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Role of some micromycetes in biosorption of heavy metals in different polluted water samples

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In this study, heavy metal biosorption potential of indigenous micromycetes was determined. Water samples were collected from different waste water points, chemical industry and tanneries effluents. Indigenous mycoflora was isolated on 2% MEA medium and purified for biomass production. Selected water samples were exposed to UV radiation to kill microbial life in them. Isolated biomass was added to respective water sample for biosorption study. Residual metal concentration was analyzed after 15 and 30 days of fungal inoculation using ICO-ES (Inductively Coupled Optical- Emission Spectrophotometer). Results obtained show that the indigenous micromycetes are significantly efficient in heavy metal biosorption. Concentration of Cr, Cd, Pb and Co reduced to 90-100, >90, 95-100 and 90-100%, respectively. While the concentration of Na and K reduced to 59-90 and 54-83%, respectively, in all water samples. Our findings show that micromycetes present in polluted water are helpful in uptake of heavy metal and can be used for water pollution remediation.

Key words: Biosorption, heavy metals, industrial effluent, micromycetes.

INTRODUCTION

The discharge of heavy metals into aquatic ecosystems has become a matter of concern in Pakistan over the last few decades. These pollutants are introduced into the aquatic systems as a result of different industrial operations. The chemically polluted water has seriously damaged the ecology of surface and ground water; eventually having serious consequences on living organisms in polluted area. Tanning, electroplating, textile, mining and metallurgical waste are the most considerable sources of environmental pollution by heavy metals. In Pakistan, there are 670 textile units dis-charging their wastes into water bodies without waste water treatment. (Andleeb et al., 2010) Owing to severe water crisis problems in Pakistan, the notorious heavy metal pollution requires a serious and even solution.

In order to minimize the effects of environmental pollution, the methods of metal removal and recovery based on biological materials have been considered. This biological approach has the great potential that contributes to the achievement of this goal and is also economical. Microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant (Prasenjit and Sumathi, 2005). The response of microorganisms towards toxic heavy metals is of importance in view of the interest in the reclamation of polluted sites (Shankar et al., 2007). Microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Hussein et al., 2003). This method is based on the use of the metal binding capacities of various biological materials, including algae, fungi and bacteria (Volesky et al., 1995; Veglio and Beolchini, 1997).

Fungi possess very well known heavy metal sorption capacity (Kapoor and Viraraghavan, 1995; Zhou, 1999). It has been demonstrated that some fungal species are typically associated with heavy metal rich substrata and can be even considered as hyper accumulators of heavy metals (Purvis and Halls, 1996).

The majority of fungi show filamentous or hyphal growth. Cell walls of fungi present a multi-laminate archi-

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tecture where up to 90% of their dry mass consists of amino or non-amino polysaccharides. The fungal cell walls can beconsidered as a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix (Yan and Viraraghavan, 2000). The cell walls are rich in polysaccharides and glycoprotein's such as glycans (1-6 and 1-3 linked D-glucose residues), chitin (1-4 linked N-acetyl-D-glucosamine), chitosan (1-4 linked D-glucosamine), mannans (1-4 linked mannose) and hosphormannans (phosphorylated mannans). Various metal binding groups, viz amine, imidazole, phosphate, sulphate, sulfhydryl and hydroxyl are present in the polymers of the call wall (Crist et al., 1981).

Both active and heat killed biomass of many filamentous fungi (*Aspergillus, Penicillium, Rhizopus* and *Mucor*) may be utilized in boisorption process (Gadd, 1990). It is expected that screening of metal tolerant fungi may provide efficient strains for metal biosorption. However, only limited studies have been conducted to screen the filamentous fungi of metal polluted soil and other habitat for their metal tolerance and biosorption potential (Hayat et al., 2002; Bai and Abraham, 2001; Rama Rao et al., 1997; Córdova et al., 2011). This trial is an effort to isolate the filamentous fungi from polluted water and use them for determination of biosorption potential.

