Toxic Metal Effect on Filamentous Fungi Isolated From the Contaminated Soil of Multan and Gujranwala

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Abstract-Considering the importance of filamentous fungi for bioremediation of wastewater and contaminated soils, this study was planned to investigate the metal tolerance potential of indigenous filamentous fungi. Certain metals are important to biological actions. However all metals, whether essential or inessential will show toxicity at certain levels. During 2012 total 17 fungi were isolated and preserved from contaminated peri-urban agricultural areas of Multan and Gujrawala for further detail investigation of heavy metal tolerance. Aspergillus niger, Aspergillus fumigatus and Aspergillus flavus isolated from both soil and water samples while Aspergillus terreus and Penicillium sp were only isolated from soil samples of Multan and Aspergillus versicolor, Aspergillus flavus, Fusarium oxysporum, Aspergillus niger which were isolated from contaminated soils and water samples while Penicillium sp was isolated from only water samples of Gujranwala. These few fungal isolates were selected for tolerance to metal Cu (SO4)2.5H2O, Cd (NO3)2, Cr (NO3)2 and Pd (NO3)2. The tolerant strains were selected with increasing metals concentration of 100ppm and compared to control in the medium. The degree of tolerance was measured by radial growth (cm) in the presence of various heavy metals and compare to the control, which contain no heavy metals. The present study investigation concludes isolates Penicillium .sp and Aspergillus flavus isolated from soil of Gujranwala show maximum tolerance index 2.1 at 100ppm toward Cr and 4.8 at 100ppm toward Cd respectively. Aspergillus Versicolor (isolated from waste water) exhibit considerable highest tolerance index toward Cu and Pb while show a sensitivity against other metals. The present study indicates that in future similar strains will be tested with other heavy metals for the confirmation of tolerance and tolerant strains will be used for bioremediation of heavy metals.

Index Terms-Filamentous fungi, bioremediation, metal tolerance, soil and water fungi, tolerance index

1.INTRODUCTION:

Heavy metals are environmental contaminants and not a new phenomenon. Contamination of heavy metals is a big problem for environment in our food supply and they act as contaminating agents [1-2].

Heavy metals are an imprecise term used to describe more than dozen elements that are metals or metalloids (elements that have both metals and non metal characteristics).

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Example of heavy metals includes cadmium, lead, mercury copper, nickel, and manganese. Generally, heavy metals have densities above 5 g/cm³ [3]. They are essential part of all living organisms and also present naturally in trace amount in our soil. Metal contamination man made sources are due to activities in industries, emissions of automobile, coal fired power generation plants [4-5].

Toxic metals are metals that are poisonous soluble compounds and they are not essential minerals and have no any biological role. Throughout the world major environmental problem is a heavy metal contamination. But due to their some technical importance they are used in many industries and waste water from these industries has perpetual toxic effects on human beings and environment [6].

Because they are constant in all parts of the environment and cannot be tarnished or destroyed easily. In ecosystem heavy metals can cause the geo-accumulation, biomagnifications and bioaccumulation. Iron, Copper, Zinc, Nickel (heavy metals) and trace elements are significant to perform the proper functions of the biological systems however their excess or deficiency can cause many disorders [7]. Industrial units working without any Environmental Impact Assessment and Planning (EIAP) in big cities of Pakistan. Due to industrial pollutants (organic, inorganic, toxic metals) soil, air and water are polluted [8]. The main issue in pakistan is increasingly becoming an environmental, health, economic and planning heavy metals contamination [9].

On the Earth's surface thin layer of material called soil having roots of plants. Soil is a most important group of microorganisms and main habitat for all species. The soil micro biota is involved in the breakdown and production of organic compounds as well as it dynamically responsible for weathering of primary minerals and plant nutrients cycling [10].

Fungi is one of the members of group of eukaryotic organisms and is classified as kingdom. Fungi that includes yeasts, molds and mushrooms and it is separated from bacteria, plants and animals. There are many differences between plants and fungi. One of the important differences is that fungal cell walls contain chitin and plants cell wall contains cellulose [11]. Due to this major difference and some other differences fungi are grouped separately in a separate kingdom and are named as *Eumycota* also known as *Eumycetes* or true fungi . They also have familiar ancestor that is a *monophyletic group*. Fungal group is different from myxomycetes and oomycetes. Soil micro biota's very important component is fungi dominating the soil biomass as compare to bacteria and depends on nutrient conditions and soil depth [12]. Fungi can store large quantity of metals [13].. Due to this property of fungi is very important for organisms which grow in polluted habitats as well as for heavy metals potential binding and removal of waste waters and other aquatic substrata in natural environments [14].

