ARSENIC (III) TOLERANCE POTENTIAL OF FUNGI ISOLATED FROM POLLUTED AND NON-POLLUTED SOILS OF PAKISTAN

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ABSTRACT: Filamentous fungi can play significant role in bioremediation due to their large surface area and metal sorption capacity. In present study, arsenic (As) tolerance of indigenous filamentous fungi was explored. Fungal strains were isolated from peri-urban soils of Multan and Gujranwala irrigated with untreated industrial or municipal effluent. Some fungal strains were also isolated from non-polluted soils of Islamabad area. Arsenic (III) tolerance potential of 18 fungal isolates was tested by growing them on Potato Dextrose Agar (PDA) medium amended with different concentrations of As (50 to 5600 mg/L). The fungal tolerance was evaluated by comparing tolerance index (TI), minimum inhibitory concentrations (MIC) and growth rate. Out of 18 isolates, 12 belonged to genus *Aspergillus*, 3 to *Fusarium*, 2 to *Curvularea* and one to *Penicillium*. The isolates *Aspergillus fumigatus* and *Fusarium oxysporum* appeared to be most tolerant to arsenic. Fungal strains isolated from Gujranwala soil exhibited more As(III) tolerance than those from Multan and Islamabad. Maximum fungal growth was observed at temperature of 30 to 35 ˚C and pH 6 to 7 under incubation period of 96-120 hrs. The isolated strains were capable of growing over a wide range of pH and temperatures and at high Arsenic concentrations. These findings show that the isolated strains are quite suitable for use in bioremediation under field conditions.

Key Words: Fungi, As (III), tolerance index, minimum inhibitory concentration

INTRODUCTION

Arsenic is a metalloid, ubiquitously found in nature. It ranks 20th in earth's crust, 14th in seawater and 12th in human body (Mandal and Suzuki, 2002) in terms of abundance. The common arsenic minerals are Arsenopyrite (FeAs S), realgar (AsS), orpiment (As_2S_3), loellingite ($FeAsS_2$) and arsenolite (Azcue et al., 1994). Arsenic naturally exist in different oxidized forms, i.e. As^{+3} (arsenite), As^{+5} (arsenate), As^{-3} (arsenide) (Smedley et al., 2002). These are mobilized by natural weathering reactions, biological activity, geochemical reactions, volcanic emissions and other anthropogenic activities.

Ground water is considered to be a major non-point natural source of arsenic pollution (Smedley and Kinniburgh, 2002) while mining, pesticides, wood-treatments and agricultural chemicals are considered point sources. Although, As exists in water bodies both in inorganic and organic forms, inorganic form of

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arsenic is most often found in water supplies (Bodek et al., 1998). Groundwater arsenic contamination has become a global water quality issue including Pakistan (WHO, 1999, 2001; ATSDR, 2002; Smith et al., 2002; PCRWR, 2007; Amini et al., 2008). Bangladesh has the highest number of people affected by arsenic poisoning (Dhar et al., 1997). According to a survey, almost 22 million people drink water having arsenic concentrations more than 50 µg/L in Bangladesh (British Geological Survey, 1999). The groundwater arsenic contaminated is not restricted to others countries of the region like India, Bangladesh, China, it is also found in Pakistan (Mandal and Suzuki, 2002; FAO, 2006). A joint research by Pakistan Council for Research in Water Resources (PCRWR), United Nations Children's Fund (UNICEF) and National Water Quality Monitoring Program (2002-2006) revealed the presence of arsenic in groundwater of many districts of Punjab and Sind Province. According to PCRWR (2007), in Punjab out of 11559 ground water samples, 38% had arsenic concentration >10mg/L (WHO permissible limits), 17% >50 mg/L and 4% contained >100mg/L. In Sindh, out of 5991 samples, 11% had >10mg/L, 3% had >50 mg/L and 1.42% had >100 ppb. Another joint study carried out by Tokyo Institute of Technology and National Institute of Advanced Industrial Science and Technology (AIST) also indicated five times higher As concentration in ground water than permissible limit in Punjab. Among the inorganic species, arsenite (As^{-3}) and arsenate (As^{-5}) are commonly found in natural waters and former is 2-10 times more toxic than later (Jain and Ali, 2000).

