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ISOLATION, CHARACTERIZATION AND BIOSORPTION OF ZINC BY INDIGENOUS FUNGAL STRAINS *ASPERGILLUS FUMIGATUS* RH05 AND *ASPERGILLUS FLAVUS* RH07

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Abstract

Heavy metal pollution of soil, water bodies and air is one of the major issues in Pakistan. In order to develop a biosorbent for removal of Zn from wastewater, a study was designed in which fungi were isolated from soil of local textile industry. Major fungal genera observed were *Aspergillus* sp., *Rhizopus* sp., *Rhodotorula* sp., *Dreshlera* sp., and *Curvularia* sp. Resistance studies were carried out on Sabouraud- dextrose agar. *A. flavus* RH07 and *A. fumigatus* RH05 showed maximum resistance for Zn (6500mg/L). *A. flavus* RH07 and *A. fumigatus* RH05 were selected and characterized for growth and Zn detoxification studies. Optimum pH and temperature for Zn detoxification were 5.0 and 28°C, respectively, for *A. fumigatus* RH05 whereas, *A. flavus* RH07 showed Zn biosorption at optimum pH 4.0 and temperature 28°C in growing condition. Non-growing biomass of *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07 served as better biosorbents. Temperature and pH were vital parameters, which affected the rate of Zn removal by both *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07. Oven dried dead biomass of both strains was best adsorbent over a wide pH and temperature range.

Introduction

Environmental pollution is a constant threat faced by humanity. Industrial effluents entering into the surface water are one of the most important sources of toxic contamination in the environment. In Pakistan, Indus river has gross metal ions such as Hg, Pb, As and Mn (Tariq *et al*., 1996). This is because industries are mostly situated near the riverbanks and their effluents are usually directly discharged into the river without any treatment.

Bioremediation is the use of natural biota and their processes for pollution reduction. It is a cost-effective process and the end products are non-hazardous. Microbial communities are of primary importance in bioremediation of metal contaminated soil and water because microbes alter metal chemistry and mobility through reduction, accumulation, mobilization and immobilization. There is current interest in the use of microorganisms for the removal of nitrogen, phosphorus, and metals from commercial and municipal waste (White *et al*., 1997; Maine *et al*., 2004; Fomina *et al*., 2005; Mahadevan *et al*., 2006). Joho *et al*., (1995) divided metal resistance mechanisms in two categories:

1. A reduced accumulation of metal ion by cell as a result of the excretion of metal chelating substances or by a defect in specific transport system.
2. A change in the intracellular distribution of the ions by binding to specific intracellular molecules.

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To date, most biological metal leaching has been carried out with chemoautotrophic bacteria such as *Thiobacillus* species. However these bacteria are acidophilic and may not tolerate the higher pH values of many industrial metal-loaded wastes including soils. Heterotrophic fungi can withstand a much wider pH range and many can produce organic acids, which can solubilize complex metal cations (Burgstaller & Schinner, 1993; Naz *et al.*, 2005). Haterotrophic fungi (*Mucor* sp., *Aspergillus* sp., *Penecillium* sp., and *Yarrowta* sp.) can remove both soluble and insoluble metal species from solution and are also able to leach metal cations from solid waste (White *et al.*, 1997; Słaba & Długoński, 2004).

Price *et al*., (2001) evaluated fungi for their ability to remove these metals from wastewater and found *A. niger* best suited for this purpose. *A. niger* was able to grow on plates amended with copper at a level five times that inhibitory to the growth of *Saccharomyces cerevisiae*. They also found that *A. niger* is capable of removing 91% of the copper and 70% of the zinc from treated swine effluent

Krauter *et al*., (1996) used commercially available nonpathogenic *Saccharomyces cerevisiae* to remove hexavalent chromium (Cr^{6+}) from ground water. They found a rapid (within 2 minutes) initial removal of $Cr^{6+}$with freshly hydrated cells (55-67 mg/h/g dry wt biomass) followed by much slower uptake (0.6-1.1 mg/h/g dry wt) that diminished with time. Under anaerobic conditions, fungal biomass removed $Cr^{6+}$with a slightly greater rate than aerobic conditions. Akhtar & Mohan, (1995) reported the potential use of Alkali-extracted mycelial biomass (biosorbant) from *A. niger* which was effective in sequestering metal ions especially Zn super (+) and Cd super (+) from lake waters from very low concentration. Similarly, chromium ions from effluent of electroplating industry (420 µg/L) could also be removed by recycling the biomass.

Metal contaminated industrial effluent, from a metal plating company, was exposed to waste activated sludge to optimize the biosorption process at laboratory scale. Metals assessed were Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Cr^{2+} and Cr^{6+}, of which Zn^{+2} was most prevalent. Biosorption rate of upto 96% were recorded for Zn within the first 15 min. It was also shown that wet sludge, utilized as biosorbent in a fully mixed process, has superior potential for metal ion biosorption from an industrial effluent (Atkinson *et al*., 1996).

The uptake of heavy metals by fungi is of industrial relevance for the removal of metals from wastewaters for environmental protection and / or subsequent recovery of the metals. Fungi are well suited for this purpose since they often exhibit marked tolerance towards metals and other adverse conditions e.g., low pH, also they have high capacities of metals binding to cell walls and may exhibit high values of intracellular accumulation (Blanquezet *et al*., 2004).

The objective of this study was to isolate fungi from the contaminated soil and evaluate them for use in bioremediation of heavy metals from industrial effluent. Study of Zn removal by tolerant fungi via., determination of Zn uptake by growing fungi, non-growing biomass and Zn removal by dead biomass was assessed. Effect of different environmental conditions eg., Zn concentration, pH and temperature on removal of Zn by *A. flavus* RH07 and *A. fumigatus* RH05 was also investigated.

Materials and Methods

Isolation and identification of *A. flavus* RH07 and *A. fumigatus* RH05: Samples of soil were taken from the Kohinoor Textile Mill, Rawalpindi. Fungal population of soil samples was determined by serial dilution method on Sabouraud dextrose agar (SDA) as described by Harley & Prescott (1993). Identification was carried out with the expertise

of the Pakistan Museum of Natural History, Islamabad. Identified strains were periodically sub-cultured and maintained on SDA slants at 4ºC.

