Pak. J. Bot., 41(5): 2583-2594, 2009.

FUNGAL TOLERANCE TO HEAVY METALS

SHAZIA IRAM, IFTIKHAR AHMAD*, BARIRA JAVED, SAEEDA YAQOOB, KULSOOM AKHTAR, MUNAWAR RAZA KAZMI* AND BADAR-UZ-ZAMAN*

Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan,
**National Agricultural Research Center, Islamabad, Pakistan*

Abstract

Peri-urban cultivated areas of many cities in Pakistan are being irrigated from municipal and /or industrial wastewater since long. Soil samples were collected from two villages of Faisalabad (Jappaywala and Kajle), three of Rawalpindi districts (Dahgal, Gorakhpur and Sihala) and from the campus of Fatima Jinnah Women University, Rawalpindi (FJWUR) for investigating the status of heavy metals, fungal diversity, and fungal tolerance to heavy metals. There was marked differentiation among the soils for various heavy metals (Zn, Pb, Cd, Ni and Co) and fungal diversity. Forty-one fungal species among the 37 genera were found in these soils. The most common fungal strains viz., *Aspergillus niger, Aspergillus* sp, *Fusarium* sp., and *Penicillium* sp., were tested for tolerance against the heavy metals Zn, Pb, Ni and Cd. The degree of tolerance was measured by minimum inhibitory concentration (MIC) in the presence of the each metal and it was compared with control containing no heavy metals. Among the isolated fungal strains of all locations *Aspergillus niger* was the most tolerant against all the tested heavy metals. It exhibit strong radial growth from 0-40ppm followed by *Aspergillus* sp., *Pencillium* sp., and *Fusarium* sp against all the tested heavy metals. Thus the heavy metals tolerant fungus *Aspergillus niger* has shown a high level of tolerance to all metals tested, which makes it attractive potential candidate for further investigation regarding its ability to remove metals from contaminated soil.

Introduction

Soil is a rich habitat containing all major groups of microorganisms (Mueller & Bills, 2004). The soil microbiota is instrumental in the degradation and synthesis of organic compounds. It is also actively involved in the cycling of plant nutrients and in the weathering of primary minerals (Parkinson & Coleman, 1991). Some of the consequences of anthropogenic pollution are transmission of diseases by water borne pathogens, eutrophication of natural water bodies, accumulation of toxic or recalcitrant chemicals in the soil, destabilization of ecological balance and human health hazards (Bridges *et al.*, 2000; Amisu *et al.*, 2003). Pakistan has the world's largest canal irrigation system, and its economy is based mainly on agriculture. However, canal water is not enough to cope with the crop water requirements and there is need of other resources of irrigation water for crop production. As the main sources of irrigation are canal and tube well water but the quality of groundwater is so poor for the sustainability of agriculture system. For meeting the present demand, use of municipal sewage water containing mainly the domestic liquid waste and industrial effluents, is becoming a common practice. Environmental pollution with heavy metals is a global concern being everywhere, though to different degrees and is specific to certain parts of the biogeosphere. Living organisms are not able to prepare and adapt rapidly to a sudden and huge load of different toxic substances. Accumulation of certain elements, especially of heavy metals with toxic effect, can cause undesirable changes in the biosphere bearing unforeseeable consequences (Djukic & Mandic, 2000). Microorganisms are nature's original recyclers, converting toxic organic compounds to harmless products, often carbon dioxide and water. Conventional techniques commonly applied to remove heavy metals from waste water and contaminated soils include chemical (precipitation, neutralization) or physical (ion exchange, membrane separation, electro dialysis and activated carbon adsorption) methods (Gadd & Griffith, 1978; Volesky 1987; Atkinson *et al.*, 1998). Currently, scientists are exploring the bioremediation techniques by exploiting microbial and associated biota within the ecosystem, to degrade, accumulate and/or remove the pollutants (Gadd 1990 & 1993; Paknikar *et al*., 1993; Khan & Khoo, 2000). Therefore the present study was undertaken to know the fungal diversity in the metal polluted soils, and to find their tolerance level at high concentration of certain heavy metals. The objective of this study was to identify and isolate fungal strains with particular tolerance toward heavy metals. This was conducted with the specific aims of establishing the relative toxicity of heavy metals to the fungal strains, the effect of metals and their concentration on the growth of fungal strains and identify the strains which demonstrates greater tolerance to heavy metals. The significance of the study is to improve soil health resulting from the heavy metal tolerant fungi and to check the tolerance of fungi against heavy metal pollutants and will be used as a biotechnological tool for bioremediation of environment. Besides it, the present study will provide a baseline and trained researchers to carry out applied research on bioremediation of soil through fungi for the betterment of environment.

Materials and Methods

Study area and samples collection: Soil samples were collected from two peri-urban agriculture areas of Chak No. 236/RB (Jappaywala and Kajle) in Faisalabad district (Fig. 1) and from three villages (Gorkhpur, Dahgal, Sihala) of Rawalpindi district (Fig. 2). These sites are irrigated by industrial and sewage polluted water. Additionally, one garden soil sample was collected from Fatima Jinnah Women University (FJWU), Rawalpindi for comparison as a normal soil. The soil samples were colleted from the upper layer not exceeding the depth of 5 cm. From each site soil samples were collected from five different spots in a field and mixed together to form a composite sample.

