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Arsenic (III) removal potential of natural and modified fungal biomass from aqueous solution

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Abstract

The removal of arsenic,a widely occurring natural poisonous metalloid, from water employing biological sorbents having low cost and higher sorption capacity has become an important field of research as arsenic is significantly endangering human health by contaminating drinking water. Filamentous fungi have gained important place as a bio-remedial due to their fine pores, large surface area and metal sorption capacity. In present study, arsenic (As-III) tolerance of 18 indigenous filamentous fungi was explored by exposing them to As concentrations of 50 to 5600 mg kg^{-1}.Out of 18 isolates, 12 belonged to genus *Aspergillus*, 3 to *Fusarium*, 2 to *Curvularea* and one to *Penicillium*. The fungal isolates (G-2, M-4, I-5) identified as *Aspergillus fumigatus* and (G-5) as *Fusarium oxysporum* showed highest As (III) tolerance. The fungal biomasses of highly tolerant fungi, untreated and treated with NaOH and $FeCl_3$, ware then assessed for their arsenic removal capacity from aqueous solutions. The fresh wet biomass of natural and treated fungus was equilibrated with aqueous solutions of varying As (III) concentrations (0-1000 mg L^{-1}).. The maximum As (III) (3.2 mg g^{-1}) was removed by $FeCl_3$-treated *Aspergillus fumigatus* (G-2) biomass followed by NaOH-treated (2.83 mg g^{-1}) and untreated biomass (2.66 mg g^{-1}). Maximum increase in As (III) removal (33.65 % over untreated) was observed in $FeCl_3$-treatmentedfungal biomass over untreated whereas NaOH treatment enhanced 22.27 %. Arsenic sorption parameters i.e. maximum sorption capacity and binding strength of fungal biomasses were calculated using Langmuir and Freundlic hsorption models. Langmuir regression coefficient (r^2) (0.97-0.99) indicated its better fitness to adsorption data than Freundlich model with r^2 values (0.85-0.93).The tested arsenic tolerant fungal strains removed significant amounts of arsenic from arsenic enriched media in laboratory conditions and may be used as an effective sorbent in arsenic removal technology from arsenic contaminated waters.

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Introduction

Arsenic, a persistent and bio-accumulative poisonous element, has potential to pollute land, water, crops and the overall environment; ultimately affecting human and animal lives. Arsenic has become a common contaminant in the environment and particularly ground water arsenic contamination has become a global environmental issue. Higher arsenic concentration in the environment, due to its toxicity and induced carcinogenetic effects, is considered a serious problem for human health. Groundwater arsenic contamination has become a worldwide water quality issue including Pakistan (WHO, 1999, 2001; ATSDR, 2002; Smith *et al.*, 2002; PCRWR, 2007; Amini *et al.*, 2008), Whereas, problem in Bangladesh is worse and has the highest number of people affected by arsenic poisoning (Dhar *et al.*, 1997). In Pakistan, a joint research conducted by PCRWR, UNICEF and National Water Quality Monitoring Program (2002-2006) revealed the presence of arsenic in Punjab and Sind provinces. According to PCRWR report (2005-2006), in Punjab out of 11559 ground water samples, 38% contained arsenic concentration more than permissible limit (>10 ppb) and in Sindh out of 5991 samples, 11% contained arsenic concentration >10 ppb. Arsenic naturally occur in different oxidized forms such asAs^{+3} (arsenites), As^{+5} (arsenate), As^{-3} (arsenides) (Smedley *et al.*, 2002). As^{+3} and As^{+5} are most commonly found forms of arsenic in natural waters and former is 2-10 times more toxic than later (Jain and Ali, 2000).

Ground waters is considered major non-point natural arsenic source of arsenic (Smedley and Kinniburgh, 2002) while mining, pesticides, wood-treatment, agriculture chemicals are considered point sources. Significant research has been carried out to provide arsenic free drinking, municipal and industrial waste water using conventional techniques based on principles of precipitation-coagulation, oxidation, membrane separation, electro-coagulation/flocculation and adsorption (Benefield and Morgan, 1990; Clifford, 1999; Zaw and Emett, 2002; Kim *et al.* 2006). Among others, adsorption principle for arsenic remediation has been extensively exercised using a wide range of sorbents. Sorbents used range from naturally occurring and synthetic minerals to agricultural products and wastes; however, bio-sorbent like fungi, algae and bacteria are preferably used as sorbent and considered environment friendly. Bio-sorption has the ability of removing heavy metals and other elements in traces from dilute aqueous solutions (Mohan and Pittman, 2007; Volesky and Holant, 1995).

