African Journal of Biotechnology Vol. 10(32), pp. 6072-6081, 4 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1949
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Role of live autochthonous fungi in removing toxic metals from tannery and textile effluents

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Accepted 3 June, 2011

Decontamination potential of two autochthonous fungi, *Aspergillus niger* and *Fusarium oxysporum,* was checked in tannery and textile effluents. The fungi grew well in both industrial effluents, *A. niger* showing a greater biomass than *F. oxysporum* in both effluents. *A. niger* showed less growth with increasing concentration of effluents while *F. oxysporum* showed increase in growth with increasing effluent concentration, with optimum mycelial biomass in 60% effluent concentration. A greater metal content was absorbed from higher concentration of effluents by both fungi. A significant amount of all metals was absorbed by both fungi, but a greater amount was absorbed from the tannery as compared to textile effluents. A greater reduction of heavy metals was observed in effluents by *A. niger*. Up to 75% reduction of Zn was caused in tannery effluents and 76% reduction of Cu in textile effluents by *A. niger.*

Key words: Autochthonous fungi, bioremediation, toxic metals, tannery effluents, textile effluents.

INTRODUCTION

The introduction of metal pollutants in various forms in the environment can pose a severe threat to the ecological systems (Jaiswal and Malik, 2000). The analysis of industrial effluents is required for two primary purposes, environmental monitoring, and monitoring for the loss of products, by-products and raw materials from an industrial process. The nature of the constituents in the effluents will be determined by the raw materials used, the type of processes employed and the efficiency with which materials are removed from the effluent, either through recovery processes or effluent treatment (Rojas and Ojeda, 2005). The major problem with metals is their persistence as they tend to persist indefinitely in the food chain (Gupta et al., 2000). Most of the industries in Pakistan do not control their wastewater effluents by processing, waste recycling or end-of-pipe treatment (Sial, 2006). There is a need to develop rapid, economical and environmentally benign technologies for the removal of metals from industrial effluents. There are certain microorganisms, which can survive in high concentrations of metals and have the potential to accumulate different metals. This is achieved by the virtue of covalent interaction of metal at cell surface or within the cell by different processes (Gadd and White, 1993; Bhanoori and Venkateswerlu, 2000). These microbes can be of immense significance in the clean up of heavy metals from the environment. The high surface to volume ratio of microorganisms and their ability to detoxify metals are among the reasons that they are considered as potential alternative to synthetic resins for remediation of dilute solutions of metals and solid wastes (Kapoor et al., 1999; Magyarosy et al., 2002). In this regard, fungi are a versatile group as they can adapt and grow under various extreme conditions of pH, temperature and nutrients availability as well as high metal concentrations (Anand et al., 2006). Considering the various mechanisms of metal resistance in fungi, it is expected that screening of metal tolerant fungi may provide strains with improved metal accumulation. Only limited studies have been conducted to systematically screen filamentous fungi from metal polluted sites for their diversity, metal tolerance and their biosorption potential (Bai and Abraham, 2003). This study was aimed at investigating the growth of indigenous fungal strains in the industrial effluents from which they were isolated and their metal removing capabilities in different concentrations of the effluents.

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Abbreviations: TDS, Total dissolved solids; **COD,** chemical oxygen demand.

Table 1. The fungi isolated from the tannery and textile effluents.

S/N	List of fungi	Tannery effluent	Textile effluent
1	*Alternaria alternatus* Berk.	+	-
2	*Aspergillus candidus* Link	+	+
3	*Aspergillus flavipes* (Bain and Sart.) Thom and Curch	+	-
4	*Aspergillus flavus* Link ex Gray	+	-
5	*Aspergillus fumigatus* Fresenius	+	-
6	*Aspergillus glaucus* (L.) Link	+	-
7	*Aspergillus heteromorphus* Bat. and Maia	+	-
8	*Aspergillus japonicus* Saito	+	-
9	*Aspergillus niger* van Teigham	+	+
10	*Aspergillus parasiticus* Speare	+	-
11	*Aspergillus terreus* Thom.	+	-
12	*Fusarium oxysporum* Schltdl.	+	+
13	*Rhizopus arrhizus* Fisch.	+	-
14	*Saccharomyces* Meyen ex Hansen sp.	+	+
15	*Trichoderma pseudokoningii* Rifai	+	+
16	*Trichoderma koningii* Oudem	+	+

MATERIALS AND METHODS

The industries selected for effluent sampling were the tanning industries of Kasur and textile industries of Faisalabad in Pakistan. The tannery effluent was collected from the main drain entering the Kasur tanneries waste management agency (KTWMA) for the treatment adjacent to the Depalpur Road, Kasur, Pakistan. The textile effluents of three large textile mills located at Sheikhupura road off Faisalabad, Pakistan were taken and mixed together.

Autochthonous fungi were isolated on Malt extract agar (MA) and Potato dextrose agar (PDA) by plating serially diluted effluent solution under aseptic conditions. The fungal strains isolated were identified in the fungal culture bank of Pakistan, University of the Punjab, Lahore, Pakistan. A list of the isolated fungi from both types of effluents is given in Table 1. Autochthonous strains of *Aspergillus niger* van Tieg. and *Fusarium oxysporum* Schlecht. were selected for the experiments, as they appeared to be the most common in these effluents.

Both types of effluents were subjected to physico-chemical analysis as such and after preparing 30 and 60% concentrations with deionized water. pH was determined by a pH meter (Phep, Hanna) while conductivity, NaCl percentage and total dissolved solids (TDS) were determined by a digital microprocessor (HI 9835, Hanna). Chemical oxygen demand (COD) was determined by the titermetric method given by Greenberg et al. (1995).

Digestion of the effluent samples was carried out with a mixture of concentrated nitric acid and perchloric acid (Greenberg et al., 1995). The digested samples were subjected to estimations of Na on a flame photometer (PFP7 and PFP7/C, England) and Cr, Cu and Zn on an atomic absorption spectrophotometer (AA-1275/ Varian, Australia).

