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Isolation and Screening of Some Filamentous Fungi with Various Trace Metals

KEYWORDS: Trace metal, Filamentous Fungi, Biodiversity, Paper mill effluent, Bioremediation.

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ABSTRACT *Trace metal (TM) pollution of soil is a worldwide problem threatening the quality of human life and a proper environment. We investigated fungal diversity of trace metal polluted site contaminated with paper mill effluent in India. Fourteen fungal strains were identified using ITS of rDNA belonged to* ***Aspergillus, Penicillium, Fusarium, Cunninghamella, Simplicilillium, Trichoderma, Rhizomucor, Cladosporium*** *and* ***Hypocrea*** *and subsequent screening was carried out to assess their TM tolerance in in vitro cultures. The results revealed that the majority of the isolates were tolerant to Ni, Cu, Zn whereas only* ***Penicillium*** *sp. and* ***Aspergillus*** *sp. were able to grow in Cd amended medium. The level of tolerance depended on the fungal isolate and the site of its origin. Minimum inhibitory concentrations (MIC) for Ni, Cu, Zn and Cd were also determined among the all tested fungal isolates. Overall,* ***Aspergillus, Penicillium, Rhizomucor, Trichoderma*** *and* ***Fusarium*** *isolates showed a strong growth in different concentrations of TM. Their MIC ranged between 20 and 25 mM for Zn, 15 and 20 mM for Ni and Cd. Some of these fungal isolates showed a high potential for further investigations regarding the mechanisms of TM tolerance and their use in mycoremediation to clean up TM polluted soil.*

Introduction

Pulp paper industries are the sixth largest effluent generating industries of the world as these generates as low as 1.5 m^3 of effluent per ton to as high as 60 M^3 tone of paper produced (Gavrilesca, 2004). Trace metals are included among the priority pollutants because of their high toxicity require suitable treatment prior to discharge into the environment. Trace metals cannot be degraded and even at low concentration cause toxicity in humans (Rao et al. 2005). Conventional methods used for removal of metals include chemical precipitation, ion exchange, membrane filtration and activated carbon adsorption. Biological methods provide an attractive alternative to conventional methods because of low operating cost, minimization of chemicals, biological sludge easily disposed and high efficiency in detoxifying very dilute effluent. According to World Health Organization (2006), the metals of most immediate concern are cadmium, cobalt, Zinc, nickel, copper, mercury and lead .Many microorganisms including fungi have been identified as superior candidate for metal bioremediation. Major advantages of fungi are its significant metal uptake ability at low anticipated price. Microbial population present in metal polluted environment have adapted and become tolerant to toxic trace metals (Melgar et al. 2007). Trace metals like Cu, Ni, Zn, Cd, Mn present in paper mill effluent can be removed by indigenous microbes isolated from effluent itself .Fungi and yeast biomasses are known to tolerate trace metals (Baldrian, 2003). The tolerance of some fungi to a variety of trace metals is well documented (Burgstaller and Schinner 1993). In some cases it produces intracellular/ extracellular enzymes to resist the metal concentration or they possess the processes of valence transformation, active uptake, complexation, crystallization and biosorption to cell walls (Wang and Chen, 2006; Zafar et al. 2007). Species like Penicillium, Aspergillus, Pseudomonas, Sporophyticus, Bacillus, Phanerochaete, etc are found to be very useful for the removal of trace metals such as chromium and nickel (Ezzourhi et al. 2009).Many Fungi develop their tolerance with specific metals and ability to influence the mobility of target metals with metal containing ores in a various ways (Fomina et al.2005).They are a versatile group, as they can adapt and grow under various extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations (Atlas,1984).They offer the advantage of having cell wall material which shows excellent metal-binding properties(Sabat and Gupta 2009). El-Morsy (2004) isolated 32 fungal species isolated from polluted water in Egypt and studied their resistance to metals and found that **Cunninghamella echinulata** biomass could be employed as a biosorbent of metal ions in waste water. The present work reports the isolation of filamentous fungi indigenous to paper mill environments contaminated with trace metals and their tolerance capacity with the different concentrations of trace metals. The trace metals MICs (minimal inhibitory concentrations) for each microorganism were determined for the selection of most tolerant strains, which can be used in bioremediation of trace metals in the contaminated site.

