Enhanced arsenic removal from aqueous medium by arsenic tolerant groundwater fungi

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Abstract

Aim : Arsenic concentration in groundwater above the acceptable level is of global concern due to its serious human health effects. Application of certain fungi, due to their amazing skill to remove arsenic from arsenic contaminated water has received attention in recent years. The main aim of this study was to isolate arsenic tolerant fungi from arsenic-contaminated groundwater and assess their ability for arsenic removal from the aquatic system *in-vitro*.

Methodology : Fungi were isolated from arsenic-contaminated groundwater (44.9–161.1 mg l⁻¹). An experiment on bioremediation/ removal of arsenic by selected highly arsenic tolerant fungi was conducted by culturing them in arsenic supplemented medium and measuring the arsenic contents in initial and after incubation with fungi using Hydride Generation Atomic Absorption Spectroscopy (HG-AAS) to find out the arsenic removal percentage.

Results : In all, ten out of forty-five fungi obtained from highly arsenic-contaminated groundwater (44.9–161.1 mg l⁻¹) were able to remove high arsenic contents (>80%) from liquid medium with >85% arsenite (by 6 fungal isolates) and >85% of arsenate (by 9 fungal isolates). In arsenic tolerance assay, all 10 test fungi grew readily in liquid medium enriched with either arsenate (10 and 20 g l⁻¹) or arsenite (2 g l⁻¹) under laboratory conditions (27±2°C). These fungi showed log phase between 5 to 10 days with stationary phase of 11-15 days. High arsenite concentration (4 g l⁻¹) however, completely inhibited the growth of 50% of these fungal isolates. Five of these isolates showed high tolerance index pattern (0.97- 0.91) as compared to others (0.22–0.13). These fungi greatly altered the pH of arsenic supplemented liquid medium (10 mg l⁻¹) as compared to the control when they were individually cultured. These arsenic tolerant fungal isolates may effectively be used for the bioremediation of arsenic from groundwater.

Interpretation : The study suggested that 10 fungal isolates were able to tolerate high arsenic concentrations (20 g l⁻¹ of arsenate and 2 g l⁻¹ of arsenite) and were able to enhance arsenic removal (more than 80%) in liquid medium at neutral pH (7.0) and 27°C temperature.

Key words: Arsenic, Arsenic removal, Groundwater fungi, Tolerance index

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Introduction

Arsenic (As), a highly toxic compound in nature, commonly occurs as a contaminant of drinking water and can be found in both ground and marine water. It is released into the environment at potentially harmful levels as a result of various natural processes (WHO, 2001). Arsenite [As(III)] and arsenate [As(V)] species of arsenic salts are known to cause high toxicity (Ratnaike, 2003). In addition to other forms, the oxides of arsenic are most hazardous for human health. These forms are extracted into the groundwater as components of geologic formations and may be present in both inorganic and organic forms in well waters. The inorganic forms of arsenic are more toxic than their organic forms (Flora, 2015). Arsenite is dominant in more reduced condition whereas arsenate is dominant in the oxidizing environment.

The arsenic contamination in groundwater has been a major health concern in many countries, including India. Long term ingestion of arsenic may result in permanent and severe damage to human health. Arsenic toxicity causes skin lesions, rhagades, and damage mucous membranes, digestive, respiratory, circulatory and nervous system (Singh et al., 2007). Moreover, it is associated with skin, liver and lung cancers (Biswas et al., 1998; Murugesan et al., 2006). World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA) have reduced the permissible limit of arsenic in drinking water from $50 \mu\text{g l}^{-1}$ to $10 \mu\text{g l}^{-1}$ (Pokhrel and Viraraghavan, 2006) as it bioaccumulates within animal tissues if consumed (Alexander et al., 2016).

Removal of arsenic from arsenic-contaminated groundwater and produce safe drinking water is a tough challenge. None of the arsenic removal methods known so far is full proof. Existing methods include oxidation (Chiu and Hering, 2000), ion-exchange, precipitation (Chow, 1997), ultrafiltration, etc. Adsorptive removal technique (Dambies, 2004) is also being used to remove arsenic from water as well as from industrial effluents. Most of the conventional removal methods are less effective, uneconomical, and environment unfriendly. They demand a high cost as they generate a massive quantity of sludge (Siddiquee et al., 2015). New and innovative water treatment technologies are necessary for the removal of heavy metal ions from wastewater. The biological treatments or bioremediation techniques have shown great promises as an alternative method for removing heavy metal ions from a polluted area (Siddiquee et al., 2015). Bioremediation is a process of using naturally living organisms to reduce or neutralize environmental pollutants. It is followed by bacteria and fungi or plants to degrade or detoxify hazardous ingredients to human health /or the environment. Maity et al. (2011) investigated some bacterial species with good efficiency of arsenic removal.