Research has been conducted for the removal of arsenic from water employing biological materials such as coconut coir pith (Anirudhan *et al.*, 2007), sea nodule, orange waste (Ghimere *et al.*, 2003), coconut husk carbon (Manju *et al.,* 1998), bone char (Sneddon *et al.*, 2005), crab shell (Niu *et al.*, 2007), powdered egg shell (Oak *et al.*, 2008) and many more. However, researchers are still struggling to get even better biological materials having ready availability, low cost and having higher sorption capacity. Low cost sorbents like algae (Gadd, 1988), fungi (Sag, 2001), bacteria (Miyatake and Hayashi, 2009) and lingo-cellulosic agricultural by-products (Mahmood-ul-Hassan *et al.*, 2015) have high bio-sorption capacity and can potentially be used for heavy metals removal. However, fungi, algae and bacteria are commonly used bio-sorbents and have shown good results against several metals (Ioannis and Zouboulis, 2004; Fedrickson *et al.*, 2000; McLean and Beveridge, 2001). 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 *Rhizopus arrhizus* (Aksu *et al.*, 1999), *Phanerochaete chrysosporium* (Say *et al.,* 2001), *Aspergillus nidulans* (Maheswari and Murugesan, 2009), *Aspergillus flavus* (Maheswari and Murugesan, 2011), *Aspergillus fumigatus* have been studied for sorption of arsenic (Sathishkumar *et al.*, 2008; Maheswari and Murugesan, 2009). Considering the importance of fungi in bioremediation, indigenous filamentous fungi were isolated from heavy metals polluted and unpolluted sites and their arsenic tolerance and removal parameters were study.

Materials and methods

Soil sampling

Soil samples were collected from peri-urban agricultural areas of Multan (30° 07′-30° 09′ North latitude and 71° 21′-71°26′ East longitude), Gujranwala(30° 06′-32° 07′ North latitude and 74° 10′ East longitude) and Islamabad (33°40' North latitude and 73°07′ East longitude). The Multan and Gujranwala sites were under untreated municipal/industrial effluents while Islamabad site was under fresh water irrigation. The collected samples were brought to Soil Environment Laboratory, National Agricultural Research Centre, Islamabad, Pakistan for further investigation. 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 ≈100 g was drawn from the 2 mm fraction and reground to obtain <200 µm fraction for physico-chemical analysis.

Physico-chemical analysis of soil

The collected samples were analyzed for basic physico-chemical parameters such as particle size distribution 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 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 characterization of fungi

Fungal growth media was prepared by dissolving 39 g potato dextrose agar (PDA) in 1 Litre deionized water (Razak *et al.*, 1999) and autoclaved at 121°C for 15 minutes. After autoclaving and cooling to room temperature, streptomycin (an antibiotic) 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 three replicates).

Distinct fungal isolates were purified by repeated streaking and incubations and identified by observing their macroscopic and microscopic characteristics as described by Barnett and Hunter (1999); Watanabe (2002) and Nyongesa et al. (2015). The colony color, texture, appearance of mycelia, shape of spores, columella and sporangiophore position and other characteristics were ascertained.

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) (0 to 5600 mg/L) at 28±2°C for 7 days and measuring radial growth periodically against control (without As (III)).

Minimum inhibitory concentrations (MIC's) and tolerance index

Minimum inhibitory concentrations (MIC's), the lowest As concentration that inhibits visible fungal growth, were determined from the As screening data. The arsenic tolerance index, ratio of radial growth of treated colony to that of the untreated colony, was gauged by growing and measuring readial growth of selected isolates on As(III) amended PDA medium @ 100 mg/L As concentration for 7 days. Tolerance index (*Ti*) was calculated using the following equation:

$$Ti = \frac{D_t}{D_u}$$

Where, D_t and D_u are diameters (mm) of treated and untreated colonies, respectively.

As (III) removal studies

Biomasses of selected fungal isolate were harvested by growing in sterilized liquid potato broth (Lab M Ltd., UK) medium in 250 mL Erlenmeyer flasks, separately. The live mycelial biomass was separated by filtrating through Whatman No.1 filter paper. The harvested biomass was then washed with double distilled water and gently squeezed with filter paper (Sathishkumar *et al.*, 2004).A portion of the collected biomass (12 g) of each arsenic resistant isolate was treated with I N NaOH and $FeCl_3$ (containing 15 mg L^{-1} of Fe) solutions, separately, in conical flasks and suspensions were shaken for 60 minutes at 125 rpm using a rotary shaker.

Subsequently, the suspensions were filtered through Whatman No.1 filter paper and gently washed with deionized distilled water to remove extra material and to bring pH in neutral rang.

Treated and untreated biomass of selected arsenic tolerant fungal strains was equilibrated with synthetic aqueous solutions of varying As (III) concentrations, i.e., 0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg L^{-1}.The treated and untreated 2 g fungal biomass was equilibrated with 100 mL arsenic solutions of each concentration at pH 6 in 250 ml conical flasks (Kapoor *et al.*, 2000). The flasks were agitated on a rotary shaker for 4 hrs at room temperature (28±2 °C). The equilibrated suspensions were filtered through Whatman # 1 filter papers and the filtrate arsenic concentrations were measured using graphite furnace Atomic Absorption Spectrophotometer (Perkin Elmer, A Analyst 800).

