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RESEARCH ARTICLE

TOLERANCE AND BIOSORPTION STUDIES ON HEAVY METAL BY FILAMENTOUS FUNGI FROM BATTERY EFFLUENT SOIL

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ABSTRACT

In the present investigation that the isolation of fungi from battery effluent soil. The maximum fifteen fungal isolates were screened and identified. These fungi were heavy metal tolerance and biosorption potential also highly performed. Growth of the fungi were tested using various concentrations of 100, 200, 300, 400 and 500 mg/ml of $MgSO_4$ and $CuSO_4$ heavy metal treated by potato dextrose liquid medium. In all the fungi were tolerate and well growth up to 300 mg/ml except the higher concentration of 400 and 500 mg/ml. Biosorption efficiency was depending upon the growth and incubation. *A.niger* showed highest biosorption potential for magnesium sulphate and copper sulphate when tolerance compared to the other fungi.

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INTRODUCTION

Human activities such as industrial production, mining, agriculture and transportation, release high amount of heavy metals from surface and ground water and soils. Accumulation of heavy metals in crop plants of great concern due to the probability of food contamination through the soil root interface. Though the heavy metal like, Cd, Pb and Ni are not essential for plant growth, they are readily taken up and accumulated by plants in toxic forms. Ingestion of vegetables irrigated with wastewater and grown in soils contaminated with heavy metals possesses a possible risk to human health and wildlife (Jan *et al*., 2011). Microorganisms like bacteria, fungi, algae and yeast are known to tolerate and accumulate heavy metals. Biosorption mechanism involved in the process may include ion exchange, co-ordination, complication, chelating, adsorption, micro precipitation, diffusion through cell walls and membrane, which differ depending on the fungi used, the origin and processing of the biomass and solution chemistry (Guibal *et al*., 1992; Churchill *et al*., 1995).

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Some heavy metals are essential for the fungal metabolism. However, essential and non-essential heavy metals are toxic for fungi. Whereas fungi have metabolic requirements for trace metals, the same metals are often, toxic at higher concentration only a few times greater than these requires (Hughes and Poole, 1991). The metals necessary for fungal growth include copper, iron, manganese, molybdenum, zinc and nickel. Non-essential metals commonly encounters include chromium, cadmium, lead, mercury and silver. Potential of filamentous fungi in bioremediation of heavy metal containing industrial effluents and wastewaters has been increasingly reported from different parts of the world (Gadd, 1993).

MATERIALS AND METHODS

Isolation of fungi from battery effluent soil samples

The battery effluent soil samples were collected from Avoor, Trichy, India. Potato Dextrose Agar (PDA) was used for culturing fungi from industrial effluent. 100 µl of the effluent was spread on the PDA plate. The inoculated plates were incubated at 27˚C for 5 days. The fungi were identified using Lactophenol cotton blue (Barnett and Hunter 1999).

Screening of fungal growth

The heavy metal tolerance of isolated fungi was evaluated at various concentrations of (100, 200, 300, 400 and 500 mg/ml) $CuSO_4$ and $MgSO_4$ supplemented in PDA and were poured in the petriplate. After the solidification, the fungi with 6 mm disc was inoculated and incubated at 27˚C for 5 days. The culture in PDA without heavy metal served as control. Reduction of radial growth rate was used as an index for metal tolerance. To compare the heavy metal tolerance of each species, a parallel Index of Tolerance (TI) were calculated as a percentage value from the ratio

$$\text{Radial growth} = \frac{\text{Radial growth rate in metal treatment}}{\text{Radial growth rate in control}}$$

The isolates exhibiting better growth after incubation were considered as heavy metal tolerant (Tahir, 2012).

Determination of selected fungal growth at different concentrations of heavy metals (Anand, *et al.*, 2006)

The heavy metal tolerance fungi such as *Aspergillus fumigates, A. flavus A. niger, Fusarium sp, Penicillium sp* and *Trichoderma sp* was evaluated at various concentrations of $CuSO_4$ and $MgSO_4$ (100, 200, 300, 400 and 500 mg/ml) individually supplemented with PD broth and 6 mm disc of each fungal culture was inoculated to Potato Dextrose (PD) broth and incubated at 27˚C for 5 days. Bioadsorption capacity, *i.e.* amount of metal ions (mg) bio adsorbed per gm (dry mass) of biomass was calculated using the following equation:

$$Q = (Ci - Cf\ m)/V$$

where Q = mg of metal ion bio adsorbed per gm of biomass, Ci = initial metal ion concentration mg/L, Cf = final metal ion concentration mg/L, m = mass of biomass in the reaction mixture gm, V = volume of the reaction mixture (Ahmad , *et al.*, 2005).

RESULTS AND DISCUSSION

Shazia *et al.,* (2013) have isolated 19 fungi from heavy metal contaminated soils. Similar researchers are *Aspergillus* sp. were frequently (12 out of 19) encountered than other fungi. *Aspergillus* sp. were observed to be the most commonly occurring in the heavy metal contaminated soils as also reported by Ahmad *et al.* (2005) and Zafar *et al.* (2007).

In the present investigation, a totally 15 fungi were isolated from the Battery effluent contaminated soil such as *Aletrnaria alternate, Aspergillus flavus, A.fumigatus, A.niger, A.versicolor, Cladosporium* sp, *Curvularia lunata, F.oxysporium, F.solani, Fusarium sp, Penicillium* sp, *Rhizpus oryzae, Rhizopus* sp, *Trichoderma harizanum*, *T. viride* and *Verticillium* sp were observed (Table 1). The selected fungi were observed at different concentration of 100, 200, 300, 400 and 500 mg/ml in $MgSO_4$ and $CuSO_4$ heavy metal individually in PD broth.