Materials and Methods

Isolation of microorganisms

Soil samples were taken from the polluted solid effluent area of the Hindustan paper Mill Corporation, Panchgram, India. The paper mill is surrounded by a vast spread of agriculture land used for paddy cultivation on its eastern side while the northern side has hill areas covered with dense forest. Once this area was full of different kinds of plants but now the dumping of solid wastes near the adjoining area resulted in the depletion of various important plant species. The study area was located between 24° 52′ N and 92° 36′ E longitudes. The altitude of the study area was 16 MSL.The serial dilution technique was carried out with 1gm of soil sample with standard dilution 10^{-4}. The aliquots of 100 µl of different dilutions were plated both onto Potato Dextrose Agar (PDA) plates. After at least 7 days of incubation at 25°C, developed colonies were picked and isolated. Purified strains were obtained by streaking repeatedly colonies in PDA medium and observation under compound microscopy. The cultures were characterized to the genus level on the basis of macroscopic characteristics (colonial morphology, colour and appearance of colony, shape) and microscopic characteristics (septation of mycelium, shape, diameter and texture of conidia).

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

The fungal DNA isolated with the Nucleic acid and protein purification kit (Macherey-Nagel, USA, MCN7444004, www.mn-net.com).The all fungi strains have been characterized by PCR with (forward) ITS1 TCCGTAGGTGAACCTGCGG and (reverse) ITS4TCCTCCGCTTATTGATATGC (White et al. 1990).

Screening and selection of trace metal-tolerant microorganisms
Purified strains were screened on the basis of their tolerance toCr^{6+}, Pb^{2+}, Zn^{2+}, Cd^{2+} and Cu^{2+}. A disk of mycelium was inoculated aseptically on PDA plates supplemented individually with 1 mM of trace metal. The metal salts used were nickel sulfate, copper sulfate, zinc sulfate and cadmium sulfate. The inoculated plates were incubated at 25°C for at least 7 days. The growth of the strains was estimated by measuring the radius of the colony extension (mm) against the control (medium without metal) and index of tolerance was determined. The index was defined as the ratio of the extension radius of the treated colony to that of the untreated colony.

Determination of minimum inhibitory concentrations (MICs)
The resistance of the selected strains to Cr^{6+}, Pb^{2+}, Zn^{2+} and Cu^{2+} was determined by the dilution method. Metal ions were added separately to PDA medium at concentrations of 1mM to 15 mM. The plates were inoculated with 8 mm agar plugs from young fungal colonies, pre-grown on PDA. Three replicates of each concentration and controls without metal were used. The inoculated plates were incubated at 25°C±2°C for 7 days. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of metal that inhibited visible growth of the isolate.

Trace metal analysis of soil
The sediment samples were dried at 105°C manually ground and sieved through a sieve of (500 μm pore size). A sample of 1 g was treated with 10 ml of aqua regia (25% HNO^3; 75% HCl). Digestion was carried out on a hot plate until dense fumes evolved and a clear solution was obtained. The clear solution was filtered through a Millipore filter (0.45 μm) and diluted to 50 ml with distilled water. A control was included by treating 10ml of aqua regia in the same way as the sediment samples. Trace metal (Ni, Cu, Zn and Cd) concentrations were determined using Atomic Absorption Spectrometry.

Statistical analysis
The experiments were set up with three replicates. Analysis of variance was performed by using statistical software (Fisher's LSD test, Method: 95.0) to compare resistance to metal among individual strains (Figures 5-8).

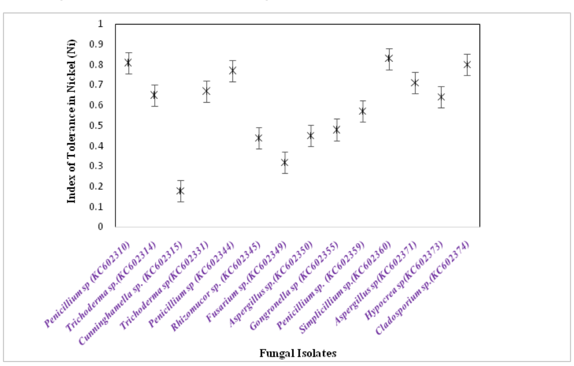

Figure 5: Nickel index of tolerance among strains. Method: 95.0 LSD percentages.

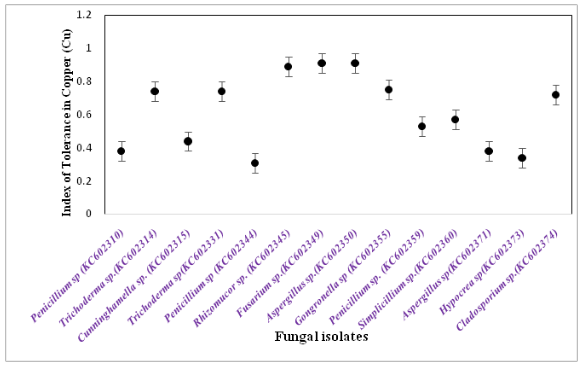

Figure 6: Copper index of tolerance among strains. Method: 95.0 LSD percentages.

