Toxic Effect of Heavy Metals on Filamentous Fungi Isolated From Contaminated Soil of Kasur

Anam Rasool[1], Shazia Iram[2]

Abstract-It is well-known that certain varieties of metals are necessary for plant growth and for varieties of biological actions. However, whether the metals are essential or non-essential, they are noxious or toxic to certain levels. The metals become toxic or hazardous when their limit exceeds from permissible limits or standards set by EPA. Sixteen fungi were isolated from heavy metal polluted sites of peri-urban areas of Kasur. Filamentous fungi isolated belonged to the genera Aspergillus spp., Aspergillusnidulans, Aspergillusochraceus, Geotrichum sp., Rhizopus sp., Curvularia spp., Fusarium sp., Aspergillusniger, Penicillium sp., Acremonium sp. and Aspergillusfumigatus. The heavy metal resistance development of Aspergillus sp., Aspergillusnidulans, Geotrichum sp., Rhizopus sp., Curvularia sp., Fusarium spp., Aspergillusniger, Penicillium sp., Acremonium sp. and Aspergillusfumigatusstrains in the presence of Pb(NO3)2, Cd (NO3)2, Cr (NO3)2 and CuSO4.6H2O was studied up to concentration of 100ppm. The adaptive performance was measured from the development frequency of the strains with time. The effect of metal type, the metal concentration and the type of strains on this adaptive performance was examined. The results discovered that the Penicillium sp., Acremonium sp., Curvularia sp. and Aspergillusfumigatuswere highly tolerant/resistant towards the metal (Pb(NO3)2, Cd (NO3)2, Cr (NO3)2 and CuSO4 .6H2O) concentrations. In future, the same fungal species will be verified with other metals for the clear understanding of tolerance.

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

1. INTRODUCTION

In all over the world Contamination of sediments and natural aquatic receptors with different pollutant is a major environmental problem.[1-3]Industrial development and urbanization particularly in developing countries have led to the accumulation of heavy metals and petroleum hydrocarbons in the environment [4-8] (Heavy metal is one of the members of ill-defined division of elements which involves of metalloids, lanthanides, actinides and transition metals that have metallic possessions [9]. The metals above the threshold perimeter are toxic and lethal [10]. Throughout the world major environmental problem is a heavy metal contamination. These metals are poisonous soluble compounds and they are not essential minerals and have no any biological role. But due to their some technical importance they are used in many industries and

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Waste water from these industries has perpetual toxic effects on human beings and environment [11]. Because they are constant in all parts of the environment and cannot be tarnished or destroyed easily. Heavy metals contamination is a major problem of our food supply [12-13].

Toxic heavy metals arriving the ecosystem may cause the geo-accumulation, bioaccumulation and biomagnifications. Different heavy metals like Fe, Cu, Zn, Ni and other trace elements are significant to perform the proper functions of all biological systems but their deficiency or excess amount could lead to a number of disorders in our environment [14].

Soil pollution is caused by the existence of noxious compounds in the soil. If the poisonous composites are present in high amounts than the standard concentrations and this eventually affects the human health and the environment. It can also be caused by the presence of xenobiotic compounds which add up to the soil through agricultural means of disposal, industrial discharges, precipitates of acidic materials and by the fallout of radioactive material [15].

Among microorganisms fungi are very important microorganism; it can tolerate heavy metals to a limit and can also help to remove heavy metals from contaminated soil. Fungi and yeast biomasses are known to tolerate heavy metals [16-19]. 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 [20].They offer the advantage of having cell wall material which shows excellent metal-binding properties. They are used for bioremediation because of their mycelia nature and ability to accumulate metals of all families.

Many fungal species such as *Rhizopusarrhizus, Penicilium spinulum* and *Aspergillus niger* have been extensively studied for heavy metals biosorption and the process mechanism seems to be dependent upon species [21-23]. The accumulation of heavy metals in agricultural soils is of increasing concern due to food safety issues and potential health risks as well as its determinate effects on soil