The experiments were set up in an aseptic culture room in the Department of Botany, University of the Punjab, Lahore, Pakistan. Volumetric conical flasks (250 ml) were used in the experiments. The experiment was set up in a completely randomized design (Steel and Torrie, 1980). Each flask was filled with 100 ml of deionized water in case of control and 100 ml of effluent in case of 100% effluent concentration. Similarly 100 ml of 30 and 60% effluent concentrations were prepared with deionized water. To each flask, malt extract (2%) was added as a C source. Five replicates of each treatment were taken. Actively growing mycelium on the margins of a culture slice of MA in the form of 1 cm discs were used as inocula. Different treatments of effluents were maintained on an orbital shaker at 28±2°C at 150 rpm for 10 days from the information on optimized culture conditions, recorded before setting up the actual experiment.

After 10 days, the samples were removed from the orbital shaker. In order to separate the fungal biomass from the supernatant, the samples were centrifuged at 10,000 rpm for 10 min at 4°C (Srivastava and Thakur, 2006). Pellets separated from the supernatant were transferred in sterile pre-weighed crucible and mycelial pellets were dried overnight at 60°C in an oven. Weight of the dried pellets in the form of dry ash of fungal mycelium was determined on a digital balance. One gram of dry ash of fungal mycelium was taken, and crushed in a pestle and mortar and acid digested as given by Srivastava and Thakur (2006). The solution obtained was used for the determination of metal content on flame photometer and AAS. The supernatant obtained was filtered through a Whatman No. 1 filter paper and processed as given by Srivastava and Thakur (2006). The metal content was determined on flame photometer and AAS as described earlier. The statistical analyses of the data were carried out using SPSS software, version 11.5.

RESULTS

Analysis of effluents

The physico-chemical analysis of the various effluent concentrations is given in Table 2. Tanning effluents were much greater in all pollution parameters especially

Table 2. Physico chemical analyses of different concentrations of industrial effluents from the leather industry, Kasur and the textile industry, Faisalabad, Pakistan.

Parameter	Type of effluent					
	Tanning effluent			Textile effluent		
	30%	60%	100%	30%	60%	100%
pH	8.20	8.27	8.30	8.41	8.46	8.50
EC (mS cm^{-1})	15.93	17.26	19.15	1.87	2.12	3.76
NaCl (%)	29.9	33.7	36.9	3.01	4.30	7.3
TDS (mg L^{-1})	6910	8000	9550	500	1000	1870
COD (mg L^{-1})	1400	1800	3400	286	571	1502
Amount of Ca (mg L^{-1})	302.6	572.3	846.3	210	374	520
Amount of Cr (mg L^{-1})	68.2	172.3	420	75	110.4	150
Amount of Cu (mg L^{-1})	30.6	51.3	83.7	58.5	94.3	120
Amount of K (mg L^{-1})	213.2	364.2	515.1	140	215	300
Amount of Na (mg L^{-1})	342.5	645.2	948.7	305	490	630
Amount of Zn (mg L^{-1})	85.3	135.1	220.3	50.7	89.5	110

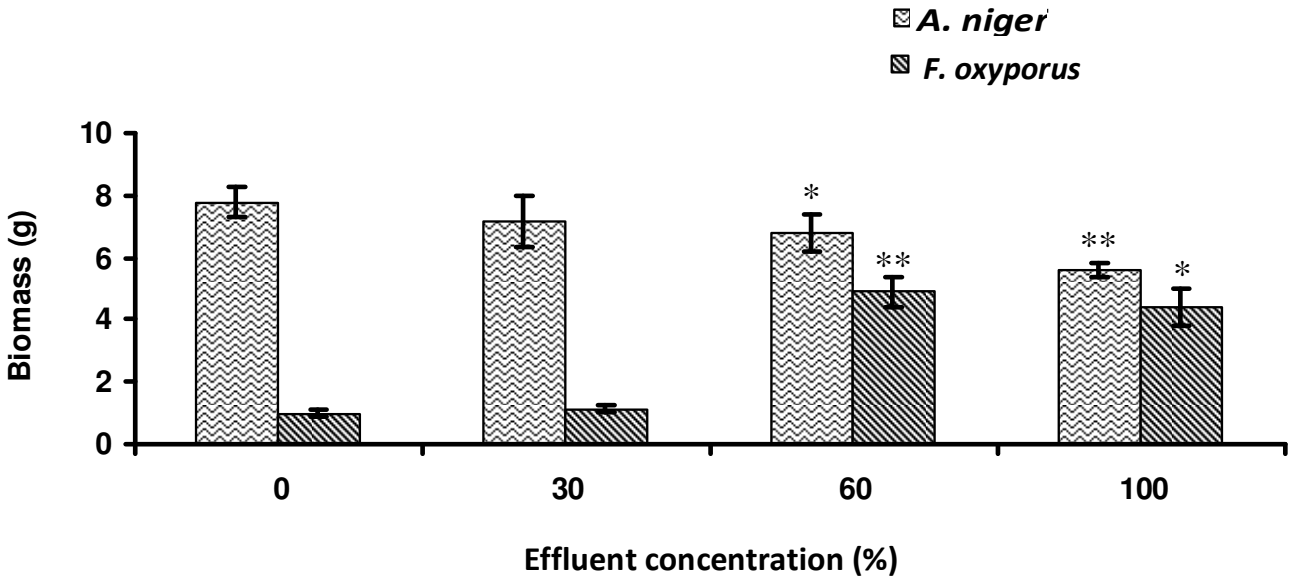

Figure 1. Biomass produced by *A. niger* and *F. oxysporum* in different concentrations of the tannery effluents for 10 days (* = significant effluent treatment at P = 0.01 level, ** = significant effluent treatment at P = 0.05 level).

conductivity, TDS and COD. The metal contents were also higher in the tanning effluent. Amount of metals was quite high in both types of effluents.