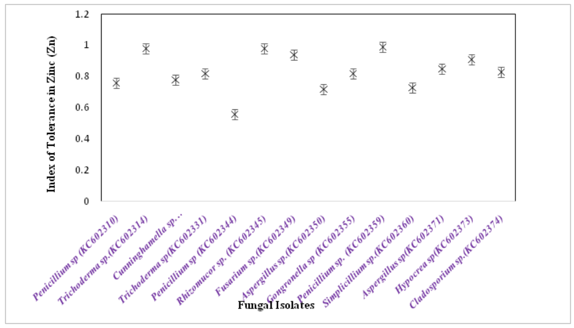

Figure 7: Zinc tolerance among strains. Method: 95.0 LSD percentages.

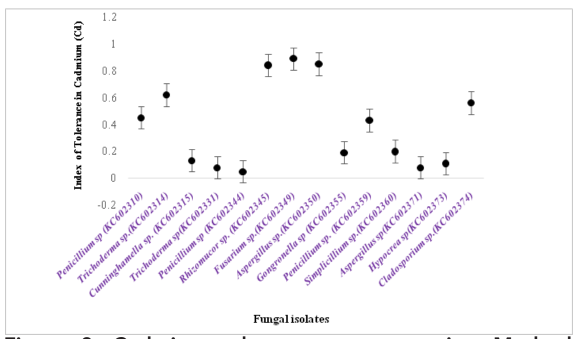

Figure 8: Cadmium tolerance among strains. Method: 95.0 LSD percentages.

Results and Discussion
Bioremediation is the elimination or reduction of toxic pollutant from the contaminated sites with the help of biological organisms such as bacteria, fungi and plants by degradation, assimilation or transpiration in the atmosphere. In the present study, Fourteen fungal strains were isolated from polluted soil contaminated with trace metals in paper mill effluents and tested with different trace metals in different concentrations. The genera like Aspergillus, Penicillium, Simplicillium, Cladosporium, Cunnighamella, Gongronella, Trichoderma, Fusarium and Hypocrea were common (Table 1). The differences between the sampled periods regarding their richness on microbial strains appear to be closely linked to the degree of trace metal pollution.

Table 1. Genetic characteristics of isolated fungal strains

Isolated Fungal strains	Acession number	Hit in NCBI database	Max indent
Penicillium sp.	KC602310	***Penicillium aculeatum.***	99%

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Trichoderma sp	KC602314	Trichoderma koningiopsis	94%
Cunninghamella sp.	KC602315	Cunninghamella sp.66-2011strain	90%
Trichoderma sp.	KC602331	Trichoderma harzianum	97%
Penicillium sp.	KC602344	Penicillium simplicissimum	98%
Rhizomucor sp.	KC602345	Rhizomucor variabilis	99%
Fusarium sp.	KC602349	Fusarium proliferatum	99%
Aspergillus sp.	KC602350	Aspergillus tamarii	98%
Gongronella sp.	KC602355	Gongronella buttleri	99%
Penicillium sp.	KC602359	Penicillium janthinellum	99%
Simplicillium sp.	KC602360	Simplicillium subtropicum.	99%
Aspergillus niger	KC602371	Aspergillus niger	99%
Hypocrea sp.	KC602373	Hypocrea lixii	95%
Cladosporium sp	KC602374	Cladosporium tenuissimum	100%

The screening test revealed heterogeneity in the trace metal tolerance of these fungi (Figures 1- 4).The tolerance level varied due o inherent variation of species. Similar results were reported by Valix et al (2001); Martino et al (2004).The most tolerant fungi which were able to grow in high concentration of the trace metals are Penicillium (KC602310), Trichoderma (KC602314), Aspergillus sp (KC602350), Fusarium sp (KC602349), Hypocrea sp (KC602373), Penicillium janthinellum (KC602344) and Cladosporium (KC602374)showed tolerant to copper and all other trace metals. These fungi may of great importance for removal of trace metals from contaminated site due to their metal tolerance. Some deuteromycetes have been reported by Ghorbani et al (2007); Zafar et al. (2007).Metals such as copper and zinc are essential to bioactivities, however, they tend to show toxicity after a certain level. Their toxicity may differ, depending on the isolate and its site of isolation. Some strains were tolerant, while others reacted negatively even at low metal ion concentrations. This could be explained by the heterogeneity of pollution in the locality from which the tested strains originated (Gadd, 2010).