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ecosystem. In most parts of Pakistan, untreated city effluent is utilized for growing vegetables around large urban settlements such as Kasur. Farmers use it as a source of irrigation water and plant nutrients. However, its continuous use may have serious environmental implications, since it also contains heavy metals. Use of untreated city effluent for irrigation without risk assessment and management could be a serious hazard, impacting soil and crop quality and ultimately human health. In Pakistan, heavy metals contaminated land is becoming an environmental and economic issue. Combination of inadequately plan effluent disposal techniques and a rapidly growing population has lead to gradual accumulation of heavy metals in the soils and water of Pakistan. According to EPA, the industrial effluents being dumped into the land, by electroplating, tanner industries and textile mills and contains toxic metals such as Cr, Ni, As, Hg, Cu, and Pb, which have contaminated the soil and the biota residing in and around soils in Pakistan [24](EPA, 1990). Over the years there have been increasing concerns over the rising level of heavy metal contamination, as they tend to persist in the environment, accumulating in the biota, thereby entering the food chain causing deleterious harm to higher animals and humans [25]. The aim of present study The aim of present study was to measure the potential of fungi to grow in presence of heavy metals and to check the tolerance potential of different species against heavy metals. For the present study soil and water samples were collected from peri-urban agricultural areas of Kasur in 2013 which is irrigated by the contaminated industrial water and containing heavy metals and toxic chemicals. Fungi were isolated from the contaminated soil and studied for their tolerance analysis.

2. Materials and Methods

2.1 Study area and samples collection

To study the toxic effect of heavy metals on filamentous fungi was the chief resolution of this study. For present investigation Total 16 (Contaminated soil 11 and 05 waste water used for irrigation) samples were collected from kasur in 2013 and were inspected to check the toxic effect of heavy metals on filamentous fungi. The water being used in Kasur for agricultural purposes contains toxins and impurities i.e. it contains heavy metals, noxious chemicals, municipal and industrial discharges from the nearby industries. From the contaminated soil fungi were isolated and preserved for further detail investigation of heavy metal tolerance. The list of soil and water samples with area code given in table 1 & 2.

Table: 1

List of fungal isolates from soil samples

Sr. no.	Fungi	Fungi code	Location
1	*Aspergillus sp.*	KS-1	Kasur
2	*Aspergillus nidulans*	KS-2	Kasur
3	*Aspergillus ochraceus*	KS-3	Kasur
4	*Geotrichum sp.*	KS-4	Kasur
5	*Rhizopus sp.*	KS-5	Kasur
6	*Curvularia sp.*	KS-6	Kasur
7	*Fusarium sp.*	KS-7	Kasur
8	*Aspergillus niger*	KS-8	Kasur
9	*Fusarium sp.*	KS-9	Kasur
10	*Aspergillus fumigatus*	KS-11	Kasur
11	*Penicillium sp.*	KS-12	Kasur

Table: 2

List of fungal isolates from water samples

Sr. no.	Fungi	Fungi code	Location
1	*Acremonium sp.*	KW-1	Kasur
2	*Aspergillus niger*	KW-2	Kasur
3	*Aspergillus fumigatus*	KW-3	Kasur
4	*Aspergillus niger*	KW-4	Kasur
5	*Aspergillus niger*	KW-5	Kasur

2.2. Sterilization of Apparatus:

Firstly, all the apparatus that was supposed to be used for experimental purposes was washed thoroughly and then was put to be sterilized in an autoclave at 121°C temperature range for about 40 minutes. And after sterilization, all the autoclaved apparatus was oven dried at 95°C.

The apparatus used for experiment included: syringes,

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McCartney bottles, distilled water, media bottles and petri dishes.

2.3.Media Preparation:

For revival of fungal cultures or isolates, the media containing Potato Dextrose Agar (PDA) was used [26].After the preparation of media, it was autoclaved for about 30 minutes at the temperature range of 121°C [27].

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 [28]. 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 [27]. This all work was done in laminar flow to avoid any kind of bacterial growth.

2.5.Identification of Fungi:

At the center of petri plates containing PDA media, a small mass of fungi was placed by taking proper precautionary measures. Then, these plates were placed in an incubator for 7 days at the temperature range of 30°C for the growth of fungal specie. After period of incubation, different colonies of fungi were observed and then they were identified by using light microscope. The identification of these fungal cultures was done on the basis of their microscopic and macroscopic features [29]. Macroscopic features includes its color, its texture, its diameter and shape and its colonial morphology while microscopic features includes presence of reproductive structures, presence of sterile mycelium, septation in mycelium, shape and structure of conidia. The identification of pure fungal isolates was done by studying literature [30-31].