Growth and biomass production of *A. niger* and *F. oxysporum* in different concentrations of effluents

Both the fungal strains exhibited a good growth in different effluent concentrations but a difference in the growth pattern was observed. *A. niger* showed a reduction in biomass content with increasing concentration of effluents whereas *F. oxysporum* showed increase in growth with increasing effluent concentration. However, the maximum biomass content of *F. oxysporum* was observed at 60% effluent concentration in both types of effluents (Figures 1 and 2).

Metal content of fungal biomass

On analysis of fungal biomass, similar patterns of metal content were observed in both fungal strains. In all cases a significantly higher amount of metal was observed in 60 and 100% effluent concentrations (Figures 3 and 4). In all concentrations, the amount of metals absorbed from the

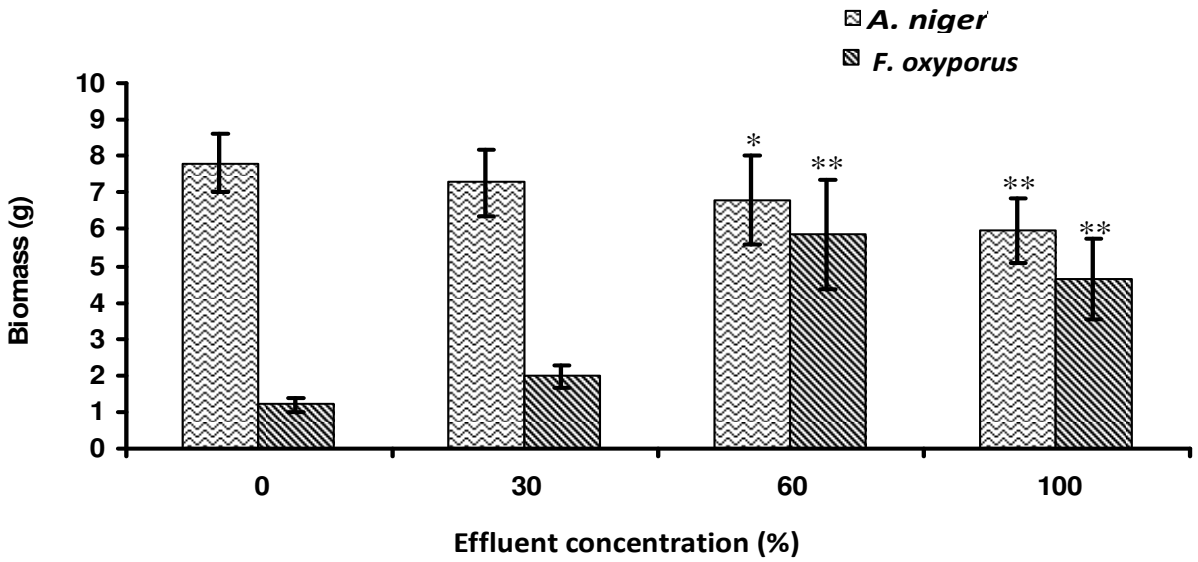

Figure 2. Biomass produced by *A. niger* and *F. oxysporum* in different concentrations of the textile effluents for 10 days (* = significant effluent treatment at P = 0.01 level, ** = significant effluent treatment at P = 0.05 level).

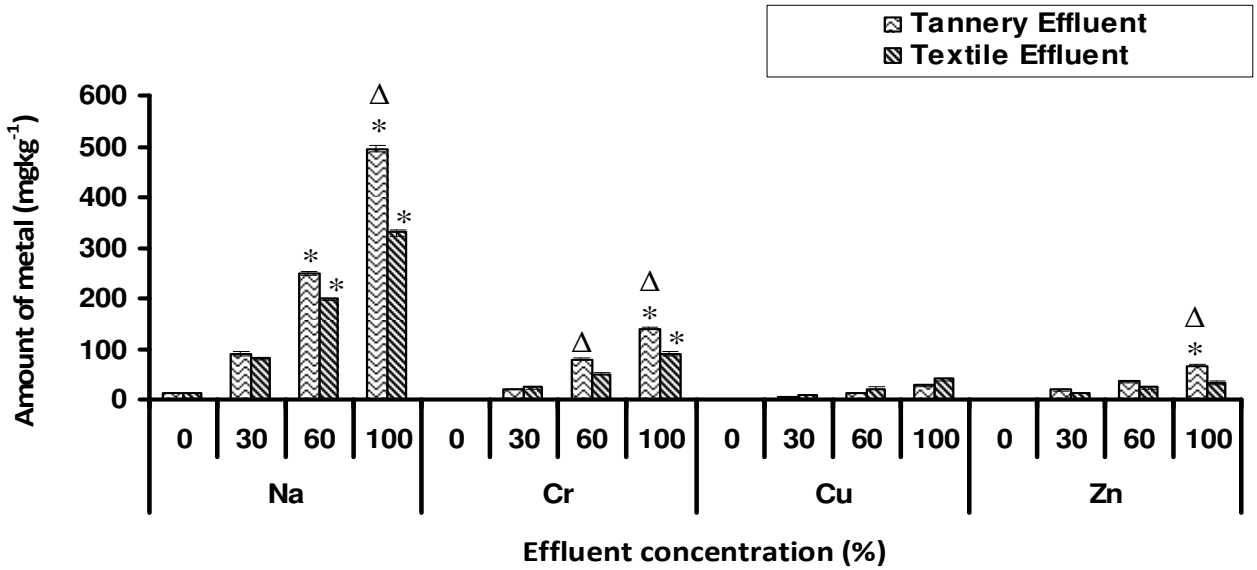

Figure 3. Metal content of *A. niger* grown in different concentrations of industrial effluents (* = significant effluent treatment at P = 0.05 level, Δ = significant metal uptake at P = 0.05 level).

tannery effluent was higher as compared to textile effluents. A higher Na and Cr uptake was observed as compared to Cu and Zn.