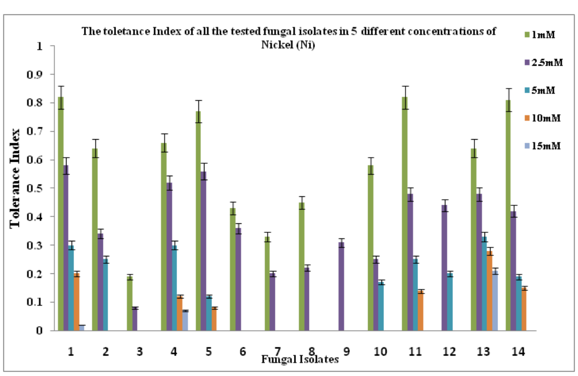

Figure 1.The tolerance Index of tested fungal strains in different concentrations of Nickel (Ni).

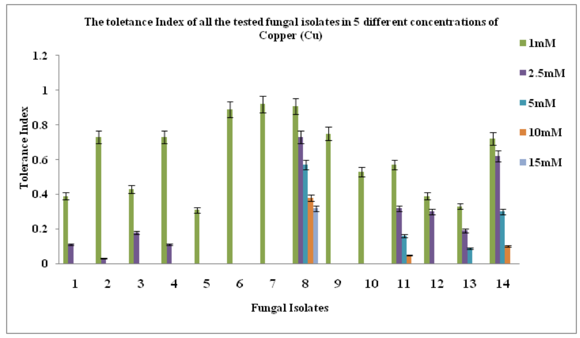

Figure2. The tolerance Index of tested fungal strains in different concentrations of Copper (Cu).

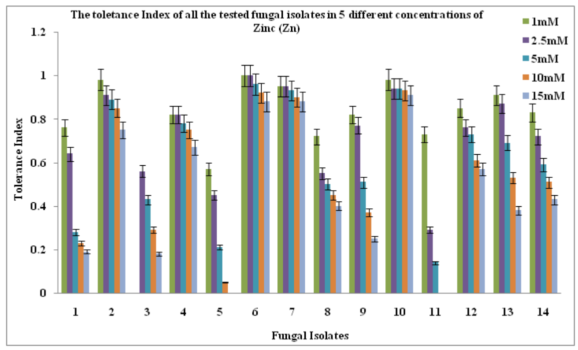

Figure 3.The tolerance Index of tested fungal strains in different concentrations of Zinc (Zn).

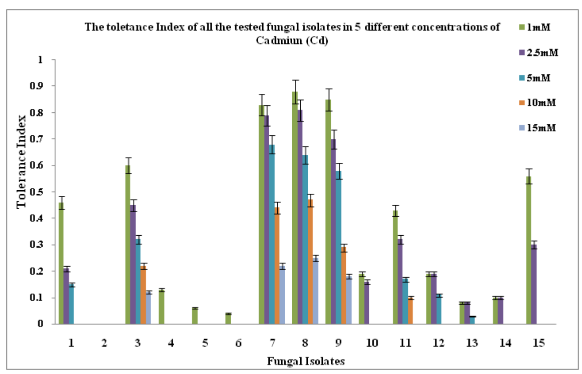

Figure 4.The tolerance Index of tested fungal strains in different concentrations of Cadmium (Cd).

Minimum Inhibitory Concentration (MIC) of the four metal ions against the studied fungal strains is shown in (Table 2). The growth rate of the fungi exhibited a long lag, and retarded rate of growth in the presence of trace metal relative to the control. At higher metal ions concentrations, most of the tested fungal strains were very tolerant and exhibited strong growth, usually exceeding the control. Higher metal ions concentration caused a reduction in growth and increased the length of the lag phase compared to the control.Immobilization can result from sorption to biomass or exopolymers, transport and intracellular sequestration or precipitation as organic and inorganic compounds (Burford et al.2003).

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Table 2. Minimum Inhibitory concentration (MIC) for tested fungal strains