Following were the identified fungal species; *Aspergillus sp., Aspergillus nidulans., Aspergillus ochraceus., Geotrichum sp., Rhizopus sp., Curvularia sp., Fusarium sp., Aspergillus niger, Penicillium sp., Acremonium sp.* and *Aspergillus fumigatus.*

2.6.Screening and Selection of Heavy Metal-Tolerant Microorganisms:

Metal solutions of all the four metals were prepared containing metal salt and PDA in separate bottles. After autoclaving the media at 121°C for 30 minutes, it was poured in autoclaved and oven dried plates in laminar flow after cooling and 30 mg/l of streptomycin was also added in the media bottles before pouring into the plates. The metal salts used were $Pb(NO_3)_2$, $Cd(NO_3)_2$, $Cr(NO_3)_2$ and $CuSO_4 \cdot 6H_2O$. All the plates contained 100 ppm of heavy metal. The plates were put for solidification of media present in it. Then, a disc of fungal specie was placed at the center of plates individually and was incubated for 7 days at 25°C. Three replicates of every strain were cultured. Screening of pure fungal isolates was done on the basis of their tolerance to Cr, Cd, Pb and Cu. The inoculated plates will be incubated at 25°C for at least 7 days. The effect of the heavy metal on the growth and physical features of the fungal isolates were tested by measuring their tolerance index. Tolerance index is estimated by measuring growth of fungal isolate in metal containing media against the fungal isolate in controlled media which is having no metal salt in it. The fungal isolates showing enhanced growth after the period of incubation were considered tolerant to the heavy metal. [32].

T. I. = Radial growth rate in metal treatment÷
Radial growth rate in control

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

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

3.RESULTS AND DISCUSSION:

Pollution of soil has increased due to industrial development and expansion of population. Heavy metals belong to transition group present in the periodic table and they have relatively high densities. Heavy metals are also known as trace elements and some are present in low quantities in the soil while some are present abundantly. In general, the elements of consideration are copper, nickel, lead, zinc chromium and cadmium [33].

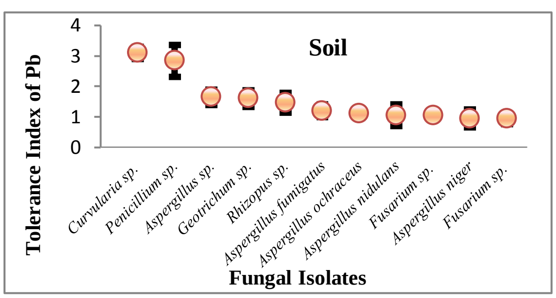
Heavy metals have directly or indirectly effects on the growth and metabolism of fungal species and sometimes they have harmful effects on growth of fungal species if exceeds from permissible limits [34]. Heavy metals existing in the environment have effects on fungal growth, its community and diminish its diversity and shifts towards more resistant species. Certain types of metals are necessary for plant growth but they all show toxic effects to the environment at certain standards. For example Chromium contamination in the environment occurs due to metal refining, tanning industries, metal electroplating, liquid fuels of power plants, weathering of rocks, brown and hard coal and due to industrial and municipal discharges [35] . Chromium is widely distributed and its concentration in the soil ranges up to 4g/kg. This concentration is easily available to micro flora in chromium rich soils. Increased industrialization and development has resulted in increased deposition of heavy metals mainly lead in the environment. Increased concentration of lead in soil and vegetative crops have attributed to lead containing products from combustion of leaded gasoline [36] and vapor waste from coal burning and smelting activities and lead arsenic pesticides and phosphate fertilizers.

In the current study, several fungal species were isolated from the polluted soil samples of Kasur, where heavy metals and other contaminants have been discharged in industrial emissions for some years. Higher volumes of heavy metals were found in soil and water samples of Kasur. Soil fungal species capable to develop in the presence of heavy metals were sequestered. The isolated fungal species belong to *Aspergillus, Geotrichum, Fusarium,*

Penicillium,Rhizopus,Curvularia and *Acremonium species.*
Total 16 fungal cultures were obtained for present study and detail investigation of heavy metal tolerance. The main purpose of the present study was to test the tolerance index of filamentous fungi of i.e., *Aspergillus sp., Aspergillus nidulans, Aspergillus ochraceus, Geotrichum sp., Rhizopus sp., Culvularia sp., Fusarium sp., Aspergillus niger, Penicilliumsp., Acremoniumsp.* and *Aspergillus fumigates* against heavy metals Cr ,Pb, Cu and Cd.