Table 1. Identification of fungal strains

S.No	Name of the fungi
1	*Alternaria alternaria*
2	*Aspergillus flavus*
3	*A.fumigatus*
4	*A.niger*
5	*A.versicolor*
6	*Cladosporium* sp
7	*Curvularia luata*
8	*F.oxysporum*
9	*F. solani*
10	*Fusarium* sp
11	*Penicillium* sp
12	*Rhisopus* sp
13	*R.oryzae*
14	*Trichoderma harzarium*
15	*T.viride*
16	*Verticillium* sp

From the analysis, $MgSO_4$ in different concentrations found in all selected fungi while $CuSO_4$ to measure only *A.niger* and *Fusarium* sp (Fig 1 and 2). Several report of *Aspergillus* sp have been used for heavy metal ion adsorption from *A. niger* (Srivastava, Thakur, 2006), *A. fumigatus* (Al-Garni, *et al.*, 2009), *A. niveus* (Karaca, *et al.*, 2010), *A. versicolor* (Cabuk, *et al.*, 2005), *A. flavus* (Akar, and Tunali, 2006), *A. terreus* (Sun, *et al.*, 2010) and *A. cristatus* (Hassan, and El-Kassas, 2012) etc. Sen and Ghosh Dastidar (2005) have also studied *Aspergillus* sp. from industrial wastewater for hexavalent chromium removal. Shazia *et al.*, (2013) also reported that *Aspergillus* isolates were the most resistant to all the metals tested, namely cadmium, copper and nickel.

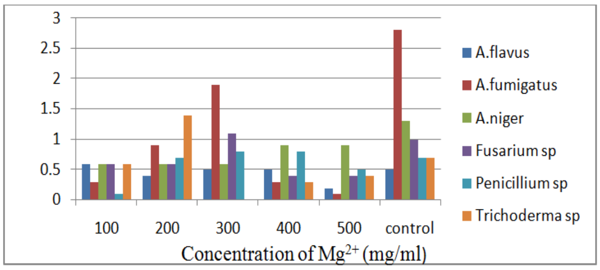

Fig. 1. Fresh biomass of Mg^{2+} at different concentration in liquid medium

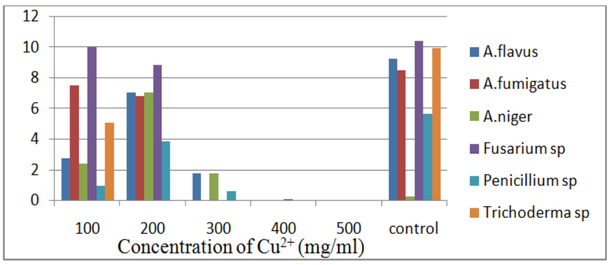

Fig 2 Fresh biomass of Cu^{2+} at different concentration in liquid medium

Siddiquee *et al.*, (2013) report *T.harzianum* uptake maximum $CuSO_4$ ion. According to Deshmokh and Rai (2005), the increase of the uptake capacity of metals may be often associated with toxicity or the increasing permeability of cell membrane because of further binding of the metal to exposed intracellular sites, causing the biomass to decrease with elevated metal exposure.

Table 2. Screening of fungal isolates for tolerance to heavy metals

Name of the Fungi	Measurement of growth (mm)	
	$MgSO_4$	$CuSO_4$
A. flavus	9	4
A.fumigatus	11	4
Aspergillus niger	10	6
Fusarium sp	8	3
Penicillium sp	9	2
Trichoderma sp	4	2

Table 3. Biosorption of $MgSO_4$ heavy metals (%) in flask trails

Name of the fungi	Treatment of growth mg/ml				
	100	200	300	400	500
A. flavus	20	61.9	27.78	18.1	15.9
A.fumigatus	14.9	28.6	24.4	10.2	7.8
Aspergillus niger	8.3	52.38	22.22	20.12	19.3
Fusarium sp	38.5	4.7	3.8	23.3	
Penicillium sp	9.5	15.6	17.3	13.2	9.1
Trichoderma sp	11.2	15.1	19.4	11.6	8.2

Table 4. Biosorption of $CuSO_4$ heavy metals (%) in flask trails

Name of the fungi	Treatment of growth mg/ml				
	100	200	300	400	500
Aspergillus flavus	7.1	6.09	-	-	-
A.fumigatus	5.8	23.3	-	-	-
A. niger	28.57	27.28	23.08	-	-
Fusarium sp	29.5	3.7	-	-	-
Penicillium sp	40.0	23.5	13.4	-	-
Trichoderma sp	1.7	-	-	-	-

Hence, the present investigation, six species viz., *Aspergillus flavus A.fumigatus, A.niger, Fusarium* sp*, Penicillium* sp and *Trichoderma* sp are tested the biosorbent study in heavy metal with $MgSO_4$ was observed. The biosorption of magnesium sulphate heavy metals from *Aspergillus flavus* was maximum percentage (27.78) at 300mg/ml, when compare to other concentration. Whereas in the minimum 3.8 percentage with *Fusarium* sp at 300mg/ml degraded in the treated copper sulphate heavy metal was maximum at 100mg/ml treated with *Penicillium* sp highly degraded when compare to other concentration because the higher concentration of heavy metal of $CuSO_4$ was highly active against all fungi. The minimum dose only suitable for degradation of Copper sulphate in the medium (Table 3 and 4; Fig 1 and 2).

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