Soil Pollution due to heavy metals not only effect the microorganisms functioning but also induces population structure alteration. Filamentous fungi show significant tolerance for heavy metals as well as for polluted habitats they are dominant organisms [15]. Fungi can gather large quantity of metals [13].

During the last three decades like many other cities of Pakistan, Multan and Gujranwala going through a period of fast industrial growth and large number of new industries has emerged in Multan and Gujranwala area such as textile, leather goods and fertilizer. These Industries not only generate solid waste and liquid and cause contamination of soil and nearby water bodies by organic and inorganic waste of these industries [16].

The present study main perpose is to determine the tolerance index of micro fungal flora of contaminated soil and water samples against four different toxic metals (Cr,Cu,Cd and Pb). For this

purposes soil and water samples were collected from area of Multan and Gujranwala. Physiochemical parameter of soil samples was analyzed to know the nature of soil and concentration of metals were also determined. The autoclaved distilled water was consumed to isolate the filamentous fungi from water and soil contaminated with heavy metal samples. PDA was used as growth media to support the fungal proliferation.

2. Materials and Methods

2.1. Sampling and sampling site

For present investigation Total 17 (Contaminated soil 10 and 07 waste water used for irrigation) samples were collected from Multan and Gujranwala peri-urban agricultural area of Pakistan. The water of both areas were contaminated by sewage and industrial effluents and contains heavy metals and toxic chemicals. Soil and water samples were collected for heavy metals analysis, filamentous fungal isolation and to check their tolerance index toward heavy metals.

2.2. Sterilization of Apparatus

Petri plates, media bottles, distilled water, McCartney bottles and syringes will be sterilized in autoclave. For sterilization purpose all apparatus will be autoclaved for 40 minutes at 121°C. After autoclaving all sterilized material will be dried in hot air oven at 95°C.

2.3. Media Preparation

Potato dextrose agar (PDA) was used as the growth media [17]. PDA (29 g) was dissolved in 750 ml of sterilized water. After that, media was autoclaved at 121°C for 40 minutes. In order to retard the bacterial growth, 30mg/L streptomycin was also added in the media [18-19](Martin, 1950; Iram *et al*., 2012).

2.4. Preparation of Plates

After autoclaving the media, it was allowed to cool at room temperature. When temperature of media was at 60°C then at that time 30 mg/lit of streptomycin was added in it to destroy the bacterial growth [18].

Then, this media was poured in petri plates and then was left over for 24 hours so that the media could get solidified. After solidification of media present in plates, these plates were placed at room temperature in an inverted position to avoid any water content [18]. (Martin, 1995). This all work was done in laminar flow to avoid any kind of bacterial growth.

2.5. Preservation and Identification of fungi

Morphological studies were carried out by classical method in which the compound microscope was used at magnification of 100X, 400X and 1000X in which characterization was possible by macroscopic characteristics (shape, diameter, quality, appearance of colony) and microscopic (conidia shape, conidia structure, presence of reproductive structures, measurement on hyphal color, septation and presence of sterile mycelium) characteristics [20]. With the help of literature fungus pure culture identification was possible [21-22]. Fungi were preserved on slants of PDA for further studies.

2.6. Screening and Toxic Metal Experiment

For the screening of metal resistant fungal isolates, PDA medium will be amended with 100ppm of (CU, Cd, Cr, Pd) and a disk of mycelium will be inoculated aseptically on amended and unamended PDA plates in triplicates. The inoculated plates will be kept in incubator at 28±1°C for 7 days. Effects of the heavy metals on the growth of the isolates will be anticipated by measure the colony diameter extension against the medium without metal (control). Metal Tolerance Index (T_i) will be calculated as the ratio of the total radius of the control colony to that of the uncontrol colony [23].

$$T_i = \frac{D_t}{Du}$$

Where the D_t is the diameter (in cm) of treated colony and D_u is the diameter (in cm) of untreated colony (cm).

3. Results and Discussion

In present investigation the resistance of fungi to heavy metals was studied by tolerance experiment. During April 2012 contaminated soils and collection of water samples was from peri- urban agricultural Multan and Gujranwala areas. Total 17 fungal cultures were obtained for present study for detail investigation of heavy metal tolerance. The main purpose of the present study was to test the tolerance index of filamentous fungi of genra i.e., *Aspergillus* , *Pencillum* and *Fusarium* against heavy metals Cr , Pb, Cu and Cd.

3.1. Heavy toxic metal content in contaminated soil and water:

3.1.1. Growth of fungal isolates at Copper:

Growth rate of various metals can be expressed by assessing the tolerance index. Tolerance index was assessed to evaluate the growth rate of fungi by the effect of heavy metal. This tolerance index was designed by measuring growth in the presence of metal (control) divided by the growth of the fungi in the same period in the absence of metal (uncontrol). The present (figure 1) regarding Cu metal content collected from Multan and Gujranwala contaminated soil and water samples shows that *Aspergillus Versicolor* show maximum tolerance index 2.88 at 100 ppm of Cu concentration in Gujranwala water and there was 2 time more growth as compare to control. While *Aspergillus flavus* show minimum tolerance index, i.e 0.5 at 100 ppm of Cu concentration in Multan water and it shows less growth as compare to control.