Fungal Isolates Ni	Accession number	MIC (Mm)			
		Cu	Zn	Cd	
Penicillium sp.	KC602310	10<MIC>15	1<MIC>2.5	10<MIC>15	2.5<MIC>5
Trichoderma sp	KC602314	10<MIC>15	1<MIC>2.5	20<MIC>25	15<MIC>20
Cunninghamella sp.	KC602315	10<MIC>15	5<MIC>10	15<MIC>20	5<MIC>2.5
Trichoderma sp.	KC602331	15<MIC>20	1<MIC>2.5	20<MIC>25	cont<MIC>1
Penicillium sp.	KC602344	15<MIC>20	1<MIC>2.5	20<MIC>25	15<MIC>20
Rhizomucor sp.	KC602345	15<MIC>20	15<MIC>25	15<MIC>25	15<MIC>20
Fusarium sp.	KC602349	5<MIC>10	1<MIC>2.5	15<MIC>25	cont<MIC>1
Aspergillus sp.	KC602350	5<MIC>10	1<MIC>2.5	15<MIC>25	5<MIC>10
Gongronella sp	KC602355	15<MIC>20	5<MIC>10	2.5<MIC>5	cont<MIC>1
Penicillium sp.	KC602359	15<MIC>20	2.5<MIC>5	20<MIC>25	1<MIC>2.5
Simplicillium sp.	KC602360	10<MIC>15	1<MIC>2.5	20<MIC>25	15<MIC>20
Aspergillus sp	KC602371	10<MIC>15	1<MIC>2.5	20<MIC>25	15<MIC>20
Hypocrea sp	KC602373	15<MIC>20	2.5<MIC>5	15<MIC>20	1<MIC>2.5
Cladosporium sp	KC602374	15<MIC>20	5<MIC>10	15<MIC>20	2.5<MIC>5

Atmospheric deposition of nickel results from the burning of fossil fuels, especially oil and its refinery products including diesel. Nickel is relatively minor contaminant of fertilizers, and historically the main source of nickel in agricultural soils came from the application of sewage sludge (Parknikar, 2003). The fungal strains which were able to grow in 15-20mM were Trichoderma sp, Penicillium sp Rhizomucor sp ,Cladosporium sp and Hypocrea sp. The other tested strains like Penicillium, Aspergillus, Cunninghamella were also to grow in MIC of 10-15mM (Table 2). Sun and shah (2007) and Rao et al. (2005) also observed that with the increasing metal concentration of trace metals, fungi can increase the rate of metal removal by saturation adsorbents concentrations by increasing mobilization of metal ions. Metals are mobilized via several mechanisms, including acidolysis (proton promoted), complexolysis (ligand promoted) and reductive mobilization by the fungal hyphae (Burford et al. 2003). Penicillium and Aspergillus showed a higher metal tolerance against Nickel (Figures 9 & 10).

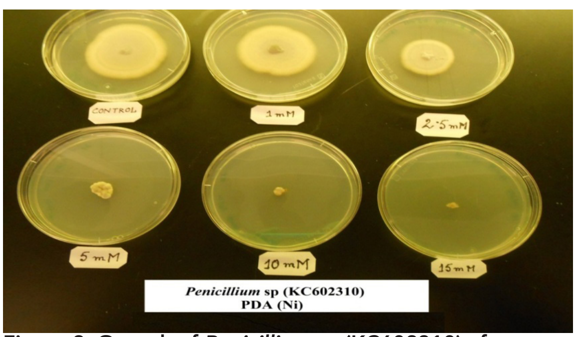

Figure 9: Growth of *Penicillium* sp (KC602310) after exposure to different concentrations of Nickel ions for 10 days.

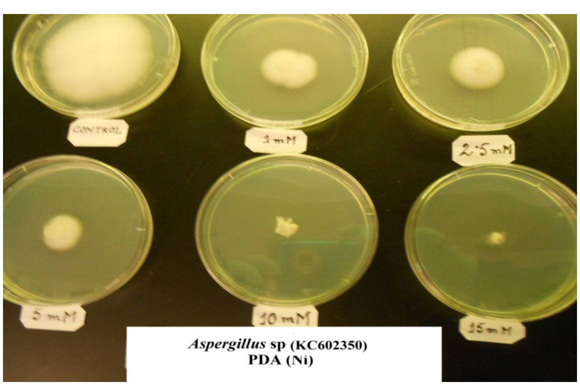

Figure 10: Growth of Aspergillus sp (KC602350) after exposure to different concentrations of Nickel ions for 10 days

