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Uptake and Reduction of Hexavalent Chromium by *Aspergillus niger* and *Aspergillus parasiticus*

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Abstract

The uptake and reduction of Cr(VI) by *Aspergillus niger* and *A. parasiticus* was studied in this journal. After 96 hours of growth, the culture solutions spiked with an initial dichromate concentration of 20 mg/l, were completely decolorized and had residual Cr(VI) concentrations of only 0.74 ± 0.55 and 1.69 ± 0.29 mg/l in *A. niger* and *A. parasiticus* cultures representing Cr(VI) removal of 96.3% and 91.6%, respectively. In the *A. niger* culture, significantly ($P < 0.01$) lower Cr(VI) concentrations were observed within 72 hours of growth compared to those of *A. parasiticus*, but in both cultures complete removal was almost achieved by 144 hours of growth. The rate of Cr(VI) removal was 0.21 ± 0.09 $mgl^{-1}hr^{-1}$ and 0.20 ± 0.07 $mgl^{-1}hr^{-1}$ for *A. niger* and *A. parasiticus*, respectively. Cellular concentrations of Cr(VI) in the two fungi increased significantly ($P < 0.05 – 0.001$) with increasing concentrations of the dichromate treatments. Although tannic acid as sole source of carbon and energy gave significantly lower Cr(VI) removal than glucose ($P < 0.001$) and acetate ($P < 0.01$), it supported the removal of about 85.0% and 68.8% of the metal ion by *A. niger* and *A. parasiticus*, respectively. The active mycelia of both fungi showed significantly ($P < 0.001$) higher Cr(VI) removal than inactivated mycelia after incubation at 30°C for 72 hours. Incubation of cell – free extracts of both fungi with NADH at 30°C for 2 hours showed Cr(VI) reduction of 68.0% and 55.5% for *A. niger* and *A. parasiticus*, respectively. These findings suggest that uptake and metabolic reduction may be the process by which the two fungi are able to tolerate the toxic effects of hexavalent chromium. However, Cr removal via uptake by the two fungal biomass was observed to be in the range of 0.5 – 1.78% only, for all the concentrations applied, which is insignificant when compared with the initial Cr concentration in the culture medium. The results obtained through this investigation indicate the possibility of treating waste effluents containing hexavalent chromium using *Aspergillus niger and A. parasiticus*.

Keywords: *Aspergillus spp*; Cr(VI) Uptake/reduction; Bioremediation

Introduction

Chromium pollution is one of the most important environmental problems in many regions of the world. Improper disposal of this heavy metal at facilities in arid and semiarid regions has led to contaminations of underlying vadose zones and aquifers [1]. Hexavalent chromium, which is highly toxic to most biological systems is generated and released to the environment through a large number of industrial operations, including tanning, metal electroplating, iron and steel and inorganic chemical industries [2] and is largely responsible for the pollution of underground water due to its high mobility in soils.

Various solutions have been proposed for bioremediation of chromium contaminated soil and water with selected microbial species. Many species of bacteria [1,3-8], yeasts [9-11] and fungi [12-16] have been investigated from this aspect, and the biosorption and bioaccumulation of chromium for bioremediation purposes have been demonstrated. Park et al. [13] had demonstrated that *Pseudomonas putida* culture was able to carry out enzymatic reduction of chromates. Cr(VI) was also shown to be quantitatively transformed to Cr(III) largely by soluble reductase activities in *Bacillus species* isolated from chromium-contaminated soil samples [5] Chromium reduction by the *Bacillus species* was not affected by sulphate and/or nitrate. The removal of chromium ions by the cyanobacterium, *Anacystis nidulans* immobilised on agar has been documented [3] Reports on chromate reduction by resting cells of sulphate-reducing bacteria particularly, *Desulfovibrio vulgaris* [17] and *Cellulomonas* sp. [4] are also available.

The biosorption processes for the removal of Cr(VI) from aqueous solutions and industrial effluents by dead fungal biomass [18] immobilized biomass of *Rhizopus arrhizus* [11,12] and fungal biomass of *Rhizopus nigricans* [14,15] have been reported. Biosorption of chromium from aqueous solutions by fungal pellets of *Aspergillus niger* has also been reported [13]; The bioremediation of chromium by the yeast *Pichia guilliermondii* [10] and *Saccharomyces cerevisiae* [9,19] has also been studied. Increased tolerance to chromium and enhanced Cr accumulation capabilities were observed among Cr-resistant mutants of *Pichia guilliermondii* obtained by means of environmental mutagenesis [10].

The mechanisms of chromium tolerance or resistance of selected microbes are of particular importance in both bioremediation and waste water treatment technologies. Studies of the various microbial uptake mechanisms for heavy metals could result in the identification of specific microbes with promising potential for application in metal

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bioremediation. Prasenjit and Sumathi [16] had demonstrated the ability of *Aspergillus foetidus* to take up chromium during the stationary phase of growth and under non-supportive growth conditions. In this work we report the uptake and metabolic transformation of hexavalent chromium by cultures of *Aspergillus niger* and *Aspergillus parasiticus*.

Materials and Methods

Fungal isolates

The fungi, *Aspergillus niger* and *Aspergillus parasiticus,* used in this study were isolated from landfill and sludge samples obtained from the Neital Shoe Factory and Tannery, Maiduguri, Nigeria. The isolation and identification of the fungi was carried out in the Mycology laboratory of the Plant Pathology Unit of the Department of Crop Science, University of Maiduguri. The fungi were identified by their colony characteristics as well as their vegetative and reproductive structures as observed under the electron microscope. Some macroscopic characteristics used for the identification include, colour of the colony, patterns of growth of colony and the byproducts released by the organisms. Some of the microscopic characteristic as viewed under the microscope include, the shape of the conidia head, pattern of arrangement of spores on the conidia, shape of the spores and shape of the conidiophores. The isolates were further confirmed in the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. The organisms were isolated and maintained on Sabouraud Dextrose Agar, SDA (Lab M, Biotech, England) at pH 5. Inoculated slants were incubated for 7 days at 30°C and then stored at 4°C until used.

Growth medium and culture conditions

For the growth of the fungi in suspension culture, a stock solution containing 0 to 20 mg/l Cr(VI) was prepared by dissolving analytical grade potassium dichromate ($K_2Cr_2O_7$) in 1 litre of sterile modified Vogel's mineral salts medium [20-23]. The solution was adjusted to a final pH of 4.5 ± 0.5 and 100 ml was used as culture medium for the growth of fungi. Spores of the two fungi were harvested from the 7-day-old culture slants by washing with 0.2% Tween-80. After vigorous shaking, inocula containing an average of 5.00×10^3 spores per ml were obtained for each of the two fungal strains.