3.1.Tolerance Analysis of Fungal Isolates of Soil and water Samples Against Pb $(NO_3)_2$

Figure 1 represents the growth rate of fungal species at the metal concentration of 100 ppm during different days. It indicates the tolerance index of fungal isolates of soil and water samples against $Pb(NO_3)_2$. It is clear from the figure that *Curvularia sp.* (KS-6) and *Penicillium sp.* (KS-12) have shown greater growth phase in the presence of 100 ppm concentration of Pb $(NO_3)_2$. So, it means that *Curvularia sp.* and *Penicillium sp.* are highly tolerant towards Pb $(NO_3)_2$as it has not affected the growth rate of these species while *Fusarium sp.* (KS-9) and *Aspergillus niger* (KS-8) are sensitive towards 100 ppm concentration of Pb $(NO_3)_2$. *Aspergillus sp.* (KS-1), *Geotrichum*sp. (KS-4), *Fusarium sp.* (KS-7), Aspergillus fumigates (KS-11), *Aspergillus ochraceus*(KS-3) and *Aspergillus nidulans*(KS-2) are moderately tolerant towards 100 ppm concentration of $Pb(NO_3)_2$.It is evident from the figure that *Acremonium sp.* (KW-1)has shown greater growth rate in the presence of 100 ppm concentration of Pb $((NO_3)_2$. So, it means that *Acremonium sp.* is highly tolerant towards Pb $(NO_3)_2$as it has not affected the growth rate of this specie while *Aspergillus niger* (KW-2),*Aspergillus fumigates* (KW-3), Aspergillusniger (KW-4), *Aspergillus niger* (KW-5) are moderately tolerant towards 100 ppm concentration of $Pb(NO_3)_2$



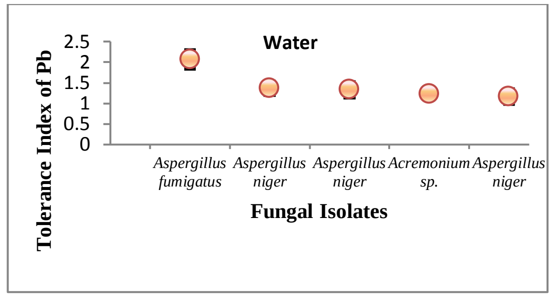
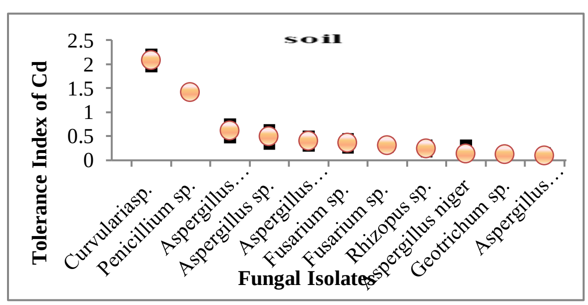


Figure 1: Tolerance index of fungal isolates of soil and water samples against Pb $(NO_3)_2$

3.2.Tolerance Analysis of Fungal Isolates of Soil and water Samples Against Cd $(NO_3)_2$

Figure 2 indicates the growth rate of fungal isolates at the metal concentration of 100 ppm during different days. It indicates the tolerance index of fungal isolates of soil and water samples against $Cd(NO_3)_2$. It has been shown in the figure that *Curvularia sp.* (KS-6) and *Penicillium sp.* (KS-12) have shown greater growth rate in the presence of 100 ppm concentration of $Cd(NO_3)_2$, it means that *Curvularia sp.* and *Penicillium sp.* are highly tolerant towards the concentration of $Cd(NO_3)_2$ as the growth rate of these species has not been affected while *Fusarium sp., Aspergillus niger* (KS-8), *Aspergillus nidulans*(KS-2) and *Geotrichum sp.* (KS-4) showed less tolerance against the 100 ppm concentration of $Cd(NO_3)_2$. *Aspergillus sp.* (KS-1), *Aspergillus fumigatus*(KS-11) and *Aspergillus ochraceus* (KS-3) are moderately tolerant against 100 ppm concentration $Cd(NO_3)_2$.It is obvious from the figure that all the species of water *Acremonium sp.* (KW-1), *Aspergillus niger* (KW-2), *Aspergillus fumigatus*(KW-3), *Aspergillus niger* (KW-4) and Aspergillusniger (KW-5)) are moderately tolerant in the presence of 100 ppm concentration of $Cd(NO_3)_2$. So, it means that $Cd(NO_3)_2$is a poisonous metal and it has retarded the growth rate of fungal isolates.