Copper is a micronutrient essential for fungal growth, playing a role as a cofactor for a range of oxidases and oxygenases, including lactases and other 'blue-copper' oxidases that are involved in pigmentation and catabolism of aromatic compounds such as lignin (Howe et al.1997; Renata and Elena 2006).The growth of all fungi tested was found decreased after addition of copper in high concentration in comparison with zinc, nickel and cadmium. The concentration of Copper, Cr (VI) and Pb were significantly high in paper mill effluent and soil and exceeded the permissible limit of BIS (1993) due to its application as catalyst, pigments, wood preservatives and corrosion inhibitors. All strains studied could not grow in higher concentrations except Rhizomucor sp (KC602345) which showed highest MIC of 15-25mM. (Figure 11).The white colour of the mycelium became blue green due to accumulation of Cu ions inside the cell wall of the tested fungi. Copper tolerance in fungi ascribed to diverse mechanisms involving trapping of the metal by cell-wall components, extracellular chelation or precipitation by secreted metabolites, and intracellular complexing by metallothioneins and phytochelatins as also described by Cervantes and Gutierrez (1994). The most of the tested strains showed a very low MIC except Cumminghamella, Gongronella sp and Cladosporium where MIC range was 5 - 10 mM. The morphology of strains was highly affected by the presence of Cu. Their mycelia became diffused compared with the control. The growth rate of fungi tested was reduced and their conidiogenesis was also slowed down. Addition of copper sulphate to the PDA resulted in the growth of the isolated fungal strains and changed the colour and morphology of the mycelium Venkateswerlu and Stotzky (1989). Cladosporium sp (KC602374),the mycelia became thick in comparison with the control accompanied with the secretion of a deep brown substance, which is probably a response to the stress imposed by the presence of high concentration of Cu in the medium (Figure 12).One of the reasons could be that in media, organic complex-forming substances may diminish the free concentration of essential metal ions and thus influence the competition between essential and toxic metals ,where metal required for nutrition could be substituted for toxic ones (Hughes and Poole,1989). The tolerance of the tested fungi to high copper concentrations could be related to metallothioneins and other thiol compounds which may be promising detoxifying agents for copper as reported by Malik (2004); Dusrun (2008).

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

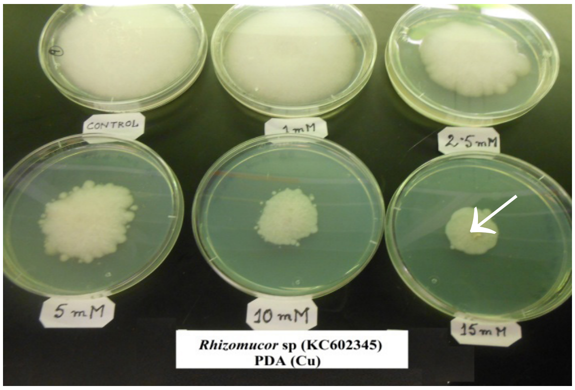

Figure 11: Growth of *Rhizomucor* sp (KC602345) after exposure to different concentrations of copper ions for 10 days.

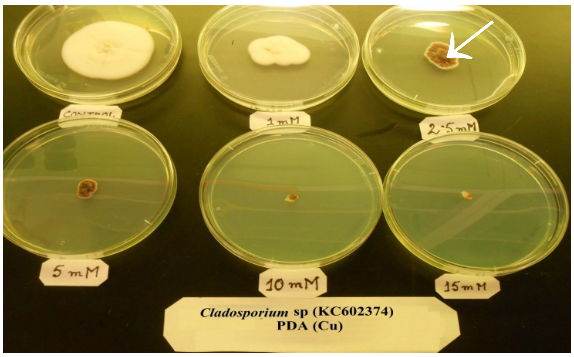

Figure 12: Growth of Cladosporium sp (KC602374) after exposure to different concentrations of copper ions for 10 days.

Zinc is essential for all organisms, although at higher concentrations it can be toxic .The majority of fungi tested were able to grow at a zinc concentration of 10mM or higher. The fungal colour and morphology were both affected by high Zn concentrations as observed by Balsalobre et al (2003). The mycelium became thick in comparison with the control. Moreover, the growth of Fusarium isolate on agar medium at high zinc concentration was accompanied with the secretion of a violet pigment which is probably a response to the stress imposed by the Zn in the medium. The zinc MIC was in the range 20 -25 mM, 15 - 20 mM, 10- 15 mM and 5-10mM for the Fusarium sp. Cadmium is highly toxic to organisms even at very low concentrations. It inhibited DNA replication and appears to make it more susceptible to nucleolytic attack (Volesky and Holan, 1995) (Figure 13). Cadmium exhibits a strong affinity to glutathione and sulfhydryl groups in proteins and can cause cellular damage.When the concentration of cadmium increased in the media, the absorbance of the fungal culture decreased as observed by Darlington and Rauser (1988) .The isolates Trichoderma, Aspergillus niger and Penicillium sp showed a high MIC with 15-20mM (Figures 14 & 15).It must also be taken into account that the contamination at the polluted sites is usually not caused by a single metal and that the selection is probably driven either by the most toxic element or by different metals acting synergistically and widely studied by (Baldrian and Gabriel 2003; Gadd and Sayer 2000; Svecova et al. 2006).