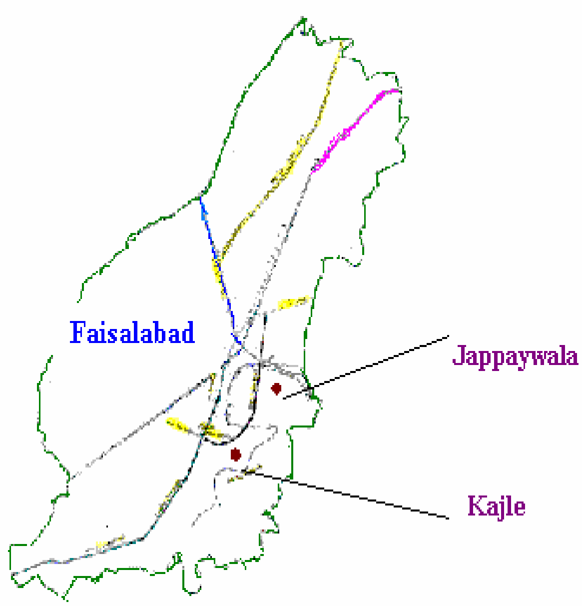

Fig. 1. Faisalabad sampling sites.

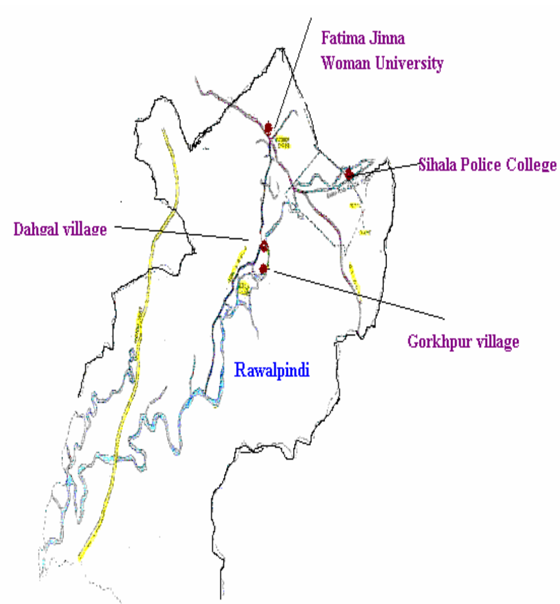

Fig. 2. Rawalpindi sampling sites

Sterilization of apparatus: Petri plates, media bottles, distilled water, Mccartney bottles and syringes were sterilized in autoclave. For sterilization purpose all apparatus were autoclaved for 40 minutes at 121°C. After autoclaving all sterilized material dried in oven at 95°C.

Media preparation: Potato Dextrose Agar (PDA) media was used for fungal cultures revival. Potatoes (200g) were peeled, sliced and boiled and then sieved through a clean muslin cloth to get a broth in which agar (7.5) and dextrose sugar (7.5) was added. The media was then autoclaved for 30 minutes at 121°C (Razak *et al*., 1999).

Preparation of plates: Poured the media in Petri-dishes and allowed to solidify for 24 hour. To suppress the bacterial growth, 30 mg/lit of streptomycin was added in the medium. Once the agar was solidified, and then put plates in an inverted position for 24 hours at room temperature (Waksman, 1922).

Enumeration of fungi: Potato dextrose agar (PDA) media (1 litre) was used for the isolation of fungi (Razak *et al*., 1999). The soil samples were processed with isolation procedure using the soil dilution plate method (Waksman, 1922). After incubation distinct colonies were counted and identified. The cultures were identified on the basis of macroscopic (colonial morphology, colour, texture, shape, diameter and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium). Pure cultures of fungi isolates were identified with the help of literature (Domsch *et al.*, 1980; Barnett & Hunter, 1999).

Preparation of slants: Prepared Potato Dextrose agar (PDA) media was poured in Mccartney bottles and placed in autoclave for 30 minutes at 121°C. After autoclaving the bottles were placed in tilt position for 24 hours. After solidification a plug of fungal culture was placed in the center of bottle under sterilized conditions. The slants were incubated at 30°C for one week. After the appearance of fungi full growth, slants put in refrigerator for further use and preservation.

Metal tolerance test for fungi: Fungal strains including *Aspergillus niger*, *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp were tested for their tolerance against different concentrations of heavy metals ($NiSO_4$, $ZnSO_4$, $CdSO_4$, Pd NO_3). Potato dextrose agar media was used for heavy metal resistance experiment. The concentrations (1, 5, 10, 15, 20, 25, 30, 35, 40 ppm) of heavy metals ($NiSO_4$, $ZnSO_4$, $CdSO_4$, Pd NO_3) were used for the selection of fungi. Incubation was conducted at 30^{o}C for two weeks (Malik & Jaiswal, 2000). The growth was monitored by measuring the culture from the point of inoculation or centre of the colony. Tolerance of fungi were studied by determination of Minimum Inhibitory Concentration (MIC) (Zafer *et al*., 2007).

Heavy metals analysis of soil: Each soil sample (1g) was taken in the conical flask (50ml), added 10ml of HNO_3:$HClO_4$ (1:2) solution (50ml) and heated for half an hour. Solutions were filtered through Whatman 1 filter paper and volume was made to 50mL by adding distilled water. Soil samples were digested in triplicates and analyzed for Zn, Cd, Co, Ni and Pb. The blank was prepared for quality assurance of samples. The blank sample contained 10ml of HNO_3:$HClO_4$ (1:2) solution and heated for half an hour and volume was made 50ml by adding distilled water. For the determination of heavy metals the atomic absorption spectrophotometer was powered on and warmed up for 30 minutes. After the heating of cathode lamp, the air acetylene flame was ignited and instrument was calibrated or standardized with different working standards (Vanloon & Lichwa, 1973).