MATERIALS AND METHODS

Polluted water samples and industrial effluents were collected from different localities of Lahore and Kasur. The collected water samples were transferred into a labeled, sterile plastic container and brought immediately to the laboratory and maintained at 4°C for further studies. Water samples were analyzed for pH, EC, CO_3^{2-}, HCO_3^-, Cl^-, BOD, COD for determination of pollution extent. Indigenous micromycetes were isolated on 2% MEA medium and purified for mass culture production. These isolated entities were identified morphologically. Analysis for heavy metals, that is, Na, K, Cr, Cd, Ni, Pb and Co was done by Inductively Coupled Optical-Emmision Spectrophotometer. Water samples were irradiated with UV light for 10 days to eliminate all microbes. Cultural mass of isolated fungal species was inoculated to these UV irradiated water samples to determine their biosorptional activity after 15 and 30 days of fungal inoculation. Following the analysis of variance (ANNOVA), means were used to find the difference. Duncans multiple range test was also used (Steel and Torie, 1980)

RESULTS AND DISCUSSION

Isolation of fungi

Fifty seven (57) species of different micromycetes were isolated from collected water samples. These belong to 17 different genera *(Aspergillus, Penicillium, Rhizopus, Paecilomyces, Curvularia, Eurotium, Fusarium, Thozetollopsis, Cephalosporium, Goidanichiella, Alternaria, Blastomyces, Trichoderma, Bacteriodiopsis, Verticillium, Strachybotrys, Chaetomium* and *Chaetosartorya)* (Table 1).

Preliminary pollution assessment

EC, CO3, HCO3, Cl, BOD and COD of collected water samples showed high contamination. Values of BOD and COD were 552 and 2969 mg/L, respectively, in tanneries effluent. These values were higher than the standard values (80 mg/L for BOD and 150 mg/L for COD) given by Government of Pakistan (1993). Similarly, values of EC were also higher in all water samples than the standard NEQ (National Environmental Quality Standards) guide line value, 1.3 ms/cm (Table 2). Temperature, pH, biomass, heavy metal concentrations are the factors that affect the biosorption process, particularly, pH (Gourdon et al., 1990) and biomass concentration (Gong et al., 2005).

Concentration of Cr and its subsequent absorption

Biosorption of Cr ranged from 94to 100% in all water samples. In tanneries effluent it was 34.008 mg/L and in chemical industrial effluent, it was 4.93 mg/L. In other water samples, it remained below permissible limit. There was 100% reduction in concentration of Cr in tanneries effluent after 30 days of fungal inoculation. In sewage water, 94% biosorption took place within 30 days of fungal inoculation (Figure 1). These results are in agreement with the finding of Mala et al. (2006) and Srivastava and Thakur (2006), who used *Aspergillus niger* for Cr biosorption and find 79-83% and 75% removal respectively in 3-4 days. In our experiment, time duration is more as compared to the above mentioned finding. It seems that more contact time with more biomass favors efficient biosorption. Ozer and Ozer (2003) studied the biosorption of Pb(II), Ni(II) and Cr(VI) ions using inactive *Saccharomyces cerevisiae* and found 270.3, 46.3 mg/g and 32.6 mg/g uptake, respectively, at 25°C. It is generally assumed that microorganisms accumulated metals on the cell wall by complexation and/or ion exchange reactions between metal ions and the charged chemical constituents of cell walls (Gupta et al., 2000). The cell surface functional groups of the fungus might act as ligands for metal sequestration (Pal et al., 2010). Amines, phosphates and carboxylic group hava a major role in Cr absorption (Narvekar and Vaidya, 2009).

Concentration of Cd and its subsequent absorption

Analytical results reveal that the average concentration of cadmium in effluents of tanneries and chemical industry was 0.009 and 0.018 mg/L, respectively, while it

Table 1. List of fungal species isolated from different polluted water.

Canal water	Pond water	Sewage water	Chemical effluent	Tanneries effluent
Aspergillus alliaceous	*Aspergillus niger*	*Alternaria tunnis*	*Alternaria porri*	*Aspergillus ochraceus*
A.kanagawaaensis	*Aspergillus* sp. C	*Aspergillus flavus*	*Aspergillus niger*	*A. sydowii*
A. niger	*Cephalosporium* sp.	*Blastomyces* sp.	*A. oryzae*	*Aspergillus* sp. D
Aspergillus sp. B	*Curvularia* lunata	*Curvularia lunata*	*Bacteridiopsis* sp.	*Chaetomium murorum*
Penicillium sp. A	*Fusarium* sp.	*Fusarium solani*	*Chaetomium* sp.	*C. botrychodes*
Penicillium sp. B	*Goidanichiella scopula*		*Curvularia lunata*	*Chaetomium cremea*
Species.1	*Paecilomyces variota*		*Strachybotrys cylindrospora*	*Curvularia lunnata*
Species. 2	*Penicillium* sp. C		*Thysanophora penicillioides*	Species 11
	Penicillium sp. D		*Trichoderma viride*	Species 12
	Penicillium sp. E		*Verticillium* sp.	Species 13
	Penicillium sp. F		Species 5	
	Rhizopus stolonifer		Species 6	
	Thozetolopsis toklaiensis		Species 7	
	Species 3		Species 8	
	Species 4		Species 9	
			Species 10	

Table 2. Values of different pollution parameters in polluted water samples.

