HEAVY METAL TOLERANCE POTENTIAL OF FUNGI ISOLATED FROM EFFLUENT DUMPING SITES OF LLOYDS STEEL INDUSTRIAL AREA OF WARDHA CITY, MAHARASHTRA.

S. S. Khandare[1]*, A.P. Kale[2], M. G. Ingale[3]

[1, 2, 3] *P.G. Department of Microbiology, Bajaj College of Science, Wardha. 442001.*

** Corresponding author- ksuhas21@gmail.com*

ABSTRACT

The soil contamination with the heavy metals is the growing concern throughout the world as the result of industrialization, agricultural and other anthropogenic activities. The fungi are the most common and efficient group of the heavy metal resistant microbial family which have potential for bioremediation of metals. This study investigates the tolerance potential of different species of indigenous fungi that isolated from effluents dumping sites of Lloyds steel industrial area of wardha city, Maharashtra. All the strains were grown on Potato dextrose agar (PDA) media and morphological characterization of all the isolates were performed by using standard microbiological protocols. Different strains were identified as *A niger, A. flavus, Fusarium sp., Curvalaria* and *Cladosporium sp.* Among them, three fungal species ie. *A niger, A. flavus* and *Cladosporium* were screened for their resistance to copper (Cu), Zinc (Zn) and Lead (Pb) in potato dextrose agar plates amended with various concentrations ranging from 200-1000 mg/lit. The results revealed that the majority of the isolates were resistant to Pb, Cu and Zn. The present study reveals that *A.niger* and *A. flavus* were the potent fungal isolates who could tolerate the highest concentration of heavy metal ie. Zn and Cu respectively with 1.79 metal tolerance index for both the heavy metals at 600 mg/lit. On the other hand both *A.niger* and *A. flavus* found to be sensitive against high concentration of Pb ie. Metal tolerance index of 0 and 0.25 respectively at 1000 mg/lit. *Cladosporium* sp. showed highest metal tolerance index of 1.75 against Pb and lowest metal tolerance index of 0.45 against Cu and Zn each. Hence heavy metals contaminated industrial soil might be considered as a precious natural source of resistant fungal strains, which can be used significantly as a bioleaching or bioremediation tool.

Keywords: *Fungi, Heavy metal, Wardha, Bioremediation.*

INTRODUCTION:

Substantial increase in industrial and agricultural growth, in addition to population escalation resulted in deterioration of environmental quality worldwide. Rapidly growing cities, more traffic on roads, growing energy consumption and waste production together with lack of strict implementation of environmental regulation are increasing the discharge of pollutants into air, water, and soil (1). Water pollution is an appalling problem, powerful enough to lead the world towards destruction. Becoming an easy solvent most of the pollutants get dissolved in water and contaminate the same.

Contamination of sediments and natural aquatic receptors with heavy metals is a major environmental problem all over the world (2-5). These inorganic micropolluants are released by effluents generated from various industries such as electroplating and metal finishing industries, metallurgy, tannery, and battery manufacturing. The introduction of heavy metal compounds into the environment generally induces morphological and physiological changes in the microbial communities (6), hence exerting a selective pressure on the microbiota (7). Generally, the contaminated sites are the sources of metal resistant micro-organisms (8). Therefore, it is important to explore autochthonous micro-organisms from such contaminated niches for the bioremediation of heavy metals since conventional processes such as chemical precipitation; ion exchange and reverse osmosis are uneconomical and inefficient for treating effluents of dilute metal concentrations (9-12).

The wastewater from metal processing industries is also polluting the environment as well as from other pollutant routes. Virtually, any industrial activity using metals have a metal disposal problem (13). Heavy metals have drastically increased in the environment and also found in nature. Elements or compounds having different properties, such as Zn, Cu, Ni, Fe, and Mn are essential trace elements in living organisms and non-essential metals such as cadmium, lead, mercury, and nickel are toxic even at low concentration (14-15). Heavy metals are non-biodegradable and persistent into the environment and accumulate into living tissues and posing a serious threat to the environment and public health. Due to this reason, they are not able to purify by the environmental compartments (soil and water). These harmful substances are carcinogenic and mutagenic in nature and are causing threats to the living beings.

Increased heavy metal contamination of soil and water environments has necessitated the need for clean-up strategies. Recently, diverse eco-friendly remediation options have been explored for the restoration of contaminated environments. These remediation options, among others, include the use of plants (phytoremediation), bacteria (bacterial bioremediation) and fungi (mycoremediation). The employability of these bio-resources (plants, bacteria and fungi) for effective bioremediation has been well reported.

Metal tolerance/resistance has been defined as the ability of an organism to survive metal toxicity by means of one or more mechanisms devised in direct response to the metal(s) concerned (16).