Extensive research has been carried out for providing solution to arsenic contaminated water. Different techniques such as precipitation-coagulation, oxidation, membrane separation, electro coagulation/ flocculation and adsorption (Benefield and Morgan, 1990; Clifford, 1999; Zaw and Emett, 2002; Kim et al., 2006) are being practiced for removal from aqueous solution. Among these, the adsorption technique has been extensively practiced using a wide range of sorbents. Conventionally, iron oxides and oxyhydroxides were widely used as As sorbents; however, bio-sorbent like fungi, algae and bacteria are preferably used as sorbent and considered environment friendly. Biosorbtion is a passive immobilization of metals by sorption mechanism and is independent of cell metabolism (Kadukova and Vircikova, 2005). It is based upon physio-chemical interactions between metals and functional groups of the cell wall (Veglio and Beolchini, 1997; Kadukova and Vircikova, 2005).

Filamentous fungi are progressively flattering as a metal sorbent for remediation of contaminated soil and water because of ubiquitous and profusion nature over a wide range of pH (Visoottiviseth and Panviroj, 2001) and are known for accumulating high amounts of metals (Morley et al., 1995). Their cell walls are largely composed of polysaccharides and proteins, which offer many functional groups such as carboxyl, hydroxyl, sulphate, phosphate and amino groups for binding of metal ions (Arica et al., 2004; Baldrian, 2003; Couto et al., 2004). Fungal species are also capable of transforming inorganic arsenic compounds

by bio-methylation into mono-methyl arsonic acid, dimethyl arsenic acid, trimethyl arsine and trimethyl arsineoxide (Adriano, 2001).

Heavy metals in polluted soils are known to affect the functions and population structure of micro-organisms. Fungi have been reported to show more tolerance to heavy metals than other microorganisms and become dominant organisms in some polluted habitats (Martino et al., 2000). Many fungal species such as *Rhizopusarrhizus* (Aksu et al., 1999), *Phanerochaete chrysosporium* (Say et al., 2001), *Aspergillus nidulans* (Maheswari and Murugesan, 2009(a)), *Aspergillus flavus* (Maheswari and Murugesan, 2011), *Aspergillus fumigates* have been studied for sorption of arsenic (Sathishkumar et al., 2008; Maheswari and Murugesan, 2009(b)).Considering the importance of fungi in bioremediation, indigenous filamentous fungi were isolated from heavy metals polluted and unpolluted sites and their arsenic tolerance were explored.

MATERIALS AND METHODS

Soil Sampling and Sampling Site

A total of 12 composite soil samples were collected from Multan, Gujranwala and Islamabad peri-urban area. Multan and Gujranwala sites were under untreated municipal /industrial effluents while Islamabad site was under fresh water irrigation. The composite soil samples were collected randomly by selecting transects of 10×10 m at each location. Then from each location, a composite soil sample was collected from four carefully mixed subsamples, taken at different random places within that transect area. Soil samples were transferred to soil Environment Laboratory, Land Resources Research Institute, National Agricultural Research Centre, Islamabad for further studies. A portion of the collected soil samples was stored in refrigerator at 4°C to ensure minimal biological activity. Isolation of fungi was carried out within 24 hours of samples collection. Rest of the portion of soils samples was air-dried, ground and passed through 2 mm sieve and then about 100 g was drawn from the 2 mm fraction and reground to obtain <200 µm fraction for physicochemical analysis.

Physico-chemical analysis of soil

The collected samples were analyzed for basic physico-chemical parameters. Particle size distribution was estimated by hydrometer method (Gee and Or, 2002), pH by making 1:1 (soil:water) suspension (Thomas, 1996), organic matter by titration method described by Nelson and Sommers (1996). Total heavy metal concentrations were measured by digesting the soil samples in a mixture of hydrogen peroxide, hydrofluoric acid, nitric acid and per chloric acid (Amacher, 1996) and analyzing on Atomic Absorption Spectrometer (AAS) (Perkin Elmer, A Analyst 800).

Isolation and morphological characterization of fungus

Fungal growth medium was prepared by dissolving 39 g potato dextrose agar (PDA) in 1 Littre deionized water (Razak et al., 1999) and autoclaved at 121°C for 15 minutes. After autoclaving and cooling (to room temperature), streptomycin was added @ 30 mg/L to suppress the

bacterial growth. Fungi were isolated by pouring 100 µl of each soil suspension (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) onto PDA plates in triplicate. Mixed multi-colonies fungi were further cultured and purified through repeated streaking on fresh PDA medium and identified using morphological, micro- and macroscopic examinations and biochemical tests (Domsch et al., 1980; Barnett and Hunter, 1999). Slides of isolates were prepared with Aniline Blue stain and were observed under the microscope at different magnifications to ascertain macroscopic characteristics precisely such as morphology of colonies, texture, color, shape, appearance and diameter of colonies, and microscopic characteristics such as presence of particular reproductive structures, septation of mycelium, structure and shape of conidia and presence of sterile mycelium as described by Barnett and Hunter (1999); Watanabe (2002) and Nyongesa et al. (2015) were observed.