Factor	Control water	Canal water	Pond water	Sewage water	Chemical effluent	Tanneries effluent
pH	8.31	6.71	7.61	5.47	3.37	8.75
EC (ms)	0.09	0.16	0.462	9.65	12.68	21.22
CO3 (m.eq/L)	0.192	0.00	0.34	0.00	2.156	2.12
HCO3(m.eq/L)	1.76	5.48	3.36	9.78	5.68	4.33
Cl (m.eq/L)	0.9	9.75	7.25	7.5	35.616	33.48
BOD (mg/L)	0.00	4.2	4.97	239.2	439.8	552.2
COD (mg/L)	0.00	268.7	271.2	954.6	2234	2969

remained below the standard permissible limits in all remaining samples (WHO, 1989). After 30 days of fungal inoculation, no Cd was detected in tanneries effluent indicating 100% biosorption (Figure 2). *Aspergillus niger* was successfully used for Cd biosorption by Barros et al. (2003). The maximum uptake capacity estimated by Bashar (2003) was close to 35 and 40 mg Cd/g DW cell for *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* yeast cells, respectively at a pH value of 5.0**.** Similarly *Rhizopus* sp. accumulated 4.33 mg Cr/g and 2.72 mg Cd/g (Zafar et al., 2007). Difference in absorbance of heavy metal might be due to the variation in binding sites available for heavy metal to bind or also it might be due to the difference in surface area of biosorbant material.

Microbes that survived on cadmium pollution could have developed a cadmium-resistant mechanism to tolerate cadmium (Abou-Shanab et al., 2007). Strains isolated from heavy metal polluted sites generally possess resistant to metals (Patra et al., 2004). *Aspergillus, Penicillium*, *Alternaria*, *Geotrichum*, *Fusarium*, *Rhizopus*, *Monilia* and *Trichoderma* were found resistance to cadmium (Zafar et al., 2007). In another study, *P. chrysosporium* was used to biosorp cadmium (II), lead (II), copper (II) and the adsorption capacities which reached 23.04, 69.77 and 20.33 mg/g dry biomass, respectively (Say et al., 2001). Our findings are in accordance with that of earlier works. Heavy metals are attached to different functional groups (amines, carboxlic acid, phosphates, etc) present in cell wall of fungal species. The roles played by amines, carboxylic acids, phosphates, sulfhydryl group and lipids in lead biosorption has been studied by Parvati et al (2006). They concluded that electrostatic attraction may be the mechanism of biosorption.

Concentration of Pb and its subsequent absorption

Effluent of tannery and chemical industry were found to contain the average concentration of Pb, 0.126 and 6.109 mg/L, respectively. In canal and sewage water, its concentration was 0.504 and 0.175 mg/L, respectively.