Arsenic removal capacity/data analysis

The amount of arsenic (mg) removed by a unit fungal biomass (g) was calculated with the following formula:

$$Q = \left(\frac{C_i i - C_f}{m}\right) V$$

Where, Q is amount of arsenic removed by the unit fungal biomass (mg g^{-1}), $C_i$and C_f are the initial (beforesorption and final (after sorption) equilibrium solution arsenic concentrations (mg L^{-1}), respectively, m is the mass of fungal biomass (g) and V is the volume (L) of aqueous solution.

All the batch experiments were performed in three replicates and means were used for plotting the experimental results. The removal of As (III) in terms of percentage was calculated by the following equation:

$$\text{As(III) Removal (\%)} = \frac{\text{Initial As(III)} - \text{Final As(III)}}{\text{Initial As(III)}} \times 100$$

Arsenic sorption parameters

Arsenic sorption parameters like maximum sorption capacity, intensity and binding strength were calculated using classical Langmuir (Langmuir, 1918) and Freundlich (Freundlich, 1906) sorption models for selected fungi.

The Langmuir sorption model quantitatively explains the build-up of a layer of molecules on a bio-sorbent surface as a function of the concentration of the bio-sorbed material in the contact liquid phase and thus describes the adsorption of adsorbate from an aqueous solution onto the number of identical sites on the surface of adsorbent as a monolayer (Dogan *et al.*, 2000). Linearized form of Langmuir adsorption equation is as under:

$$\frac{C}{q} = \frac{1}{Kb} + \frac{1}{b} C_e$$

Where b and k are constants; associated with adsorption capacity and energy, q (mg/kg) is the amount of metal adsorption per unit weight of sawdust. A plot of C_e/q versus C_e yields a straight line with slope $1/b$ by the intercept $1/Kb$.

Freundlich isotherm model describes non-ideal sorption which involves adsorption heterogeneously and it may be derived by assuming a logarithmic decrease in the adsorption enthalpy with the increase in the occupied sites fraction. The isotherm can be evaluated from the experimental data by plotting log (qe) against log (C_e). A plot of log (qe) against log (Ce) yield a straight line and 1/n equals to slope and Log KF equals to an intercept (Santhi *et al.*, 2010). The linear form of Freundlich isotherm equation is

$$Log\ X = \log KF + \frac{1}{n} \log C_e$$

Where, X, denotes the amount adsorbed per gram of the adsorbent, C_e denotes the equilibrium concentration, while KF and 1/n are constants. KF is the measure of adsorptive capacity and is a function of energy of adsorption and temperature, and 1/n indicates the adsorption intensity (Uddin, 2007; Khan, 2005).

Statistical analysis

Data was recorded and analyzed using standard statistical techniques like Microsoft Office Excel 2007 and Statistix 10.

Results and discussion

Soil characteristics

All the 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).

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	8.3	7.8	7.87
Mean O.M. (%)	1.2	1.18	0.9
Average Textural Class	Loam	Silt Loam	Silt Loam
Mean heavy metals/metalloids contents (mg/kg)			
As	11.25	11.54	4.51
Pb	136	42.24	72.25
Cd	5.7	14.51	2.27
Cu	159.5	41.74	60.5
Cr	181.75	170	62.25
Ni	101.75	113.5	41.75

The analysis of soil samples indicated higher metals content in the soils sampled collected from peri-urban areas under untreated municipal/industrial effluent. The mean total arsenic concentrations in Gujranwala, Multan and Islamabad soils were11.25, 11.54 and 4.51 mg kg^{-1}, respectively, whereas in soils As >5 mg kg^{-1} isconsidered contaminated background level (Smith *et al*., 1998; Mandal and Suzuki, 2002). Mean cadmium concentrations were above the recommended permissible limits (3 mg kg^{-1}described by Council of European Community, 1986) in Gujranwala and Multan, 5.7 to 14.51 mg kg^{-1}, respectively and total soil copper, chromium, and nickel contents in almost all samples were also higher than CEC permissible limits (50 mg $kg^{-1}$for Cu, 100 mg $kg^{-1}$for Cr, and 75 for Ni).

Table 2. Minimum Inhibitory Concentrations (MIC) of isolated fungi able to grow on PDA media amended with Arsenic (III).

Origin	Fungal strain	MIC(mg/L)	Reduction in colony growth (%)
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
	Curvularealunata	400	12.43
Islamabad Soil	*A.niger*	100	46.35
	A.flavus	100	29.34
	C. lunata	300	18.07
	Penicilliumsp.	100	50.10
	A. fumigatus	1600	16.10
	F. chlymadosporium	100	63.60
	A. paraciticus	100	53.65

Fungal biodiversity

A total of 18 predominant fungal strains were selected, 11 from the metal contaminated soils of Gujranwala and Multan and seven (7) from non-contaminated soils collected from Islamabad, were used in this study (Table 2). The fungal isolates belonged to four genera*viz. Aspergillus, Fusarium, Curvularea* and *Penicillium*. The most dominant genera were *Aspergillus* (12 out of 18) and followed by *Fusarium* (3 out of 18).

Table 3. Langmuir and Freundlich isotherm model parameters of *Aspergillus fumigatus* (G-2, M-4 and I-5) and *Fusarium oxysporum* (G-5) biomasses.