A batch system of cultivation in batch reactors using 150-ml Erlenmeyer flasks on a rotary shaker (200 to 300 rpm) at pH 4 to 5 and temperature of 30°C for 96 hours was used for growing the fungi. The culture media in the bioreactor were inoculated with an average biomass of 50 mg per liter of inoculum of the fungal strains. Culture media without chromium treatment, 0 mg/l Cr(VI), served as control for the growth experiment.

Determination of Cr(VI) removal in the fungal culture

The removal of chromate from the culture solutions was determined by estimating the residual Cr(VI) in the culture media after the 96 hour growth period as previously described by Shugaba et al. [21-23] Cr(VI) uptake and reduction during growth was followed by loss of the yellow to orange colour of the dichromate solution and by the quantitative decrease in Cr(VI) concentration in culture [6,24,25] also, as part of the Cr(VI) reduction studies, the concentrations of trivalent chromium, [Cr(III)], and total chromium, Cr_T were also determined in the culture medium [26] Samples of the spent culture solutions were filtered using a membrane filter before measuring the Cr(VI), Cr(III) and Cr_T concentrations.

The role of metabolic activity in Cr(VI) removal by the two fungi was evaluated as described by Desjardin et al. [27]. Active and inactivated mycelia of *A. niger* and *A. parasiticus* were incubated in culture medium spiked with 50 mg/l Cr(VI) under the same conditions for 72 hours. Inactivation of the mycelia was achieved by autoclaving at 121^{0}C for 20 minutes. In order to determine the effects of carbon sources (electron donors) on chromium removal by the two fungi, glucose, acetate and tannic acid were applied separately as sole carbon and energy sources in fresh culture media containing 20 mg/l Cr(VI).

Determination of Cr(VI), Cr(III) and Cr_T concentrations in culture medium

The concentration of hexavalent chromium, Cr(VI), in the culture media was determined spectrophotometrically using 1, 5-diphenylcarbazide as complexing agent [24,28-30]. One milliliter (1 ml) of 0.2 % w/v of 1, 5-diphenylcarbazide solution (prepared in 95% ethanol and 1 ml of $^1/_5$ H_2SO_4) was added to 1 ml of the sample solution. The solution was allowed to stand for 10 minutes after which the absorbance of the purple-coloured solution was read at 540 nm. Cr(VI) concentration was extrapolated from a standard curve prepared from standard solutions of potassium dichromate.

Cr(III) and Cr_T were determined using atomic absorption spectrophotometer with Zeeman graphite furnacve as described by Xu et al. [31]. For Cr(III) determination, about 20 ml of the filtered medium was passed through anion exchange resin at a flow-rate of 1.0 ml/min, and then it was collected for the determination of trivalent chromium, Cr(III), concentration by Atomic Absorption Spectrophotometer (AAS).

Determination of total chromium uptake by fungal biomass

For the determination of total chromium uptake by the fungi, the mycelia were acid digested [10,32]. The cells were separated from the culture media by filtration, washed with water and dried. The dry cells were weighed and then suspended in a known volume of concentrated nitric acid, kept for 30 min at room temperature, and heated gently on a heating mantle (at 60°C for 30 min). After cooling to room temperature, hydrogen peroxide (H_2O_2) solution was added (in a ratio of 7:3 v/v nitric acid/H_2O_2) and the samples were heated again for 15 minutes and then centrifuged at 1500 x g. The supernatant was then used to determine the total chromium Cr_T concentration using Atomic Absorption Spectrophotometer, AAS (Perkin Elmer, Japan Co, Ltd.) with Zeeman graphite furnace.

Determination of Cr(VI) reducing activity of cell–free extracts of fungal mycelia

The ability of the two fungi to reduce hexavalent chromium in solution was studied using cell – free extracts of exponentially grown mycelia. The cell-free extracts were prepared by homogenizing the mycelia in 10 ml of 50 mM Tris–HCl buffer (pH 7.0) and then centrifuged at 3000 x g. The supernatant was then tested for ability to reduce Cr (VI). The Cr(VI) reducing activity was determined by measuring the decrease in Cr(VI) concentration [24] after incubation for 2 hours. The activity was assayed at 30°C in a reaction mixture containing 0.1 mM NADH (40 µl), 50 mM Tris – HCl buffer of pH 7.0 (1.8 ml) 0.05 mM $K_2Cr_2O_7$ (100 µl) and 600 µl of 0.2% 1, 5 – diphenylcarbazide reagent and the absorbance was read at 540 nm against a reference sample incubated without the extract.

Measurement of fungal biomass concentration

The biomass concentration in the culture media was measured

by collecting the biomass on pre-dried Whatman No. 1 filter paper, washing the biomass with distilled water and drying to constant weight at 70°C [20]. The biomass concentration was calculated as follows:

$$\text{Biomass concentration (mg/l)} = \frac{A-B}{V}$$

Where A = weight of the dry cells + filter paper

B = weight of the filter paper, and

V = Volume of culture media (l)

Measurement of specific growth rate

Culture turbidity was used to determine the specific growth rates (μ) of fungal cultures growing in shake – flask batch cultures [33]. Erlenmeyer flasks containing 100 ml of the growth medium and Cr(VI) at concentrations of 5 – 20 mg/l were inoculated with 2 ml of exponential – phase culture grown in identical medium (initial cultures absorbance of 0.1 – 0.4 nm). The fungal cultures were then incubated at 30°C on a rotary shaker at 200 rpm and changes in turbidity were determined at 24 hours interval for five days. Culture turbidity was measured spectrophotometrically by taking the absorbance of the growing cultures at 560 nm. The specific growth rate, μ (hr^{-1}) was determined by dividing the change in turbidity by the time interval within which growth was assessed.

Statistics

The data obtained in the study were analyzed by one-way analysis of variance, (ANOVA) and the Student t-test (using GraphPad Instat statistical program). Differences between means were considered significant at values of P<0.05.