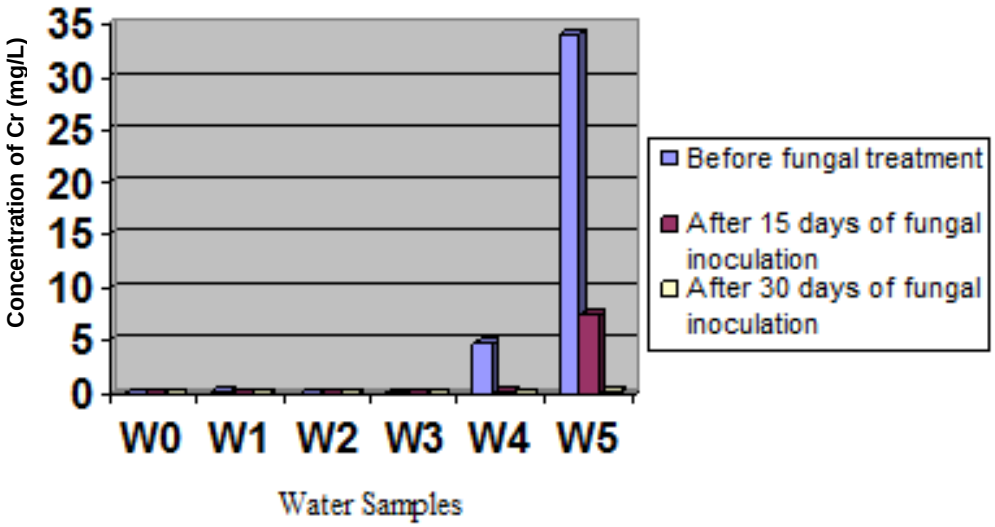

Figure 1. Effect of isolated fungi after 15 and 30 days of fungal inoculation on chromium concentration. W0 = Control water; W1 = Canal water; W2 = Pond water; W3 = Sewage water; W4 = Chemical industrial effluent; W5 = Tannery effluent.

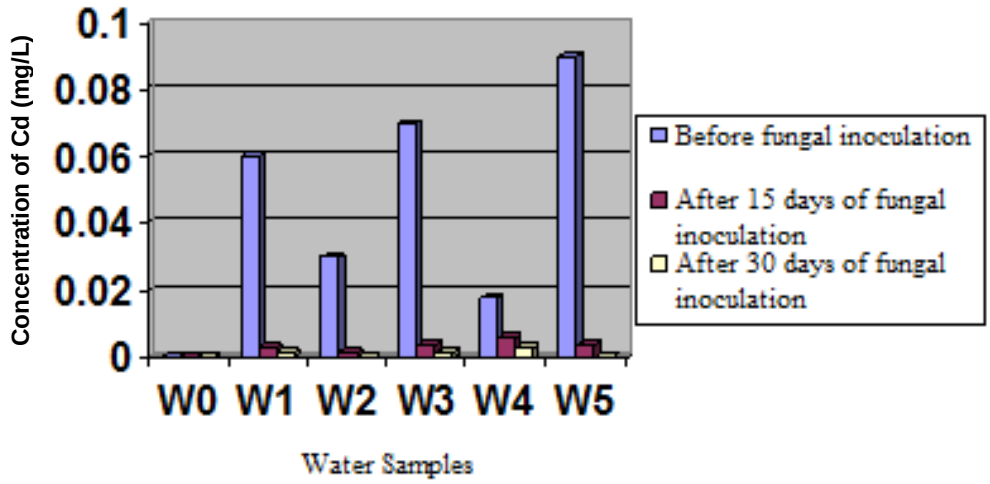

Figure 2. Effect of isolated fungi after 15 and 30 days of fungal inoculation on Cd concentration. W0 = Control water; W1 = canal water; W2 = pond water; W3 = sewage water; W4 = chemical industrial effluent; W5 = tannery effluent.

Biosorption of Pb in our findings ranged from 96-100% in all water samples after 30 days of fungal inoculation (Figure 3). According to Haluk and Ulki (2001), Ni(II) and Pb(II) uptake capacities of a lignolytic white-rot fungus, *Phanerochaete chrysosporium* were 55.9 mg Ni/g and 53.6 mg Pb/g of biomass, respectively. *Aspergillus fumigatus* was found to be suitable biosorbent for Pb ions, especially when the metal content in the aqueous solution was in the concentration of 100 mg/L. Limin et al. (2009) studied the mechanism of Pb biosorption by *S. cerevisiae* by SEM-EDX, FTIR and XPS methods. They find that cell wall play an important role in adsorption due to many spots on it. They concluded by FTIR analysis that COOH, C=O, C-O and N-H are the main active binding sites for the adsorption of Pb and Pb combines with functional group containing C, N, O and P. They concluded that it mainly resulted from ion exchange and surface complexation.