Treatments	*Langmuir parameters			**Freundlich parameters		
	Max. Sorption Capacity Q (mg g^{-1})	Bindingstrength b (Lmg^{-1})	Correlation Coefficient r^2	Sorption capacity (mg g^{-1})	Sorption intensity 1/n (Lmg^{-1})	Correlation Coefficient r^2
Aspergillus fumigatus (G-2) Biomass						
Untreated	3.1949	0.313	0.97	1.879	0.545	0.90
NaOH	3.3784	0.296	0.98	2.071	0.492	0.90
$FeCl_3$	3.6101	0.277	0.98	2.165	0.475	0.88
Fusarium oxysporum(G-5) Biomass						
Untreated	2.7624	0.362	0.99	1.607	0.648	0.89
NaOH	3.3223	0.301	0.99	1.77	0.611	0.85
Fe Cl_3	3.8462	0.26	0.96	1.804	0.607	0.85
Aspergillus fumigatus (M-4) Biomass						
Untreated	3.8462	0.26	0.97	1.612	0.676	0.96
NaOH	4.0000	0.25	0.97	1.636	0.647	0.93
$FeCl_3$	3.9526	0.253	0.98	1.696	0.617	0.88
Aspergillus fumigatus (I-5) Biomass						
Untreated	3.1250	0.32	0.98	1.574	0.655	0.87
NaOH	3.5211	0.284	0.98	1.745	0.613	0.87
$FeCl_3$	4.1494	0.241	0.98	1.779	0.866	0.87

*Q, maximum uptake capacity of the adsorbent (mgg^{-1}); b, Langmuir binding constant (Lmg^{-1})

**Freundlich constant (kf)- adsorption capacity (Lmg^{-1}) ; n, Freundlich constant related to adsorption intensity.

The widespread occurrence of *Aspergillus* species in heavy metals contaminated soils has also been 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 was also reported in literature around the world (Gadd *et al*, 1993). There was more fungal biodiversity in non-contaminated soils than those of contaminated soils. In non-contaminated soils four genera (*Aspergillus, Curvularea, Penicillum* and *Fusarium*) were observed while in contaminated soil two genera from each (Gujranwala- *Aspergillus and Fusarium* and Multan- *Aspergillus*and *Curvularea)* were observed. The environmental stress due to enhanced heavy metals concentration could reduce the microbial species; however, simultaneously it can increase the population of surviving species (Griffioen, 1994).

Giller *et al.* (1998) reported that heavy metals concentration in soils may increase the fungal diversity up to a moderate level and higher levels may cause a sharp decrease.

Tolerance potential

The different fungal isolates demonstrated different As tolerance and isolates from contaminated soils had relatively 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 concentrations of selected As tolerant fungal isolates are presented in Table 2. *Aspergillus fumigatus* showed maximum MIC, either isolated from contaminated sites, i.e., Gujranwala

(2400 mgL^{-1}) and Multan (2000 mg L^{-1}) or uncontaminated site, i.e., Islamabad (1600 mg L^{-1}). However, least MIC value was 100 mg L^{-1} for most of the isolates. The fungi isolated from heavy metals contaminated environments (Gujranwala and Multan), normally showed higher MIC than those isolated from unpolluted area (Islamabad) and reaffirmed the earlier observation of Yazdani *et al.* (2010).

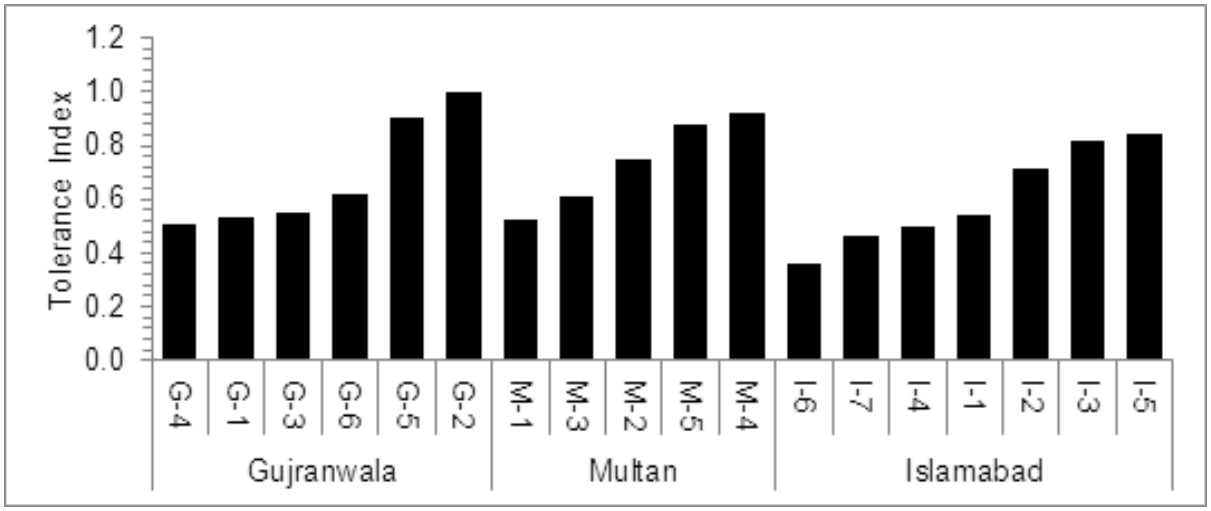

Fig. 1. Tolerance index of fungal isolates at 100 mg/ L As (III) concentration.