Metal tolerance by filamentous fungi has been associated with their sites of isolation, toxicity of the metal tested, its concentration in medium, and the isolate's competence (17). Contaminated sites are known as principal sources of metal-resistant species (18) with indigenous fungal strains isolated from heavy metal contaminated sites exhibiting notable tolerance for high heavy metal concentrations.

Several metal-tolerant filamentous fungi (*Rhizopus*, *Trichoderma*, *Aspergillus*, *Penicillium*, and *Fusarium*) have been isolated from multiple heavy metal contaminated soils.

The present research was designed to isolate and characterize metal tolerant fungi from polluted environments of Wardha city. The objective of this study is to isolate and identify the fungal strains and to study the effect of various concentrations of heavy metals on fungal isolates. However, utility of this approach under field conditions under diverse environmental conditions need to be tested.

MATERIALS AND METHODS:

Selection of site

Wardha, one of the important city in western vidarbha region of Maharashtra suffering from different pollution load due to population and industrialization. Industrial effluents dumping sites of Lloyds steel industrial area of wardha was selected as a sampling site. Soil samples considered as contaminated sites for the present study were collected aseptically in poly bag and were stored at 4^0C before further analysis.

Isolation of fungal strains

Fungal strains were isolated from soil using conventional dilution plate culture technique. One gm of soil was suspended in 10 ml of sterilized distilled water and then serially diluted to 10^{-4} dilution. 1ml of such diluted sample was plated on Potato dextrose agar (PDA) growth media and incubated at 25-30°C for six days. Distinct single colonies with sufficient distance from other colonies were obtained (19). These distinct colonies of a particular fungal strain were collected and studied further.

Identification of fungal isolates

All isolated strains were identified on the basis of macroscopic (colonial morphology, color, texture, shape, diameter, and appearance of colony) and microscopic characteristics (conidial shape, conidiophores shape, vesicle shape, sterigmata position etc Properly grown fungal cultures were preserved under refrigerator at 4°C as stock cultures for further studies

Assessment of metal tolerance of isolated fungal strains

Purified isolates were screened on the basis of their tolerance to Lead (Pb), Copper (Cu) and Zinc (Zn). Lead nitrate, Copper sulphate and zinc sulfate metal salts were selected for running all experiments. A fungal culture was inoculated aseptically on PDA plates supplemented individually with 200, 400, 600, 800 and 1000 mg/lit of each heavy metal. The inoculated plates were incubated at 25°C for 6 days. The effect of the heavy metal on the growth of the isolates tested was estimated by measuring the radius of the colony extension (mm) against the control (medium without metal) and the index of tolerance was determined. The index is defined as the ratio of the extension radius of the treated colony to that of the untreated colony. The Tolerance Index (TI) was calculated from the fungal growth in the presence of metals, divided by the fungal growth in the control (containing no heavy metals) plate under standard condition (20).

$$\text{Tolerance Index (TI)} = \frac{\text{Diameter of the colony in presence of heavy metals}}{\text{Diameter of the colony in the absence of heavy metals}}$$

RESULT AND DISSUCION:

In this study, isolation and characterization of metal tolerant fungi from effluents dumping sites of Lloyds steel industrial area of wardha city was done. Fungal strains were isolated from soil using conventional dilution plate culture technique. All isolated strains were identified on the basis of macroscopic (colonial morphology, color, texture, shape, diameter, and appearance of colony) and microscopic characteristics (conidial shape, conidiophores shape, vesicle shape, sterigmata position etc.). Fungi isolated belonged to the genera *Aspergillus, Curvalaria, Fusarium* and *Cladosporium.* Results of the morphological characteristics are depicted in Table 1. Out of these genera, *Aspergillus* and *Cladosporium* were used to assess their metal tolerance ability based on the results of initial screening.

Purified isolates were screened on the basis of their tolerance to Cu and Zn. Fungal culture was inoculated aseptically on PDA plates supplemented individually with 200, 400, 600, 800 and 1000 mg/lit of each heavy metal. Lead nitrate, Copper sulphate and Zinc sulfate metal salts were selected. The effect of the heavy metal on the growth of the isolates tested was estimated by measuring the radius of the colony extension (mm) against the control (medium without metal) and the determination of the index of tolerance. Data revealed that *A.niger* and *A. flavus* were the potent fungal isolates who could tolerate the highest concentration of heavy metals ie. Zn and Cu respectively with 1.79 metal tolerance index for both the heavy metals at 600 mg/lit. On the other hand both *A.niger* and *A. flavus* found to be sensitive against Pb with metal tolerance index of 0 and 0.25 respectively at 1000 mg/lit. Hence as the concentration of Pb increased the sensitivity of both the isolates also gets increased. As far as the *Cladosporium sp.* is concerned it showed highest metal tolerance index of 1.75 against Pb and lowest metal tolerance index of 0.45 against Cu and Zn each. In broad sense each fungal isolate found to tolerate all the heavy metals up to 1000 mg/lit except *A. niger* who found totally sensitive against Pb (metal tolerance index zero) at 1000 mg/lit. Each isolate showed dramatic variation in their metal tolerance index against tested heavy metals. The results of metal tolerance index of fungal isolates are depicted in Table 2 and Figure 1.