Results and Discussion

Changes in Cr (VI) concentration during fungal growth

Figure 1 shows the changes in hexavalent chromium concentrations during growth of *A. niger* and *A. parasiticus*. Cr(VI) was significantly (P<0.001) reduced in both cultures every 24 hours with decolouration of the characteristic pale yellow colour of the dichromate. At 96 hours, the solution became completely colourless, and exhibited a Cr(VI) concentration of only 0.74 ± 0.55 and 1.69 ± 0.29 mg/l in *A. niger* and *A. parasiticus* cultures, respectively. Thus, with the initial dichromate concentration of 20 mg/l up to 96.3 % and 91.6 % Cr(VI) removal was achieved within 96 hours by *A. niger* and *A. parasiticus,* respectively. The significant reduction in media Cr(VI) concentration following fungal growth in both cultures suggest that *A. niger* and *A. parasiticus* are

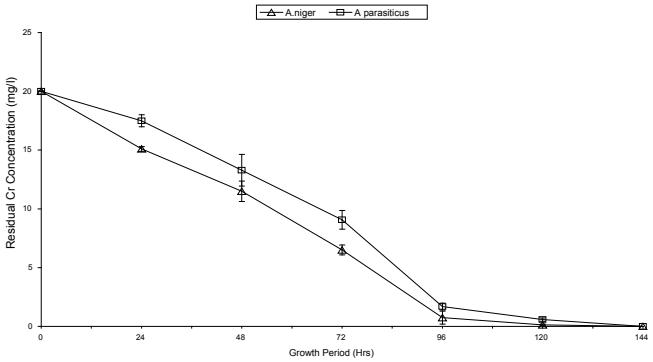

Figure 1: Changes in Cr(VI) concentration during fungal growth in culture solutions treated with hexavalent chromium at initial concentration of 20 mg/l.

both capable of removing this toxic metal in solutions. *A. niger* culture showed significantly (P<0.01) lower Cr(VI) concentration compared to that of *A. parasiticus* within 72 hours of growth, suggesting that it is more efficient than *A. parasiticus*. However, both organisms showed complete removal of Cr(VI) from their media by 144 hours of growth. The rate of Cr(VI) removal was 0.21 ± 0.09 $mgl^{-1}hr^{-1}$ and 0.20 ± 0.07 $mgl^{-1}hr^{-1}$ for *A. niger* and *A. parasiticus*, respectively.

Chromium removal using bacterial chromate reductase has been reported by several workers [5,6,25,29,]. The complete decolouration and clearance of up to 20 mg/l Cr(VI) from the culture media of *A. niger* and *A. parasiticus* suggests that these organisms may have the enzymic capabilities for converting Cr(VI) to Cr(III), thus indicating their ability for adaptation. The observed decrease in Cr(VI) concentration in culture media of *A. niger* and *A. parasiticus* may be related to a metabolic reduction of Cr(VI) ions, a metabolic uptake of Cr(VI) with no reduction, or a passive uptake of Cr(VI) with no reduction (i.e. biosorption). Metabolic uptake with reduction [27,25,29,34] as well as active uptake without reduction (bioaccumulation) and passive uptake (biosorption) of metal ions, including hexavalent chromium [27,35,36] are known processes of bioremediation by microorganisms.

The cellular concentration of Cr(VI) in the two fungi (Figure 2) increased significantly (P<0.05–0.001) with increasing concentration of Cr(VI) treatment; suggesting that the two organisms take up chromium ions from the media solution. Biosorption and bioaccumulation are processes by which microorganisms remove metal ions from solutions and industrial effluents [11,35-37]. *Aspergillus* mycelia have particularly been found to be efficient for removing zinc and thorium from solutions and polluted water [38,39]. In this study *A. parasiticus* had significantly (P<0.001) higher cellular Cr(VI) concentration (587.56 ± 47.17 μg/g) compared to *A. niger* (82.12 ± 3.45 μg/g) at the highest Cr(VI) treatment (20 mg/l). Thus *A. parasiticus* accumulates more chromium than *A. niger*.

Accumulation of high concentrations of Cr(VI) up to 34 mg/g dry weight by some microorganisms has been reported [40]. The mechanism of Cr(VI) – resistance and reduction may differ in microbial community from group to group or from strain to strain within a group [41] In some organisms Cr(VI) reduction takes place intracellularly because the enzyme is membrane-bound, and in such cases the substrate is taken up from the medium into the cells [34]. The rate of Cr(VI) uptake and its subsequent conversion to Cr(III) differ from organism to organism and reduced substrate uptake is said to be one of the mechanisms of chromium resistance in many microorganisms [42,43].

The significant difference in intracellular Cr(VI) concentration between *A. niger* and *A. parasiticus* may therefore be due to differences in the rate of chromium uptake between the two organisms and/or rate of conversion to Cr(III). It is likely that *A. parasiticus* may have taken up so much Cr(VI) that it could not convert to Cr(III) since intracellular accumulation of Cr(VI) may also disturb the normal functioning of microbial cells [34]. In studies of chromium toxicity and accumulation, Kshminska et al. [10] reported diverse, individual and complex mechanism of chromium bioremediation in the cells of different strains of the yeast *Pichia guilliermondii*. They observed that all the investigated strains responded to Cr(VI) treatment with substantial changes in protein profile patterns, which included both induction and suppression of certain polypeptides. In particular they

observed the induction of a 62 kDa as well as the suppression of a 59 kDa proteins upon Cr(VI) treatment and suggested that these proteins are involved in Cr(VI) bioremediation by *P. guilliermondii*.

Influence of carbon source on Cr(VI) reduction

The effect of carbon source on chromium reduction in cultures of *A. niger* and *A. parasiticus* is shown in Figure 3. Throughout the growth period, Cr(VI) concentrations were lower in cultures containing glucose compared to those with acetate and tannin as carbon sources; the difference being significant (P<0.001) between 72 and 120 hours. Cultures containing acetate also showed significantly (P<0.01) lower Cr(VI) compared to those with tannic acid as carbon and energy source (electron donor). This finding suggests that glucose and acetate as carbon and energy source support Cr(VI) reduction better than the tannin Figure 4. Glucose and acetate are known electron donors for Cr(VI) reduction by several organisms; indeed, about 2700 mg/l glucose was utilized to reduce Cr(VI) from 20 mg/l to 12 mg/l in 24 hours, and that Cr(VI) reduction ceased when glucose was nearly depleted within the 24 hours growth period [44]. According to Prasenjit and Sumathi [16], the initial rate of chromium uptake is enhanced by higher biomass concentrations and the presence of glucose. In an earlier study, it was shown that Cr(VI) reduction by pure culture of *S. thermocarboxydus* NH50 was more rapid with glycerol as sole carbon source than with glucose [27].