Method proposed by Price *et al*., (2001) was used for determination of metal tolerance by these isolates. Inoculation was done by cutting 5mm plug from the margin of growing colonies and then this was placed on the surface of metal amended agar plates. After inoculation,these plates were incubated at 28ºC for 6 days and their diameters were measured. *A. flavus* RH07 and *A. fumigatus* RH05 showed high resistance in metal amended agar. Ability of these two strains to remove and develop a biosorbent for Zn detoxification from assimilated effluent was studied in various conditions.

Effect of metal concentration, pH and temperature on biosorption by growing biomass: Biosorption was determined by adding different concentrations of metal ion (3000-7000mg/L) in 100mL of Sabouraud-dextrose broth (SDB), which were inoculated with spore suspension of *A. flavus* RH07 (1.2x105 spore/mL) and *A. fumigatus* RH 05 (1.0x105 spore/mL). Flasks were placed on orbital shaker (100rpm) at 28ºC and pH 5.0. Effect of different pH (4.0-8.0) were studied in 100mL of SDB, containing 4000mg/L Zn at 28ºC. Effect of various temperatures (28, 34 and 40ºC) on removal of metal was also determined at pH 5.0, in the above prescribed condition.

Samples of all experiments were analyzed identically, in duplicate, i.e., samples of each day were filtered by Whatmann filter paper no.1. Biomass was dried at 55ºC in oven upto constant weight and after drying, it was weighed to determine the effect of different cultural conditions on the growth of fungal strains. Metal content was analyzed after digestion with direct air-acetylene flame spectrophotometer, Solar Unicam (Clesceri *et al*., 1989).

Zn adsorption by non-growing live biomass: In order to obtain non-growing biomass of *A. flavus* RH07 and *A. fumigatus* RH05, spore suspensions of both strains were inoculated in 200 mL of SDB in 500 mL conical flasks. These flasks were incubated at 28ºC for 4 days on an orbital shaker (100 rpm). Small pellets, ranging from 1-3 mm in diameter were formed, which were centrifuged, filtered though Whatmann filter paper no. 1, and then washed several times with 0.1 M sodium citrate buffer (pH 5.0). The washed pellets were kept at 4ºC before utilization in adsorption experiments (Al-Asheh & Duvnjat, 1995).

Zn uptake by both strains was determined by using a method similar to Avery *et al*., (1996). Non-growing live biomass was washed with MOPS buffer (0.1 M, pH 5.0). Washed pellets were suspended in $ZnCl_2$ solution, prepared and added to attain a range of Zn from 0 to 500 mg/L in 250 mL conical flasks. To each flask, 5 mL of glucose (5 mM) was added and incubated at 34ºC for 6 days in an orbital shaker (100 rpm). Each day, a sample from every concentration was removed, harvested by centrifugation (5,500 *g* x 15 minutes) and filtered through 0.45 µm filter paper. Filtrate was digested with 1+1 HNO_3, filtered and analyzed for Zn uptake on an atomic absorption spectrophotometer.

Zn adsorption by oven-dried fungal biomass: Non-living biomass of *A. flavus* RH07 and *A. fumigatus* RH05 were prepared by oven drying. Both strains were grown in SDB (pH 5.0), for 4 days at 28ºC, in an orbital shaker (100 rpm). Biomass was harvested by filtration through Whatmann filter paper no. 1, and rinsed several times with distilled deionized water, dried at 55ºC, and weighed every 15 minutes, till the weight became constant. After pulverization to a geometric mean particle size of 30-40 mesh, it was

stored in a dessicator at room temperature. For determination of adsorption by oven-dried biomass, methods of Zhang *et al*., (1998) and Fogarty *et al*., (1999) were used.

Effect of Zn concentration, temperature and pH on adsorption by oven-dried fungal biomass: To study the effect of Zn concentration on adsorption to dead biomass, $ZnCl_2$ solutions (50 mL), covering a range from 100-400 mg/L at pH 5.0, were prepared in distilled deionized water in 100 mL flasks. To each flask, 0.4 g dried biomass was added and kept at an orbital shaker (100 rpm) at 28ºC.

To ascertain the effect of temperature on adsorption, a temperature range of 28 - 40ºC was studied in 100 mg/L Zn concentration in a similar manner to the previous experiment. A pH range of 4.0-7.0 was used to study the effect of pH on adsorption by non-living biomass of *A. flavus* RH07 and *A. fumigatus* RH05 in 100 mg/L Zn concentration at 28ºC in an orbital shaker (100 rpm).

In all adsorption experiments, 5 mL sample was drawn and filtered through Whatmann filter paper no.1. Filtrate was digested with 0.5 mL 1+1 HNO_3. Zn removal was detected on an atomic absorption spectrophotometer. In order to test if there was any adsorption of metal to the container, control experiments without adsorbent were carried out.

Results

Identification of fungal isolates: Fungal species identified from soil were *A. fumigatus, A. flavus, A. niger,* six other *Aspergillus* sp., two *Rhizopus* sp., and one *Curvularia* sp., *A. fumigatus* RH05 and *A. flavus* RH07 were able to grow till 6500 mg/L of Zn among all the isolates.

Growth conditions for *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07 were optimized. On the basis of the results, further studies for Zn removal were performed at the optimum pH 5.0 and 28°C.

Effect of Zn concentration, pH and temperature on biosorption by growing biomass: No growth was observed at Zn concentrations of 6000 and 7000 mg/L in both strains. Zn removal by *Aspergillus flavus* 07 was 11.63$\pm$0.035% at 4000 mg/L. Although there was a significant increase in biosorption at various concentrations, but 4000 mg/L was the optimal concentration for Zn removal by *Aspergillus flavus* RH07. Zn removal (24.00±0.088%), at various concentrations tested was the best seen at 4000 mg/L, by *Aspergillus fumigatus* RH05 (Fig. 1).