Roane and Pepper reported that the differences in resistance levels were probably due to the potential variation in the mechanism of resistance (21). Massaccesi et al. reported that various filamentous fungi isolated from the sediments of industrially polluted streams removed 63 to 70% Cd during a 13-day growth period (22).

Metals are very crucial for growth and development of living organisms. They are directly or indirectly involved in various biochemical pathways and have important roles in different functional processes of life. Although metals are highly essential for life systems and metals like Na, Mg, Ca, K, Fe, Cu, Mn, Zn, Ni, Co etc, have been found to have important functions in metabolism of living organisms, many metals (e.g. Cd, Hg, Al, Au, Ag, Pb) have no apparent essentiality. In the present study the three fungal species selected from industrial effluent dumping site showed differential response towards the heavy metals Pb, Cu and Zn in relation to their growth, colony formation and some other parameters. Such differential response is dependent on sensitivity and tolerance towards metals as seen in cases of yeasts and other saprophytic fungi (23-24).

Table 1: Morphological characteristics of fungal isolates

Sr. No	Isolate code	Colonies on PDA	Microscopic Characters	Fungal species identified
1	S1	Black	Conidial heads were large globose, dark brown, which become radiate	A niger
2	S2	Velvet Black	Conodiophores brown geniculate producing conidia in sympodial order	Curvalaria
3	S3	White	Hyphae are hyaline and septate conidiophores	Fusarium sp.
4	S4	Yellow -green	Septate hyphae, dichotomous branching	A. flavus
5	S5	Olivaceous-brown to blakish-brown	Branched ramoconidia and chain of conidia	Cladosporium

Table 2: Tolerance index of fungal isolates on PDA amended with various concentrations of metals.

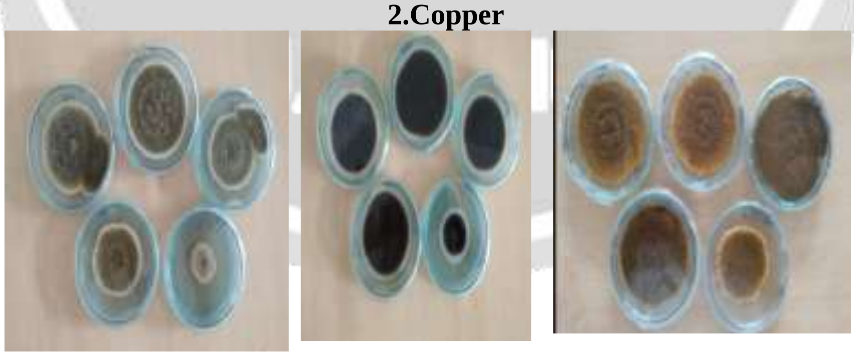
Heavy metal	Concentration of Heavy metals (mg/lit)	A. flavus	A. niger	Cladosporium
		Metal tolerance index ZOI (mm)		
Pb	200	1.5	1.5	1.5
	400	1.50	1.56	1.25
	600	1.56	1.66	1.5
	800	1.53	1.75	1.75
	1000	0.25	0	1
Cu	200	1.75	1.5	1
	400	1.75	1.7	1.5
	600	1.79	1.7	1.5
	800	1.50	1.45	0.75
	1000	1	1.45	0.45
Zn	200	1.75	1.5	1
	400	1.75	1.7	1.5
	600	1.79	1.7	1.5
	800	1.50	1.45	0.75
	1000	1	1.45	0.45

Fig 1: Heavy metal tolerance of fungi at different concentrations of metals (200-1000 mg/lit)

1.Lead

A. flavus **A. niger** **Cladosporium**

2.Copper



A. flavus **A. niger** **Cladosporium**

3.Zinc

A. flavus **A. niger** **Cladosporium**

CONCLUSION

The present study concludes that effluent waste from Lloyds steel industry of wardha region contains the three heavy metal tolerant fungus species. Our findings indicate that these indigenous fungal species exhibited remarkable tolerance in heavy metal-rich media and have the ability to resist higher concentrations of metals. *Aspergillus* and *Cladosporium* isolates were the most resistant to all the metals tested, which may make them promising candidates for further investigations regarding their ability to remove metals from contaminated environments. Hence, indicate the bioremediative potentials inherent in the indigenous filamentous fungal species.