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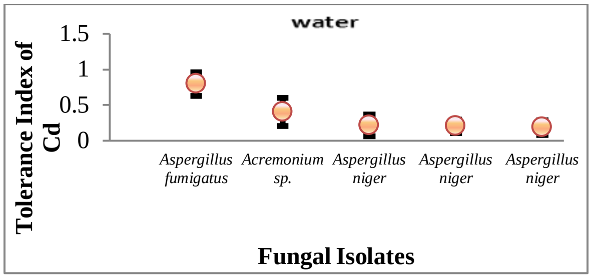
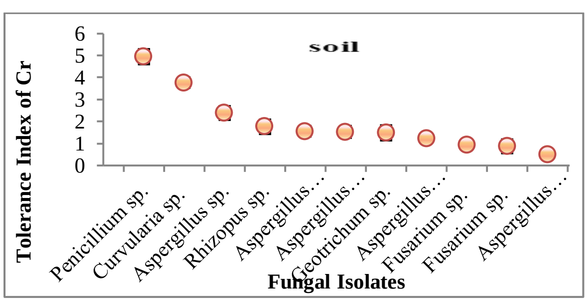


Figure 2: Tolerance index of fungal isolates of soil and water samples against Cd $(NO_3)_2$

3.3.Tolerance Analysis of Fungal Isolates of Soil and water Samples Against Cr $(NO_3)_2$

The growth rate of fungal isolates at the metal concentration of 100 ppm during different days has been represented in figure 3. It indicates the tolerance index of fungal isolates of soil and water samples against Cr $(NO_3)_2$. It has been indicated that *Penicillium sp.* (KS-12) *Curvularia sp*. (KS-6) and *Aspergillus sp*. (KS-1) have shown greater growth rate in the presence of 100 ppm concentration of Cr $(NO_3)_2$. So, it means that *Curvularia sp.* and *Penicillium sp.* are highly tolerant towards Cr $(NO_3)_2$as this metal has not affected the growth rate of these species.*Fusarium sp.* (KS-7), Fusarium sp. (KS-9) and *Aspergillus fumigatus*(KS-11) are sensitive towards 100 ppm concentration of Cr $(NO_3)_2$. Chromium has retarded the growth of these species. The tolerance index of fungal isolates of water samples against Cr $(NO_3)_2$represent that *Acremonium sp.* (KW-1), *Aspergillus niger* (KW-2), *Aspergillus fumigatus*(KW-3), Aspe rgillusniger (KW-4) and *Aspergillus niger* (KW-5) all have shown greater growth rate in the presence of 100 ppm concentration of Cr $(NO_3)_2$. So, it means that these are highly tolerant towards Cr $(NO_3)_2$as it has not affected the growth rate of these species.



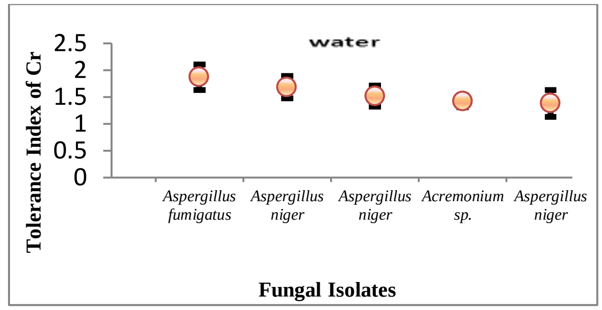
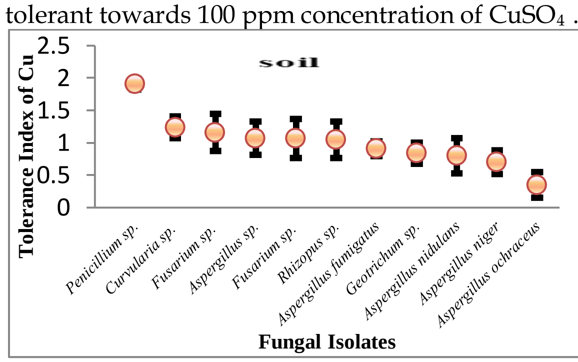


Figure 3: Tolerance index of fungal isolates of soil and water samples against Cr $(NO_3)_2$

3.4.Tolerance Analysis of Fungal Isolates of Soil and water Samples Against $CuSO_4.6H_2O$