However, the fact that Cr(VI) concentration was significantly (P<0.001) reduced by about 85.0 % (*A. niger*) and 68.8 % (*A. parasiticus*)

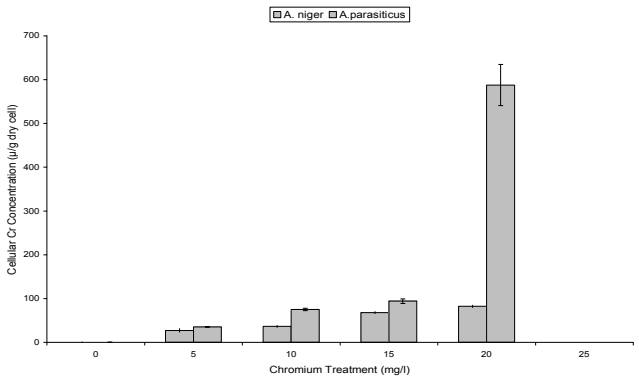

Figure 2: Cellular Cr(VI) concentration at 96 hours of growth.

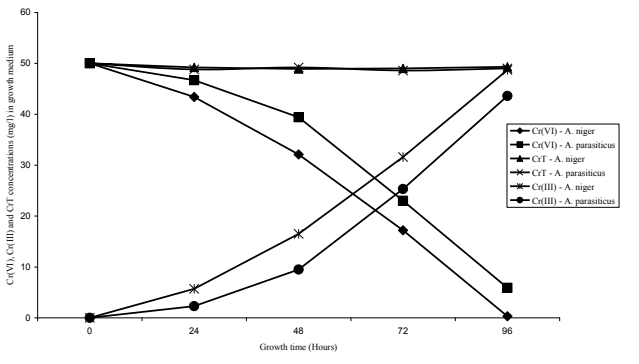

Figure 3: Concentrations (mg/l) of Cr(VI), Cr(III) and total chromium (CrT) in culture medium during fungal growth for 96 hours.

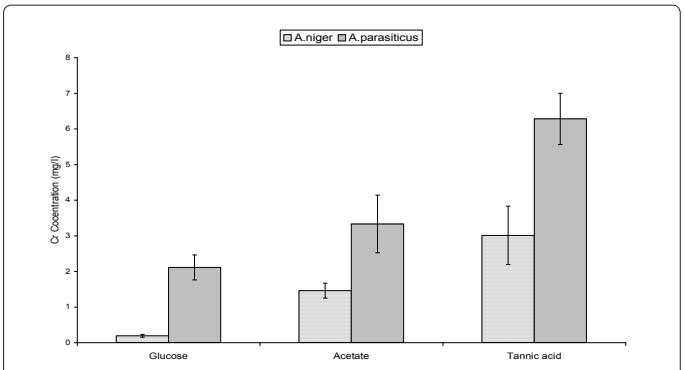

Figure 4: Influence of carbon source on Cr(VI) removal from culture solution by the fungi.

	A. niger		*A. parasiticus*	
No cells	Active	Inactive	Active	Inactive
49.27 ± 0.24	16.72 ± 1.25 (66.1%)	40.44 ± 0.97 (17.9%)	22.83 ± 1.03 (53.7%)	41.30 ± 1.13 (16.2%)

Values are presented as Means ± S. D of triplicate determinations.
Mean values are statistically different (P< 0.05).
Figures in parentheses represent percentage Cr(VI) removal.

Table 1: Residual concentration of Cr(VI) (mg/l) in solution after 72 hours of incubation at 30^{0}C in Vogel's mineral salt medium spiked with Cr(VI) at initial concentration of 50 mg/l.

after 96 hours of growth in media with tannic acid as sole carbon and energy source shows that these fungi are capable of utilizing tannins as carbon source or electron donors for Cr(VI) removal in solutions. The tannic acid, being a complex polyphenol, may not be readily available for use by the organisms; it may therefore have to be degraded to simpler, less toxic products, before it can be used to fuel Cr(VI) reduction. Indeed, it was reported that microorganisms breakdown complex tannins to simpler and less toxic products, which they could use as nutrients for growth [45,46] The growth of *Fusarium flocciferum* [47] the *Penicillium* strain Bi 7/2 [46], *Lactobacillus hilgardii* and *Lactobacillus plantarum* [46,48] on tannins such as protocatechuic, gallic, ferullic and syringic acids has been described. These organisms were able to grow on the tannins after degrading them into smaller less toxic products, which they used as source of energy and carbon for growth and metabolism.

Cr(VI) removal by active and inactive fungal biomass

The results presented in Table 1 show that in the media with active mycelia, *A. niger* culture had significantly (P< 0.05) lower Cr(VI) concentrations (16.72 ± 1.25 mg/l) compared to the active *A. parasiticus* culture (22.83 ± 1.03 mg/l); representing 66.6% and 54.3% Cr(VI) removal, respectively. However, there were no significant (P>0.05) differences in residual Cr(VI) concentrations in the media containing the inactivated mycelia of these fungi. For both organisms, the media with active cells had significantly (P<0.001) lower Cr(VI) concentrations after the 72 hours of incubation compared to those with inactivated mycelia. Also, for both fungi, the residual Cr(VI) concentrations were significantly lower in media with the active mycelia (P<0.001) and inactivated mycelia (P<0.05) compared to media incubated without the mycelia. In the media incubated with the inactivated mycelia, the Cr(VI) removal was 19.1% and 15.7% for *A.*

niger and *A. parasiticus*, respectively. There was no significant decrease in Cr(VI) concentration in the absence of the mycelia.

These observations suggest that Cr(VI) removal by *A. niger* and *A. parasiticus* is mainly a metabolic process involving either a metabolic uptake and reduction or metabolic uptake with no reduction (i.e. bioaccumulation). In addition, the significant decrease in Cr(VI) concentration in the presence of inactivated mycelia compared to values obtained in the absence of mycelia suggests that a passive uptake (biosorption) may also be involved in the removal process for Cr(VI). Bioaccumulation is a growth dependent process by which microbial cells sequester metals ions intracellularly by an active metabolic process; it is therefore mediated only by the living biomass [27,35]. In biosorption, the microbial biomass binds substantial amounts of metal ions by a passive process mediated by living and non – living biomass [27,35-37].

The mechanisms of biosorption mainly involve cell surface complexation, ion–exchange, chelation, and microprecipitation [49]. A large number of microorganisms are known to bind a variety of heavy metals with varying degrees of affinity. However, fungal biomasses exhibit a high percentage of cell wall material, with excellent metal – binding properties [37,49]. Among the fungi that have shown excellent potential of metal biosorption, *Aspergillus* and *Rhizopus* species have been well studied [13-15,37,50].