Different pH levels, within the range of 4.0 to 8.0, were studied for their effect on Zn biosorption by both strains, *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07, using 4000 mg/L Zn at 28°C. No growth of both strains was observed at pH 6.0, 7.0 and 8.0. However, at pH 4.0, 4.5 and 5.0, *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07 removed Zn. *Aspergillus flavus* RH07 biosorbed 23.00$\pm$0.083% Zn at pH 4.0 and *Aspergillus fumigatus* RH05 showed 7.61±0.019% at same pH. Effect of pH on growth of both strains, during biosorption of Zn, showed maximum growth of both strains at pH 4.0 (Fig. 2).

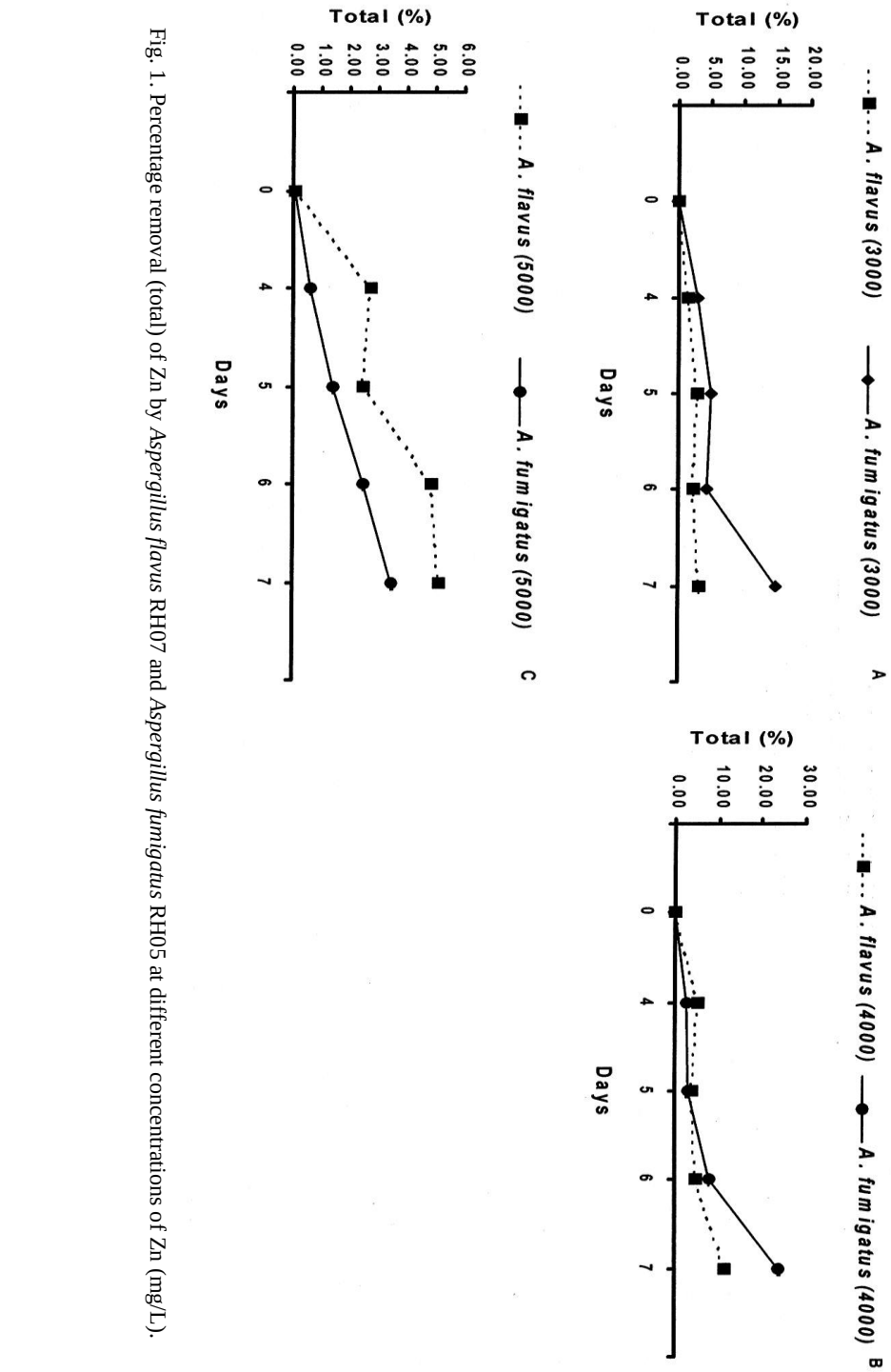

Fig. 1. Percentage removal (total) of Zn by *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 at different concentrations of Zn (mg/L).