Figure 4 represents the growth rate of fungal species at the metal concentration of 100 ppm against $CuSO_4$ $.6H_2O$ for soil and water samples. It is clear from the figure that *Penicillium sp*. (KS-12) have shown greater growth phase in the presence of 100 ppm concentration of $CuSO_4$ $.6H_2O$. So, it means that *Penicillium sp*. is highly tolerant towards $CuSO_4$ $.6H_2O$ as it has not affected its growth rate while *Curvularia sp*. (KS-6), *Fusarium sp*. (KS-7), *Aspergillus sp*. (KS-1) and *Fusarium sp*. (KS-9) are moderately tolerant towards 100 ppm concentration of CuSO4 .6H2O. *Geotrichum sp*. (KS-4),*Aspergillus fumigatus*(KS-11), *Aspergillus ochraceus* (KS-3) and *Aspergillus nidulans*(KS-2)*Aspergillus niger* (KS-8) are less tolerant towards 100 ppm concentration of $CuSO_4$ $.6H_2O$. Fungal isolates of water samples against CuSO4.6H2O that *Acremonium sp*. (KW-1) and *Aspergillus fumigatus*(KW-3) has shown greater growth rate in the presence of 100 ppm concentration of $CuSO_4$ $.6H_2O$. So, it means that these species are highly tolerant towards CuSO4.6H2O as it has not affected the growth rate of this specie while the species of *Aspergillus niger*are moderately tolerant towards 100 ppm concentration of $CuSO_4$ $.6H_2O$



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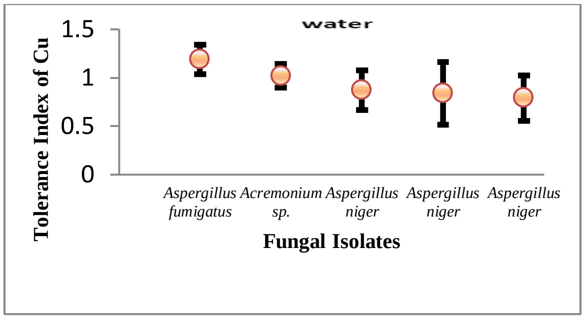

Figure 4: Tolerance index of fungal isolates of soil and water samples against $CuSO_4 .6H_2O$

The species of *Curvularia* and *Penicillium* showed highly tolerance while the species of *Aspergillus, Rhizopus, Fusarium* and *Geotrichum* showed less resistance towards Cd $(NO_3)_2$ in soil samples while in water samples *Aspergillus fumigatus* and *Acremonium sp*. were moderately tolerant towards Cd $(NO_3)_2$.

The species of *Penicillium* and *Curvularia* showed highly tolerance while the species of *Rhizopus* and *Geotrichum* were moderately tolerant and the species of *Aspergillus* and *Fusarium* were less tolerant towards Cd $(NO_3)_2$in soil samples while in water samples *Aspergillus fumigatus* was highly tolerant towards Cd $(NO_3)_2$than *Acremonium sp*. and *Aspergillus niger*.

Penicillium sp showed highly tolerance whiles the species of *Aspergillus, Rhizopus, Fusarium* and *Curvularia* were moderately resistant while and *Geotrichum sp*. showed less resistance towards $CuSO_4 .6H_2O$ in soil samples while in water samples *Aspergillus fumigatus* and *Acremonium sp* were highly tolerant towards $CuSO_4 .6H_2O$.

The species of *Penicillium* and *Curvularia* showed highly tolerance while the species of *Rhizopus* and *Geotrichum* were moderately tolerant and the species of *Aspergillus* and *Fusarium* were less tolerant towards Pb $(NO_3)_2$ in soil samples while in water samples *Aspergillus fumigatus* was highly tolerant towards Pb $(NO_3)_2$ than *Acremonium sp*. and *Aspergillus niger.*

Most plentiful species at the contaminated sites were found to be of *Aspergillus species*. According to the exploration of [32]. on filamentous fungi sequestered from contaminated soils and their results presented that fungi belonged to genera *Aspergillus* were most richly present in all the contaminated sites. The modifications between the experimented locations concerning their productivity on microbial isolates seem to be closely interconnected to the amount of heavy metal contamination. In general, contamination of soil and water by heavy metals may lead to a reduction in microbial variety.

Isolates of the similar species can present a noticeable modification in the levels of metal tolerance. The difference in the metal tolerance may be due to the presence of different forms of tolerance practices or resistance mechanisms showed by dissimilar isolates.

The effect of Co, Cu, Pb, Cr and Zn on the growth of *Curvularia* isolated from the industrialized city of Saudi Arabia at concentrations of 500, 1000 and 2000 μg/ml were determined [37]. *Curvularia* presented good colony growth on heavy metal media at 500μg/ml in association to controlled media.

In summary, it seems that the levels of the heavy metals tolerance attained by investigation of fungi are related to metal levels which can be anticipated to achieve in the natural environments.