Reduction in various pollution parameters

Insignificant changes in pH and conductivity were observed. Some reduction in NaCl percentage was observed in the tannery effluents by both the fungi. A greater reduction in conductivity and NaCl percentage was observed by *A. niger* as compared to *F. oxysporum* (Figures 5 and 6). Similarly, a greater reduction in COD of the sample was observed by *A. niger* in both types of effluents (Figure 7).

Reduction in metal content of the effluents

In both types of effluents, *A. niger* caused a greater reduction in metal content of effluents as compared to *F.*

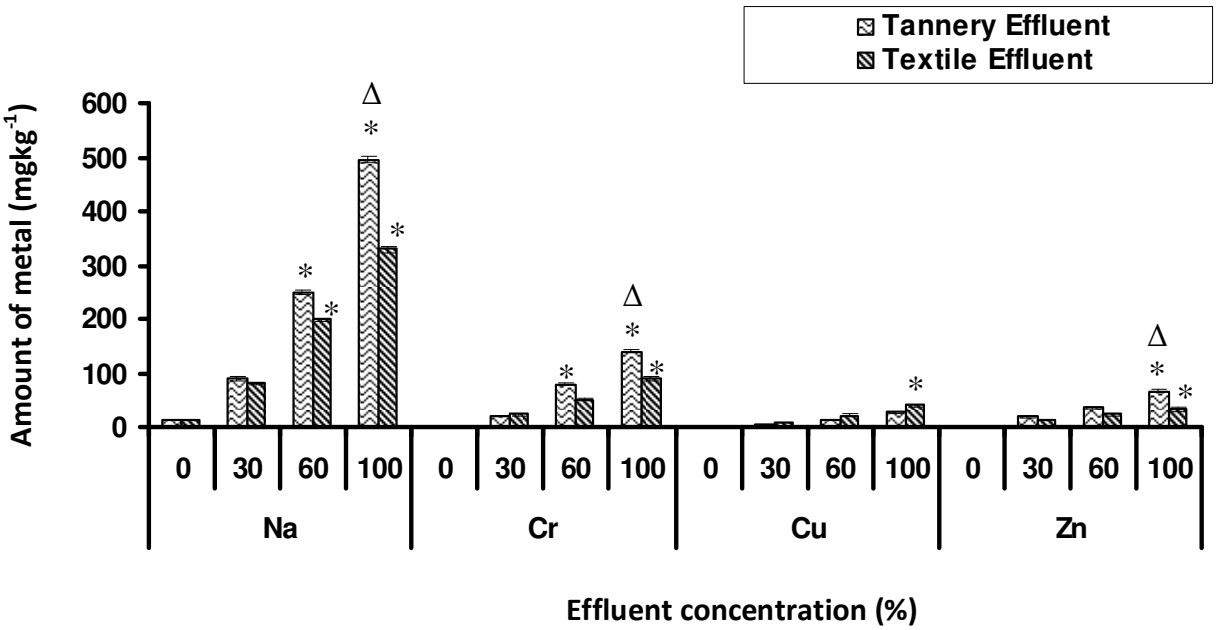

Figure 4. Metal content of *F. oxysporum* grown in different concentrations of industrial effluents (* = significant effluent treatment at P = 0.05 level, Δ = significant metal uptake at P = 0.05 level).

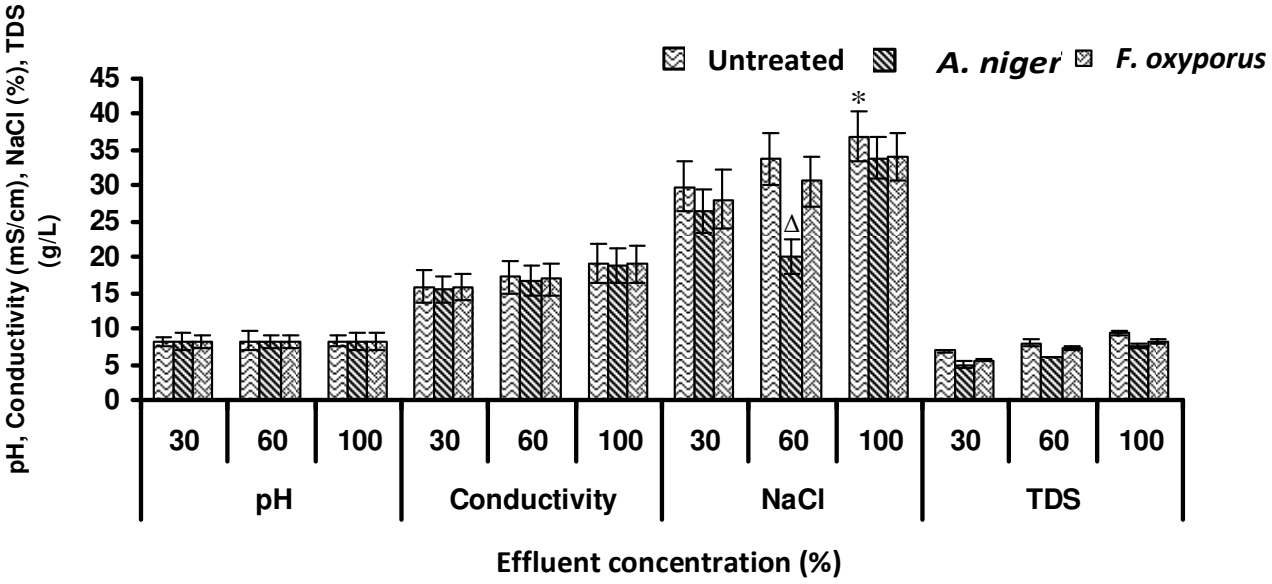

Figure 5. Changes observed in pH, conductivity NaCl percentage and total dissolved solids (TDS) after growing *A. niger* and *F. oxysporum* in tannery effluents for 10 days (* = significant effluent treatment at P = 0.05 level, Δ = significant fungal treatment at P = 0.05 level).

