewater up to desired limits. Heavy metal resistant microbes might be present in heavy metal contaminated sites. The resistance and efficiency of microbes for removal of heavy metals vary greatly. Therefore, there is need to isolate and screen heavy metal tolerant fungi from heavy metals contaminated sites. The bio-sorption of heavy metals using various live and heat treated fungi has been studied. These studies showed that the bio-sorption capacity of the heat treated cells might be greater, equivalent or less than that of their living counterparts. The present study attempts to isolate and screen heavy metal (Pb and Cr) tolerant fungi and to evaluate their efficiency to remove heavy metals from liquid media under laboratory conditions.

## MATERIALS AND METHODS

**Study area and samples collection:** Soil samples were collected from nearby regions of UCIL factory, the source of MIC gas causing Bhopal gas tragedy. The sample was collected from the dried solar extraction pond of the UCIL Pvt Ltd. These samples were brought to laboratory and kept in refrigerator at 4°C for further processing.

**Isolation of Fungi:** Fungal isolates were isolated from samples of soil by serial dilution method using potato dextrose agar containing 25 ppm of chromium. A serial dilution of each soil sample was made up to $10^6$ and one ml of dilution $10^4$ and $10^6$ was added in sterilized petri plates in duplicate. The 1000 ppm stock solutions of Cr were made in double distilled water using $K_2Cr_2O_7$. The stock solution of heavy metals was sterilized separately through bacteriological filters and added to sterilized potato dextrose agar (PDA) medium to make the concentration at 25 ppm. Twenty milliliter of PDA medium containing 25 ppm of chromium was poured in these petri plates and incubated at 28°C for 48 h. The colonies of predominant genera of fungi were picked up and purified by pour plate method.

**Determination of minimum inhibitory concentration (MIC):** Tolerance to heavy metals was determined as the minimum inhibitory concentration (MIC) against the test fungi. SDA medium was prepared and amended with various amounts of heavy metals to achieve the desired concentration ranging from 0.05 to 0.5 mg $ml^{-1}$. Each heavy metal plate was subdivided into four equal sectors and an inoculum of test fungi was spotted in duplicate on metal and control plates (plates without metal). The plates were incubated at 29 ± 1 °C for 2–5 d to observe the growth of fungi on the spotted area. MIC was defined as the minimum inhibitory concentration of the heavy metal that inhibited visible growth of test fungi.

**Biosorption of Cr from synthetic medium:** The fungi were cultured in filamentous form under aerobic conditions for 3 days in shake flasks (125 rpm). The biomass was harvested by filtration through a 150µm sieve. The biomass was thoroughly washed with distilled deionized water to remove residual growth medium. The washed biomass (live biomass) was used immediately thereafter. Briefly, 1–5 g of biomass of the above fungi was added to 100 ml metal solution (pH- 5.0) supplemented with 100, 250, 500 and 1000 mg/l concentration of chromium. Inoculated flasks were incubated on rotatory shaker (150 rpm) at 30 °C for 4h with control containing spore inoculated medium without metal. After incubation, concentration of heavy metal in fungal inoculated medium and control was determined to check any significant heavy metals reduction by fungi. The solution was centrifuged at 8000 rpm for 30 min to separate the biomass. The supernatant and control (metal solution without biomass) were digested in 67% HNO3. The digested solution was evaporated and solids were re-dissolved in 0.1 M HCl. The heavy metal concentration was analyzed by atomic absorption spectrophotometer (GBC- 932 Plus). All samples were analyzed in triplicate.

