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Biosorption of heavy metals by some soil fungi

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Abstract :

Heavy metal pollution is one of the main problems due to increasing technological development. Heavy metals are generated from electroplating, textile, chemical industries and metallurgical industries. Heavy metals are held in soil due to adsorption, chemical reaction and ion exchange of soil. Microorganisms play a vital role in heavy metal biosorption from polluted soil and water. In the present study an average values of physico-chemical parameters were analysed from two sampling stations. Four fungal strains were identified from two different river soil samples. The uptake of heavy metal magnesium sulphate by *Aspergilus flavus* and *A.niger* in 10ppm concentration is more than 20ppm concentration. The FT-IR spectra of degradation products displayed peaks at different positions indicating the complete break- down of black rose.

Key Words : Biosorption, FTIR, fungi, heavy metal

INTRODUCTION

The problems of the ecosystem are increasing with developing technology. Heavy metal pollution is one of the main problems. Though industrial use of water is very low as compared to agriculture, it has a significant impact on water quality. Among various industries, electroplating (Magnesium, Zinc), textile, chemical and metallurgical industries play a vital role in the contamination of the environment. Another important risk is the accumulation of these substances in the soil. Heavy metals are held in soil as a result of adsorption, chemical reaction and ion exchange. The toxicity caused by heavy metals is generally due to binding with the biomolecules, as as result of strong coordinating abilities (Afal and Wiener, 2014). Metal toxicity is divided into three categories they are blocking the essential biological functional groups of molecules, displacing the essential metal ion in biomolecules and bring conformational changes in the metabolic pathways. In general, exposures are divided into two classes: acute exposure and chronic exposure. Acute exposure refers to contact with a large amount of the heavy metal in a short period. Chronic exposure refers to contact with low levels of heavy metal over a long period of time. Mercury is a global pollutant with complex and unusual chemical and physical properties. The major natural source of mercury is the degassing of the Earth's crust, emissions from volcanoes and evaporation from natural bodies of water. Magnesium sulphate is widely used in agriculture, gardening and medical science. Magnesium, Zinc-responsive transcription factors are found in fungi, mammals, fish, and possibly plants, suggesting that the transcriptional control of genes involved in zinc homeostasis is of universal importance. Microbes executes major role in heavy metals biosorption from polluted soil and water. The heavy metals have cumulative and toxicity due to which they have a hazardous effect not only to soil but also to human beings. The present article deals with biosorption of some heavy metals by some selected fungi.

MATERIALS AND METHODS

Heavy metal contaminated soil samples were collected from the Papanadu, Orathanadu Tk, Thanjavur Dt and Poondi Village, Papanasam Tk, Thanjavur Dt river soils. Soil samples were collected in sterile polythene bags, and the samples were stored at 4°C in the refrigerator for further work. The fungi were isolated using Potato Dextrose Agar (PDA). The soil samples were diluted serially to isolate the fungi from each sample. The colonies growing on PDA plates with different morphology were counted separately. The slide was observed under a compound microscope. Microphotography of the individual fungal species was taken by using Olympic Binocular microscope.. Identification of fungi was done by using standard manuals of soil fungi by (Gillman 1967; Domsch, *et al.*, 1980; Barnett and Hunter, 1999). Prepared PDA medium was poured in screw cap tubes and sterilized by autoclaving. After autoclaving the tubes were placed in slanting position till solidification. Mercuric chloride, Zinc sulphate and Magnesium sulphate were purchased from Hi-Media, Mumbai, India

Screening of fungal isolates for tolerance to heavy metals

Determination of heavy metal bio absorption by filamentous fungi was made by using standard method. The uptake of heavy metal by fungal biomass was calculated using the following equation:

$$qe\,(mg/g) = \frac{C \times V \times 1000}{W}$$

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qe: concentration of heavy metal accumulated by fungal biomass, (mg/g)
C: concentration of heavy metal (gm)
V: (ml) the volume of the aqueous medium
W: (g) is the dry weight of the fungal biomass.