oxysporum (Figures 8 and 9). A significant reduction in case of all toxic metals was observed, the level of reduction being greater in raw effluent (100% effluent). In tannery effluents, in 100% concentration, *F. oxysporum* caused a reduction of greater than 40% of all toxic metals whereas *A. niger* indicated a selective behavior towards metals, causing greater reduction in Cr and Zn content (Figure 10). In the textile effluents too, *F. oxysporum* caused a reduction of greater than 40% in all metals except Zn whereas in all concentrations *A. niger* caused a greater reduction, more significantly in case of Cr and Cu (Figure 11). In general, percentage reduction in metal content increased with increasing concentration of effluents, and was the maximum in case of raw effluents

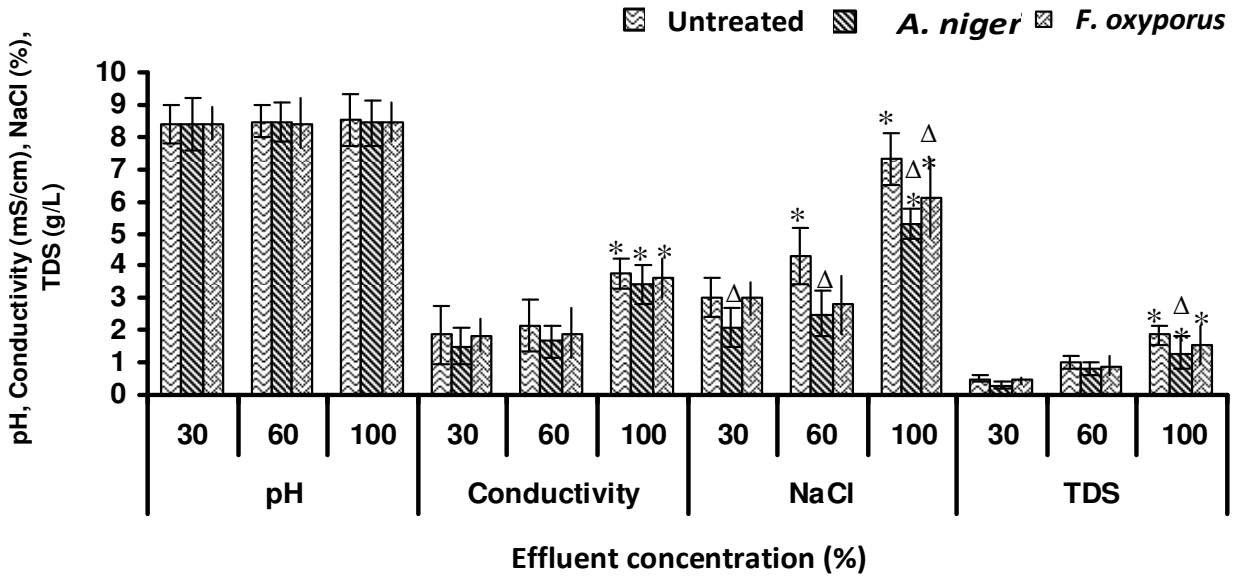

Figure 6. Changes observed in pH, conductivity NaCl percentage and total dissolved solids (TDS) after growing *A. niger* and *F. oxysporum* sp. in textile effluents for 10 days (* = significant effluent treatment at P = 0.05 level, Δ = significant fungal treatment at P = 0.05 level).

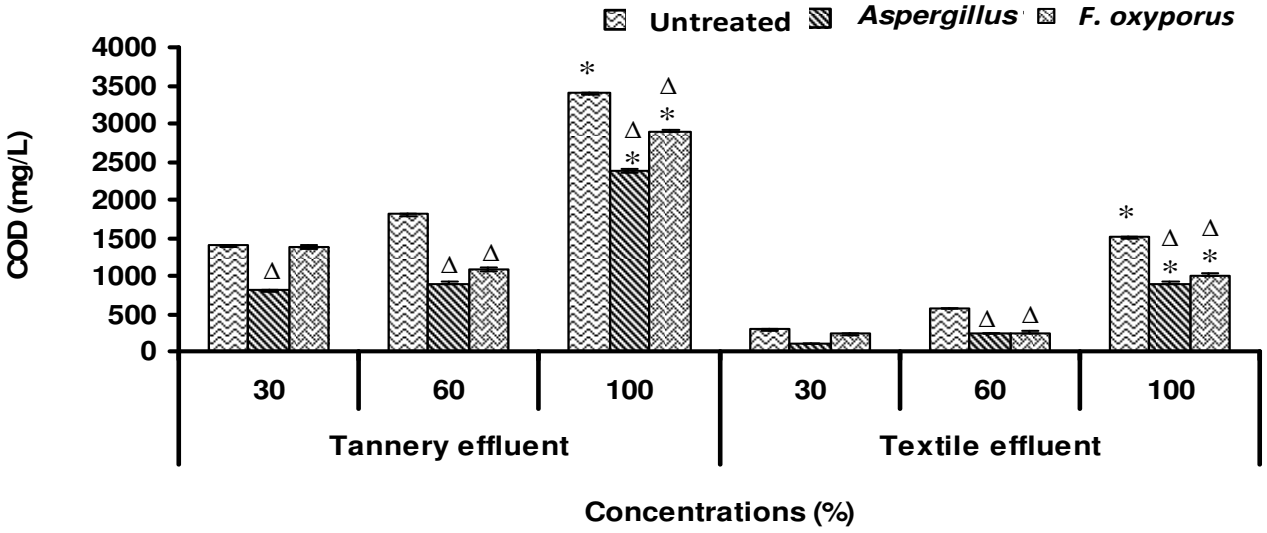

Figure 7. Changes observed in chemical oxygen demand (COD) of tannery and textile effluents after growing *A. niger* and *F. oxysporum* for 10 days (* = significant effluent treatment at P = 0.05 level, Δ = significant fungal treatment at P = 0.05 level).

in 100% concentration (Figures 10 and 11). In the raw effluents (100% concentration), a reduction of at least 50% was achieved for all metals by both the fungi.