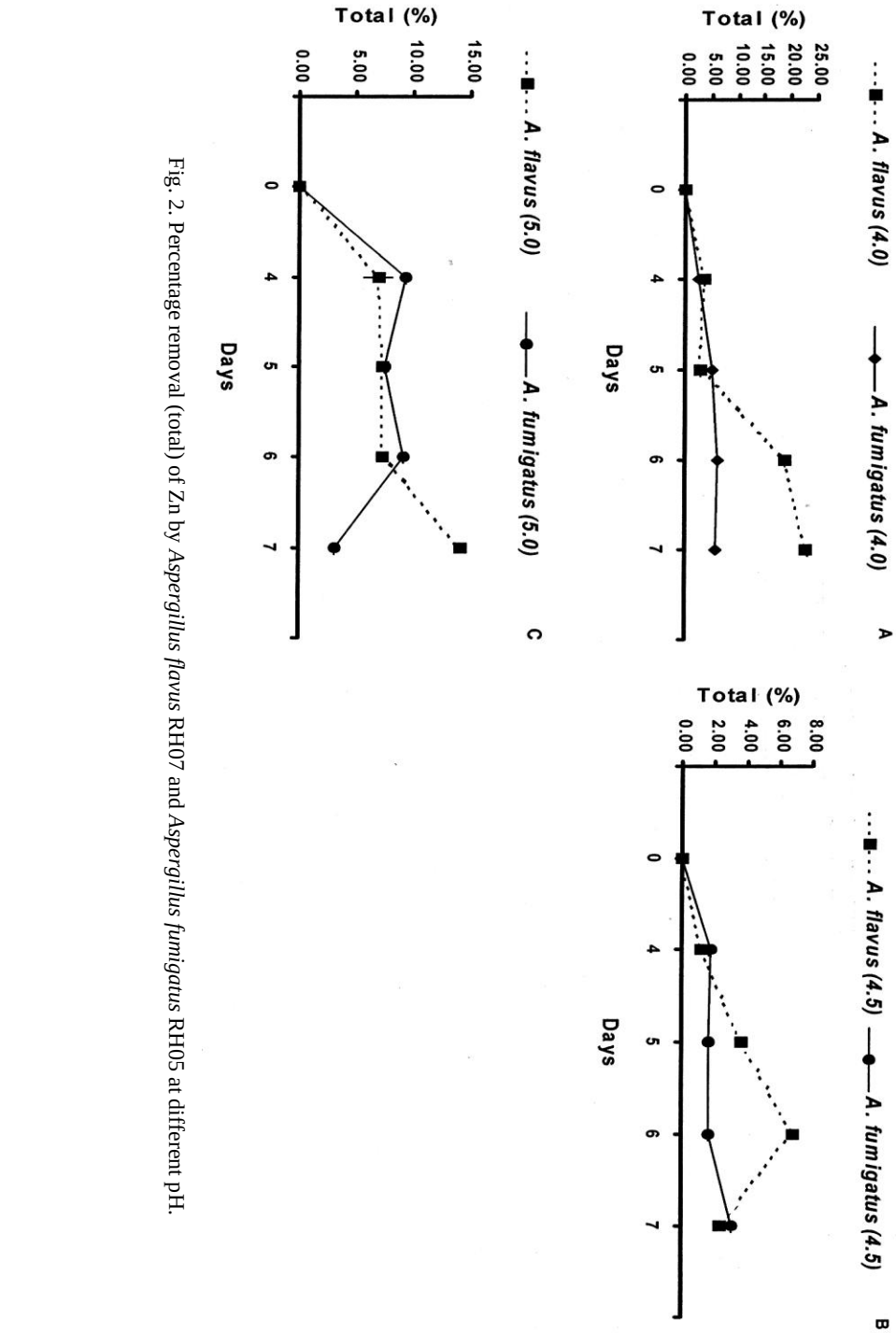

Fig. 2. Percentage removal (total) of Zn by *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 at different pH.

Both strains of *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07, showed a similar trend, with regard to different temperatures (28, 34 and 40°C), and optimal temperature for biosorption by both these strains were found to be 28°C (Fig. 3).

Zn adsorption by non-growing live biomass: Increase of Zn concentrations resulted in reduced adsorption by both strains. *Aspergillus flavus* RH07 removed 40.89±0.675% from 100 mg/L Zn. Under same pH and temperature condition, non-growing pellets of *Aspergillus fumigatus* RH05 removed 59.72±0.454% at 100 mg/L Zn after 6 days incubation.

Comparison of *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07 revealed an identical pattern of Zn removal by both the strains, however, *Aspergillus fumigatus* RH05 removed greater concentration of Zn within the same time span of 6 days (Fig. 4).

Effect of Zn concentration, pH and temperature on adsorption by oven-dried biomass: With increasing concentration of Zn, adsorption by *Aspergillus flavus* RH07 decreased. Best adsorption (49.74±0.038%), was seen at 100 mg/L Zn, after 90 minutes. At 200, 300 and 400 mg/L Zn, this adsorption was reduced to 34.71±0.233%, 33.65±0.238% and 26.75±0.089%, respectively. Non-living biomass of *Aspergillus fumigatus* RH05 adsorbed 59.81±0.355% Zn at 100 mg/L, after 120 minutes. A pattern similar to the one observed for *Aspergillus flavus* RH07, was exhibited, an increase in Zn concentration brought about a decrease in adsorption. When dead biomass of both *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07 were compared for Zn adsorption at different Zn concentrations. *Aspergillus fumigatus* RH05 showed better adsorption percentages at all the studied concentrations of Zn (Fig. 5).

pH range of 4.0 to 7.0 was used to determine effect of changing pH on the adsorption of Zn by non-living biomass of both *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07, at 28°C and 100 mg/L Zn. *Aspergillus flavus* RH07 showed best adsorption (82.38±1.035%) at pH 5.0, after 75 minutes, closely followed by 81.41±1.410% after 60 minutes at pH 6.0. Increasing the pH beyond 6.0 resulted in decrease of adsorption, while increasing pH from 4.0 to 6.0, brought about an increase in the adsorption. *Aspergillus fumigatus* RH05 adsorbed 88.51±1.515% Zn at pH 6.0 after 90 minutes, and 86.16±1.985% at pH 5.0. However, better adsorption by non-living biomass was observed for *Aspergillus fumigatus* RH05 at pH 6.0, as compared to that demonstrated by *Aspergillus flavus* RH07 at pH 5.0 (Fig. 6).

Non-living biomass of *Aspergillus flavus* RH07 showed maximum adsorption (59.24±1.100%) at 28°C, using 100 mg/L Zn at pH 4.0, after 30 minute incubation. At 40°C, 46.38±0.930% adsorption was seen after 30 minutes, but then a rapid desorption occurred, and after 120 minutes, only 3.87±0.180% Zn remained adsorbed to the non-living biomass (Fig. 7).

On the other hand, *Aspergillus fumigatus* RH05 showed maximum adsorption (around 65%) at 40°C after 30 minutes, followed by desorption. At 28°C, *Aspergillus fumigatus* RH05 removed 63.84±2.385% Zn by adsorption after 30 minute incubation.

A comparative analysis of percentage adsorption by both *Aspergillus fumigatus* RH05 and *Aspergillus flavus* RH07 revealed an almost similar pattern, with good Zn removal at 28°C, within 30 minutes. *Aspergillus fumigatus* RH05 showed better results, not only at 28°C, but also at 40°C.