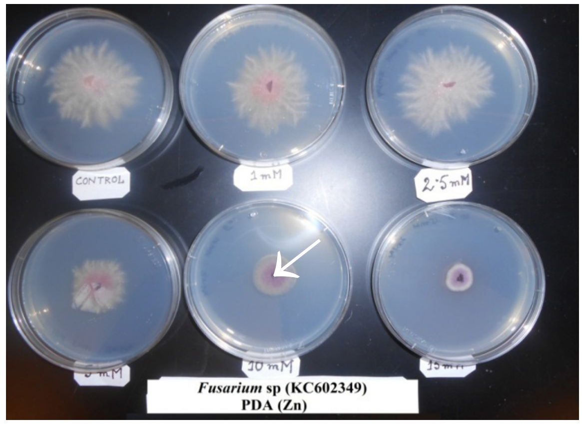

Figure 13: Growth of *Fusarium* sp (KC602349) after exposure to different concentrations Zinc ions for 10 days.

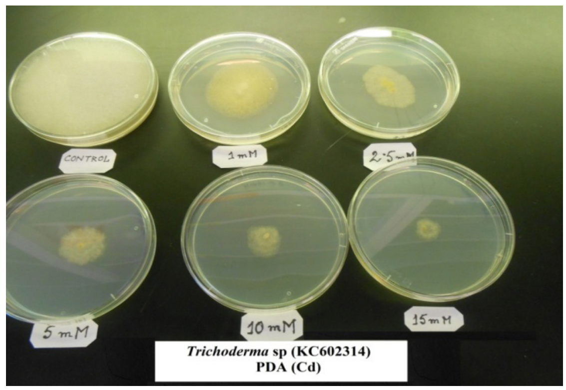

Figure 14: Growth of *Trichoderma* sp (KC602314) after exposure to different concentrations of Cadmium ions for 10 days.

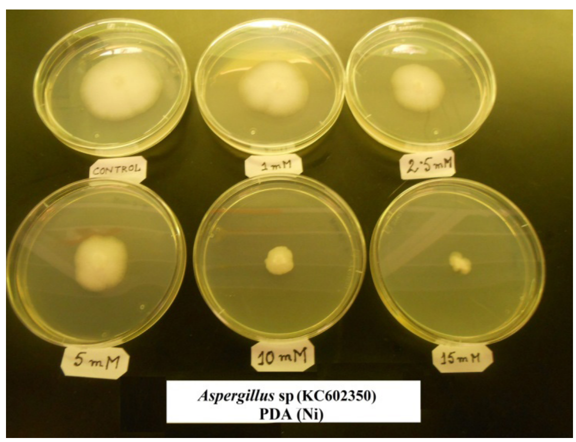

Figure 15: Growth of *Aspergillus* sp (KC602350) after exposure to different concentrations of cadmium ions for 10 days.

Conclusion

Our findings indicate that fungal populations isolated from trace metal-contaminated sites have the ability to resist higher concentrations of trace metals like Zinc, Copper, Nickel and Cadmium. The tolerance and the resistance of the strains depended much more on the fungus tested than on the sites of its isolation. The study also reveals the MIC of different filamentous fungi which would be important tool for future bioremediation program in the contaminated site. This variation in MIC may be explained by the development of tolerance or adaptation of the fungi to trace metals. The isolated fungal starins like **Aspergillus, Penicillium, Rhizomucor,**

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Trichoderma, Fusarium, Cladosporium, Hypocrea were the most tolerant to all the metals tested. So these isolated fungi could be of great interest for habititation of polluted site contaminated with various trace metals. These strains were the found tolerant to all metals as tested and thereby making them most promising strains for further investigation and biomonitoring by their vital ability to remove trace metals from contaminated paper mill soil and environments.