Cr(VI) – reducing activity of fungal extracts

The residual Cr(VI) concentrations of the reaction mixture incubated with the cell-free extracts of both organisms decreased significantly ($P<0.001$) within 2 hours, from an initial concentration of 7.35 ± 0.05 mg/l to 2.35 ± 0.17 mg/l and 3.27 ± 0.07 mg/l for *A. niger* and *A. parasiticus,* respectively. This shows Cr(VI) reduction of 68.0 and 55.5% for *A. niger* and *A. Parasiticus*, respectively. The observation of Cr(VI) reduction by the cell-free extracts of the two fungi suggests that metabolic uptake and reduction to Cr(III) may be the process by which these organisms carry out bioremediation of Cr(VI) in solutions. The conversion of Cr(VI) to Cr(III) has been recognized as one of the several mechanisms by which Cr(VI)-resistant organisms tolerate the toxic effects of chromium [25,29,34]. Cr(VI) – resistant organisms are known to produce chromium reductase, which mediates the transfer of electrons from NAD(P)H to Cr(VI), converting it to Cr(III) [25,29,51]. The abilities of *Aspergillus* and *Penicillium* species to massively reduce Cr(VI) to Cr(III) have earlier been reported [26,52-55].

Chromium removal via uptake by the fungal biomass

In Table 2 are results showing Cr uptake determined by multiplying the concentration of Cr in the fungal cells grown for 96 hours at 30°C by the fungal biomass concentration. Cr accumulation by the two organisms ranged from 0.5 – 1.78%, for all the concentrations applied; the percentage Cr uptake decreases with increasing concentrations of Cr(VI) treatment in the growth medium. These chromium uptake values are insignificant when compared with the initial Cr concentration in the culture medium. Similar observations on Cr uptake by fungal biomass of *Aspergillus* (strain Ed8) and *Penicillium* (strain H13) were reported by Acevedo-Aguilar et al. [26] leading to suggestion that Cr uptake by these fungal strains was almost negligible, as compared to the total Cr present in the growth medium. They reported a total Cr uptake of only about 0.06% and 0.04% by the fungal biomass of the *Aspergillus* sp. (strain Ed8) and the *Penicillium* sp. (strain H13), respectively after a growth period of 96 hours in a medium containing initial Cr content of 2500 µg. Thus, very little of the Cr in the culture medium was incorporated into the biomass, indicating that the decrease of Cr(VI) in the culture media of strains Ed8 and H13 resulted from its quantitative reduction to Cr(III) rather than from its uptake by the biomass.

In an earlier study, however, Park et al. [52] reported that when synthetic wastewater containing Cr(VI) was placed in contact with

Cr(VI) Treatment (µg/ml)	Cr concentration in mycelia (µg/g dry biomass)		Mean Biomass Concentration (g/ml)		Amount of Cr removed by fungus(µg)		% Cr removed from medium	
	A. niger	*A. parasiticus*	*A. niger*	*A. parasiticus*	*A. niger*	*A. parasiticus*	*A. niger*	*A. parasiticus*
5	26.93 ± 4.75	35.02 ± 1.07	0.0033	0.0024	0.089	0.084	1.78	1.68
10	36.18 ± 2.98	75.07 ± 2.79	0.0025	0.0013	0.091	0.098	0.91	0.98
15	67.57 ± 3.81	93.86 ± 5.33	0.0013	0.0010	0.088	0.094	0.57	0.63
20	82.12 ± 3.45	587.57 ± 47.17	0.0012	0.0002	0.099	0.118	0.50	0.59

Table 2: Chromium uptake by the biomass of the fungal strains after 96 hours of growth.

Growth Period (Hours)	*A. niger*			*A. parasiticus*		
	Cr(VI)	CrT	Cr(III)	Cr(VI)	CrT	Cr(III)
0	50.0 ± 0.12	50 ± 0.02	0.0	50 ± 0.10	50 ± 0.05	0.0
24	^{a}43.4 ± 1.03	49.2 ± 0.05	^{a}5.7 ± 0.11	^{a}46.7 ± 0.08	48.8 ± 0.19	^{a}2.3 ± 0.05
48	^{b}32.1 ± 0.22	48.9 ± 0.15	^{b}16.5 ± 0.07	^{b}39.4 ± 0.09	49.2 ± 0.31	^{b}9.5 ± 0.75
72	^{c}17.2 ± 0.85	49.0 ± 1.00	^{c}31.6 ± 0.45	^{c}23.0 ± 0.61	48.6 ± 0.72	^{c}25.3 ± 1.01
96	^{d}0.3 ± 0.01	49.3 ± 0.97	^{d}48.7 ± 0.52	^{d}5.9 ± 0.06	49.0 ± 0.84	^{d}43.6 ± 0.88

Values are presented as Mean ± S.D of triplicate determinations
Mean values with different superscripts along a column are statistically different ($P < 0.05$)

Table 3: Concentrations (mg/l) of hexavalent chromium [Cr(VI)], total chromium (CrT) and trivalent chromium [Cr(III)] in fungal cultures during the 96 hours growth period.

dead fungal biomass of *Aspergillus niger*, the Cr(VI) was completely removed from aqueous solution, whereas Cr(III), which was not initially present, appeared in aqueous solution Table 3. By using desorption and X-Ray Photoelectron Spectroscopy (XPS) studies they showed that most of the Cr bound onto the biomass was in the trivalent form and postulated that the main mechanism of Cr(VI) removal was a redox reaction between Cr(VI) and the dead fungal biomass, which is quite different from previously reported mechanisms. It was also reported that *Trichoderma inhamatum*, grown in a batch culture system, exhibited a remarkable capacity to tolerate and completely reduce Cr(VI) to Cr(III) from initial concentrations of 0.83 up to 2.43 mM [56] and that the specific and volumetric rates of Cr(VI) reduction by this fungus decreased as the initial Cr(VI) concentration increased.

It has been reported that in some chromate-resistant yeasts, the general mechanism of Cr(VI) resistance is related to limited ion uptake rather than to chemical reduction of toxic species of the metal ion . However, other yeasts such as *Candida maltosa* [57] showed some ability to reduce Cr(VI) and also the capability to accumulate chromium in the biomass. Prasenjit and Sumathi [16] had reported 97% decrease in hexavalent chromium (initial concentration, 5 µg/g) at the end of 92 hours of growth in *Aspergillus foetidus* culture, possibly due to its reduction to Cr(III) and/or complexation with organic compounds released due the metabolic activity of the fungus. Thus, variations in metal tolerance may be due to the presence of different types of tolerance processes or resistance mechanisms exhibited by different isolates. Sun and Shao [58] had demonstrated that both intracellular bioaccumulation and extracellular biosorption contributed to the high resistance of *Penicillium* sp. Psf-2 to lead. Sintuprapa et al. [59] also suggested that ion exchange and intracellular accumulation in the form of polyphosphate precipitation are the mechanisms of Zn^{2+} uptake by living cells of *Penicillium* sp. The resistance of the bacterium *Ralstonia mettalidurans* CH34 to Pb is mediated by a P-type ATPase, which can transport lead out of the cell. Copper resistance in *Aspergillus niger* is due to an active process involving copper metallothionein synthesis [60].