Results and Discussion

Diversity of fungi: It is well known that a long-time exposure of water and sediment to heavy metals can produce considerable modification of their microbial populations, reducing their activity and their number. In the present study, soil samples collected from Agricultural fields of Faisalabad and Rawalpindi where heavy metals and other pollutants have been emitted in industrial effluents for several years (Zafar *et al*., 2007). Different 41 species of fungi were isolated and identified from the collected soil samples (Table 1). *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp., *Penicillium* sp., and *Rhizopus* sp., were present and dominant in all the soil samples. Metal resistant fungi (*Aspergillus*, *Penicillium*, *Alternaria*, *Geotrichum*, *Fusarium*, *Rhizopus*, *Monilia* and *Trichoderma)* can be isolated from the agricultural soils polluted with industrial and municipal water (Nazina *et al*., 2002). Abundance and activities of microflora in soil strata are controlled by the availability of water, nutrients, pH, concentration of metal ions, hydrodynamic communication with the ground surface and so on (Okoronkwo *et al*., 2005). Environmental stresses brought about by the contamination could be a reason for the reduction in microbial species but increasing the population of few surviving species (Griffioen, 1994). In Jappaywala, Faisalabad sample the frequency percentage of fungi was 15% while in Kajle, Faisalabad the frequency percentage was 15.9%. Among the Rawalpindi soil samples, the Sihala soil sample appeared to have the lowest (10.3%) frequency percentage of isolated fungi (Fig. 3). The soil samples collected from polluted sites were more affected by wastewater irrigation which affected the population densities of fungi. The differences between the sampled sites regarding their richness on microbial isolates appear to be closely linked to the degree of heavy metal pollution. Generally, pollution of soil and water by heavy metals may lead to a decrease in microbial diversity. This is due to the extinction of species sensitive to the stress imposed, and enhanced growth of other resistant species. As the Fig. 4 shows the colony-forming unit of garden soil sample from Fatima Jinnah Women University, Rawalpindi (FJWUR), Gorkhpur and Dahgal were higher than that of Sihala (Rawalpindi district), Jappaywala and Kajle (Faisalabad district). The increasing toxicity of urban wastes due to rapid industrialization make the use of abandoned municipal waste dump hazardous for agricultural purposes. The source of pollutant as well as long periods of exposure are also the important factors regulating the stress and fungal adaptation (Zafar *et al*., 2007. Less information has been gathered in Pakistan on the impact of heavy metals on fungal diversity in peri urban agriculture lands. More research is needed to provide information on the effect of untreated wastewater on the fungal diversity in peri urban agriculture soil, especially since peri urban agriculture is very important for sustainable use of resources.

Table 1. Diversity of fungi in the soil samples of Faisalabad and Rawalpindi districts.

Fungal species	Faisalabad			Rawalpindi		
Acremonium sp.	+	+	+	-	+	+
Acremonium furcatum	+	+	+	-	+	+
Acremoniella sp.	+	-	-	-	+	+
Alternaria alternata	-	+	-	-	-	+
Aspergillus flavus	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	+
Aspergillus. fumigatus	-	-	-	+	-	-
Arthrobotrys sp.	-	-	-	-	+	-
Botrytis sp.	-	-	-	-	+	+
Cheatomium sp.	+	+	+	-	+	+
Chromelosporium sp.	-	-	+	-	-	-
Chrysosporium sp.	-	-	+	-	-	-
Cunninghamella sp.	-	-	+	-	-	-
Dreschlera sp.	-	-	-	-	+	+
Epicocum nigrum	-	-	-	+	-	-
Fusarium sp.	+	+	+	+	+	+
Geotricum sp	-	-	+	+	-	+
Gliocladium sp.	-	-	+	-	+	-
Gonatobotrys sp.	-	-	-	-	+	+
Histoplasma sp.	-	-	-	-	+	-
Hypomyces sp.	+	+	+	-	+	+
Leptographium sp.	-	-	+	-	-	-
Microsporum sp.	-	+	-	-	-	+
Monilia sp.	+	-	+	-	+	-
Mycosphaerella sp.	-	-	+	-	-	-
Necteria sp.	+	-	-	-	+	-
Odiodendron sp.	+	+	-	-	-	-
Pacilomyces sp.	+	+	-	-	+	-
Penicillium sp.	+	+	+	+	+	+
Periconia sp.	-	-	+	-	-	-
Phytophthra sp.	-	+	-	-	+	-
Rhizomucor sp.	-	-	-	+	-	-
Rhizopus sp.	+	+	+	+	+	+
Rhizopus oryzae	+	-	-	-	+	-
Rhopalomyces sp.	-	-	+	-	-	-
Sclerotium sp.	-	-	+	-	+	-
Stachybotrys sp.	-	-	-	-	+	+
Trichoderma sp.	+	+	-	-	+	-
Torula sp.	-	+	+	-	-	+
Verticillium sp.	-	+	-	+	-	-
Ulocladium sp.	-	-	-	+	-	-