Screening and selection of As (III) tolerant fungi

Purified isolates were screened for As (III) tolerance by growing on PDA medium amended with varying concentrations of As (III) (50 to 5600 mg/L) at 28+1°C for 7 days and measuring radial growth periodically against control (without As (III) (Mukherjee et al., 2013; Visoottiviseth and Panviroj, 2001).

Minimum inhibitory concentrations (MIC's)

Minimum inhibitory concentrations (MIC's), the lowest As concentration that inhibits visible fungal growth, were determined from the As screening data (Andrews, 2001).

Arsenic tolerance index

The tolerance index, ratio of radial growth of treated colony to that of the untreated colony, was measured by growing selected isolates on As amended PDA medium (100 mg/L As concentration) for 7 days (Rasool and Irum, 2014).

Tolerance index (Ti) was calculated using the following equation:

$$Ti = \frac{Dt}{Du}$$

Where, Dt is diameter (mm) of treated colony; and, Du is diameter (mm) of untreated colony (Ezzouhri, 2009).

Optimization of fungal growth conditions

Growth conditions of arsenic tolerant fungal isolates were optimized (to obtain maximum biomass) by growing in 200 mL PD broth at different pH (4, 5, 6, 7, 8 and 9) and temperatures (25, 30, 35, 40oC) for 48, 72, 96, 120, 144 hrs.The harvested mycelia were thoroughly washed with sterilized deionized distilled water and weighed for wet and dry (65 oC) biomasses.

RESULTS AND DISCUSSION

Soil Properties

All the three soils used in this study were non-saline (0.17 to 0.43 mS/cm), alkaline in reaction (Mean pH- 7.8 to 8.3), and moderate in organic matter content (0.90% to 1.2%) (Table 1). The heavy metals and metalloids analysis of soil samples indicated higher contents of potentially toxic metals in the soils of sampled area. The mean of total Arsenic concentrations was 11.25

Table 1. Physiochemical characteristics and heavy metals/ metalloids of soils used for fungal isolation

Sampling Site	Gujranwala	Multan	Islamabad
Mean EC (mS/cm)	0.42	0.43	0.17
Mean pH	8.30	7.80	7.87
Mean O.M. (%)	1.20	1.18	0.90
Average Textural Class	Loam	Silt Clay Loam	Silt Loam
Mean heavy metals/metalloids contents (mg/kg)			
As	11.25	11.54	4.51
Pb	136.00	42.24	72.25
Cd	5.70	14.51	2.27
Cu	159.50	41.74	60.50
Cr	181.75	170.00	62.25
Ni	101.75	113.50	41.75

mg/kg in Gujranwala, 11.54 mg/kg in Multan and 4.51 mg/kg in Islamabad soils (In soils As < 5 mg kg-1 is considered uncontaminated background level (Mandal and Suzuki, 2002; Smith et al., 1998)). Mean cadmium concentrations were above the recommended permissible limits (3 mg/kg chromium, and described by Council of European Community (CEC), 1986) in Gujranwala and Multan, 5.7 to 14.51 mg/kg, respectively. Total soil copper, chromium and nickel contents in almost all samples were higher than CEC permissible limits (50 mg/kg for Cu, 100 mg/kg for Cr, and 75 mg/kg for Ni) as shown in Table 1.

Fungal Biodiversity

A total of 11 predominant fungal strains were isolated from the metal contaminated soils collected from Gujranwala and Multan while 7 from non-contaminated area of Islamabad

Table 2. Minimum Inhibitory Concentrations (MIC) of fungi grown on PDA media amended with Arsenic (III)

Origin	Fungal Strain	MIC (mg/L)	Reduction*
Gujranwala Soil	*F. chlamydosporium*	200	47.14
	A. fumigatus	2400	0
	A. ochreus	100	45.38
	A. niger	100	49.31
	F. oxysporum	2000	10.44
	A. terreus	200	38.49
Multan Soil	*A. flavus*	100	48.37
	A. ochreus	400	25.28
	A. niger	200	38.68
	A. fumigatus	2000	7.78
	Curvularea lunata	400	12.43
Islamabad Soil	*A. niger*	100	46.35
	A. flavus	100	29.34
	C. lunata	300	18.07
	Penicillium sp.	100	50.10
	A. fumigatus	1600	16.10
	F. chlymadosporium	100	63.60
	A. paraciticus	100	53.65