Among microorganisms, fungal biomass offers the advantage of having a high percentage of cell wall material with excellent metal-binding properties [35-37]. Many fungi and yeast, particularly the genera *Rhizopus*, *Aspergillus*, *Streptoverticillum* and *Saccharomyces* have shown excellent potential for metal biosorption and bioaccumulation. The entry of all metal ions into the plasma membrane and cytoplasm takes place via the cell wall, which consists of a variety of polysaccharides and proteins that offer a number of active sites for metal binding. Differences in the cell wall composition among different species of microorganisms and the intra species differences can thus cause significant differences in the type and amount of metal binding to them. Fungi generally show greater resistance to heavy metals than many bacterial species [61] because mycelial growth gives the organism a larger surface area, which provides greater protection to sensitive organelles of the fungi. In addition, fungi are eukaryotic cells, which contain more genes that provide other means of dealing with inhibitory compounds such as toxic metals.

Regeneration of fungal cells after initial exposure to Cr(VI)

Tables 4 and 5 show the specific growth rates and biomass concentrations, respectively, for both fungi after sub-culturing from the Cr(VI) treated cultures into non-chromium media. The specific growth rates for both organisms sub-cultured from all the treatments were not significantly (P>0.05) different. Similarly, the biomass concentrations of both organisms were not significantly (P>0.05) different, except for those of *A. parasiticus* sub-cultured from the 20 mg Cr(VI)/l treatment which showed significantly (P<0.05) lower biomass compared to the rest.

Biotechnological exploitation of microbial biosorption and bioaccumulation technology for removal of heavy metals from wastewater and industrial effluents depends on the efficiency of the

Cr(VI) Treatment	Specific growth rate, µ hr^{-1}	
	Aspergillus niger	*Aspergillus parasiticus*
Control 0 mg/l	0.27 ± 0.02	0.21 ± 0.06
5 mg/l	0.29 ± 0.04	0.18 ± 0.01
10 mg/l	0.25 ± 0.02	0.22 ± 0.03
15 mg/l	0.24 ± 0.01	0.18 ± 0.06
20 mg/l	0.25 ± 0.05	0.17 ± 0.02

Values are mean ± S.D. of four determinations (P>0.05).

Table 4: Specific growth rate, µ (hr^{-1}) of *A. niger* and *A. parasiticus* regenerated from hexavalent chromium treated cultures into chromium-free medium.

Cr(VI) Treatment (mg/l)	Biomass concentration (g/l) in Cr(VI) treated cultures		Biomass concentration (g/l) in Cr(VI)-free medium after initial exposure to Cr(VI)	
	A. niger	*A. parasiticus*	*A. niger*	*A. parasiticus*
0	^{a}3.344 ± 0.010	^{a}2.982 ± 0.013	^{a}3.311 ± 0.050	^{a}2.926 ± 0.024
5	^{a}3.332 ± 0.030	^{a}2.352 ± 0.017	^{a}3.246 ± 0.048	^{a}2.900 ± 0.037
10	^{b}2.520 ± 0.023	^{b}1.303 ± 0.034	^{a}3.331 ± 0.025	^{a}2.856 ± 0.028
15	^{c}1.330 ± 0.018	^{b}1.000 ± 0.016	^{a}3.229 ± 0.031	^{a}2.853 ± 0.045
20	^{c}1.200 ± 0.015	^{c}0.180 ± 0.015	^{a}3.297 ± 0.023	^{b}2.740 ± 0.023

Values are presented as Mean ± S.D. of four determinations.
Mean values with different superscripts along a column are significantly different (P < 0.05).

Table 5: Biomass concentrations of *A. niger* and *A. parasiticus* regenerated from hexavalent chromium treated cultures in a chromium-free medium.

regeneration of the microbial biomass for reuse in multiple cycles [35]. In bioremediation of metal ions, the performance of living microbial biomass depends much on cell age and toxic effects of heavy metals may result in cell death [36]. Hexavalent chromium is highly toxic to a lot of microorganisms, and like other heavy metal ions such as Cu(II), Pb(II), Zn(II), etc. it may cause structural damage in cells and consequently a permanent effect on cell growth [34,62].

The results obtained in this study show that although *A.niger* and *A. parasiticus* were exposed to concentrations of Cr (VI), which severely inhibited their growth; these organisms were capable of regenerating with specific growth rates (μ) and biomass comparable to those of the chromium-free control organisms. This observation suggests a remarkable potential for application of these fungi in chromium biotreatment. Sani et al. [62] had reported similar observation with *Desulfovibrio desulfuricans* where toxic concentration of the heavy metal ions, Cu(II), Pb(II) and Zn(II) utterly inhibited growth, but did not kill the cells of the organism. Although Ezzouhri et al. [63] reported 10 – 15 mM of Cr(VI) as the Minimum Inhibitory Concentration (MIC) for *Aspergillus species*, the results obtained however revealed that the response of the isolates to heavy metals depended on the metal tested, its concentration in the medium and on the isolate considered. They observed that various genera and also isolates of the same genus did not necessarily have the same heavy metal tolerance. Thus, variation in metal tolerance among fungal species may be due to the presence of one or more strategies of tolerance or resistance mechanisms exhibited by fungi.