## RESULTS AND DISCUSSION

**Isolation of Fungi:** Prolonged exposure of soil fungi to elevated heavy metals content has developed resistance in them[7]. In the present study, a total of twelve soil fungi were isolated from the heavy metal contaminated soils. The isolates were characterized and identified by fine tuning their morphological characteristics with those described by Barnett and Hunter (1999). The isolated fungal isolates belonged to genera *Aspergillus niger, A. flavus, Aspergillus sp., Rhizomucor miehei, Fusarium solani, Penicillium chrysogenum, Rhizopus sp., Trichoderma sp., Fusarium sp. and Geotrichum sp*. The *Aspergillus sp*. appeared to be the most commonly occurring in the heavy metals contaminated soils as also reported elsewhere[8-9]. *A. niger* strains produced initially wooly white colonies which quickly became black with conidial production while *A. flavus* produces dark green color colonies. *P. chrysogenum* produces grey color colonies and *R. miehei* produces white cottony colonies on PDA.

**Minimum Inhibitory Concentration:** The results of present study depicted that all tested isolates showed different tolerance behavior. Some isolates were sensitive, moderately tolerant and tolerant. Common and dominant metal-resistant fungi isolated from polluted soils belong to the genera of *Aspergillus sp., Rhizomucor miehei, Penicillium* and *Fusarium* which showed different minimum inhibitory concentration (MIC) for chromium (Table 1). The MIC values suggest that the resistance level against chromium was dependent on the isolates. *Aspergillus niger* isolated from the soil samples showed the highest tolerance towards each metal salts. The *Rhizomucor miehei* showed tolerance towards chromium followed by *Fusarium sp., Penicillium sp.* and *Rhizopus sp*.

**Table 1:** Minimum inhibitory concentration (MIC) of heavy metals for isolated tolerant fungal species

| S.N. | Test fungi | Minimum inhibitory concentration (mg $ml^{-1}$) |
|---|---|---|
| 1 | *Aspergillus niger* | 850 |
| 2 | *Aspergillus sp. 1* | 550 |
| 3 | *Aspergillus sp. 2* | 400 |
| 4 | *Rhizomucor miehei* | 600 |
| 5 | *Fusarium sp.* | 200 |
| 6 | *Penicillium sp.* | 150 |
| 7 | *Rhizopus sp.* | 125 |

Among all isolates studied, the most tolerant isolate belonged to the genus *Aspergillus*, with a MIC of 400 to 850 mg/l. Similar results were reported by Price *et al.*,[10], who showed that *Aspergillus* was better to grow or tolerate heavy metals as compared to other fungi. *Penicillium* and *Fusarium* isolates were less tolerant to chromium (up to 200 mg/l). The growth pattern appears to suggest tolerance development or adaptation of the fungi to the presence of heavy metals. At lower metal ions concentrations, the tested fungal isolates were very resistant and exhibited strong growth, usually exceeding the control. Higher metal ions concentration caused a reduction in growth and increased the length of the lag phase compared to the control. If the growth of fungi in a metal-free medium was observed after a day, metal prolonged the lag-phase, depending on the metal used and its concentration. In some cases, the fungus grew relatively quickly, even after a long lag phase. A reduction in the growth rate is a typical response of fungi to toxicants[7], whereas the lengthening of the lag phase is not always present. The morphology of isolates was highly affected by the presence of Cr. Their mycelia became diffuse compared with the control. The variation in the metal tolerance may be due to the presence of one or more strategies of tolerance or resistance mechanisms exhibited by fungi. It must also be taken into account that the contamination at the polluted sites is usually caused by a combination of metals and that the selection is probably driven either by the most toxic element or by more different metals acting synergistically[11].

**Biosorption studies:** Biosorption of toxic metals is based on ionic species associating with the cell surface or extra cellular polysaccharide, proteins and chitins[12]. Fungi showing high tolerance to toxic metals may be useful in metal recovery systems. In the present study heavy metal-tolerant *Aspergillus niger* and its less tolerant counterparts *Aspergillus sp.1* and *2* were evaluated for their biosorption potential for Cr. Biosorption of Cr was studied at 100, 250, 500 and 1000 ppm initial metal concentration. Maximum biosorption from metal solution occurred at 500 ppm initial concentration.