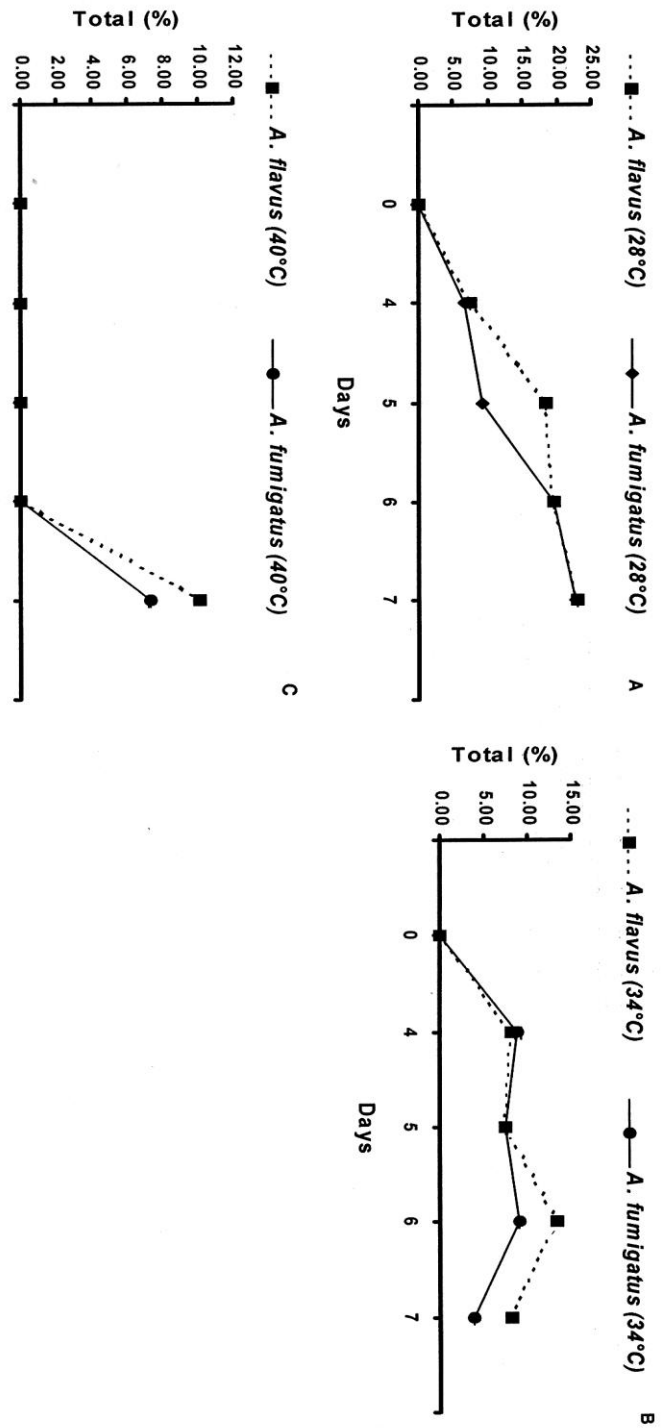

Fig. 3. Percentage removal (total) of Zn by *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 at different temperatures.

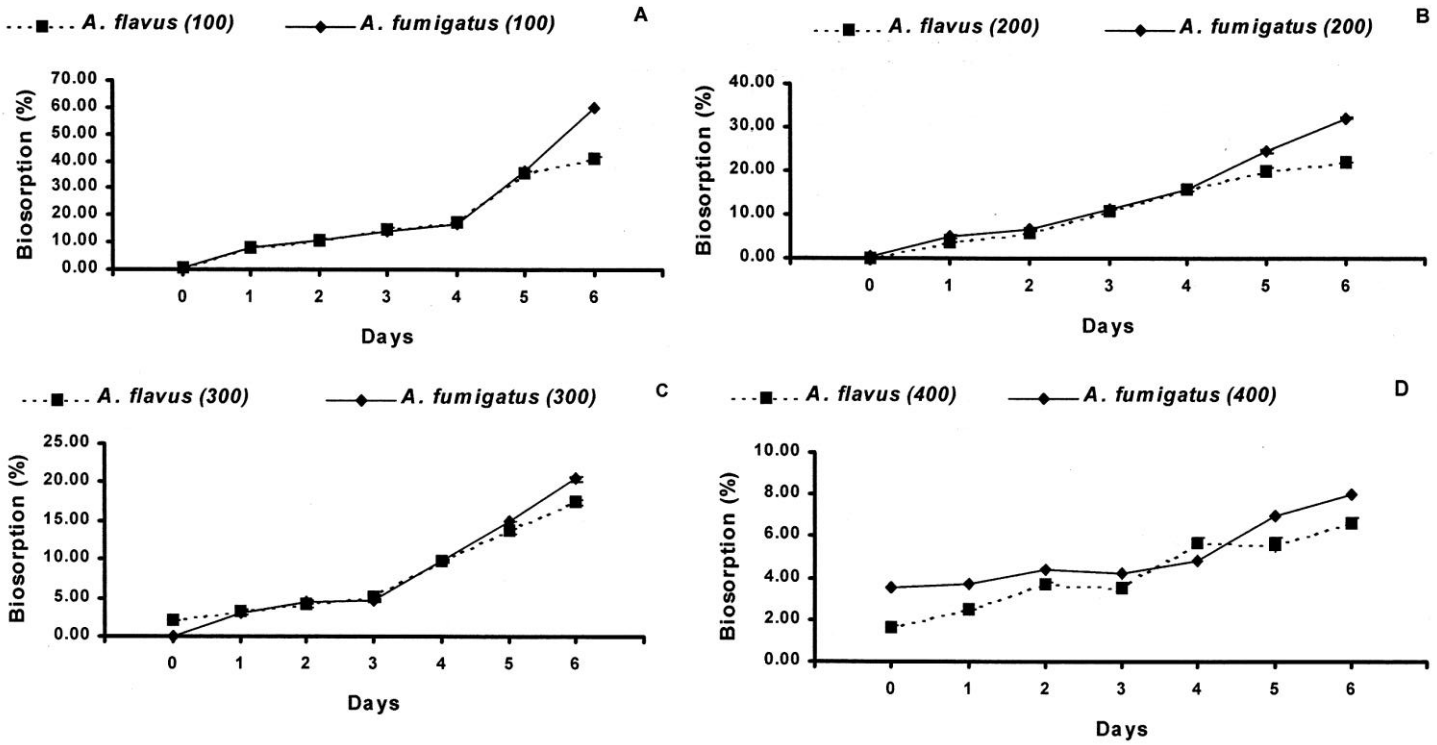

Fig. 4. Percentage biosorption of Zn by non-growing biomass of *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 at different concentrations.