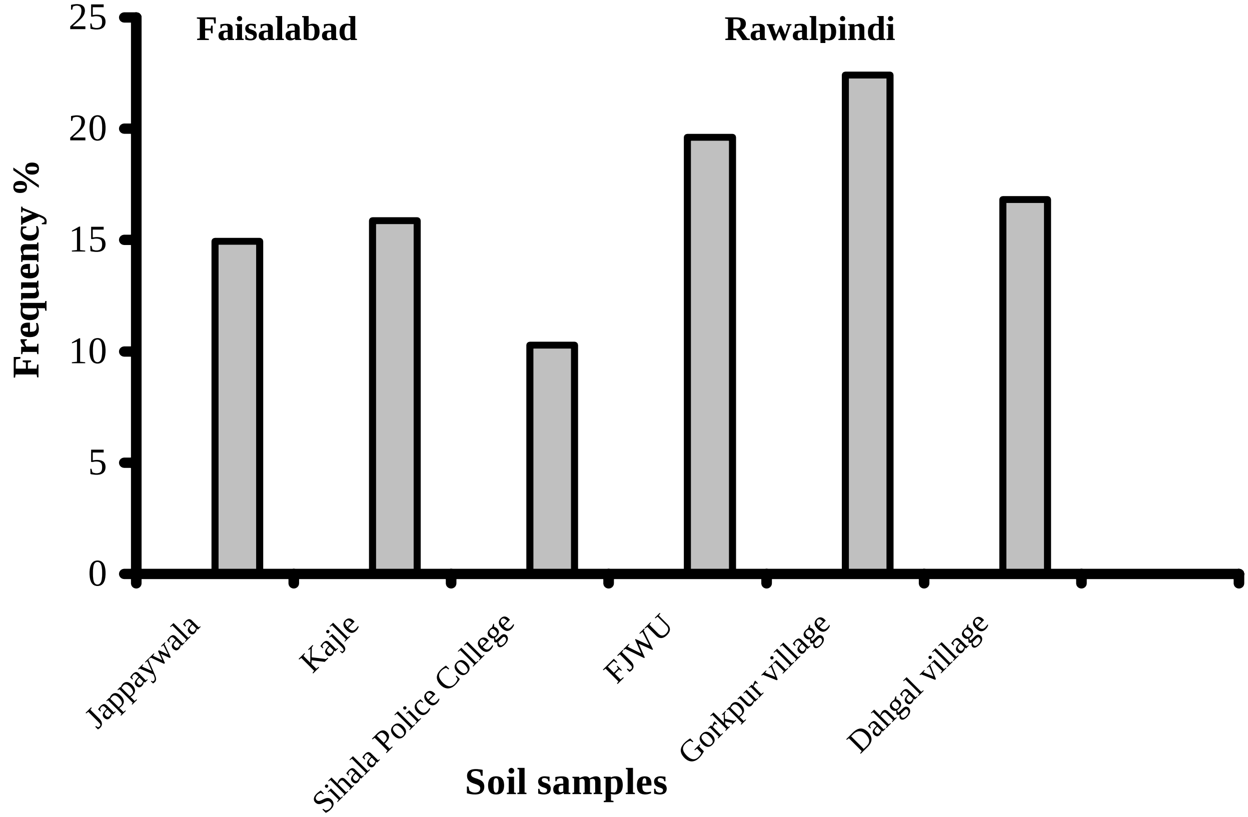

Fig. 3. Frequency (%) of isolated fungi in soil samples from Faisalabad and Rawalpindi districts.

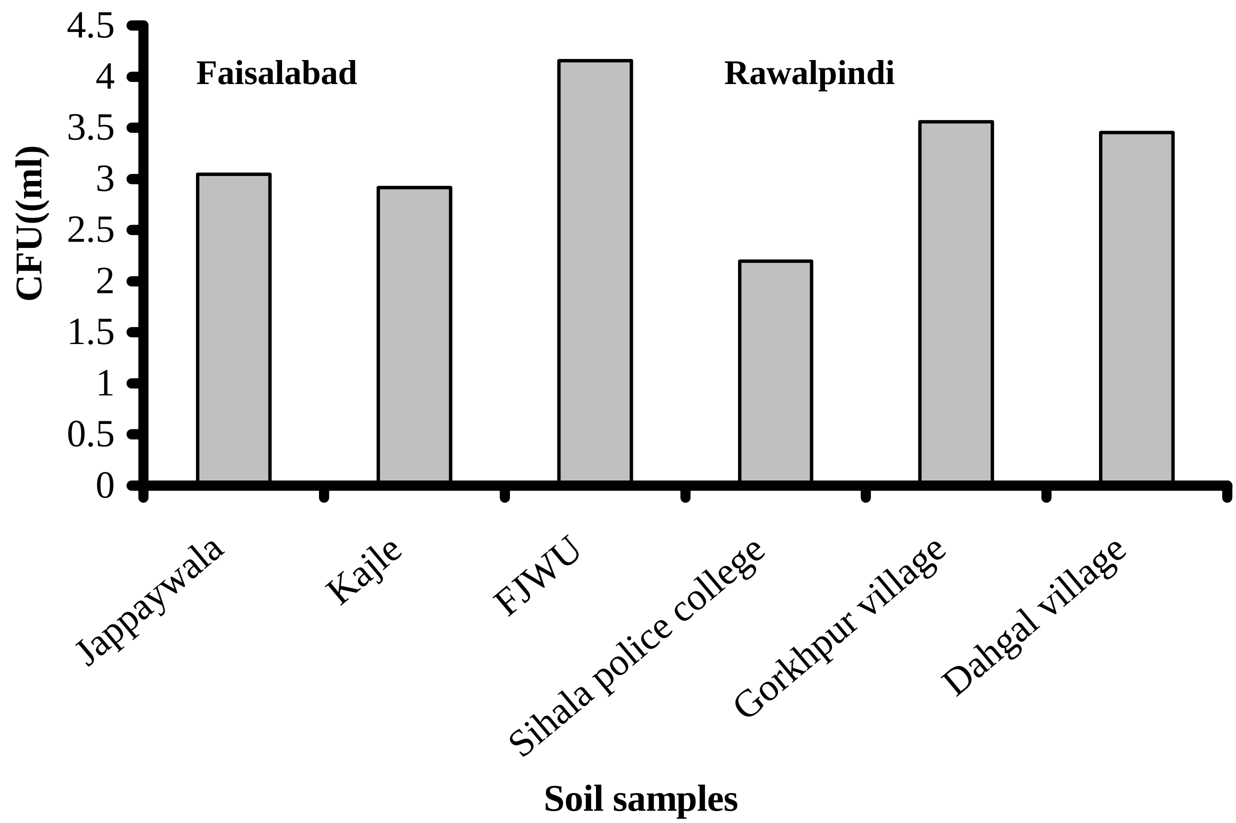

Fig. 4. Colony forming units (CFU) of fungi in soil samples from Faisalabad and Rawalpindi districts.