Concentration of Ni and its subsequent absorption by filamentous fungi

The value of Ni was far below the standard permissible

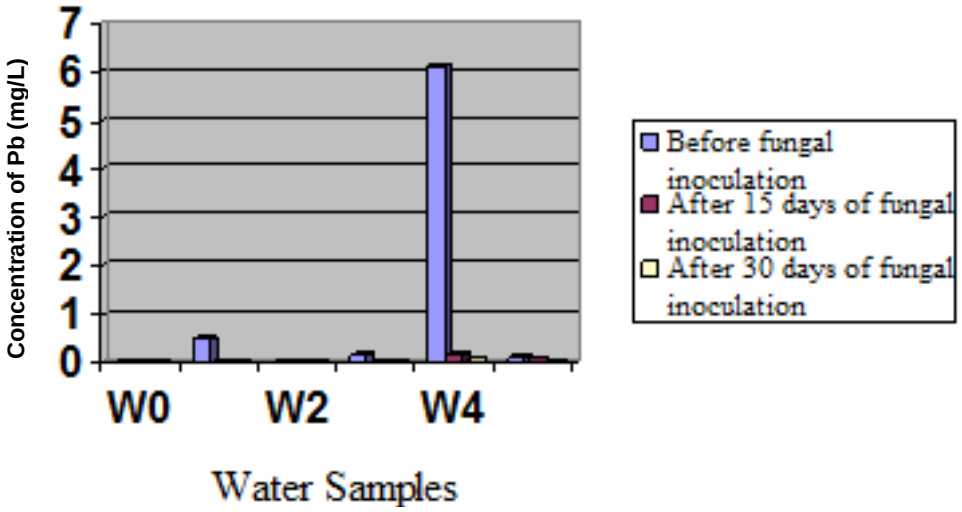

Figure 3. Effect of isolated fungi after 15 and 30 days of fungal inoculation on Pb concentration. W0 = Control water; W1 = canal water; W2 = pond water; W3 = sewage water; W4 = chemical industrial effluent; W5 = tannery effluent.

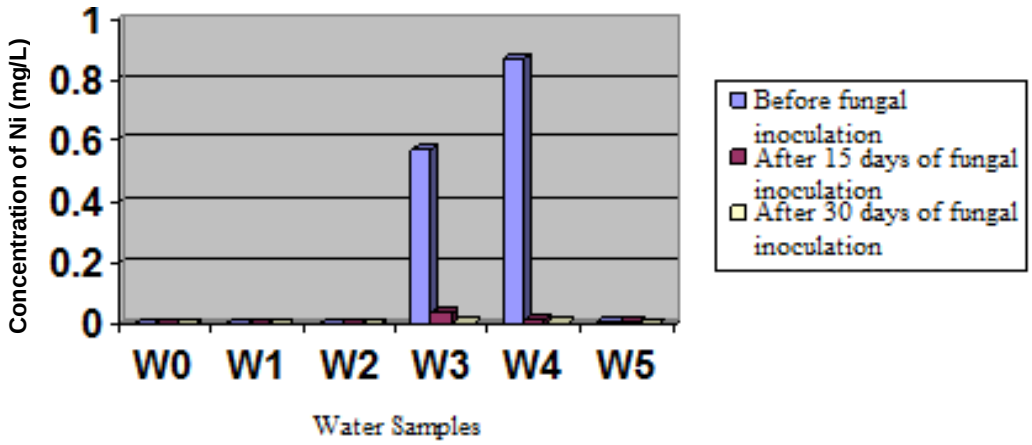

Figure 4. Effect of isolated fungi after 15 and 30 days of fungal inoculation on Ni concentration. W0 = Control water; W1 = canal water; W2 = pond water; W3 = sewage water; W4 = chemical industrial effluent; W5 = tannery effluent.

value except in sewage and chemical industrial effluent. In sewage water, 99% biosorption took place after 30 days of fungal inoculation. In chemical industrial effluent concentration of Ni was 0.007 mg/L after 91% biosorption by fungi after 30 days of fungal inoculation (Figure 4).

Nickel (Ni) uptake capability from aqueous solutions has also been studied by Mogollon et al. (1998) in a filamentous fungal strains group of *Rhizopus* sp., *Penicillium* sp. *Aspergillus* sp., *Trichoderma* sp., *Byschoclamyss* sp. and *Mucor* sp. The metal uptake of a *Rhizopus* sp. strain, which has the highest uptake capacity, was corroborated by electron microscopy.

Fungal mycelium pellets of *A. niger* 405 were used for adsorption of different metal ions, that is, Cu^{2+}, Zn^{2+}, Ni^{2+} and total Cr by Filipović-Kovačević (2000). Fungus *A. niger* 405 showed a good affinity for binding of Cu^{2+}, Zn^{2+} and Ni^{2+} ions. It was probably due to the absence of competition processes between metals and biomass. Their interaction takes place at active sites on biomass cell wall where solid and liquid phase equilibrium occurs.