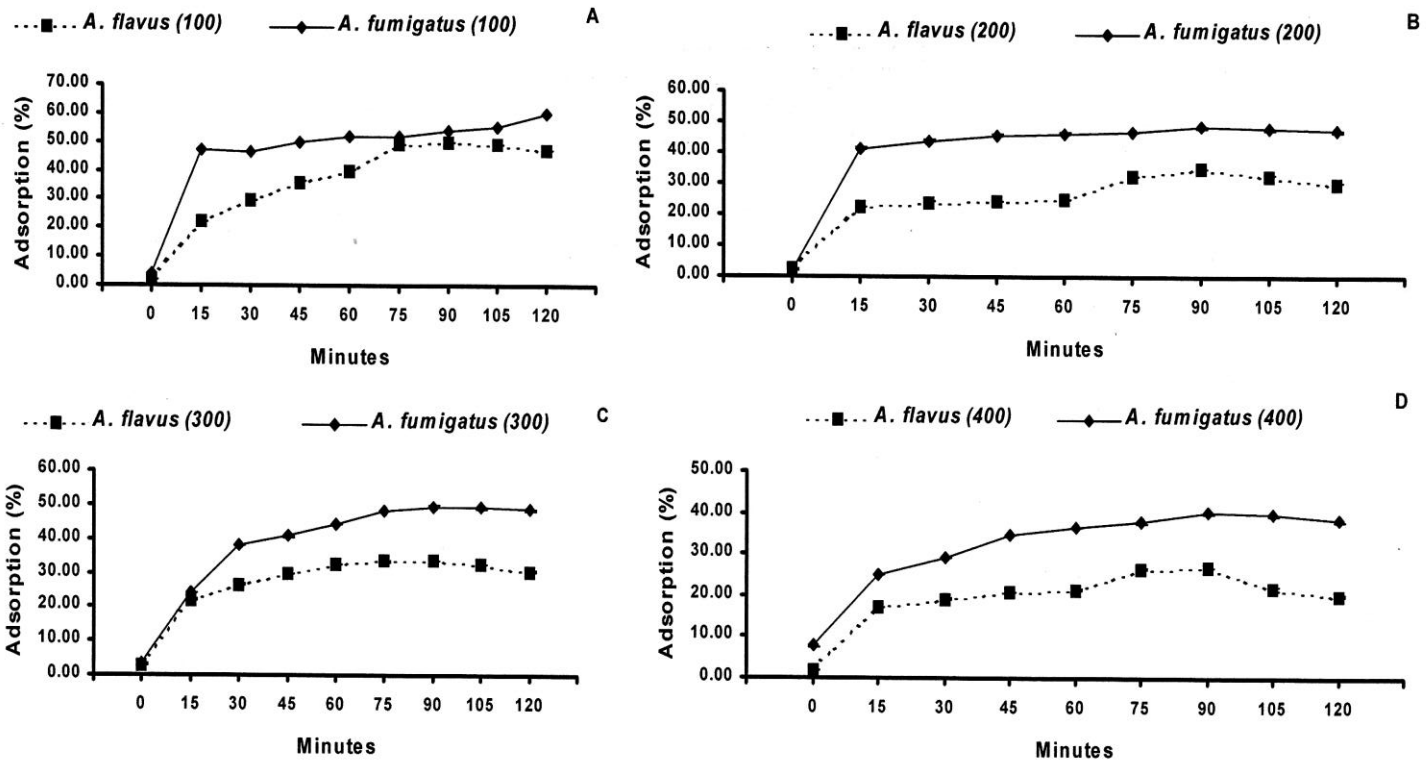

Fig. 5. Percentage adsorption of Zn by oven dried biomass of *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 at different concentrations.