Table 2. Minimum inhibitory concentration (MIC) of heavy metals against test fungi.

Locations	Fungal species	Minimum inhibitory concentration (mg L-^{1})			
		ZnSO	$PbNO_3$	$NiSO_4$	$CdSO_4$
Faisalabad					
Jappaywala	*Aspergillus* niger	30	`15	10	1
	*Aspergillu*s sp.	15	10	1	1
	Penicillium sp.	1	5	1	1
	Fusarium sp.	1	10	1	1
Kajle	*Aspergillus* niger	20	5	10	1
	*Aspergillu*s sp.	1	1	10	1
	Penicillium sp.	1	1	1	1
	Fusarium sp.	1	1	1	1
Rawalpindi					
Dahgal	*Aspergillus niger*	10	1	1	1
	Aspergillus sp.	5	1	1	1
	Penicillium sp.	1	1	1	1
	Fusarium sp.	1	1	1	1
FJWU	*Aspergillus niger*	15	1	10	1
	Aspergillus sp.	5	1	1	1
	Penicillium sp.	1	1	5	1
	Fusarium sp.	1	1	5	1
Gorakhpur	*Aspergillus niger*	25	10	15	1
	Aspergillus sp.	1	1	5	1
	Penicillium sp.	1	1	1	1
	Fusarium sp.	25	1	1	1
Police College Sihala	*Aspergillus niger*	15	1	20	1
	Aspergillus sp.	1	1	1	1
	Penicillium sp.	1	1	1	1
	Fusarium sp.	15	1	1	1

MIC: Minimum inhibitory concentration refers to the concentration of heavy metals (mg L-^{1}) at which fungi showed inhibition in growth

Tolerance of fungi: Common and dominant metal-resistant fungi isolated from waste-water treated soils belong to the genera of *Aspergillus*, *Penicillium* and *Fusarium* which showed different Minimum inhibitory concentration (MIC) for Zn, Pb, Ni, Cd (Zafer *et al*., 2007). The MIC values suggest that the resistance level against individual metals was dependent on the isolates. *Aspergillu niger* isolated from the soil samples of Faisalabad showed the highest tolerance towards each metal salts. The *Fusarium* sp., of Jappaywala, Faisalabad showed tolerance towards $PdNO_{3,}$>$ZnSO_{4,}$ $NiSO_4$> $CdSO_4$. *Aspergillus niger* of Rawalpindi district soil samples (Gorakhpur) showed highest tolerance followed by *Aspergillus* sp, *Penicillium* sp while *Fusarium* sp., of Gorakhpur, Rawalpindi showed the highest MIC against $ZnSO_4$ (Table 2). Fungi have greater potential for remediation by virtue of their aggressive growth, greater biomass, production and extensive hyphal reach in the soil. Moreover fungi have been widely used in bioremediation of industrially polluted soils and waters, specifically in the removal of hydrocarbons and heavy metals (Akhtar & Mohan, 1995; Khan, 2001; Potin *et al*., 2004). Several researchers have reported the use of *Aspergillus niger* , *Aspergillus* sp , *Penicillium* sp and *Fusarium* sp to remove heavy metals Cr , Zn , Ni , Pd and Cd and checked their tolerance ability to see their tolerance towards $CdSO_4$, $ZnSO_4$, $PdSO_4$ and $NiSO_4$ in the Soil (Gadd., 1990; Fourest *et al*., 1994 ; Bai & Abraham, 2001; Teskova & Petrov, 2002). Similar study was reported by by Price *et al*., (2001) who showed that *Aspergillus niger* was better to grow or tolerance heavy metals as compared to other fungi. Results of the present investigations shows that *Aspergillus niger* and *Aspergillus* sp., were more tolerant as compared to *Penicillium* sp., and *Fusarium* sp (Zn > Ni > Pd >Cd). The occurrence of various fungi such as *Aspergillus, Rhizopus, Penicillium, Fusarium, Chaetomium, Geomyces* and *Paecilomyces* species in the soil polluted by heavy metals (Cu, Cd, Pd, As and Zn) has also been reported by other worker (Babich & Stotzky, 1985; Gadd, 1993). The variation in the metal tolerance might be due to the presence of one or more types of tolerance strategies or resistance mechanisms exhibited by different fungi. At least one representative of two major classes of isolated fungi, deuteromycetes (*Aspergillus*) showing various levels of metal tolerance were evaluated in this preliminary in vitro investigation. Tolerance of toxic metals is based on ionic species associating with the cell surface or extra cellular polysacharides, proteins and chitins (Volesky, 1990). Our preliminary findings indicate that fungi from soil contaminated with heavy metals have metal tolerance potential similar to that of other fungi and could be exploited for metal removal from aqueous metal solution. Further investigations are needed to optimize the conditions for metal removal from multimetal aqueous solutions and diluted wastewaters for large scale operation.