Concentration of Co and its subsequent absorption

Analytical results revealed that the average concentration of cobalt in effluents of chemical industry, tannery industry and sewage water were 0.054, 0.006 and 0.006 mg/L, respectively. After 30 days of fungal inoculation Co

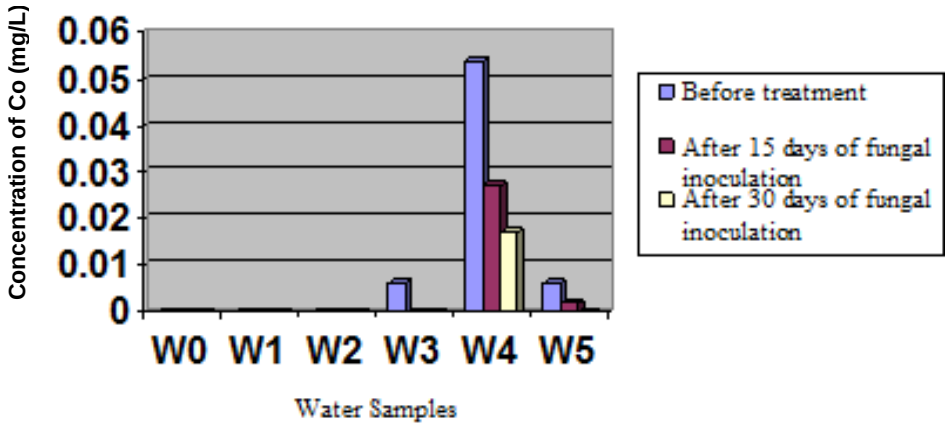

Figure 5. Effect of isolated fungi after 15 and 30 days of fungal inoculation on Co concentration. W0 = Control water; W1 = canal water; W2 = pond water; W3 = sewage water; W4 = chemical industrial effluent; W5 = tannery effluent.

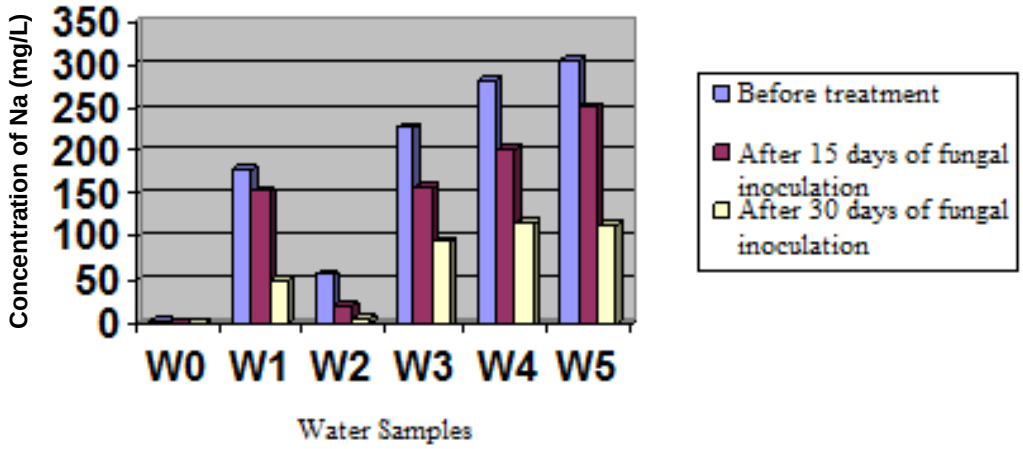

Figure 6. Effect of isolated fungi after 15 and 30 days of fungal inoculation on Na concentration. W0 = Control water; W1 = canal water; W2 = pond water; W3 = sewage water; W4 = chemical industrial effluent; W5 = tannery effluent.

was not detected in any water sample except in chemical industrial effluent where concentration of Co was 0.017 mg/L after 30 days of fungal treatment (Figure 5).

Pal (2006) isolated the fungi belonging to *Aspergillus, Mortierella, Paecilomyces, Penicillium, Pythium, Rhizopus* and *Trichoderma,* from serpentine soil of Andaman (India) for cobalt-resistance. Eleven out of total 38 isolated fungi which tolerated >6.0 mM Co(II) were evaluated for cobalt biosorption using dried mycelial biomass. Maximum Co(II)-loading (1036.5 microM/g, 60 min) was achieved with *Mortierella* SPS 403 biomass, which removed almost 50% of 4.0 mM cobalt from the aqueous solution. Co(II)-sorption kinetics of *Mortierella* SPS 403 biomass was fast and appreciable quantities of metal 562.5 microM/g was adsorbed during first 10 min of incubation. The metal biosorption capacity of the isolate was accelerated with increasing cobalt concentration. According to Volesky and Kuyucak (1989) Co biosorption take place mainly due to ion exchange method and COOH group, a functional group in cell wall, which play an important role in cobalt binding.