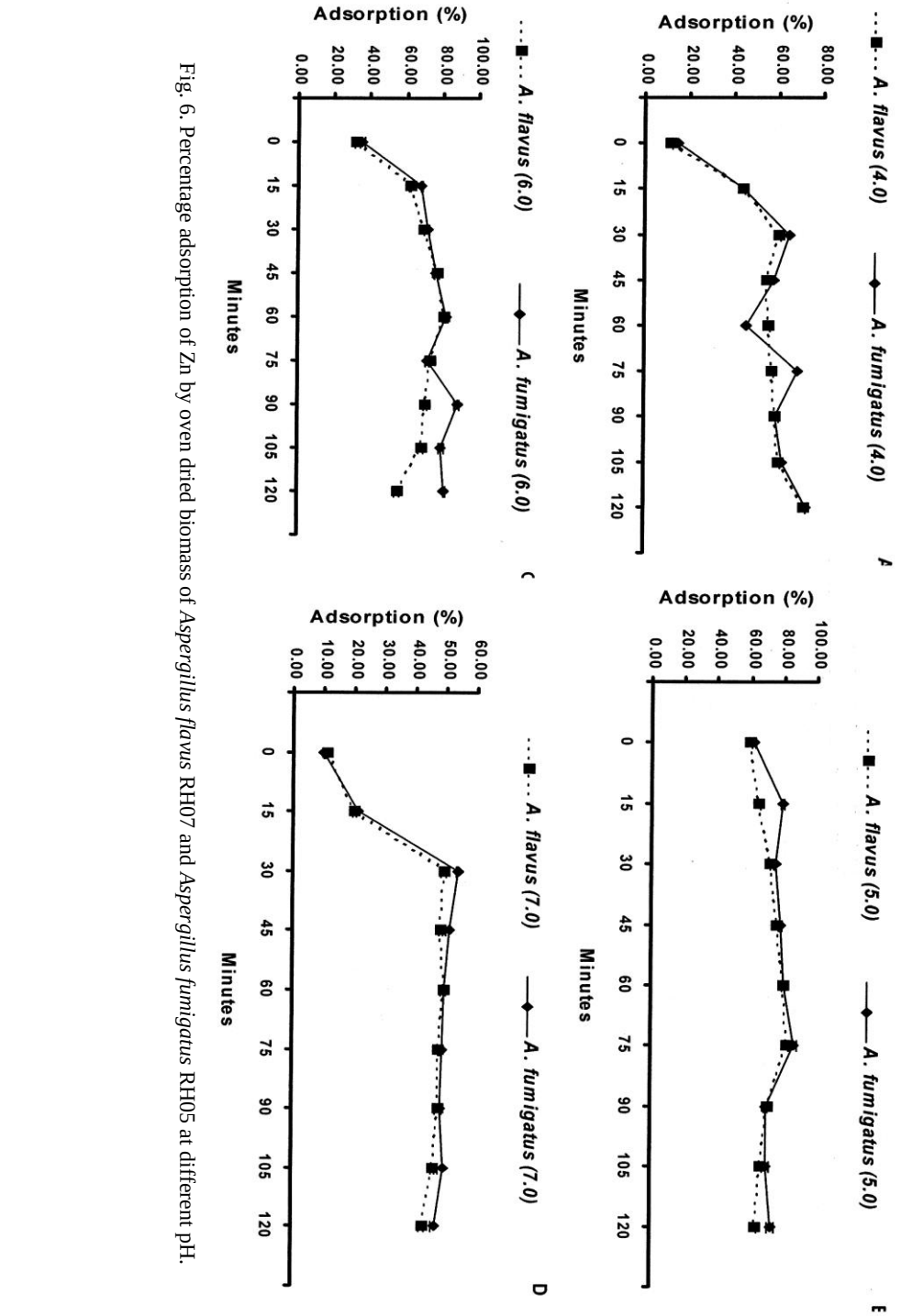

Fig. 6. Percentage adsorption of Zn by oven dried biomass of *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 at different pH.

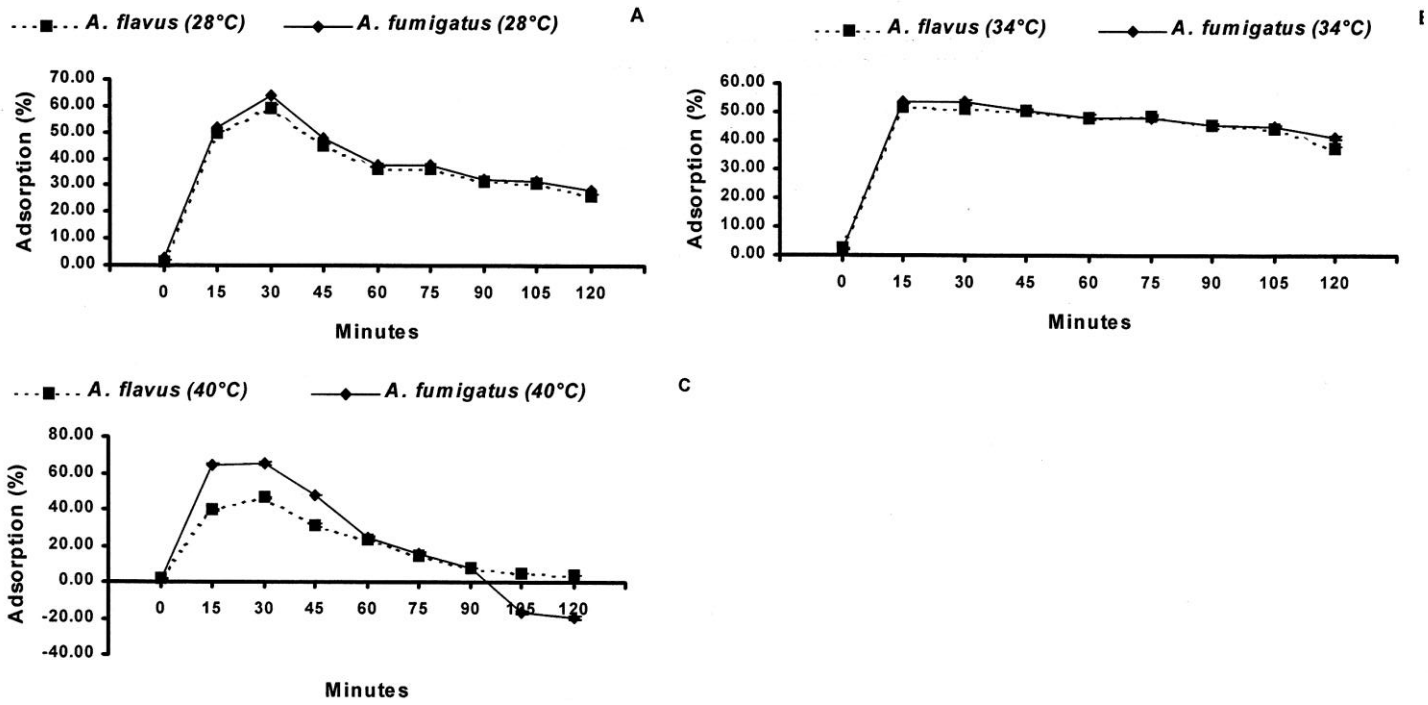

Fig. 7. Percentage adsorption of Zn by oven dried biomass of *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 at different temperatures.

Discussion

Fungi are used widely in industrial fermentation and bioremediation (Akhtar & Mohan, 1995). Fungi were preferred over other organisms because they are easier to remove from liquid substrates. When the resistance of these fungal species was tested on metal amended agar plates, they grew at sufficiently high levels of Zn. Price *et al*., (2001) tested different fungi by growing them on 5mM and found that all of the fungi tested were able to grow. However *A. niger* grew better and obtained a colony diameter of 84.5 mm in 7 days. Sayer *et al*., (1995), also observed that *A. niger* can solubilize Zn, when grown on malt extract agar amended with 0.4% (w/v) $Zn_2 (PO_4)_2$. *A. fumigatus* RH05 and *A. flavus* RH07 were selected because they showed highest resistance against Zn. Both strains removed Zn from media containing up to 5000mg/L of Zn. However, Chen & Lin (2004) reported 96-98% Zn removal by sulphur oxidizing bacteria.