** in colony growth (%) at 100 mg/L As (III) conc.*

(Table 2). The fungal isolates were belonged to four genera, i.e., *Aspergillus, Curvularea, Fusarium* and *Penicillium*. The most common genera was *Aspergillus* (12 out of 18), followed by *Fusarium* (3 out of 18). The widespread occurrence of *Aspergillus* species in heavy metals contaminated soils was also reported by Ahmed et al.(2005) and Zafar et al.(2007).The occurrence of different fungi such as *Aspergillus, Penicillium, Rhizopus, Fusarium, Curvularia* etc. in soils polluted with heavy metals have, also, been reported in literature around the world (Gadd et al., 1993). There was more fungal biodiversity in non-contaminated soil than those of contaminated soils. In

non-contaminated soil four genera, i.e., *Aspergillus, Curvularea, Fusarium* and *Penicillum* were observed while in contaminated soil only two genera from each (*Gujranwala – Aspergillus, Fusarium,* Multan – *Aspergillus* and *Curvularea*) were observed. Although, environ-mental stresses due to enhanced heavy metals concentration could be a reason for the reduction in microbial species, at the same time it can increase the population of surviving species (Griffioen, 1994). Giller et al. (1998) reported that heavy metals concentrations in soils may lead to increase in fungal diversity up to moderate levels whereas higher levels may cause a sharp decrease.

Tolerance Potential

The present study demonstrated that different fungal isolates had different As tolerance and isolates from contaminated soils had relative higher tolerance than those isolated from non-contaminated soils. The tolerance was evaluated by comparing Minimum Inhibitory Concentrations (MICs) and Tolerance Index.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration, the lowest As concentration inhibiting visible fungal growth (Xu et al., 2012) of 18 selected fungal isolates is presented in Table 2. *Aspergillus fumigatus* showed maximum MIC either isolated from Gujranwala (2400 mg/L), or Multan (2000 mg/L) and Islamabad (1600 mg/L). However, least MIC value was 100 mg/kg for most of the isolates. The fungi isolated from environments contaminated with heavy metals, for examples Gujranwala and Multan, normally showed higher MIC than those isolated from unpolluted area (Islamabad) and confirmed the earlier observation of Yazdani et al. (2010). The longtime exposure to heavy metals contaminated environment may produce significant change and reduce their activity and number ; however, contrarily enhance the relative population of resistant species (Iram et al., 2009). The MIC values suggest the resistance level of isolate against the element under consideration (Zafar and Aqil, 2007).

Tolerance Index

Tolerance Index (TI), ratio of extended radial growth of a treated colony to that of untreated colony, was assessed at 100 mg/L As (III) concentration and illustrated in (Figure 1). Similar to MIC, *Aspergillus fumigatus* (G-2, M-4 and I-5) showed maximum tolerance index. Radial growth of *Aspergillus fumigatus* (G-2), obtained from Gujranwala, was similar on As amended PDA medium and un-amended and exhibited maximum tolerance index i.e.1.00 followed by M-4, G-5, M-5 and I-5 with TI's of 0.92, 0.90, 0.88 and 0.84, respectively (Figure 1). Different orders of tolerance were demonstrated (Figure 1). Overall, the fungus isolated from more contaminated soils (Gujranwala) had higher TI than those obtained from less contami-