Heavy metal contents of soil: Heavy metals are environmental contaminants are not a new phenomenon. They are essential part of all living organisms and are present in trace amount in soil naturally. The man made sources of metal contamination are mainly associated with certain industrial activities, agricultural practices, automobile emissions, coal fired power generation plants, municipal incinerators (Rattan *et al*., 2002; Marshall *et al*., 2003). Soil samples collected from the both sites of Faisalabad showed very little variation in the level of heavy metal contamination (Fig. 5). The differences in heavy metals level of soil was due to use of different quantity and quality of sewage and sludge, level and type of industrialization near the sampling sites, proximity of field from main road and highways, presence of thermal power plants and use of different types of agrochemicals containing heavy metals (Norra *et al*., 2001). The present investigation indicates the type and extent of heavy metal pollution in the soils of Faisalabad and highlighted the most probable, natural / anthropogenic / farming practices related, causes for these problems. In Faisalabad there are some large and medium scale industries and tanneries near Jappaywala and Kajle village and they discharge their effluents on land through irrigation. The average concentration of lead Zn, Pb, Cd, Ni were higher in Faisalabad samples as compared to those in Rawalpindi soil samples (Table 3). Zinc is a ubiquitously emitted element and its concentration was higher in the garden soil FJWUR. Among its sources, are combustion of fossil fuels and wood, metal production, cement production, fertilizers, abrasion of construction materials, material abrasion in traffic, urban reuse, solid wastes, sewage sluges, etc (Oyedele *et al*., 1995). This means that instead of many sources, one or only a few are responsible for the high concentration of Zn in FJWU. The concentration of Pb was higher in Sihala, Rawalpindi soil sample. In Sihala the police training college owns a vast agriculture land in which seasonal vegetables and fruits are grown and sewage water is being used for irrigation for more than ten years on this site. Application of NPK fertilizers and industrial effluents in fields are the main source of lead (Oyedele *et al*., 1995). The two sites of Rawalpindi Gorkhpur and Dahgal village) are situated between river Sawan and Adiala road that run parallel to each other. The untreated industrial and municipal effluent of twin villages pollutes the river Swan and they use the sewage water for the irrigation. The concentration of cadmium is higher in the soil sample of Dahagal village. High levels of Cd in soil due to the application of phosphetic fertilizers, sewage water and industrial effluents and metal based pesticides in vegetable crops. Farming practice that is a likely source of Cd is the application of phosphate fertilizers.

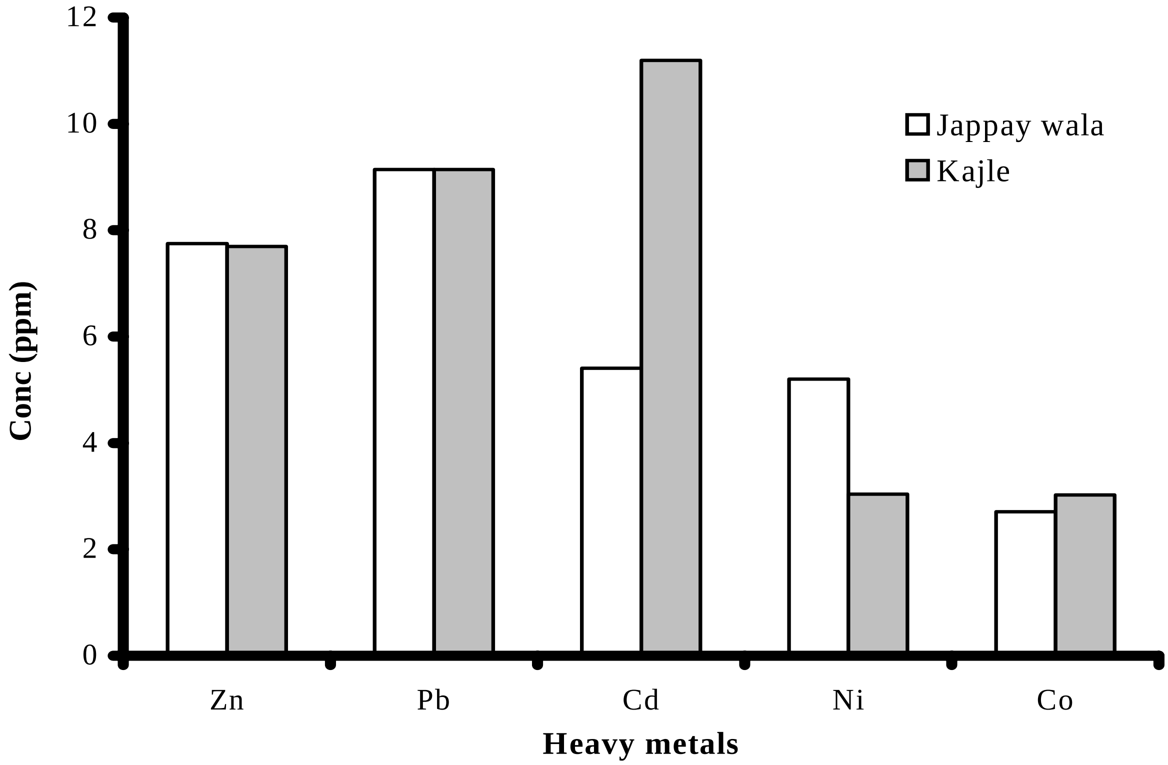

Fig. 5. Concentration of heavy metals in Faisalabad location's soil samples (as by acid digestion).