With an increase of Zn content, not only Zn removal was reduced, but also the growth of both *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 were inhibited at 6000 and 7000 mg/L. In contrast to the present findings, Price *et al.,* (2001) reported that with an increasing Zn concentration from 0.01 to 1.0 mM, Zn absorption, as well as adsorption by *Aspergillus niger* increased after 7 days at 28°C, as it removed 66% Zn from 0.01 mM $ZnSO_4$ and 40% from 0.1 mM $ZnSO_4$ in broth.

Removal of Zn varied with alteration in pH, with live and growing *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05. Optimal pH for both strains was different for growth and Zn removal, 5.0 and 4.0, respectively. Biosorption per biomass dry weight of *Aspergillus niger* was found to be a inverse function of pH, decreasing with increasing pH.

Maximum Zn uptake by both fungal strains was observed at 28°C, which was also the optimal growth temperature. Increase in temperature led to reduction of Zn removal. Zn removal was also observed at 40°C. Brady & Duncan (1994), determined optimal temperature for Cu^{2+} removal in *Saccharomyces cerevisiae* to be 25-30°C, with decrease in metal uptake at 40°C, and an increase over the temperature range of 15-35°C. High temperatures have been suggested to affect the integrity of the cell membranes, and hinder compartmentalization of metal ions, leading to reduced metal uptake.

Adsorption was the main mechanism responsible for Zn removal by *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 (unpublished data). Huang *et al.,* (1988) reported the major accumulation mechanism for Zn^{2+} removal in *Saccharomyces cerevisiae* was adsorption. Bacteria and algae have also been reported to be capable of accumulating metal ions extracellularly or internally, however, adsorption remains the dominant mechanism for metal uptake from aqueous solutions (Wang & Shen, 1995; Chong *et al.*, 2000; Tam *et al.*, 2001)

Efficiency of dead cells is reported to be equivalent, or greater than, the live fungal cells. Therefore, dead biomass was studied for Zn removal, as it offers many advantages. Metal removal system is not subject to toxicity, it has no requirement of growth media and nutrients. Biosorbed metal ions can be easily desorbed, and dead biomass can be reused. Oven dried biomass of both strains showed higher Zn removal, as compared to live cells. Non-living biomass of *Aspergillus fumigatus* RH05 demonstrated more adsorption as compared to *Aspergillus flavus* RH07. Percentage adsorption in both strains reduced with increase in Zn concentration from 100-400 mg/L. In case of *Saccharomyces* strain MAS95, an opposite effect has been reported; that with an increase in metal

concentration, uptake also increased within first 10 minutes of incubation (Afshan *et al*., 2001). *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05, also showed a similar adsorption pattern; Zn adsorption was maximum in the first 15-30 minutes, and reached equilibrium after 60 minutes.

Adsorption by both strains was highly pH dependant. Adsorption increased with an increase in pH from 4.0 to 6.0, but decreased at pH 7.0. A similar effect of pH on metal adsorption by dried (non-living) biomass was reported for a fern, *Azolla filiculoides*, which showed an increase in Cr^{6+} adsorption at pH 2.0-3.0, and a limited sorption at neutral pH (Zhao & Duncan, 1997). Optimal pH range for adsorption of Zn by *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05 was 5.0 to 6.0, which is similar to the pH range for Zn adsorption by dried, non-living and granulated biomass of *Streptoverticillium cinnamoneum* (Puranik & Paknikar, 1997) and *Sargassum* for Cu^{2+} (Volesky *et al*., 2003). At pH 6.0, *Aspergillus fumigatus* RH05 removed 88% Zn from aqueous solution.

Aspergillus flavus RH07 and *Aspergillus fumigatus* RH05, demonstrated good adsorption over the temperature range of 28-40°C. *Aspergillus fumigatus* RH05 showed maximum adsorption (64.53%) within 15 minutes at 40°C, however, after 30 minutes, desorption took place. The increase in uptake with temperature could be due to the increase in the energy of the system, that facilitates the attachment of Zn^{2+} to the surface of the cells, and later on desorption could result from the distortion of some sites of the cell surface available for metal adsorption, as suggested by Al-Asheh & Duvnjak (1995).

Removal of Zn by non-living biomass was greater as compared to that by growing mycelium of both the strains. Huang & Huang (1996) suggested that an increase in metal biosorption, after pretreatment of the biomass, could be due to the removal of surface impurities and exposure of available binding sites for metal biosorption.

Fungal pellets (non-growing biomass) of *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05, were used as biosorbent, in order to compare their capacity to remove Zn, with growing cells and non-living biomass. Non-growing biomass showed a higher percentage of Zn removal, as compared to the growing cells of both *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05. In contrast, dead biomass of both the strains demonstrated more Zn removal capacity, as compared to non-growing and growing biomass. Pellets of wood rotting *Basidiomycetes* have been demonstrated to have sorped Cu^{2+} from 100 mg/L Cu solution at a pH range of 3.5-4.0, and among these *Basidiomycetes*, Cu removal (mg/g dry weight) observed was 8.77, 6.29, 5.08 and 4.77 for *Oudemansiella mucida*, *Lepista nuda*, *Pycnoporus cinnabarinus* and *Pleurotus ostreatus*, respectively (Gabriel *et al.*, 2001). In the present study, non-growing cells showed reduction in Zn uptake with an increase in Zn^{2+} concentration of aqueous solution, with equal weight of mycelium.

Addition of glucose to salt solution of Zn^{2+}, with non-growing mycelia of *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05, resulted in higher biosorption. This increase could be due to an increase in the cell's activities, including biosorption, caused by availability of glucose as a source of energy to the cell.

After analyzing various types of biomass of both *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05, oven dried biomass proved superior, as compared to growing and non-growing mycelia. pH and temperature also played a vital role in the biosorption of Zn, not only by growing cells, but also by non-living cells. The study has

also demonstrated that both *Aspergillus flavus* RH07 and *Aspergillus fumigatus* RH05, possessed a potential that could be used for bioremediation of Zn contaminated waste water and soil.

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