DISCUSSION

Removal of heavy metals by biosorption techniques using fungi have been employed to remove metals such as Cd, Cu, Fe, Ni, Pb, Ra, Th and U from aqueous solutions (Ahluwalia and Goyal, 2007; Mungasavalli et al., 2007). Biosorption is considered to be the removal of substances from solution by inactive, dead biological materials, while bioaccumulation is described as intracellular pollutant accumulation (Gadd, 2009; Wang and Chen, 2009). Both living and dead fungal cells possess a remarkable ability for taking up toxic and precious metals from aqueous solutions (Kapoor et al., 1999; Fu and Viraraghavan, 2001; Wang and Chen, 2009). When the heavy metal uptake involves its passage into the cell

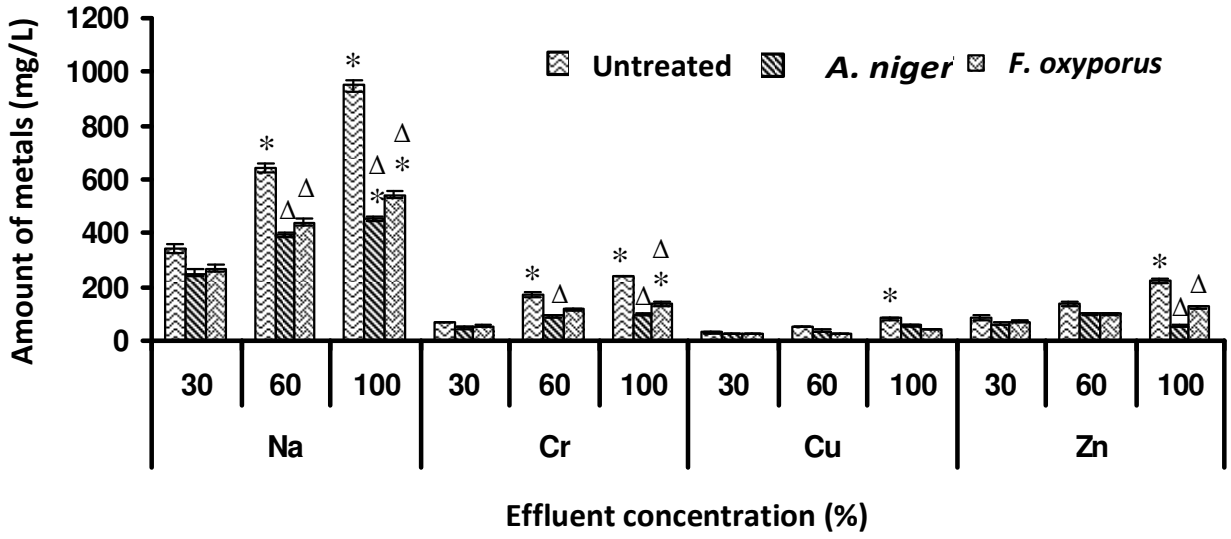

Figure 8. Amount of metals in tannery effluents after growing *A. niger* and *F. oxysporum* in different concentrations of tannery effluents for 10 days (* = significant effluent treatment at P = 0.05 level, Δ = significant fungal treatment at P = 0.05 level).

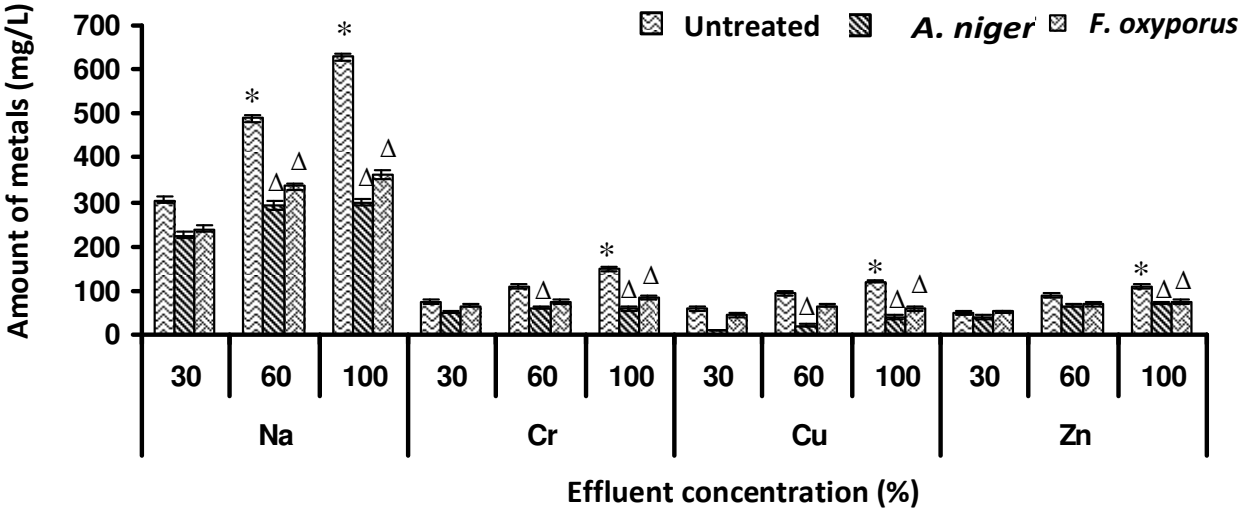

Figure 9. Amount of metals in tannery effluents after growing *A. niger* and *F. oxysporum* in different concentrations of textile effluents for 10 days (* = significant effluent treatment at P = 0.05 level, Δ = significant fungal treatment at P = 0.05 level).