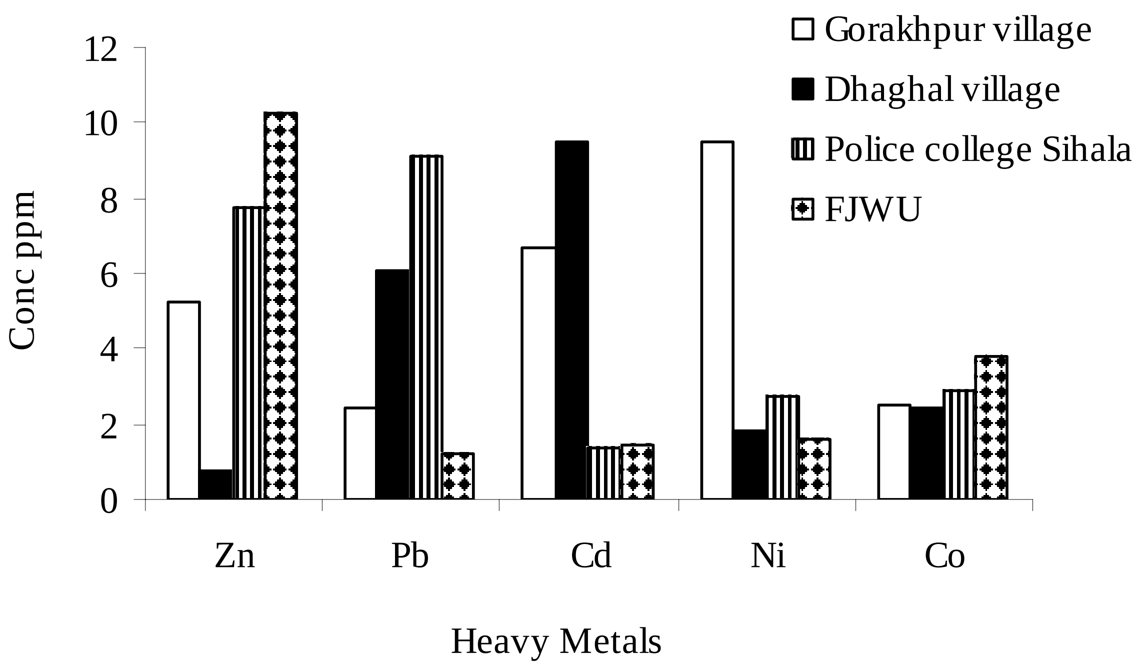

Fig. 6. Concentration of heavy metals in Rawalpindi location's soil samples (as by acid digestion).

According to previous reports (Rothbaum *et al*., 1986) higher concentration of Cd is because of super phosphate fertilizers. The nickel concentration was higher in Gorakpur village (Fig. 6). Atmospheric deposition of nickel results from the burning of fossil fuels, especially oil and its refinery products including diesel. Land disposal of fly ash from coal-fired power generation is the largest single input of nickel to soils. Nickel is relatively minor contaminant of fertilizers, and historically the main source of nickel in agricultural soils came from the application of sewage sludge (McGrath & Loveland, 1992).

Table 3. Average and range of heavy metals in soil samples of Faisalabadand and Rawalpindi district.

Heavy metals	Metal Conc (mg kg-1)							
	Faisalabad				Rawalpindi			
	Mean	S.D	Min-Max	Median	Mean	S.D	Min-Max	Median
Zn	7.7	± 0.042	7.6-7.7	7.72	6.00	± 4.06	0.74-10.25	6.51
Pb	9.1	± 0.00	9.1-9.1	9.14	4.72	± 3.60	1.21-9.14	4.26
Cd	8.3	± 4.09	5.41-11.19	8.30	4.74	± 4.00	1.37-9.46	4.07
Ni	4.1	± 1.53	3.04-5.2	4.12	3.90	± 3.76	1.61-9.5	2.25
Co	2.8	± 0.219	2.71-3.02	2.86	2.88	± 0.623	2.41-3.77	2.67

Mean ± S.D

Conclusions

The present study shows that the soil samples of Faisalabad locations showed higher concentration of heavy metals as compared to that from Rawalpindi district. The isolated fungi of Faisalabad sites were more tolerant to heavy metals. Our conclusion indicates that fungal population isolated from heavy metal contaminated sites has the ability to resist higher concentration of metals. A comparative level of metal resistance was also shown by filamentous fungi originated from unpolluted sites. The tolerance and resistance of the isolates depended much more on the fungus tested than on the site of its isolation. This variation may be explained by the development of tolerance and adaptation of the fungi to heavy metals. *Aspergillus* was the most resistance to all the metals tested, which make them promising candidates for further investigations regarding their ability to remove metals from contaminated environment. The present study indicates that isolated fungi of contaminated soils can be used as bioremediation agents.

Acknowledgments

The authors are thankful to National Agricultural Research Center (NARC), Islamabad, Pakistan for providing all research support to find out the fungal diversity and their metal tolerance and to determine the heavy metal contents in the soil samples.

References

Akhtar, M.N. and P.M. Mohan. 1995. Bioremediation of toxic metal ions from polluted lake-waters and industrial effluents by fungal biosorbent. *Curr. Sci.*, 69(12): 1030-1038.

Amisu, K.O., A.O. Coker and R.D. Isokpehi. 2003. *Acrobacter butzlieri* strains from poultry abattoir effluent in Nigeria. *East Afri. Medi. Journal*, 80: 218-221.

Atkinson, B.W., F. Bux and H.C. Kasan. 1998. Consideration for application of biosorption technology to remediate metal-contaminated industrial effluent. *Water Safe*, 24: 129-35.

Babich, H and G. totzky. 1985. Heavy metal toxicity to microbe mediated ecological processes: a review and potential application to regulatory policies. *Environ Resour.*, 36: 111-137.

Bai, S and T.E. Abraham. 2001. Biosorption of chromium (VI) from aqueous solution by *Rhizopus nigricans*. *Bioresour Technol.*, 79: 73-81.

Barnett, H.L. and B.B. Hunter. 1999. *Illustrated genra of imperfect fungi.Fourth edition*. Prentice Hall Inc.

Bridges, O., J.W. Bridges and J.F. Potter. 2000. A generic comparison of the airborne risks to human health from landfill and incinerator disposal of municipal solid waste. *The Environ.*, 20(4): 325-334.

Djukic, D. and L. Mandic. 2000. Microorganisms and technogenic pollution of agroecosystem. *Acta. Agricul..Serbica.*, 5(10): 37-44.