Fungi have more potential for remediation due to different features of their violent development, greater biomass, manufacturing and widespread hyphal spread in soil. Furthermore, fungi have been extensively used in bioremediation of industrially contaminated soils and waters, specifically in the elimination of hydrocarbons and heavy metals.

[38]studied 32 fungal species isolated from contaminated water in Egypt for their tolerance to heavy metals and described that *Cunninghamela echinulata* biomass could be used as a bio-sorbent of metal ions in discarded water.

[39]. described favorable bio-sorption for cadmium and chromium by two filamentous fungal species, Aspergillus sp. and *Rhizopus sp*., isolated from metal polluted agronomic zone. It has also been described that *Rhizopus stolonifer* and *Cunninghamela blakesleana* are the two extreme common zygomycete species that have achieved tolerance contrary to copper, cadmium, nickel, lead and cobalt, and are resistant to these metals even in medium with high amounts. The current work reports the categorization of metal resistant microorganisms isolated from contaminated environments. The heavy metal tolerance for each microorganism was resolved and assortment of the utmost resilient strains can be used in bioremediation of heavy metals.

It could be determined that soil and water fungal species of metal polluted territory have established resistance to noxious metals and perhaps improved metal bio-sorption capability. Further comprehensive studies are required to improve the circumstances for extreme bio-adsorption of heavy metals from multi -metal solution and wastewater.

4. CONCLUSION:

From the current research following points were concluded that in the present study soil irrigated with waste water contains tolerant fungus. The species of *Curvularia* and *Penicillium* are highly tolerant to heavy metals as compared to the species of *Aspergillus, Rhizopus, Fusarium* and *Geotrichum*. No morphological changes observed in the tested fungal species at the given metal concentration It is affirmed that the response of the isolates to heavy metals depended on the metal concentration in the medium and on the isolate considered and can be used further for the purposes of bioremediation. Detailed investigation is needed for heavy metal tolerance analysis with other metals.

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REFERENCES

[1] Baldrian, P. Interactions of heavy metals with white-rot fungi. Enzyme Microbiological Technology. 32: 78-91, 2003.

[2] Gavrilesca, M. Removal of heavy metals from the environment by biosorption. Eng. Life Sciences. 4(3): 219-232, 2004.

[3] Malik, A. Metal bioremediation through growing cells. Environment International.30: 261-278,2004.

[4] Adveniyi A A. Determination of cadmium, copper, iron, lead, manganese and zinc in water leaf (talinum triangular) in dumpsite, Journal of Environment International 22: 259-262, 1996.

[5] Bamgbose O, Sibanjo O. Comparative assessment of three methods for cholorinated hydrocarbon determination in fish, Mig. Journal of Science 32: 169-174, 1998.

[6] Ngodigha E N, Olayimiko F O, Oruwari B M, Ekweozor E K E, Whekhe S N. Toxic effects of crude oil in organs and blood cells of west Africa dwarf goat, Nigeria Vitinary Journal 20: 82-91, 1999.

[7] Yamasoet M A, Artaxo P , Miguel A, Allen A G. Chemical composition of aerosol particle from direct emission of vegetation fire in Amazon basin. Water soluble spices and trace elements, Atmospheric Environment 34: 1641-1653,2000.

[8] Adeniyi A A, Folab J A. Determination of total petroleum hydrocarbons and heavy metals in soils with in the vicinity of facilities handling refined petroleum products in logos metropolis, Journals of Environment International 28: 79-82, 2002.

[9] Zevenhoven, R. and Kilpinen, P. Control of pollutants in flue gases and fuel gases. Teknillinenkorkeakoulu41:454-456, 2001.

[10] Gadd. Biosorption of Lead, Cadmium, and Zinc by Citrobacter Strain. Biotechnology Progress. 15: 228-237,1992.

[11] Anonymous. Bioremediation of Arsenic, Chromium, Lead and Mercury. United states Environment Protection Agency, Office of solid waste and emergency response technology innovation office Washington DC, 2004.

[12] Gholizadeh, A., Ardalan, M., Mohammadi, M.T., Hosseini, H.M. and Karimian, N. Solubility test in some phosphate rocks and their potential for direct application in soil. Journal of World Applied Science,6:182-190,2009.

[13] Khair, M.H. Toxicity and accumulation of copper in Nannochloropsisoculata(Eustigmatophycea, Heterokonta) Journal of World Applied Science,6 :378-384, 2009.

[14] Ward, N.I. Environmental analytical chemistry. In: Trace Elements. (eds): F.W. Fifield and P. Haines. Academic and Professional, UK, 1995.