References

1. Oliver DS, Brockman FJ, Bowman RS, Kieft TL (2003) Microbial reduction of hexavalent chromium under vadose zone conditions. J Environ Qual 32: 317-324.
2. Gao Y, Zeng Q, Guo J, Cheng J, Ellis BE, Chen JG (2007) Genetic characterization reveals no role for the reported ABA receptor, GCR2, in ABA control of seed germination and early seedling development in Arabidopsis. Plant J 3: 1-10.
3. Khattar JIS, SarmaTA, Singh DP (1999) Removal of chromium ions by agar immobilized cells of the cyanobacterium Anacystis nidulans in a continuous flow bioreactor – a highly pH dependent phenomenon. Enzyme Microb Technol 25: 564–568.
4. Sani RK, Peyton BM, Smith WA, Apel WA, Petersen JN (2002) Dissimilatory reduction of Cr(VI), Fe(III), and U(VI) by Cellulomonas isolates. Appl Microbiol Biotechnol 60: 192-199.
5. Camargo FA, Okeke BC, Bento FM, Frankenberger WT (2003) In vitro reduction of hexavalent chromium by a cell–free extracts of Bacillus sp. ES 29 stimulated by Cu^{2+}. Appl Microbiol Biotechnol 62: 569 – 573.
6. Horton RN, Apel WA, Thompson VS, Sheridan PP (2006) Low temperature reduction of hexavalent chromium by a microbial enrichment consortium and a novel strain of Arthrobacter aurescens. BMC Microbiol 6: 5.
7. Li Y, Low GK, Scott JA, Amal R (2007) Microbial reduction of hexavalent chromium by landfill leachate. J Hazard Mater 142: 153-159.
8. Srivastava S, Ahmad AH, Thakur IS (2007) Removal of chromium and pentachlorophenol from tannery effluents. Bioresour Technol 98: 1128-1132.
9. Zetic VG, Stehlik-Tomas V, Grba S, Lutilsky L, Kozlek D (2001) Chromium uptake by Saccharomyces cerevisiae and isolation of glucose tolerance factor from yeast biomass. J Biosci 26: 217-223.
10. Kshminska H, Jaglarz A, FedorovychD, Babyak L, Yanovych D, et al. (2003) Bioremediation of chromium by the yeast Pinchia guilliermondii: toxicity and accumulation of Cr(III) and Cr(VI) and the influence of riboflavin on Cr tolerance. Microbiol Res 158: 59–67.
11. Poljsak B, Pocsi I, Raspor P, Pesti M (2010) Interference of chromium with biological systems in yeasts and fungi: a review. J Basic Microbiol 50: 21-36.
12. Niyogi S, Abraham TE, Ramakrishna SV (1998) Removal of Cr(VI) ions from industrial effluents by immobilized biomass of Rhizopus arrhizus. J Sci and Industr Res 57: 809–816.
13. Park CH, Keyhan M, Wielinga B, Fendorf S, Matin A (2000) Purification to homogeneity and characterization of a novel Pseudomonas putida chromate reductase. Appl Environ Microbiol 66: 1788-1795.
14. R SB, Abraham TE (2001) Biosorption of Cr (VI) from aqueous solution by Rhizopus nigricans. Bioresour Technol 79: 73-81.
15. Bai RS, Abraham TE (2002) Studies on enhancement of Cr(VI) biosorption by chemically modified biomass of Rhizopus nigricans. Water Res 36: 1224-1236.
16. Prasenjit, B, Sumathi S (2005) Uptake of chromium by Aspergillus foetidus. Journal of Material Cycles and Waste Management 7: 88–92.
17. Chardin B, Giudici–Orticoni MT, De Luca G, Guigliarelli B, Bruschi M (2003) Hydrogenases in sulfate–reducing bacteria function as chromium reductase. Appl Microbiol Biotechnol 63: 315 – 321.
18. Bai S, Abraham TE (1998) Studies on biosorption of Cr(VI) by dead fungal biomass. J Sci and Ind Res 57: 821 – 824.
19. Krauter P, Martinelli R, Williams K, Martins S (1996) Removal of Cr(VI) from groundwater by Saccharomyces cerevisiae. Biodegradation 7: 277–286.
20. Withers JM, Swift RJ, Wiebe MG, Robson GD, Punt PJ et al. (1998) Optimization and stability of glucoamylase production by recombinant strains of Aspergillus niger in chemostat culture. Biotechnol Bioeng 59: 407– 418.
21. Shugaba A, Nok AJ, Ameh DA, Lori JA (2010) (a) Studies on the growth of some filamentous fungi in culture solutions containing hexavalent chromium. International Journal of Biotechnology and Biochemistry 6: 715–722.
22. Shugaba A, Nok AJ, Ameh DA, Lori JA (2010) (b) Bioremediation of hexavalent chromium and tannic acid in synthetic tannery wastewater using free and calcium–alginate immobilized spores and mycelia of Aspergillus niger and Aspergillus parasiticus. Bioremediat J 14: 142–149.
23. Shugaba A, Nok AJ, Ameh DA, Lori JA (2011) Studies on some growth related changes in cultures of Aspergillus niger and Aspergillus parasiticus treated with hexavalent chromium and tannic acid. International Journal of Biotechnology and Biochemistry 7: 251–264.
24. Filipovic–Kovacevic Z, Sipos L, Briski F (2000) Biosorption of chromium, copper, nickel, and zinc ions onto fungal pellets of Aspergillus niger 405 from aqueous solutions. Food Technol Biotechnol 38.
25. Ackerley DF, Gonzalez CF, Park CH, Blake R 2nd, Keyhan M, et al. (2004) Chromate-reducing properties of soluble flavoproteins from Pseudomonas putida and Escherichia coli. Appl Environ Microbiol 70: 873-882.
26. Acevedo-Aguilar FJ, Espino-Saldana AE, Leon-Rodriguez IL, Rivera-Cano ME, Avila-Rodriguez M, et al. (2006) Hexavalent chromium removal in vitro and from industrial wastes, using chromate-resistant strains of filamentous fungi indigenous to contaminated wastes. Can J Microbiol 52: 809-815.
27. Desjardin V, Bayard R, Huck N, Manceau A, Gourdon R (2002) Effect of microbial activity on the mobility of chromium in soils. Waste Manag 22: 195-200.
28. Aksu Z, Egretli G, Kutsal T (1999) A comparative study for the biosorption characteristics of chromium (VI) on Ca-alginate, agarose and immobilized C. vulgaris in a continuous packed bed column. J Environ Sci Health 32: 295 - 316.
29. Ackerley DF, Gonzalez CF, Keyhan M, Blake R 2nd, Matin A (2004) Mechanism of chromate reduction by the Escherichia coli protein, NfsA, and the role of different chromate reductases in minimizing oxidative stress during chromate reduction. Environ Microbiol 6: 851-860.
30. Balasubramanian S, Anuradha k (1999) Characterization of tannery effluents and the correlation between TDS, BOD and COD. J Environ Sci Health 34: 461– 478.
31. Xu W, Hai C, Cheng Y, Gao S, Wang F (2006) A Novel Pumpkin Rich in Chromium Lowers Hyperglycemia and Enhances Translocation of Glut 4 in Alloxan-induced Diabetic Rats. J Med Sci 3: 1–10.
32. Munoz AH, CoronaFG, Wrobel K, Soto GM, Wrobel K (2005) Subcellular distribution of aluminium, bismuth, cadmium, chromium, copper, iron, manganese, nickel and lead in cultivated mushrooms (Agaricus bisporus and Pleurotus ostreatus). Biol Trace Elem Res 106: 265–277.
33. Trinci APJ (1972) Culture turbidity as a measure of mould growth. Transaction of the British Mycological Society 58: 467–473.