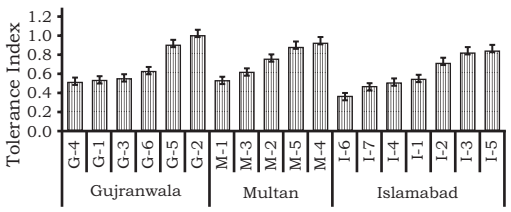


Figure 1. Tolerance Index of Fungal isolates at 100 mg/ L As (III) concentration

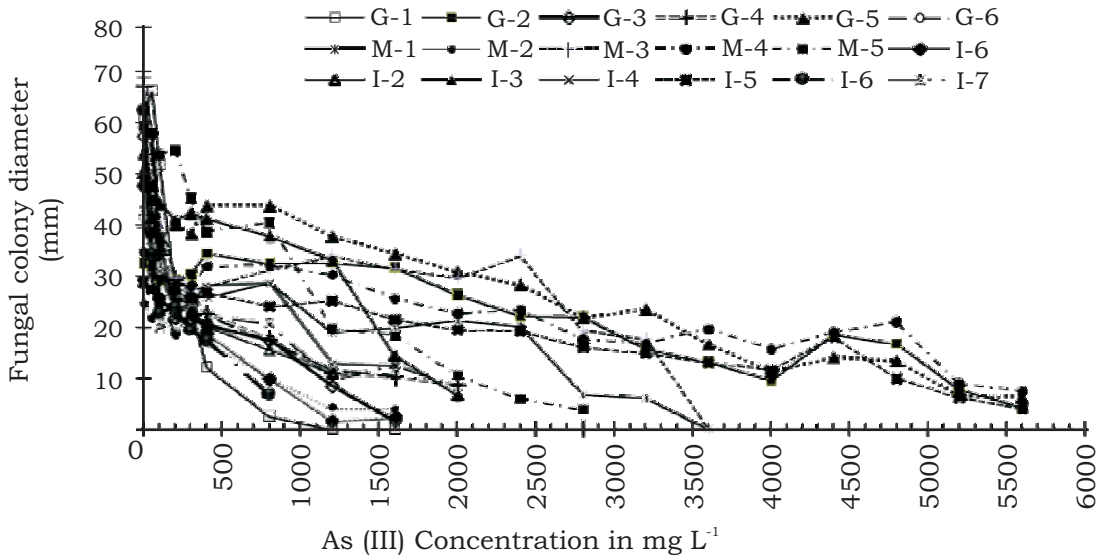
nated soils (Multan) and uncontaminated (Islamabad) soil. Heavy metals concentrations in the original environment may trigger the evolution for higher As tolerance (Krznaric et al., 2009). In contrast, *Aspergillus fumigatus* (I-5), isolated from unpolluted soil of Islamabad, showed also exceptionally high tolerance and 16 % growth reduction was observed at 100 mg/L As concentration. It reveals that fungal resistance mainly depends on the biological functions of the strain rather than pollution level (Iram et al. 2009; Baldrian and Gabriel, 2002). *Fusarium chlamydosporium, Aspergillus niger, Aspergillus ochreus* relatively showed less tolerance.

Effect of As concentrations on Fungal Growth

Most of the isolates (11 out of 18) showed exponential reduction in radial growth with increase in As concentration from 0 to 300 mg/L (Figure 3). Afterwards, these isolates showed gradual reduction in growth and after 2000 mg/L, no growth was observed in these strains. While four strains (two from Gujranwala - *A. fumigatus* and *F. oxysporum*; one from Multan - *A. fumigatus* and one from Islamabad - *A. fumigatus*) showed growth even up to As concentration of 5600 mg/L. Valix and Loon (2003) have, also, observed similar reductions in growth of *Aspergillus* sp, which was more resistant to Cr at higher metal concentrations. Further, the results of our study are also comparable with those reported by Yoshida et al. (2006), Iram et al. (2012), Akhter et al. (2013).

In general, strains isolated from contaminated sites are more tolerant than those isolated from natural environments (Massaccesi et al., 2002; Malik, 2004). However, in the present study the A. fumigatus showed more As tolerance irrespective of origin showing that the fungal specie is more important than site of isolation as has also been reported by Rudawska and Leski, 1998; Iram et al., 2009 and Fazli et al., 2015.

Figure 3. Effect of As (III) concentrations on growth of fungal isolates



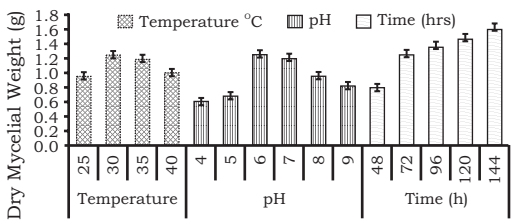
Optimal growth conditions

Optimal growth conditions (pH, temperature and time) were appraised for most As tolerant fungal isolates for biomass production for possible use in As bioremediation studies. The results showed that all tested fungi are capable of growing at wider range of pH values from 4 to 9 (Figure 3) when incubated at 30oC. Maximum dry biomass production at pH 6 and 7 confirmed the earlier observation of Gautam et al. (2011) and the general belief that fungi grow maximum at neutral pH (Mukherjee et al., 2013). However, there are reports that reveal that fungi can grow well under alkaline conditions as well (Kezia et al., 2011). Growth of almost all selected isolates was maximum at 30 oC. A relative large increase in fungal growth was observed till 96 hour and then there was minimal increase in growth till 144 hour.

Conclusion

In the present study, 11 fungal strains were isolated from heavy metal contaminated sites and most of them exhibited the ability to grow under high As(III) concentrations. While one isolates, obtained from non-contaminated soil performed almost equally good. The fungal isolate, *A. fumigatus* showed highest As (III) tolerance regardless of origin. As (III) tolerant isolates will be further evaluated for their sorption potential and possible use for bioremediation of As (III) contaminated soil and waters. The strains were capable of growing under a wide range of pH and temperatures, also, showing potential for use under controlled and field conditions.