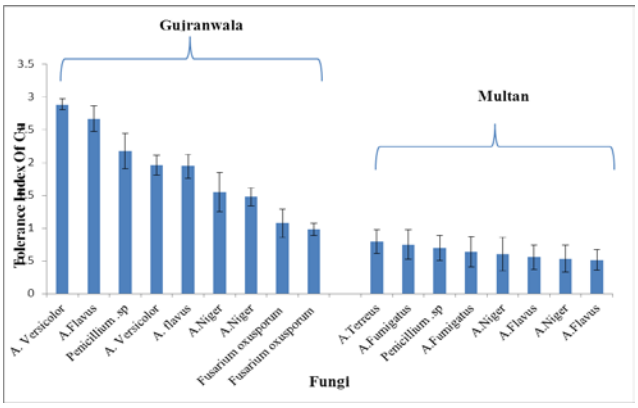

Figure 1: Tolerance Index of fungi isolated from Multan and Gujranwala samples against Cu

3.1.2. Growth of fungal isolates at Chromium:

The present study regarding Cr metal content collected from Multan and Gujranwala contaminated soil and water samples shows that *Penicillium sp* show maximum tolerance index 2.1 at 100ppm of Cr concentration in Gujranwala soil and it shows 1 time more growth as compare to control. *Aspergillus niger* show minimum tolerance index 0.5 at 100ppm of Cr concentration in Multan water and it shows less growth as compare to control. (Figur 2).

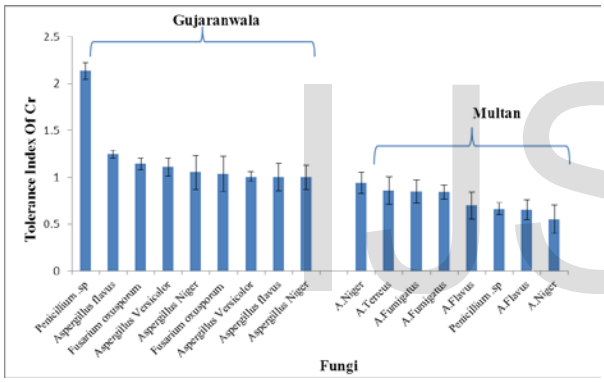

Figure 2: Tolerance Index of fungi isolated from Multan and Gujranwalasamples against Cr

3.1.3.Growth of fungal isolates at Cadmium:

The present study as regards Cd metal content collected from Multan and Gujranwala contaminated soil and water samples shows that *A. Flavus* show maximum tolerance index 4.8 at at 100ppm of Cd concentration in soil of Gujranwala and it shows 3 time more growth as compare to control and *Aspergillus niger* show minimum tolerance index 0.2 at 100ppm of Cd concentration in water and it shows less growth as compare to control(Figure 3). Means growth decline shows significant change on growth behaviour.

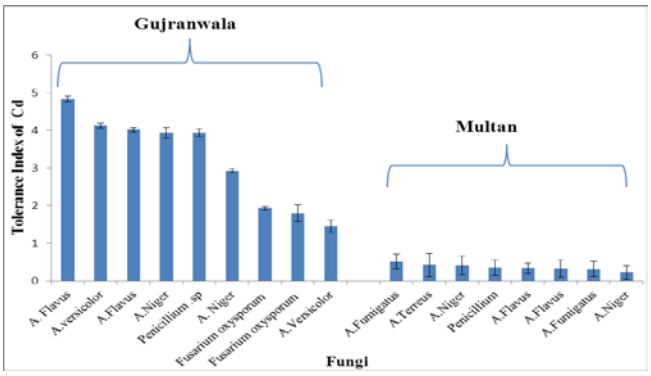

Figure 3: Tolerance Index of fungi isolated from Multan and Gujranwala samples against Cd

3.1.4.Growth of fungal isolates at Lead:

The figure (4) shows Pb metal content collected from Multan and Gujranwala contaminated soil and water samples shows that *Aspergillus versicolor* show maximum tolerance index 2.6 at 100ppm of Pb concentration in water of Gujranwala and it shows 2 time more growth as compare to control and *Aspergillus niger* show minimum tolerance index 0.5 at 100ppm of Cu concentration in water and it shows less growth as compare to control.