across the cell membrane through the cell metabolic cycle, the mode of metal uptake is referred to as active uptake while metal uptake by both active and passive modes can be termed as bioaccumulation. In this study, live cultures of *A. niger* and *F. oxysporum* were used to remove heavy metals from actual tanning and textile effluents in different concentrations. In all treatments, a greater amount of metals was removed by *A. niger* as compared to *F. oxysporum.* The growing *Aspergillus versicolor* was shown to accumulate heavy metals and a dye, both singly and in combination with heavy metal or/and dye levels using molasses as a C and energy source in a batch process (Tasten et al., 2010). In this study, malt extract in 2% concentration was used as a C source for the fungi but any cheap source like by products of the sugar industry may also be utilized at mass level.

The cell surfaces of microorganisms are negatively charged owing to the presence of various anionic structures, which gives microorganisms the ability to bind

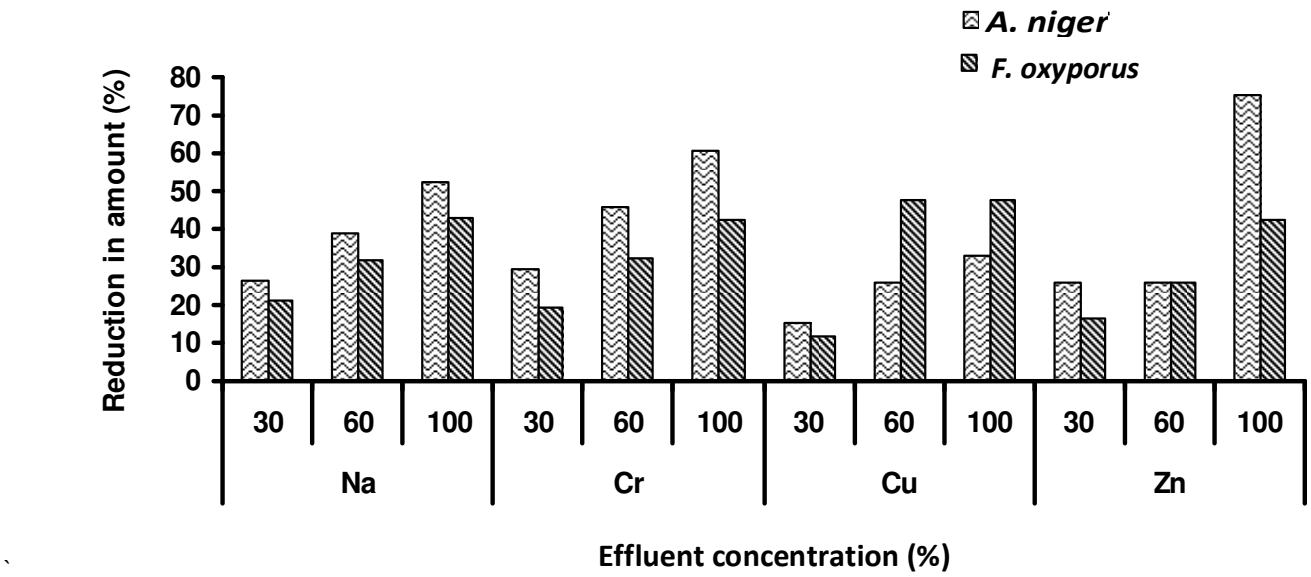

Figure 10. Percentage reduction of toxic metals observed in different concentrations of tannery effluents after growing *A. niger* and *F. oxysporum* for 10 days.

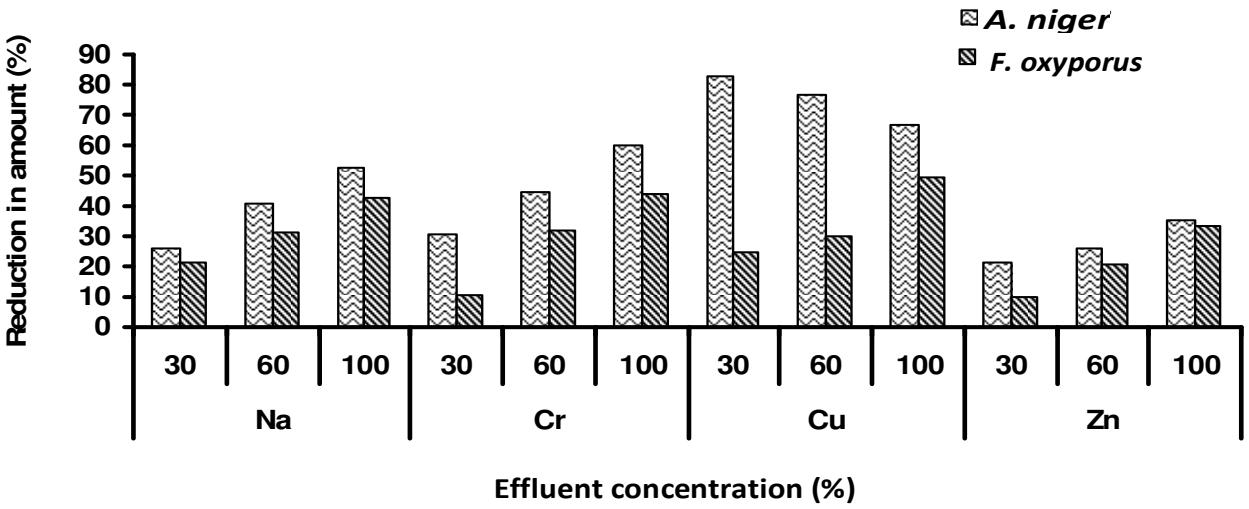

Figure 11. Percentage reduction of toxic metals observed in different concentrations of textile effluents after growing *A. niger* and *F. oxysporum* for 10 days.