Figure 3. Average dry weight values of As (III) tolerant isolates (G-2, G-5, M-4 and I-5) at different, Temperatures, pH and Time durations



REFERENCES

Adriano, D. C. 2001. Trace elements in terrestrial environments. Biogeochemistry, bioavailability and risks of metals. 2nd edition. Springer Science+Business Media, LLC, New York, USA. 229.

Ahamed, S., M. K. Sengupta, A. Mukherjee, M. A. Hossain, B. Das, B. Nayak, A. Pal, S.C. Mukhejee, S. Pati, R. N. Dutta, G. Chattejee, R.Srivastava and D. Chakraborti. 2006. Arsenic groundwater contamination and its health effects in the state of Uttar Pradesh (UP) in upper and middle Ganga plain, India: A severe danger. Sci. Tot. Env. 370: 310-322.

Akhter, S., M. Mahmood-ul-Hassan, R. Ahmad, V. Suthor and M. Yasin. 2013. Metal tolerance potential of filamentous fungi isolated from soils irrigated with untreated municipal effluent. Soil Environ. 32(1): 55-62.

Amacher, M. C. 1996. Nickel, Cadmium and Lead. In: Methods of Soil Analysis, Part 3, SSSA: 739-768.

Amini, M., K. C. Abbaspour, M. Berg, L. Winkel, S.J. Hug, E. Hoehn, H. Yang and C.A. Johnson. 2008. Statistical modeling of global

geogenic fluoride in groundwater. Environ. Sci. Techol.; 42(10): 3662-3668.

Andrews J. M. 2001. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48 (Suppl) 1:5-16. Arica, M.Y., G. Bayramoglu, M. Yilmaz, C.O. Gen, and S. Bektas. 2004. Biosorption of Hg2+, Cd2+ and Zn2+ by Ca– alginate and immobilized wood rotting fungus Funalia trogii. J. Hazard. Mater. 109:191–199.

ATSDR. 2002. Arsenic Toxicity: Case studies in environmental medicine. Agency for Toxic Substances and Disease Registry. USA.

Baldrian, P. and J. Gabriel. 2002. Copper and cadmium increase laccase activity in Pleurotus ostreatus. FEMS Microbiol Lett. 206(1):69–74.

Baldrian, P., 2003. Interactions of heavy metals with white-rot fungi, Enzyme. Microb. Technol. 32:78–91.

Barnett, H.L. and B.B. Hunter. 1999. Illustrated genera of Imperfect fungi. 4th ed. Prentice Hall Inc.

Benefield, L.D. and J.S. Morgan. 1990. Chemical precipitation in water quality and treatment, in: R.D. Letterman (Ed.) AWWA. 4th ed. McGraw-Hill. New York.

Bodek, I., W. J. Lyman, W.F. Reehl and D.H. Rosenblatt. 1998. Environmental Inorganic Chemistry: Properties, Processes and Estimation Methods. Pergamon Press, USA.

British Geological Survey, 1999. Groundwater Studies for Arsenic Contamination in Bangladesh. Final Report. In: British Geological Survey, Mott MacDonald Ltd., London,UK..

Clifford, D.A. 1999. Ion-exchange and inorganic adsorption, in: Water Quality and Treatment: A Handbook of Community Water Supplies. 5th ed. American Water Works Association. McGraw-Hill. New York.

Couto, S.R., S.A. Sanroman, D. Hofer and G.M. Gubitz. 2004. Stainless steel: A novel carrier for the immobilization of the white rot fungus Trametes hirsutefor decolorization of textile dyes. Bioresour. Technol. 95: 67–72.

Dhar, R.K., B. K. Biswas, G. Samanta, B. K. Mandal, D. Chakraborti, S. Roy, A. Jafar, A. Islam, G. Ara and S. Kabir. 1997. Groundwater arsenic calamity in Bangladesh. Current Science.73: 48-59.

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

Ezzouhri, L., E. Castro, M. Moya, F. Espinola and K. Lairini. 2009. Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. Afr. J. Microbiol. Res. 3:35-48.

FAO/WHO. 2001. Codex alimentarius commission. Food additives and contaminants. Joint FAO/ WHO Food Standards Program 2001. FAO VialedelleTerme di Caracalla, Rome, Italy.

FAO. 2006. Arsenic contamination of irrigation water, soil and crops in Bangladesh: risk implications for sustainable agriculture and food safety in Asia. ISBN 974-9746-88-2.