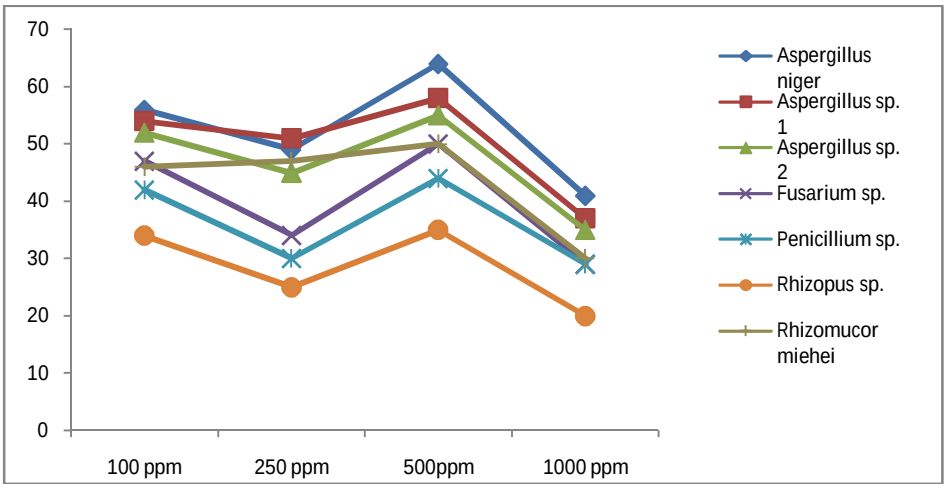


**Figure 1:** Biosorption potential of isolated species against different concentration of chromium salt

The Cr biosorption activity was checked out with all the isolated tolerant fungal isolates. The concentration of Cr biosorbed from the medium was maximum in case of *Aspergillus niger* 64 percent as compared to other fungal isolates (Figure- 1). Results indicate that the ability of *Aspergillus niger* was highest to accumulate or uptake Cr at initial concentration of 500 ppm Cr. Sorption of Cr (64 percent) by *Aspergillus niger* exceeded with the sorption values of 58 and 55 percent by less tolerant counterpart i.e. *Aspergillus sp.1* and *2* respectively. The Cr uptake with initial concentration of 500 ppm Cr, the capacity of *Rhizomucor miehei* and *Fusarium sp.* was 50 and 53 percent followed by *Penicillium sp* (44 percent) and *Rhizopus sp.* (35 percent). At higher concentrations of 1000 ppm the biosorption capacities were found to be declined. Biosorption was influenced by the initial metal concentration. This factor and other factors such as contact time, biomass dosage, temperature and pH are known to influence biosorption of metals (Fourest *et al.*, 1994; Kapoor and Viraraghavan, 1997; Zhou, 1999; Bai and Abraham, 2001; Yan and Viraraghavan, 2003). Varying levels of metal biosorption by different fungi such as *Rhizopus nigricans*, *Mucor*, *Penicillium* etc. have been reported by these authors, but the data on biosorption of various metals varied from study to study due to differences in the biomass concentration and treatment, biosorption conditions including pH and methods employed.

## CONCLUSION

In this study, Chromium resistant microorganisms were isolated from heavy metal contaminated environments, and the applicability of their heavy metal removal from industrial wastewater was evaluated at a laboratory scale. Our preliminary findings indicate that fungi from soil contaminated with heavy metals have chromium biosorption potential could be exploited for metal removal from aqueous metal solution. From the contaminated soil six fungal isolates were isolated out of which *Aspergillus niger* has shown the excellent ability to biosorb the Cr (VI) up to 64 percent. From the study, it can be concluded that fungi have high potential to remove the heavy metals from the toxic environment. Therefore, large number of fungi can be isolated and cultured in order to reduce the high toxic concentration of heavy metals. So, the fungal isolates have a great potential to remediate not only industrial effluents, but can also be used for bioremediation of other waste waters and Cr contaminated sites as well. These serve as an eco-friendly tool in major aspects of bioremediation by which the environment can be cleaned up and ultimately leads to maintain a healthy life.

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