metal cations (Chen and Hao, 1998). Electron microscopy and cell fractionation studies showed that 70 to 80% of the accumulated copper was associated with the cell wall. Binding of copper on the cell surface immobilized the metal making it less distributed (available) in the medium, thereby reducing its toxicity. This allowed the organism to further resume its normal growth (Anand et al., 2006). The fungi in this study exhibited a good growth in tannery and textile effluents probably due to the fact that the excess metal content was surface immobilized on their mycelium. Most of such studies have only been carried out in synthetic metal solutions and not real industrial effluents (Malik, 2004). One study has been performed in a real tanning effluent using the fungus *Fusarium solani* (Prigione et al., 2009). Actual tannery and textile effluents were used in this study because the impact of such work and implications in future research can only be foreseen if the technology has some feasibility at the actual contaminated site. Different concentrations of effluents were prepared to observe the tolerance level of both fungi. However, a good growth was observed in effluents without dilution.

The difficulties existing for biosorption application urge people to consider applying the hybrid technology which

comprises of various processes to treat real effluents. Consequently, application of living cells rather than dead cells for biosorption has gained attention again (Malik, 2004). Biosorption experiments require hardly 2 to 3 days for most part of the metal to be adsorbed and often a two day period is thought to be optimum for reaching equilibrium. In this study, a growth of 10 days was taken as standard because the culture conditions were optimized before starting the actual experiment. A 12 days period was found to be adequate for growth after which a stationary phase started to occur in fungal biomass. Thus, a period of 7 to 10 days is enough for the fungus to grow and absorb/immobilize multi-metal contaminated effluents.

Fungal strains have been isolated from tannery effluent and process parameters are optimized in presence of toxic form of Cr VI with biotechnological methods for its removal from tannery effluent and soil (Srivatava and Thakur, 2006). Live or dead fungal biomass can be used for the removal of toxic metal ions (Kapoor et al., 1999). The use of dead cells seems to be more advantageous than using live cells in metal ion removal (Gadd, 1993). The biosorption of Pb, Cd and Cu on pretreated *A. niger* was approximately 320, 260 and 350% times higher than the comparable biosorption by live biomass. However, live biomass was able to remove nickel more effectively than pretreated biomass, indicating that *A. niger* biomass may have taken up some nickel intracellularly (Kapoor et al., 1999). A reduction of at least 40% in metal content was achieved in raw effluents by *A. niger*. The reduction was above 70% in case of Zn. In raw effluents, the reduction in metal content was in the order of Zn>Cr>Na>Cu in case of tannery effluents, and in the order of Cu> Cr>Na>Zn in case of textile effluents. It seems to be due to the fact that the amount of Zn in tannery effluents was almost double that of textile effluents, whereas the amount of Cu was much greater in textile than in tannery effluents. Although, Na was in the greatest amount in both effluents, its reduction did not conform to its amount. There are reports of live microbial systems for the purpose of remediation of contaminated soils and waters (Kratochvil et al., 1998). Malik (2004) advised to use growing microbes as a feasible alternative to pure biosorptive removal of metal contaminants from complex Industrial effluents. Isolating fungi from polluted environments would provide the metal resistant strains appropriate for the bioremediation purpose (Zucconi et al., 2003; Malik, 2004). In a study of fungi isolated from the tanning effluent on EQ-Cr by Prigione et al. (2009), *F. solani* was selected for biosorption experiments since it was the only fungus able to grow *in vitro* into the tanning effluent, demonstrating a real adaptation to such polluted environment. They have given recommendation that for obvious applicative advantages, in the future it would be very ineresting to assess the Cr (III) active uptake capacity of this fungus. *A. versicolor* has been shown capable of accumulating heavy metals such as Cr (VI), Cu (II), and Ni (II) (Tasten et al., 2010).

A. versicolor had high tolerance to the heavy metals tested, especially to Cr (VI). But Cu (II) uptake was noticeably lower than the other two, such as 2.08 mg g^{-1} at 37.6 mg L^{-1} initial Cu (II) concentration (Tasten et al., 2010). In this study, *A. niger* and *F. oxysporum,* both showed a good affinity for all toxic metals in effluents without selection. *A. niger* showed a better metal removal potential than *F. oxysporum.* The amount of metal removed depended on the amount present in the untreated effluents.

Despite the current interest in microbial detoxification of effluents, relatively little work has been carried out with characterization of metal uptake by filamentous fungi, particularly when the heavy metals are present at different and low concentrations (Tsekova et al., 2010). Brierley (1990) indicated that microbial removal of cationic metal ions is most effective when metal concentrations range from less than 1 to about 20 mgL^{-1}. Gadd (1990) suggested that at high metal concentrations encountered in wastewaters, the metal uptake by the active mode does not contribute significantly to the total uptake by fungal microorganisms. Dursun et al. (2003) investigated Cu (II) and Cd (II) bioaccumulation properties of *A. niger* in an enrichment medium and observed no microbial growth or Cu (II) uptake over 100 mg L^{-1} initial Cu (II) concentration. Contradictory to earlier workers, this study indicated that the indigenous fungi were not only capable of tolerating 100% effluent concentration, but were also able to grow, show biomass increase and take up metals by both the active and passive modes.

Conclusion

This study indicates a practical application of indigenous fungi in bioreactors to treat industrial waste water, where such fungi can be allowed to grow in aerated effluents in the presence of any cheap C source. After toxic metal removal, the mycelium can easily be separated and processed for metal recycling or can be disposed off in a much smaller volume.

ACKNOWLEDGEMENT

This work is a part of a project no. 1032-08, sponsored by HEC (Higher Education Commission), Pakistan.

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