FAO. 2007. Remediation of Arsenic for Agriculture, Sustainability,

Food security and Health in Bangladesh. (Accessed at http://www.fao.org/3/a-ap102e.pdf on 01-12-2015)

Gadd, G. M. and C. White. 1993. Microbial treatment of metal pollution -a working biotechnology Trends Biotechnol. 11:353-359.

Gadd, G. M., M. M. Gharieb, L. M. Ramsay and J. A. Sayer., A. R. Whatley and C. White. 1993. Fungal Processes for Bioremediation of Toxic Metal and Radionuclide Pollution. J. Chem. Technol. Biotechnol. 71: 364-5.

Ge, W., D. Zamri, H. Mineyama and M. Valix. 2011. Bioaccumulation of heavy metals on adapted Aspergillusfoetidus. Adsorption. 17:901–910.

Giller, K. E., E.Witter and S. P. Mcgrath. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biology and Biochemistry. 30(10-11): 1389–1414.

Iram, S., I. Ahmad, B. Javed, S.Yaqoob, K.Akhtar, M. Kazmi and Badar-uz-Zaman. 2009. Fungal tolerance to heavy metals. Pak. J. Bot. 41(5): 2583-2594.

Iram, S., A. Arooj and K. Parveen. 2012. Tolerance potential of fungi isolated from polluted soil of Multan. Pak. J. Bio. Env. Sci. 2:27-34.

Jain, K. and I. Ali. 2000. Arsenic occurrence, toxicity and speciation techniques. Water Res. 34 : 4304–4312.

Kadukova, J. and E. Vircikova. 2005. Comparison of differences between copper bioaccumulation and biosorption. Env. Int. 31 (2):227–232.

Kim, D.H., K.W. Kim and J. Cho. 2006. Removal and transport mechanisms of arsenics in UF and NF membrane processes. J. Water Health. 4 (2): 215–223.

Krznaric, E, N. Verbruggen, J. H. Wevers, R. Carleer, J. Vangronsveld and J.V. Colpaert. 2009. Cd-tolerant Suillusluteus: a fungal insurance for pines exposed to Cd. Environ Pollut. 2009. 157: 1581–1588.

Maheswari, S. and A. G. Murugesan. 2011. Removal of arsenic (III) from aqueous solutions using Aspergillus flavus isolated from arsenic contaminated sites. Ind. J. Chem. Technol. 18: 45-52.

Maheswari, S. and A.G. Murugesan. 2009 (a). Remediation of arsenic in soil by Aspergillus nidulans isolated from an arsenic-contaminated site. Environ. Technol. 30(9):921–926.

Maheswari, S. and A.G. Murugesan. 2009 (b). Biosorption of arsenic (III) ion from aqueous solution using Aspergillus fumigatus isolated from arsenic contaminated site. Desalination and Water Treatment. 11:1-3, 294-301

Malik, A. and R. Jiswal. 2000. Metal resistance in pseudomonas strains isolated from soil treated with industrial wastewater. World J. Microb. Biot. 30: 261- 278.

Mandal, B.K and Suzuki, K.T. 2002. Arsenic round the world: a review. Talanta. 58:201-235.

Martino, E., K. Turnau, M. Girlanda, P. Bonfate and S. Perroto. 2000. Ericoid mycorrhizal fungi from heavy metal polluted soils: their identification and growth in the presence of zinc ions. Mycological Research. 184:338-44.

Morley, G.F. and G.M. Gadd. 1995.

Sorption of toxic metals by fungi and clay minerals. Mycological Research. 99:1429-1438.

Fazli, M., N. Soleimani, M. Mehrasbi, S. Darabian, J. Mohammadi and A. Ramazani. 2015. Highly cadmium tolerant fungi: their tolerance and removal potential. Journal of Environmental Health Science & Enginee-ring.13:1-9.

Mukherjee, K. K., D. Das, A. C. Samal and S. C. Santra. 2013. Isolation and characterization of Arsenic tolerant fungal strains from contaminated sites around urban environment of Kolkata. .J. Env. Sci. and Food Techn. 7(5): 33-37.

Nyongesa, B.W., S. Okoth, and V. Ayugi. 2015. Identification Key for Aspergillus species isolated from Maize and soil of Nandi County, Kenya. Advances in Microbiology.5: 205-229.

PCRWR. 2007. Arsenic Monitoring and Mitigation in Pakistan. Pak. Council of Research on Water Resources. Annual report 2005-2006. 136-140.

Rasool, A. and S. Irum. 2014. Toxic Metal Effect on Filamentous Fungi Isolated from the Contaminated Soil of Multan and Gujranwala. J. Biores. Manage. 1(2) 38-51.