Concentration of Na and its subsequent absorption by filamentous fungi

Higher concentration (307.5 m.eq/L) of Na ions was detected in tanneries effluent and relatively low amount (56.3 m.eq/L) in Pond water sample. Maximum biosorption (90%) occurred in pond water sample. Reduction in Na ions concentration in other water samples range from 59-73% (Figure 6). Na uptake by fungi was also reported

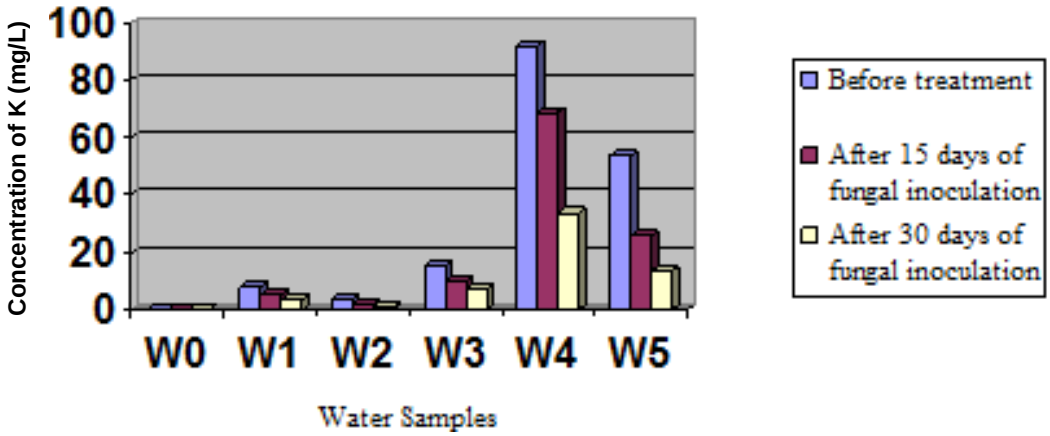

Figure 7. Effect of isolated fungi after 15 and 30 days of fungal inoculation on K concentration. W0 = Control water; W1 = canal water; W2 = pond water; W3 = sewage water; W4 = chemical industrial effluent; W5 = tannery effluent.

by Benito *et al* (2004). They investigated that fungi have an absolute requirement for K and Na^+. Na^+ uptake in *Ustilago maydis* and *Pichia sorbitophila* was found to exhibit a fast rate, low K_m, and apparent independence of the membrane potential. Further studies on *Saccharomyces cerevisiae and U. maydis* revealed that the *acu1* and *acu2* genes encode transporters that mediate high-affinity K^+ uptake in addition to Na^+ uptake. The fungal high-affinity Na^+ uptake mediated by ACU ATPases is functionally identical to the uptake that is mediated by some plant HKT transporters. Our results are in agreement with these findings. High biosorption of sodium and potassium is might be due to the synergic effect of fungal species as each water sample was inoculated by all indigenous species isolated from that sample.

Concentration of K and its subsequent absorption by filamentous fungi

Higher amount (92.26 m.eq/L) was found in Itehad Chemical Industrial effluent and very low amount (3.22 m.eq/L) in pond water. Concentration of K in tanneries effluent was 53.5 m.eq/L. Fungal mass absorbed 40 m.eq/L potassium from chemical industrial effluent and 59 m.eq/L from tannery effluent. Reduction in concentration of potassium ranged from 54 to 83% in all water samples (Figure 7). This removal of K ion from solution is just like that of Na removal. Fungus has high affinity for K as well as for Na. K uptake is also ACU ATPases mediated phenomenon (Benito et al., 2004).

Conclusion

This study shows that fungi can efficiently remove heavy metals from industrial effluents. This study also emphasizes the importance and need for carrying out extended test for the compatibility of biosorption to a specific industrial effluent with specific fugal entity. As regards the cost of fungal species, it can be obtained cheaper or at low cost from the respective industrial effluent. The findings of the study indicate that biosorption is a promising technology for removal of heavy metal especially chromium and cadmium from effluent. However, further studies with respect to metal-biosorbent specificity, applicability to other various types of metal-laden effluents and large scale studies will help fine-tune the biosorption technology for large-scale application.

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