The long term 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).

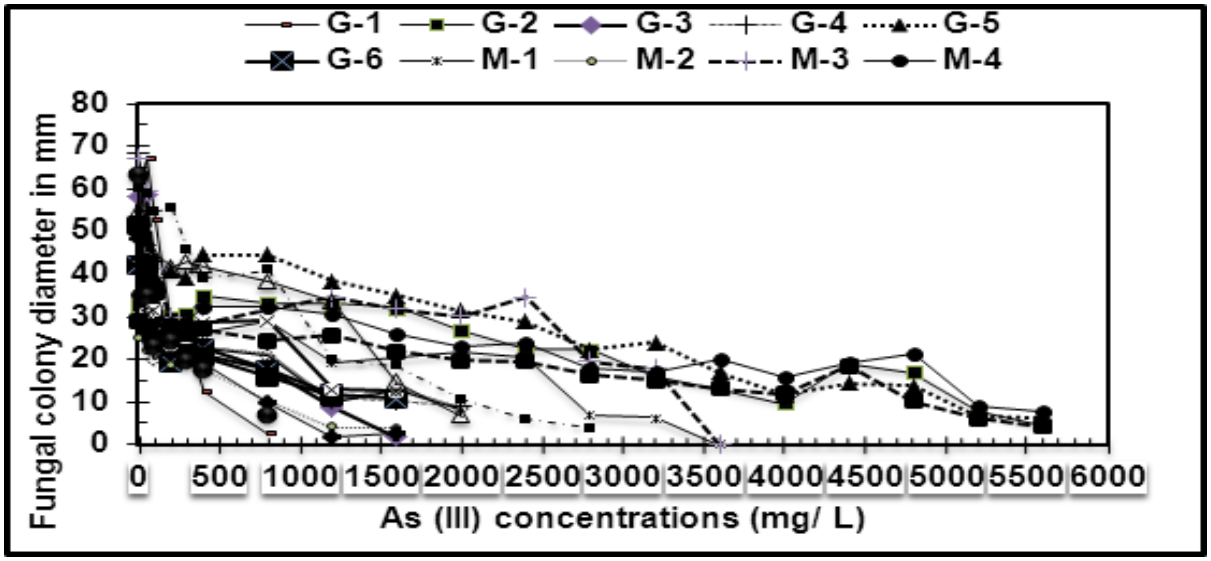

Fig. 2. Effect of As (III) concentrations on growth of fungal isolates.

Tolerance index

Tolerance Index (TI), assessed at 100 mg kg^{-1}As (III) concentration, of As tolerant fungal isolates is illustrated in Fig 1. *Aspergillus fumigatus* (G-2, M-4 and I-5) showed highest tolerance index. Radial growth of *Aspergillus fumigatus* (G-2) was similar on As amended and without As PDA media and exhibited maximum tolerance index i.e.1.0 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. Although, different orders of tolerance were demonstrated (Fig. 1) by different isolates, the fungus isolated from more contaminated soils

(Gujranwala) had higher TI than those obtained from less contaminated 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, showed also exceptionally high tolerance and only 16% growth reduction was observed at 100 mg kg^{-1}As concentration, which indicates 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.