[15] Hayat, S., Ahmad, I., Azam, Z.M., Ahmad, A., Inam, A. and Samiullah. Effect of long term application of oil refinery wastewater on soil health with special reference to microbiological characteristics. Bioresource Technology. 84:159-163, 2002.

[16] Khan, A.G. Relationship between chromium biomagnification ratio accumulation factor and mycorrhizal in plants growing on tannery effluents polluted soils. Journal of Environment International. 26: 417-423, 2001.

[17] Baldrian, P. Interactions of heavy metals with white-rot fungi. Enzyme Microbiological Technology. 32: 78-91, 2003.

[18] Gavrilesca, M. Removal of heavy metals from the environment by biosorption. Eng. Life Sciences. 4(3): 219-232, 2004.

[19] Anand, P., Isar, J., Saran, S. and Saxena, R.K. Bioaccumulation of copper by Trichodermaviride. Bioresource Technology. 97: 1018-1025, 2006.

[20] Gupta, R., Ahuja, P., Khan, S., Saxena, R.K. and Mohapatra, H. Microbial biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions. Curr. Science. 78(8): 967-973, 2000.

[21] Zhou, J.L. and Kiff, R.J. The uptake of copper from aqueous solution by immobilized fungal biomass. Journal of Chemical Technology and Biotechnology,52: 317-330, 1991.

[22] Hafez, N., Abdel-Razek, A.S. and Hafez, M.B. Accumulation of some heavy metals on Aspergillusflavus. Journal of chemical Technology and Biotechnology,68:19-22, 1997.

[23] Kapoor A, Viraraghavan T, Cullimore DR.Removal of heavy metals using the fungus Aspergillus niger., Regina Water Research Institute, University of Regina, Canada, 1998.

[24] EPA. Drafts report Govt. of Punjab, Lahore. Environmental Protection Agency, Govt. of Punjab, Lahore, Pakistan: 51, 1990.

[25] Zhang, L., Zhao, L., Yu, Y. and Chen, C. Removal of lead from aqueous solution by non-living NpzipousNigricans. Water Resource. 32: 1437-1444,1990.

[26] Razak, A.A., Bachman, G. and Rrag, R. Activities of microflora in soils of upper and lower Egypt. The African Journal of Mycology and Biotechnology. 7: 1-19, 1999.

[27] Martin, J.H. On the origin of colonies of fungi developing on soil dilution plates. Transaction of British Mycological Society. 38: 298–301, 1995.

[28] Martin, J.P. Use of acid rose Bengal and streptomycin in plate method for estimating soil fungi. Soil Science. 69: 215-232, 1950.

[29] Zafar, S., Aqil, F. and Ahmed, I. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresource Technology. 98: 13, 2006.

[30] Domsch, K.H., W. Gams and T.H. Anderson. Compendium of soil fungi. Volume1. Academic Press, 1980.

[31] Barnett, H.L. and Hunter, B.B. Illustrated genera of imperfect fungi, Fourth edition. Prentice Hall Inc, 1999.

[32] Ezzouhri, L. Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier. Morocco, African Journal of Microbiology Research. 3: 1-14, 2009.

[33] De-haan, F.A., Zwerman, M., Bolts, G., and Bruggenwert, H. Pollution of soil in soil chemistry: A. basic elements, M.G.M (eds) Elsvierpubl, Amsterdam, Netherland, 192-271, 1987.

[34] Gadd, G.M.The uptake of heavy metals by fungi and yeasts: the chemistry and physiology of the process and applications for biotechnology. In: Eccles Hunt D. S (eds) Immobilisation of ions by bio-sorption Ellis Horwood Ltd, Chichester. pp: 135-147, 1986).

[35] Badura, L. Chrom w środowisku i jegooddziaływanienaorganizmyżywe. ZeszytyNaukowe PAN 5, 1993.

[36] Lagerwerf, J.V. Agriculture and quality of our environment. 85, 1967.

[37] Hashem, A.R. Effect of heavy metal ions on the mycelial growth of some fungi isolated from the Soil of Al-Jubail Industrial City, Saudi Arabia, Department of Botany and Microbiology, College of Science. King Saud University, 1995.

[38] El-Morsy, E.M. Cunninghamellaechinulataa new biosorbent of metal ions from polluted water in Egypt. Mycologia. 96(6): 1183-1189, 2004.

[39] Zafar, S., Aqil, F. and Ahmed, I. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresource Technology. 98: 13, 2006).