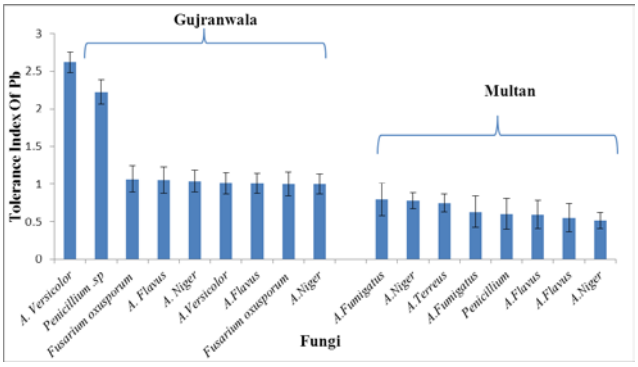

Figure 4: Tolerance Index of fungi isolated from Multan and Gujranwala samples against Pb

Heavy metal long term exposure in water can produce a significant change in microbial populations, their number and activity reduce [24].The high content of heavy metal in treated (control) soil is likely due to long term application of waste water containing heavy metals [25].

Determination of tolerance index toward the heavy metal indicated tolerance capability of individual isolate. Some were sensitive, moderately tolerant and tolerant. This tolerance index was taken as the measured growth in the presence of metal (control) divided by the fungi in the same period in the absence of fungi (uncontrol).

The tolerance index of species of *Aspergillus flavus*, *Aspergillus versicolor*, *Fusarium* oxysporum , *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus fumigatus* and *Penicillium sp* were observed in the particular conditions .

The results of present study *Aspergillus* was the dominant genera among the Multan and Gujranwala soils and water and exhibit highest tolerance index toward soil and water. Because in Pakistan environmental and economic issues is heavy metals contaminated land, as combination of poorly plan effluent disposal techniques and a rapidly growing population has lead to gradual accumulation of heavy metals in soil and water of Pakistan. In Pakistan the EPA has reported the heavy metals including lead, arsenic, chromium, mercury and zinc etc. level in effluents and soil near tanneries and textile mill greatly exceed the safety limit levels standardized by the National Environment and Quality Standard (NEQS) [26].

In the present work evaluated the difference in metal resistance among different isolates of fungus from contaminated soil and water of Multan and Gujranwala.

Similar study reported by[19].highest tolerance of Cr in soil because heavy metals enter the soil and get fixed to the soil components that tends to accumulate large quantities of heavy metals in soil which persist and have long lasting effects in the soil.

According to previous reports [27] the higher concentration of Cd is because of super phosphate fertilizers.

According to [28] in the water samples Pd and Cu concentration was above the permissible limits respectively. Heavy metal long term exposure in water can produce a significant change in microbial populations, their number and activity reduce [24].

Copper metal at high concentration is toxic to for *A. terreus* and *A. alternata* high concentration of Cu metal is toxic as their growth decrease with increase in Cu concentration.

Similar results of fungi were also observed from metal contaminated agricultural soil belonged to genera *Aspergillus, Fusarium Penicillium, Alternaria, Geotrichum,* and *Trichoderma* showed a significantly heavy metals tolerance. The minimum concentration of Cu isolate depending on the (MIC) ranged from 0.6 to 9 mg/ml [29].

According to the research of [30]. *Aspergillus niger* was found to accumulate more of lead in the mycellial mat as the concentration increased and when compared to the age of cells on 7th day, mycellial mats were found to accumulate more of the lead. *Aspergillus niger* was seen to grow even at 100mg/l concentration without any inhabitation.

The high metal tolerant species most probably have developed the physiological adaptation mechanism for surviving in elevated Cd concentrations [31-32]. According to these results genera *Aspergillus* have high capacity to biosorb Cd as well as other heavy metals [33-29-34].Similar metal tolerance differences among the isolates of the same genus have also been observed in this study.

In the present investigation no general pattern found which could fit all the strains. Every strains of same species can have different physiological adaptation to react differently even with same metal at same concentration in water and soil of Multan and Gujranwala.

4.Conclusion

It is concluded from our research that *Aspergillus* is the main dominant and wide occurring genera in heavy metal contaminated samples which indicates its resistance towards harmful heavy metals and have the ability to show highest tolerance index toward copper, cadmium and lead . The resistance and tolerance of the isolates depend more on the fungus tested as compare to on the sites collection. This variation could be explained by tolerance development or fungi adaptation to heavy metals. From all the collected samples the Gujranwala soil and water show more tolerance toward the heavy metals as compared to Multan area.

This study recommends that the species of *Aspergillus* and *Penicillium* found in the soil and water samples of Gujranwala and Multan should be utilized for the bioremediation process. Fungi have been widely used in bioremediation of industrially polluted soils and waters, specifically in the removal of hydrocarbons and heavy metals [26].The results obtained confirmed that isolates response to heavy metals depend on metal tested concentration in medium and also on isolate under concern.

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