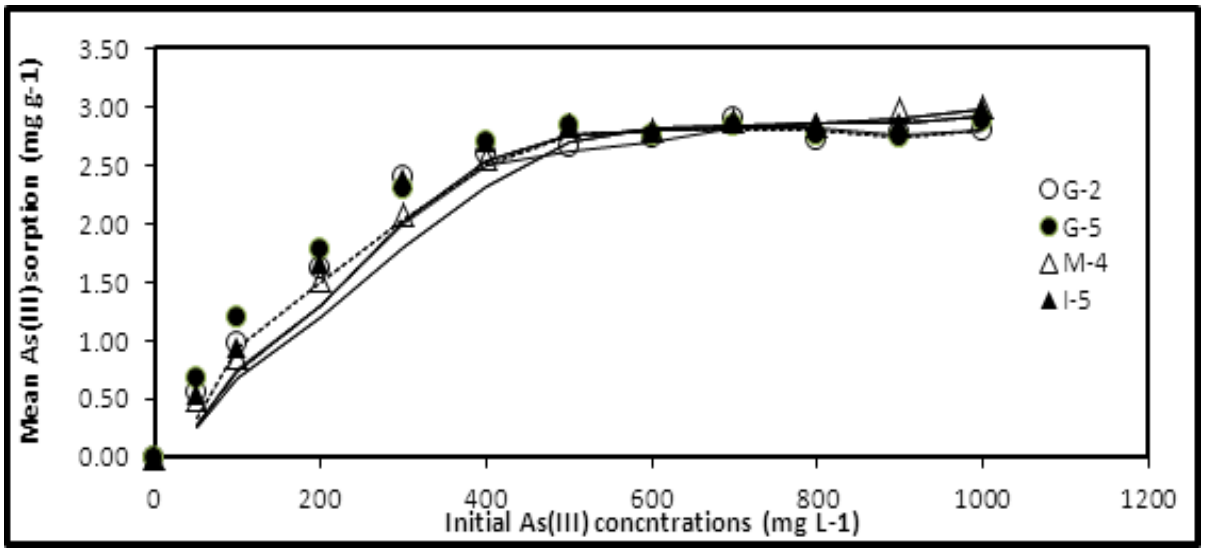

Fig. 3. Mean comparative As (III) removals by *Aspergillus fumigatus* (G-2, M-4 and I-5) and *Fusarium oxysporum* (G-5) biomasses.

Effect of As (III) concentrations on fungal growth

With increase in As (III) concentration from 0 to 300 mg L^{-1}, in majority of the isolates (11 out of 18) exponential reduction in radial growth was observed (Fig. 2). Afterwards, these isolates showed gradual reduction in growth and after 2000 mg L^{-1}, almost 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^{-1}. Valix and Loon (2003) have, also, observed similar fungal growth reductions trend in case 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). Customarily, strains isolated from contaminated sites are more tolerant than those from natural environments (Massaccesi *et al.*, 2002; Malik, 2004), while our results revealed that the *A. Fumigatus* showed maximum As tolerance irrespective of origin. However, the resistance was higher in fungi isolated from Gujranwala and Multan soils.

Arsenic removal capacity

As (III) removal capacities were calculated using mass balance equation. $FeCl_3$-treated G-2 strain biomass removed maximum As(III) (3.2 mg g^{-1}) followed by NaOH-treated (2.83 mg g^{-1}). While untreated biomass of the same strain removed 2.66 mg g^{-1} arsenic. The maximum increase in As (III) removal, i.e., 33.65 % was observed when the biomass was treated with $FeCl_3$ over untreated whereas NaOH treatment enhanced 22.27% As removal over untreated. The highest As (III) removal by untreated, NaOH- and FeCl3-treated *Fusarium oxysporum* (G-5) biomass was 2.56, 3.02 and 3.39 mg g^{-1}, respectively. The maximum increase, due to $FeCl_3$-treatment in As (III) removal by G-5 *(Fusarium oxysporum)* was 28.16% over untreated whereas

increase due to NaOH-treatment was 19.26 %. Similarly, $FeCl_3$-treated biomass of M-4 (*Aspergillus fumigatus*), isolated from Multan, also showed good increase in As (III) removal (3.27 mg g^{-1}) over NaOH-treated (2.93 mg g^{-1}) and untreated biomass (2.72 mg g^{-1}). The biomass treated with $FeCl_3$ removed up to 63.37% more As (III) than untreated while increase in As (III) removal due to NaOH treatment was relatively less, i.e., 22.48%. Arsenic (III) removal capacities of I-5 isolate (*Aspergillus fumigatus*), obtained from non-contaminated soil of Islamabad, also behaved almost similarly to those obtained from contaminated soils. The maximum As (III) removal was with $FeCl_3$-treated biomass (3.20 mg g^{-1}(31.95% more than untreated), whereas NaOH-treated removed 2.85 mg g^{-1} (27.91% more than untreated). In general, a linear increase in As (III) removal was observed up to 300 mg L^{-1} arsenic concentrations and then there was a gradual increase from 300 to 700 mg L^{-1} As (III) and afterwards there was almost no increase (Fig. 3). The results also indicate that fungal strains belonged to *Aspergillus fumigatus*, either isolated from heavy metals contaminated soils or from non-polluted soil were capable to grow and remove As (III) at higher concentrations. This means that the fungus specie is more important than site of isolation.

Langmuir and Freundlich Adsorption Isotherms

Arsenic sorption parameters i.e. maximum sorption capacity and binding strength of treated and untreated fungal biomasses were calculated using classical sorption models, i.e., Langmuir and Freundlich and are as presented in Table 3. Higher values of Langmuir regression coefficient (r^2) (0.97-0.99) indicated its better fitness to adsorption data than that of Freundlich model having regression coefficient value (0.85-0.93) (Table 3). Maximum As (III) sorption capacity,using Langmuir equation, obtained for G-2 (*Aspergillus fumigatus*) $FeCl_3$-treated fungal biomass was 3.61 mg As(III) g^{-1} followed by 3.38 mg As (III) g^{-1} for NaOH-treated and 3.19 mg As (III) g^{-1} biomass for untreated biomass. The maximum Langmuir sorption capacity of isolate G-5 biomass was 3.846 mg g^{-1} when treated with $FeCl_3$ whereas, 3.322 and 2.762 mg g^{-1} when treated with NaOH and untreated, respectively. The maximum sorption capacity observed in case of *Aspergillus fumigatus* (M-4) was 3.846, 4.00 and 3.953 mg g^{-1} for untreated, NaOH- and $FeCl_3$-treated biomasses, respectively. *Aspergillus fumigatus* (I-5) also showed equally good maximum sorption capacity when treated with $FeCl_3$ (4.149 mg g^{-1}) and slightly less sorption was observed when treated with NaOH (3.521 mg g^{-1}) but was better than untreated (3.125 mg g^{-1}) as shown in Table 3.The overall results of As (III) removal revealed that the $FeCl_3$-treated fungal biomass was more efficient in removing As (III) from aqueous solutions. $FeCl_3$ treatment improved As (III) removal up to 37.56 % whereas NaOH-treatment enhanced 22.23% As (III) removal over untreated biomass.