Domsch, K.H., W. Gams and T.H. Anderson. 1980. *Compendium of soil fungi*. London, England: Academic Press.

Fourest, E., C. Canal and J.C. Roux. 1994. Improvement of heavy metal biosorption by mycelial dead biomass (*Rhizopus arrhizus*, *Mucor miehei* and *Penicillium chrysogemum*): pH control and cation activation. *FEMS Microbiol. Review*., 14: 325-332.

Gadd, G.M and A.J. Griffiths. 1978. Microorganisms and heavy metal toxicity. *Micro Ecol*., 4: 303-317.

Gadd, G.M. 1990. Heavy metal accumulation by bectria and other microorganisms. *J. Ann. Experi*., 46: 834-840.

Gadd, G.M.1993. Interaction of fungi with toxic metals. *New Phytol*., 124: 25-60.

Griffioen, W.A.J. 1994. Characterization of a heavy metal tolerant endomycorrhizal fungus from the surroundings of a zinc refinery. *Mycorrhiza*., (4): 197-200.

Khan, A.G and C.S. Khoo. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosynthesis*, 41: 197-207.

Khan, A.G. 2001. Relationship between chromium biomagnifications ratio accumulation factor and mycorrhizal in plants growing on tennery effluent polluted soils. *J. Environ. International*, 26: 417-423.

Malik, A and R. Jaiswal. 2000. Metal resistance in Pseudomonas strains isolated from soil treated with industrial wastewater. *World. J. Microbial Biotechnol*., 16: 108-117.

Marshall, F., R. Agrawal, D. Lintell, D.S. Bhupal, R.B. Singh, P. Mukherjee, C. Sen, N. Poole, M. Agrawal and S.D. Singh. 2003. '*Heavy Metal Contamination of Vegetables in Delhi'*. Executive Summary of Technical Report.

McGrath, S.P. and P.J. Loveland. 1992. *The Soil Geochemical Atlas of England and Wales*. UK.

Mueller, G. M and G.F. Bills. 2004. Introduction inventory and monitoring methods: In: *Biodiversity of fungi*: (Eds.): G.M. Mueller, G.F. Bills and M.S. Foster. Elsevier Academic Press San Diego. 1-4.

Nazina, T.N., A.A. Grigoryan, Y. Xue, E.V. Novikova, M.V. Ivanov. 2002. Phylogenetic diversity of aerobic saprtrophic bacteria isolated from Daqing Oil Field. *Microbiol*., 71(1): 91-97.

Norra, S., A. Weber, U. Kramar and D. Stüben. 2001. Mapping of Trace Metals in Urban Soils: The Example of Mühlburg/Karlsruhe, Germany. *J. Soil Sedimen*., 1(2): 77-93.

Okoronkwo, N.E., J.C. Igwe and E.C. Onwuchekwa. 2005. Risk and health implications of polluted soils for crop production. *African J. Biotechnol*., 4(13): 1521-154.

Oyedele, D.J., I.B. Obioh, J.A. Adejumo, A.F. Oluwole, P.O. Aina and O.I. Asubiojo. 1995. Lead contamination of soils and vegetation in the vicinity of a lead smelter in Nigeria. *The Sci. Total Environ*., 172: 189-195.

Paknikar, K.M., U.S. Palnitkar and P.R. Puranik. 1993. In: *Biohydrometallurgical Technologies* (Eds.): A.E. Torma, M.L. Apel and C.L. Brierely. The Minerals, Metals and Materials Society, TMS Publications, Wyoming, USA.II: 229-236.

Parkinson, D. and D.C. Coleman. 1991. Microbial Communities, activity and biomass. *Agricul. Ecosystem. Environ*., 34(1-4): 3-33.

Potin, O., C. Rafin and E. Veiguie. 2004. Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs) contaminated soil by filamentous fungi isolated from the soil international. *Biodeterio Biodegrad*., 54(1): 45-52.

Price, M., J. Classen and G. Payne. 2001. *Aspergillus niger* absorbs copper and zinc from swine wastewater. *Bioresour. Technol*., 77(1): 41-49.

Rattan, R.K., S.P. Datta, S. Chandra and N. Saharaan. 2002. 'Heavy metals in Environments-Indian Scenario'. *Fertile. New*., 47: 21-26 and 29-40.

Razak, A.A., G. Bachman and R. Farrag. 1999. Activities of microflora in soils of upper and Lower Egypt. *The African J. Mycol. Biotech*., 7(1): 1-19.

Rothbaum, H.P., R.L. Goguel A.E. Johnson and G.E.G. Mattingly. 1986. Cadmium accumulation in soils from long continued applications of superphosphate. *J. Soil Science*, 37: 99-107.

Teskova, K. and G. Petrov. 2002. Removal of heavy metals from aqueous solution using *Rhizopus* delemar mycelia in free and polyurethane- bound form. *Zeits fur Naturforschung*, 57(7-8): 629-633.

Vanloon, J.C. and J. Lichwa. 1973. A study of atomic absorption determination of some important heavy metals in fertilizers and domestic sewage plant sludges. *Environ Letter*., 4: 1-8

Volesky, B. 1987. Biosorbents for metal recovery. Trends Biotechnol., 5: 96-101.

Volesky, B. 1990. Biosorption and biosorbents. In: Biosorption of Heavy Metals. (Ed.): B. Volesky. CRC Press, Boston.

Waksman, S.A. 1922. A method of counting the number of fungi in soil. *J. Bacteriol*., 7(3):339-341.

Zafar, S., F. Aqil and I. Ahmad. 2007. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural. *Soil Bioresour. Technology*, 98: 2557-2561.

(Received for publication 13 September 2008)