Razak, A.A., G. Bachman and R. Farrag. 1999. Activities of microflora in soils of upper and lower Egypt. Afr. J. Mycol. and Biotech. 7:1-19.

Rudawska, M. and T. Leski.1998. Aluminium tolerance of different Paxillus involutus Fr strains originating from polluted and non-polluted sites. Acta Societatis Botanicorum Poloniae. 67:115–122.

Sathishkumar, M, A. R. Binupriya, K. Swaminathan, J. G. Choi and S. E. Yun. 2008. Bio-Separation of Toxic Arsenate Ions from Dilute Solutions by Native and Pretreated Biomass of Aspergillus fumigatus in Batch and Column Mode: Effect of Biomass Pretreatment. Bull Environ. Contam. Toxicol. DOI 10.1007/s00128-008-9496-4.

Say, R., N. Yilmaz and A. Denizli. 2003. Biosorption of cadmium, lead, mercury, and arsenic ions by fungus Penicillium Purpurogenum. Sep. Sci. Technol. 38 (9): 2039–2053.

Say, R., A. Denizli and M.Y. Arica. 2001. Biosorption of cadmium (II), lead (II) and copper with the filamentous fungus Phanerochaete chrysosporium. Bioresour. Technol. 76: 67–70.

Smedley, P.L., D. G. Kinniburgh. 2002. A review of the source, behavior and distribution of arsenic in natural waters. Appl. Geochem. 17:517-568.

Smedley, P.L., H. B. Nicolli, D. M. J. Macdonald, A. J. Barros and J.O. Tullio. 2002. Hydro geochemistry of arsenic and other inorganic constituents in ground waters from La Pampa, Argentina. Appl. Geochem. 17 (3):259–284.

Smith, A.L., P.A. Lopipero, M.N. Bates and C.M. Steinmaus. 2002. Arsenic epidemiology and drinking water standards. Science. 296: 2145– 2146.

Smith, E., R. Naidu and A.M. Alston. 1998. Arsenic in the soil environment; a review. Advan. Agron. 64:149-195.

Thomas, G.W. 1996. Soil pH and soil acidity. In: Methods of Soil Analysis. Part 3. Chemical Methods. Ed. J.M. Bartels. SSSA-

ASA, Medison, Wisconsin,USA: 475-490.

Valix, M. and L.O. Loon. 2003. Adaptive tolerance behavior of fungi in heavy metals. Miner. Eng. 16: 193–198

Veglio, F. and F. Beolchini. 1997. Removal of metals by biosorption: a review, Hydrometallurgy. 44: 301–316.

Visoottiviseth, P. and N. Panviroj. 2001. Selection of fungi capable of removing toxic arsenic compounds from liquid medium. Sci. Asia. 27: 83–92.

WHO. 2003. Source and behaviour of arsenic in natural waters. In: Ch. 1. United Nations synthesis report on arsenic in drinking water.

WHO. 2001. Arsenic and Arsenic Compounds. Environment Health Criteria. 2nd ed. World Health Organization. Geneva.

WHO. 1999. Arsenic in drinking water. Fact sheet No.210. (Accessed: http://www.knowledgebank.irri.org/factsheetsPDF/Health_and_Nutrition/whoFS_arsenic.pdf. on 2-10-2015).

Xu, X, L. Xia, Q. Huang, J-D. Gu, W. Chen. 2012. Biosorption of cadmium by a metal-resistant filamentous fungus isolated from chicken manure compost. Environ Technol. 33:1661–1670.

Yazdani, M., C. K.. Yap, F. Abdullah and S.G.Tan. 2010. An in vitro study on the adsorption, absorption and uptake capacity of Zn by the bioremediator Trichodermaatroviride. Environment Asia. 3:53–59.

Yoshida, N., R. Ikeda and T. Okuno. 2006. Identification and characterization of heavy metal resistant unicellular algae isolated from soil and its potential for phytoremediation. Bior. Tech. 97: 1843-1849.

Zafar, S., F. Aqil and I. Ahmad. 2007. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bior. Tech.. 98:2557–61.

Zaw, M. and M.T. Emett. 2002. Arsenic removal from water using advanced oxidation processes, Toxicol. Lett. 133 (1): 113–118.

AUTHORSHIP AND CONTRIBUTION DECLARATION

S. No	Name	Contribution to the paper
1.	Mr. Abdul Rehman Khan	Overall Management of the article, Data collection, data entry and analysis
2.	Dr. Muhammad Mehmood-ul-Hassan	Conceived the idea, Technical in put at every step, Overall Management of the article,
3.	Dr. Rizwan Ahmed	Technical in put at every step, Supervision, Methodology,
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