Conclusion

Most of the fungal strains, isolated from contaminated sites, exhibited the ability to grow under high As (III) concentrations. While one isolate, obtained from non-contaminated soil performed almost equally good. *A. fumigatus* showed highest As (III) tolerance regardless of origin and could be used for bioremediation of As (III) contaminated soil and waters. Four fungal isolates G-2, M-4 and I-5, identified as *Aspergillus fumigatus* and G-5, identified as *Fusarium oxysporum,* were capable of removing significant amount of As (III) from the aqueous solution. While treatment of wet fungal biomass with NaOH and $FeCl_3$ further increase the As removal capacity from aqueous solution.

The $FeCl_3$-treatment proved more efficient in removing As (III) from aqueous solutions than that of NaOH treatment. There was no considerable difference in adsorption of As (III) by fungal isolates from contaminated and uncontaminated soils. Langmuir model described better sorption data that Freundlich model. It is clear from the study that fungal biomass has very high As removal capacity and is an effective bio-remedial.

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References

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

Ahamed S, Sengupta MK, Mukherjee A, Hossain MA, Das B, Nayak B, Pal A, Mukhejee SC, Pati S, Dutta RN, Chattejee G, Srivastava R, Chakraborti D. 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. Scienceof the Total Environement **370,** 310-322.

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

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

Amini M, Abbaspour KC, Berg M, Winkel L, Hug SJ, Hoehn E, Yang H, Johnson CA. 2008. Statistical modeling of global geogenic fluoride in groundwater. Journal of Environmental Science and Technology. **42(10),** 3662-3668.

Arica MY, Bayramoglu G, Yilmaz M, Gen CO, Bektas S. 2004. Biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} by Ca^{-} alginate and immobilized wood rotting fungus Funaliatrogii. Journal of Hazardous Materials. **109,** 191–199.

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

Azcue JM, Nriagu JO, Schiff S. 1994. Role of Sediment Porewater in the Cycling of Arsenic in a Mine-Polluted Lake. Environment International **20,** 517-527.

Baldrian P. 2003. Interactions of heavy metals with white-rot fungi, Enzyme. Microbiology Technology **32,** 78–91.

Barnett HL, Hunter BB. 1999. Illustrated genera of Imperfect fungi. 4th Ed. Prentice Hall Inc.

Benefield LD, Morgan JS. 1990. Chemical precipitation in water quality and treatment, in: R.D. Letterman (Ed.) AWWA. 4th Ed. Mc Graw-Hill. New York.

Bodek I, Lyman WJ, Reehl WF, Rosenblatt DH. 1998. Environmental Inorganic Chemistry: Properties, Processes and Estimation Methods. Pergamon Press, USA.

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

Cernansky S, Urik M, Seve J, Littera P, Hiller E. 2007. Biosorption of arsenic and cadmium from aqueous solution. African Journal of Biotechnology **6,** 1932-1934.

Chatterjee A, Das D, Chakraborti. 1993. A study of ground water contamination by arsenic in the residential area of behala, Calcutta due to industrial pollution. Environmental Pollution **80,** 57-65.

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

Couto SR, Sanroman SA, Hofer D, Gubitz GM. 2004. Stainless steel: A novel carrier for the immobilization of the white rot fungus Trameteshirsute for decolorization of textile dyes. Bioresource Technology **95,** 67–72.

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

FAO/WHO. 2001. Codex alimentarius commission. Food additives and contaminants. Jooint FAO/WHO Food Standards Program 2001. FAO Vialedelle Terme 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. Available at www.fao.org/3/a-ap102e.pdfon 01-12-2015

Gadd GM, White C. 1993. Microbial treatment of metal pollution -a working biotechnology? Trends in Biotechnology.**11,** 353-359.

Gadd GM, Gharieb MM, Ramsay LM, Sayer JA, Whatley AR, White C.. 1993. Fungal Processes for Bioremediation of Toxic Metal and Radionuclide Pollution. Journal of Chemical Technology and Biotechnology **71**, 364-5.

Ge W, Zamri D, Mineyama H, Valix M. 2011. Bioaccumulation of heavy metals on adapted *Aspergillus foetidus*. Adsorption.**17,** 901–910.

Giller KE, Witter E, McGrath SP. 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, Ahmad I, Stuben D. 2009. Analysis of mines and contaminated agricultural soil samples for fungal diversity and tolerance to heavy metals. Pakistan Journal of Botany **41(2),** 885-895.