34. Middleton SS, Latmani RB, Mackey MR, Ellisman MH, Tebo BM, et al. (2003) Cometabolism of Cr(VI) by Shewanella oneidensis MR-1 produces cell-associated reduced chromium and inhibits growth. Biotechnol Bioeng 83: 627-637.

35. Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H (2000) Microbial biosorbents: Meeting chanllenges of heavy metal pollution in aqueous solutions. Curr Sci 78: 967–973.

36. Yan G, Viraraghavan T (2000) Effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii*. Water SA 26: 119–123.

37. Kapoor A, Vivraraghavan T (1998) Biosorption of heavy metals on Aspergillus niger: Effect of pretreatement. Bioresourc Technol 63: 109–113.

38. Gadd GM, White C (1989) Removal of thorium from simulated acid process streams by fungal biomass. Biotechnol Bioeng 33: 592-597.

39. Gadd GM (1990) Heavy metal accumulation by bacteria and other microorganism. Cell Mol Life Sci 46: 834–840.

40. Srinath T, Verma T, Ramteke PW, Garg SK (2002) Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Chemosphere 48: 427-435.

41. Francisco R, Alpoim MC, Morais PV (2002) Diversity of chromium – resistant and -reducing bacteria in a chromium – contaiminated activated sludge. J Appl Microbiol 92: 837–843.

42. Silver S, Walderhaug M (1992) Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. Microbiol Rev 56: 195-228.

43. Czako-Ver K, Batie M, Raspor P, Sipiczki M, Pesti M (1999) Hexavalent chromium uptake by sensitive and tolerant mutants of Schizosaccharomyces pombe. FEMS Microbiol Lett 178: 109-115.

44. Wang YT, Xiao C (1995) Factors affecting hexavalent chromium reduction in pure cultures of bacteria. Water Res 29: 2467–2474.

45. Bhat TK, Singh B, Sharma OP (1998) Microbial degradation of tannins--a current perspective. Biodegradation 9: 343-357.

46. Rodriguez H, de las Rivas B, Gomez-Cordoves C, Munoz R (2008) Degradation of tannic acid by cell-free extracts of Lactobacillus plantarum. Food Chem 107: 664-670.

47. Mendonca E, Martins A, Anselmo AM (2004) Biodegradation of natural phenolic compounds as single and mixed substrates by Fusarium flocciferum. Electronic J Biotechnol 7: 30–34.

48. Alberto MR, Farias ME, Manca De Nadra MC (2001) Effect of gallic acid and catechin on Lactobacillus hilgardii 5w growth and metabolism of organic compounds. J Agric Food Chem 49: 4359-4363.

49. Fourest E, Roux JC (1992) Heavy metal biosorption by fungal mycelial products: Mechanisms and influence of pH. Appl Microbiol Biotechnol 37: 399–403.

50. Zhou JL (1999) Zinc biosorption by *Rhizopus arrhizus* and other fungi. Appl Microbiol Biotechnol 51: 686-693.

51. Upreti RK, Shrivastava R, Chaturvedi UC (2004) Gut microflora and toxic metals: chromium as a model. Indian J Med Res 119: 49–59.

52. Park D, Yun YS, Jo JH, Park JM (2005) Mechanism of hexavalent chromium removal by dead fungal biomass of Aspergillus niger. Water Res 39: 533-540.

53. Acevedo Aguilar FJ, Wrobel K, Lokits K, Caruso JA, Coreno Alonso A, et al. (2008) Analytical speciation of chromium in in-vitro cultures of chromate-resistant filamentous fungi. Anal Bioanal Chem 392: 269-276.

54. Fukuda T, Ishino Y, Ogawa A, Tsutsumi K, Morita H (2008) Cr(VI) reduction from contaminated soils by Aspergillus sp. N2 and Penicillium sp. N3 isolated from chromium deposits. J Gen Appl Microbiol 54: 295-303.

55. Coreno-Alonso A, Acevedo-Aguilar FJ, Reyna-Lopez GE, Tomasini A, Fernandez FJ, et al. (2009) Cr(VI) reduction by an Aspergillus tubingensis strain: role of carboxylic acids and implications for natural attenuation and biotreatment of Cr(VI) contamination. Chemosphere 76: 43-47.

56. Morales-Barrera L, Cristiani-Urbina E (2008) Hexavalent chromium removal by a Trichoderma inhamatum fungal strain isolated from tannery effluent. Water Air Soil Pollut 187: 327–336.

57. Ramirez-Ramirez R, Calvo-Mendez C, Avila-Rodriguez M, Lappe P, Ulloa M, et al. (2004) Cr(VI) reduction in a chromate-resistant strain of Candida maltosa isolated from the leather industry. Antonie Van Leeuwenhoek 85: 63-68.

58. Sun F, Shao Z (2007) Biosorption and bioaccumulation of lead by Penicillium sp. Psf-2 isolated from the deep sea sediment of the Pacific Ocean. Extremophiles 11: 853 – 858.

59. Sintuprapa W, Thiravetyan P, Tanticharoen M (2000) A possible mechanism of Zn^{2+} uptake by living cells of Penicillium sp. Biotechnol Lett 22: 1709–1712.

60. Kermasha S, Pellerin F, Rovel B, Goetghebeur M, Metche M (1993) Purification and characterization of copper-metallothioneins from Aspergillus niger. Biosci Biotechnol Biochem 57: 1420 – 1423.

61. Guest RK, Smith DW (2002) A potential new role for fungi in a wastewater MBR biological nitrogen reduction system. J Environ Engr Sci 1: 433–437.

62. Sani RK, Peyton BM, Brown LT (2001) Copper – induced inhibition of growth of *Desulfovibrio dusulfuricans* G-20: Assessment of its toxicity and correlation with those of Zinc and lead. Appl Environ Microbiol 67: 4765–4772.

63. Ezzouhri L, Castro E, Moya M, Espinola F, et al. (2009) Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. Afr J Microbiol 3: 35 – 48.

64. Coulibaly L, Gourene G, Agathos NS (2003) Utilization of fungi for biotreatment of raw wastewaters. Afr J Biotechnol 2: 620 – 630.

65. Sun F, Shao Z (2007) Biosorption and bioaccumulation of lead by Penicillium sp. Psf-2 isolated from the deep sea sediment of the Pacific Ocean. Extremophiles 11: 853 – 858.

66. Gao H, Liu Y, Zeng G, Xu W, Li T et al. (2008) Characterization of Cr(VI) removal from aqueous solutions by a surplus agricultural waste- rice straw. J Hazard Mater. 150: 446-452.