The methanolic extract of *A.niger* samples was taken, fine powder was prepared after evaporation and sieved with 0.07 and 0.500 mm mesh . The FT-IR spectra were recorded in mid IR region 4000-400 cm^{-1} at the resolution of cm^{-1} using a sophisticated computer controlled FT-IR Perkin Elmer spectrometer with He-Ne laser as reference. Air back ground spectrum was recorded before each sample.

RESULTS

Physicochemical analyses of the soil collected from rivers were recorded, and are presented in Table 1. From the soil sample collected from river soil, four fungal strains were isolated. They were identified based on the morphology (microphotograph) and colony characteristics. The identified main fungal isolates included *Asperillus flavus, A. terreus, A. niger* and *T.viride* (Table-2; Plate-I). *A. flavus, A. terreus, A. niger and T.viride* were screened for their heavy metal tolerance by growing on medium amended with heavy metals such as zinc sulphate, mercuric chloride and magnesium sulphate amended medium separately. Growth and uptake of heavy metals by fungi were determined by measuring the biomass. The results showed various level of difference between the fungi and the concentrations (Table 3-4; Plate I).

The uptake of heavy metal such as magnesium sulphate by *A. flavus* was found to be 50.0ug/g in 10ppm concentration. In 20ppm concentration the degradation efficiency of *A. flavus* by the fungi was observed as 43 % (Table 3-4; Plate I). *Aspergillus niger* inoculated with 10ppm concentrations of heavy metals showed that the uptake of magnesium sulphate was 55.8 %. While in 20ppm concentration magnesium sulphate uptake was 43.2 %. Comparison of FTIR spectrum of the control dye with extracted metabolites after complete degradation clearly indicated the biodegradation of Black rose by *A.niger*. The results of FT-IR analysis of Black rose sample obtained after degradation showed various peaks. The FT-IR spectra of Black rose displayed peaks at 3753.99, 3424.47, 2367.16, 2339.46, 2170.46, 1593.16, 1404.94, 1337.82, 1119.87, 926.27, and 619.15 cm-1, for N-H stretching vibrations, N-H stretching vibrations, Sulfur compounds, Unsaturated nitrogen compounds, C-H bending respectively. However, the FT-IR spectra of degradation product displayed peaks at different positions indicating the complete breakdown of Black rose. The results are presented in Table-5; Figure-1

Table 1: Physico chemical properties of soil samples

S.No	Name of the parameter	Average Value
1.	pH	7.16
2.	Organic Carbon (%)	0.21
3.	Organic Matter (%)	0.40
4.	Phosphorus (Kg/ac)	3.52
5.	Potassium(Kg/ac)	129.1
6.	Zinc (ppm)	0.62
7.	Copper (ppm)	0.23

Table2: Total numbers of colonies of fungi isolated from Papanadu and Poondi river soils

S. No	Fungal isolates	Place of Sample collection	Total number of colonies		Percentage Contribution of fungus (%)
			Papa nadu	Poo ndi	
1	*Aspergillus flavus*	4	4	8	22.25
2	*A. terreus*	5	5	10	27.75
3	*A.niger*	3	7	10	27.75
4	*Trichoder ma viride*	4	4	8	22.25
	Total	16	20	36	100.00

Table 3: Effect of different concentrations of heavy metal on *A.flavus*

Heavy metal	Concen tration (ppm)	Mycelial biomass (g)	Dry biomass (g)	%of growth
Zincsul	10	5.4	2.7	50.0
phate	20	3.9	2.2	43.5
Magnesium	10	5.2	2.4	53.8
sulphate	20	3.7	1.9	48.6
control	-	6.4	2.9	54.6

Table 4 Effect of different concentrations of heavy metal on *Aspergillus niger*

Heavy metal	Concen tration (ppm)	Mycelial biomass (g)	Dry biomass (g)	%of growth
Zinc sul	10	5.9	2.6	55.8
phate	20	3.7	2.1	43.2
Magnesium	10	5.6	2.2	60.0
sulphate	20	3.9	1.9	51.2
Control	-	6.9	2.6	72.3