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

Kadukova J, Vircikova E. 2005. Comparison of differences between copper bioaccumulation and biosorption. Environment International. **31(2),** 227–232.

Kim DH, Kim KW, Cho J. 2006. Removal and transport mechanisms of arsenics in UF and NF membrane processes. Journal of Water Health.**4** (2): 215–223.

Krznaric E, Verbruggen N, Wevers JH, Carleer R, Vangronsveld J, Colpaert JV. 2009. Cd-tolerant Suillusluteus: a fungal insurance for pines exposed to Cd. Environmental Pollution. **157,** 1581–1588.

Maheswari S, Murugesan AG. 2011. Removal of arsenic (III) from aqueous solutions using *Aspergillus flavus* isolated from arsenic contaminated sites. Indian Journal of Chemical Technology. **18,** 45-52.

Maheswari S, Murugesan AG. 2009. Remediation of arsenic in soil by *Aspergillus nidulans* isolated from an arsenic-contaminated site. Environemental Technology **30(9),** 921–926.

Maheswari S, Murugesan AG. 2009. 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, Jiswal R.2000. Metal resistance in pseudomonas strains isolated from soil treated with industrial wastewater. World Journal of Microbiology and Biotechnology **30,** 261- 278.

Mandal BK, Suzuki KT.2002. Arsenic round the world: a review. Talanta **58,** 201-235.

Martino E, Turnau K, Girlanda M, Bonfateand P, Perroto S. 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 GF, Gadd GM. 1995. Sorption of toxic metals by fungi and clay minerals. Mycological Research **99,** 1429-1438.

Mukherjee KK, Das D, Samal AC, Santra SC. 2013. Isolation and characterization of Arsenic tolerant fungal strains from contaminated sites around urban environment of Kolkata. Journal of Environemental Science and Food Technology **7(5),** 33-37.

PCRWR. 2007. Arsenic Monitoring and Mitigation in Pakistan. Pakistan Council of Research on Water Resources. Annual Report 2005-2006.136-140.

Razak AA, Bachman G, Farrag R. 1999. Activities of microflora in soils of upper and lower Egypt. African Journal of Mycology and Biotechnology **7,** 1-19.

Sathishkumar M, Binupriya AR, K. Swaminathan JG, Choi, Yun SE. 2008. Bio-Separation of Toxic Arsenate Ions from Dilute Solutions by Native and Pretreated Biomass of *Aspergillus fumigatusin* Batch and Column Mode: Effect of Biomass Pretreatment. Bull Environment. Contam. Toxicol. http://dx.doi.org/10.1007/s00128-008-9496-4.

Sag Y. 2001. Biosorption of heavy metals by fungal biomass and modeling of fungal biosorption: A review. Purification Methods **30(1),** 1–48.

Say R, Yilmaz N, Denizli A. 2003. Biosorption of cadmium, lead, mercury, nd arsenic ions by fungus *Penicillium purpurogenum*. Separation Science and Technology **38(9),** 2039–2053.

Smedley PL, Kinniburgh DG. 2002. A review of the source, behavior and distribution of arsenic in natural waters. Applied Geochemistry **17,** 517-568.

Smedley PL, Nicolli HB, Macdonald DMJ, Barros AJ, Tullio JO. 2002. Hydro geochemistry of arsenic and other inorganic constituents in ground waters from La Pampa, Argentina. Applied Geochemistry **17(3),** 259–284.

Smith AL, Lopipero PA, Bates MN, Steinmaus CM. 2002. Arsenic epidemiology and drinking water standards. Science **296,** 2145– 2146.

Smith E, Naidu R, Alston AM. 1998. Arsenic in the soil environment; a review. Advances in Agronomy **64,** 149-195.

Suzuki TM, Bomani JO, Matsunaga H, Yokoyama T. 2000. Preparation of porous resin loaded with crystalline hydrous zirconium oxide and its application to the removal of arsenic. Reactive and Functional Polymers **43(1–2),** 165–172.

Thomas GW. 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, Loon LO. 2003. Adaptive tolerance behavior of fungi in heavy metals. Minerals Engineering **16**, 193–198.

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

Visoottiviseth P, Panviroj N. 2001. Selection of fungi capable of removing toxic arsenic compounds from liquid medium. Science 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. Available at www.knowledgebank.irri.org/factsheetsPDF/Health_and_Nutrition/whoFS_arsenic.pdf.on2-10-2015.

Xu X, Xia L, Huang Q, Gu JD, Chen W. 2012. Biosorption of cadmium by a metal-resistant filamentous fungus isolated from chicken manure compost. Environmental Technology **33**, 1661–1670.

Yazdani M, Yap CK, Abdullah F, Tan SG. 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, Ikeda R, Okuno T. 2006. Identification and characterization of heavy metal resistant unicellular algae isolated from soil and its potential for phytoremediation. Bioresource Technology **97,** 1843-1849.

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

Zaw M, Emett MT. 2002. Arsenic removal from water using advanced oxidation processes. Toxicology Letters. **133(1),** 113–118.