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REFERENCE

Atlas, R.M. (1984). Use of microbial diversity measurements to assess environmental stress. In: MJ Klug,CA Reddy (eds) Current Perspectives in Microbial Ecology. American Society for Microbiology, Washington, DC, pp 540-545. | Baldrian, P. (2003). Interactions of trace metals with white-rot fungi. Enzym. Microb. Technol 32 : 78-91. | Baldrian P and Gabriel, J.(2003). Lignocellulose degradation by Pleurotus ostreatus in the presence of cadmium. FEMS Microbiol. Lett 220:235-240. | Balsalobre, L., De Siloniz, M.I. and Valderrama, M.J.(2003). Occurance of yeasts in municipal wastes and their behavior in presence of cadmium copper and zinc. J.Basic. Microbiol. 43: 185-193. | Burford, E.P., Fomina, M., and Gadd G.M. (2003). Fungal involvement in bioweathering and biotransformation of rocks and minerals. Mineralogical Magazine 67: 1127-1155. | Burgstaller, W, and Schinner, F. (1993). Leaching of metals with fungi. J Biotechnol 27: 91-116. | Cervantes, C. and Gutierrez-Corona, F. (1994). Copper resistance mechanisms in bacteria and fungi.FEMS Microbiol Rev 14:121-137. | Darlington, A, B. and Rauser, W.E. (1988). Cadmium alters the growth of the mycorrhizal fungus Paxillus involutus: a new growth model accounts for changes in Branching. Can. J. Bot 66: 225-229. | Dursun, A.Y. (2008). A comparative study on determination of the equilibrium, kinetic and thermodynamic parameters of biosorption of copper (II) and lead (II) ions onto pretreated Aspergillus niger. Biochem. Eng. J. 28: 187–195 | El-Morsy, E.M. (2004). Cunninghamella echinulata a new biosorbent of metal ions from polluted water in Egypt. Mycologia 96: 1183-1189. | Ezzourhi, L., Castro, E. and Moya, M. 2009).Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. Afr J Micr obiol Res 3:35-48. | Fomina, M.A., Alexander, I.J. and Colpaert, J.V. (2005). Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. Soil Biol Biochem 37: 851-866. | Gadd, G.M. (2010). Metals, minerals and microbes: Geomicrobiology and bioremediation. Microbiology, 156: 609-643. | Gavrilesca, M. (2004). Removal of trace metals from the environment by biosorption. Eng. Life Sci 4: 219-232. | Ghorbani, Y., Oliazadeh, M. and Shahvedi, A. (2007). Use of some isolated fungi in biological leaching of aluminum from low grade bauxite. Afr J Biotech11, 1284-1288. | Howe, R., Evans R.L. and Ketteridge, S.W. (1997). Copper-binding proteins in ectomycorrhizal fungi. New Phytol 135: 123-131. | Hughes, M.N. and Poole, R.K. (1989). The functions of metals in microorganisms. Metals and Microorganisms. London: Chapman and Hall. | Malik, A. (2004). Metal bioremediation through growing cells. Environ. Int 30:261-278. | Melgar, M.J., Alonso, J. and Garcia, M,A. (2007). Removal of toxic metals from aqueous solutions by fungal biomass of Agaricus macrospores. Sci. Total. Environ 385:12-19. | Parknikar, K.M., Pethkar, A.V. and Puranik, P.R. (2003).Bioremediation of Metalliferous wastes and products using inactivated microbial biomass. Ind. J. Biotechnol 2:426-443. | Rao, K. R., Rashmi, K. and Latha, J.N.L (2005). Bioremediation of toxic metal ions using biomass of Aspergillus fumigates from fermentative waste, Ind. J. Biotech 4: 139-143. | Renata, V. and Elena, S. (2006). Metal tolerance of yeasts isolated from water, soil and plant environments. J. basic Microbiol. 46:145-152. | | Sayer, J.A. and Gadd, G.M. (1997). Solubilization and transformation of insoluble metal compounds to insoluble metal oxalates by Aspergillus niger. Mycological Research 101: 653-661. | Sabat, J. and Gupta, N. (2009). Iron ore solubilization by Penicillium restrictum: effect of carbon source and incubation days. Am-Euras. J. Agron 2: 43-44. | Svecova, L., Spanelova, M. and Kubal, M. (2006). Cadmium, lead and mercury biosorption on waste fungal biomass issued from fermentation industry. I. Equilibrium studies. Separation and Purification Technology 52, 142-153. | Sun, F. and Shao, Z. (2007). Biosorption and bioaccumulation of lead by Penicillium sp. Psf-2 isolated from the deep sea sediment of the Pacific Ocean. Extremophiles 11: 853-858. | Valix, M., Tang, J.Y. and Malik, R. (2001). Heavy metal tolerance of fungi. Minerals Eng., 14: 499-505. | Volesky, B. and Holan, Z.R. (1995). Biosorption of heavy metals. Biotechnol. Prog 11: 235-250. | Venkateswerlu, G and Stotzky, G. (1989). Binding metals by the cell walls of Cunninghamella blakesleeana growing in the presence of copper or cobalt. Appl Microbiol Biotech 31:619-625. | Wang, J. and Chen, C. (2006). Biosorption of heavy metals by Saccharomyces cerevisiae: a review. Biotechnol Adv 24:427–451. | White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for Phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ ,White TJ(eds) PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, pp. 315-322. | Zafar, S., Aqil, F. and Ahmad, I, (2007). Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agriculture soil. Bioresour. Technol 98: 2557-2561. |