Table 5: Fourier Transforms-Infrared Spectroscopy (FT-IR) Analysis of *A.niger*

S.no	Peak area	Component name	Groups
1.	3753.99	Primary, free two bands	N-H stretching vibrations
2.	3424.47	Primary, free two bands	N-H stretching vibrations
3.	2367.16	S-H stretching vibrations	Sulfur compounds
4.	2339.46	S-H stretching vibrations	Sulfur compounds
5.	2170.46	Isocyanides	Unsaturated nitro -gen compounds
6.	1593.16	Primary	N-H bending vibrations
7.	1404.94	Tertiary alcohols	O-H bending and C-O stretching
8.	1337.82	Primary alcohols	O-H bending and C-O stretching
9.	1119.87	Secondary alcohols	O-H bending and C-O stretching
10	926.27	Alkane,mono substituted (vinyl)	C-H bending
11.	619.15	Alkane tri substituted	C-H bending

Fig -1

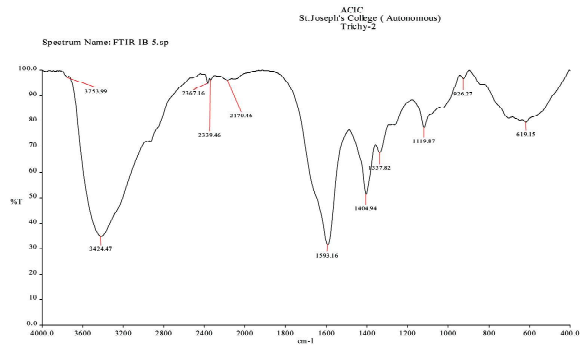


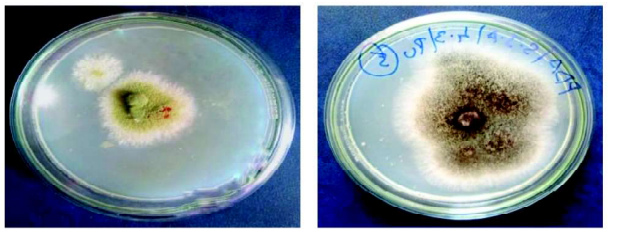
Plate-I

Effect of 10% and 20% concentration of Heavy Metal by Biosorption of some fungi

Mercury chloride

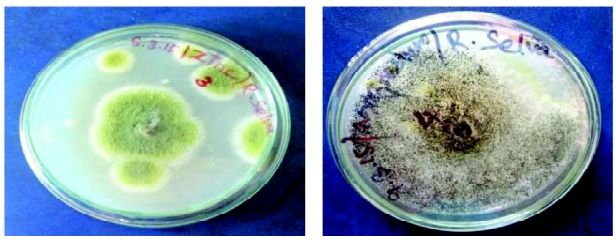
A.flavus *A.niger*

Zinc sulphate



A.flavus *A.niger*

Magnesium sulphate



A.flavus A.niger

DISCUSSION

Increased use of metals and chemicals in process industries has resulted in generation of large quantities of effluent that contain high level of toxic heavy metals. Since they do not undergo biodegradation, they persist in soil for a long time and pose environmental and health problems. These metals enter into human beings and animals through food chain and cause many metabolic disorders (Malik, 2004; Chuah *et al*., 2005). Bioremediation of heavy metals involving microorganisms could be brought about by employing methods such as bio-accumulation, biosorption, bio-precipitation and uptake by purified biopolymers from microbial cells (Churchill, 1995) The present study is an attempt to isolate and screen heavy metal tolerant to (Zinc sulphate and Mercury chloride) fungi. Their efficiency to absorb heavy metals from the media was also evaluated under laboratory conditions. Thus, in the present investigation four isolates *Aspegillus niger, A. flavus, A. terrus* and *Trichoderma viride* were identified from both of the soil. Similarly seventy six fungal isolates tolerant to heavy metals were isolated from samples of sewage, sludge and industrial effluent contaminated with heavy metals such as Pb, Cd, Cr and Ni using standard methods (Solarsk *et al*., 2009). It has also been reported that *Aspergillus niger* biosorption reflected the similar pattern of biosorption (Ahmad *et al*., 2005**).** Fourier-Transform Infrared Radiation (FTIR) is proved to be a reliable and sensitive method for detection of molecular changes in the compounds from the heavy metal by fungi. Diem *et al* (1999) studied the spectral peaks obtained from FTIR spectra of living organisms and reported their application in determination of heavy metal biosorptions

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