REFERENCES

1. Agrawal M (2005) Effects of air pollution on agriculture: An issue of national concern. Natl. Acad. Sci. Lett, 28: 93-106.
2. Baldrian T, Gabriel J (2003) Lignocellulose degradation by *Pleurotus ostreatus* in the presence of cadmium. FEMS Microbiol. Lett., 220(2): 235-240.
3. Gavrilesca M (2004). Removal of heavy metals from the environment by biosorption. Eng. Life Sci. 4(3): 219-232.
4. Malik A (2004). Metal bioremediation through growing cells. Environ. Int. 30: 261-278.
5. Srivastava S, Thakur IS (2006). Biosorption potency of *Aspergillus niger* for removal of chromium(VI). Curr. Microbiol. 53: 232-237.
6. Vadkertiova R, Slavikova E (2006). Metal tolerance of yeasts isolated from water, soil and plant environments. J. Basic Microbiol. 46: 145-152.
7. Verma T, Srinath T, Gadpayle RU, Ramteke PW, Hans RK, Garg SK (2001). Chromate tolerant bacteria isolated from tannery effluent. Bioresour. Technol. 78: 31-35.
8. Gadd GM (1993). Interactions of fungi with toxic metals. New Phytol. 124: 25-60.
9. Kapoor A, Viraraghavan T (1995). Fungal biosorption- An alternative treatment option for heavy metal bearing wastewaters: A review. Bioresour. Technol. 53 : 195-206.
10. Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H (2000). Microbial biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions. Curr. Sci. 78 (8): 967-973.
11. Pagnanelli F, Petrangeli MP, Toro L, Trifoni M, Veglio F (2000). Biosorption of metal ions on *Anthrobacter sp*.: Biomass characterization and biosorption modelling. Environ. Sci. Technol. 34(13): 2773-2778.
12. Gavrilesca M (2004). Removal of heavy metals from the environment by biosorption. Eng. Life Sci. 4(3): 219-232.
13. Das N, Vimala R, Karthika P. Biosorption of heavy metals-An overview. Indian Journal of Biotechnology 2008; 7: 159-69.
14. Grąz M, Pawlikowska-Pawlęga B, Jarosz-Wilkołazka A. Growth inhibition and intracellular distribution of Pb ions by the white-rot fungus Abortiporus biennis. International Biodeterioration and Biodegradation 2011; 65:124-9.
15. Poli A, Salerno A and Laezza G. Heavy metal resistance of some thermophiles: Potential use of α- amylase from *Anoxybacillus amylolyticus*as a microbial enzymatic bioassay. Resource Microbiology 2009; 160: 99-106.
16. Zafar S, Aqil F, Ahmad I. Metal tolerance and biosorptionpotential of filamentous fungi isolated from metalcontaminated agricultural soil. *Bioresour Technol*.2007;98:2557–2561.
17. Ruta L, Paraschivescu C, Matache M, Avramescu S, FarcasanuIC. Removing heavy metals from synthetic effluents using“kamikaze” *Saccharomyces cerevisiae* cells. *Appl MicrobiolBiotechnol*. 2010; 85:763–771.
18. Gadd GM, Sayer GM. Fungal transformation of metals andmetalloids. In: Lovely DR, ed. *Environmental Microbe–MetalInteractions*. American Soc Microbiol; 2000:237–256.19.

19. Trivedy RK, Goel PK (1984) Chemical and biological methods for water pollution studies (Vol. 215). Karad, India: Environmental publications

20. M. Valix, L.O. Loon Adaptive tolerance behavior of fungi in heavy metals Miner. Eng., 16 (2003), pp. 193-198

21. Roane TM, Pepper LL (2000). Microbial responses to environmentally toxic cadmium. Microb. Ecol. 38: 358-364.

22. Massaccesi G, Romero MC, Cazau MC, Bucsinszky AM (2002). Cadmium removal capacities of filamentous soil fungi isolated from industrially polluted sediments, in La Plata (Argentina). W. J. Microbiol. Biotechnol. 18(4): 817–820.

23. Ortiz DF, Kreppel L, Speiser DM, McDonald G, Ow DW (1992) Heavy metal tolerance in the yeast requires an ATP-binding cassette-type vacuolar membrane transporter. EMBO J., 11(10): 3491-3499.

24. Gadd GM (1993) Tansley review no. 47: interactions of fungi with toxic metals. New Phytol, 124:25±60.