Bioremediation of land and water resource contaminated with toxic compounds especially with arsenic using fungi as a remedy has received great attention because fungi are omnipresent in nature and are often the overriding organisms

(Visoottiviseth and Panviroj, 2001; Vala, 2010). Fungi are present in different environments and can tolerate high amount of arsenic, show fast growth with high cell wall binding and high metal uptake capacities (Vala and Sutariya, 2012; Rodriguez et al., 2013). The arsenic volatilization ability of filamentous fungi has also been demonstrated by many scientists earlier (Nam et al., 2019; Shardendu et al., 2017; Shrivastava et al., 2011; Vala, 2010; Visoottiviseth and Panviroj, 2001; Urik et al., 2007; Cernansky et al., 2009). The groundwater derived for public drinking was found to be dominated by a limited number of fungi such as *Acremonium*, *Exophiala*, *Penicillium*, however, the opportunistic and allergenic *Aspergilli* or pathogenic fungi are rarely encountered (Gottlich et al., 2002). Some of them occur throughout the entire drinking water system and are capable of surviving in water for longer periods. The marine water fungi have also shown a high capacity to tolerate significant concentration of arsenic (Vala, 2010) but no preceding efforts have been made so far to isolate and characterize As-tolerant fungi from groundwater. In view of this, the present study was undertaken with the main aim to evaluate a few potent As-tolerant fungal isolates from arsenic-contaminated groundwater that can be used for arsenic remediation in the future.

Materials and Methods

Arsenic tolerance assay of arsenic tolerant fungi (ATF): Ten out of forty-five fungal isolates collected from arsenic-contaminated groundwater (data not shown) from Arunachal Pradesh and Assam, Northeast India were used to test As-tolerance ability by culturing them at increasing concentrations of inorganic arsenic (10 g l^{-1} and 20 g l^{-1} of arsenate and 2 g l^{-1} and 4 g l^{-1} of arsenite). The culture disk (4 mm) from 7-days-old pure cultures of each fungal isolate were inoculated individually in 250 ml conical flasks containing 100 ml of yeast extract peptone (YEP) broth of above-mentioned arsenic concentrations. All flasks were incubated at $27 \pm 2^\circ\text{C}$ in a rotary shaker (100-150 rpm). The level of tolerance was measured as compared to the control (without arsenic) for all isolates. Each experiment was conducted in triplicates. Tolerance Index (TI) of fungal isolates was also calculated with fungal biomass obtained in presence of arsenic divided by fungal biomass in absence of arsenic in the same period (Shrivastava et al., 2011). The tolerance index value was used as a measure of proportionate arsenic tolerance capacity of arsenic tolerant fungi to shortlist the highly arsenic tolerant fungi among them.

Growth rate of arsenic tolerant fungi in arsenic medium:

Effect of arsenic on the growth of all arsenic tolerant fungi was estimated as compared to the control (without arsenic). Inoculum (4 mm disk) from the 7-days-old culture of 10 arsenic tolerant fungi was inoculated in yeast extract peptone broth containing arsenite and arsenate (10 mg l^{-1} of concentration) separately. Each experiment was conducted in triplicates. All flasks were incubated at $27 \pm 2^\circ\text{C}$ on a rotary shaker at 100-150 rpm for 15 days. Fresh biomass weight was measured daily up to 15 days during the incubation period and the growth curve was plotted.

Arsonic removal studies by selected arsonic tolerant fungi and their effect on pH of liquid culture medium: The highly arsonic-tolerant fungi selected from the above experiment were used for arsonic removal assay using the aqueous medium. Seven days old pure culture of each test fungi was inoculated into yeast extract peptone broth (300 ml) containing Chloramphenicol and arsonic (10 mg l^{-1} each). The experiment was performed in triplicates along with 2 controls i.e. positive control (without arsonic and with fungus) and negative control (without fungus and with arsonic). All flasks were incubated at $27 \pm 2^\circ\text{C}$ in a rotary shaker at 100-150 rpm. After 15 days of incubation, the total biomass of test fungi was filtered through a 0.45 mm filter. The initial pH and pH of culture filtrate after 15 days of incubation period were measured along with the two controls. Filtrates were finally analyzed for arsonic concentration through Hydride Generation Atomic Absorption Spectroscopy (Lab India AA 7000) at Defence Research Laboratory, Defence Research and Development Organisation, Tezpur, Assam, India. Hydride Generation Atomic Absorption Spectroscopy can only read arsonite hence the arsonite containing media filtrates were analyzed directly while potassium iodide and ascorbic acid (5% w/v each) were added to the media to reduce arsonate to arsonite for analysis (Aleksandar *et al.*, 2009; Visoottiviseth and Panviroj, 2001). The arsonate concentration of the media filtrate was assumed to equal to arsonite levels. The arsonic removal was calculated as the difference between initial arsonic contents and arsonite or arsonate contents after analysis.

Effect of different pH and temperature on arsonic removal of highly arsonic tolerant fungi: The effect of different pH (4-9) and temperature (27°C and 37°C) was tested for their arsonic removal capacity using selected highly arsonic tolerant fungi following the method of Visoottiviseth and Panviroj (2001). Seven -days-old pure culture (4 mm disks) of each highly arsonic tolerant fungus was inoculated into a conical flask containing yeast extract peptone broth (300 ml) supplemented with 10 mg l^{-1} of antibiotic (Chloramphenicol) and 10 mg l^{-1} of arsonate or arsonite. The pH of arsonic-containing yeast extract peptone medium was adjusted

to 4, 5, 7 or 9 using lactic acid and sodium. There were 4 sets of experiments conducted which consisted of: (1) arsonate with pH 4, 5, 7 and 9 at 27°C , (2) similar as (1) at 37°C , (3) arsonite with pH 4, 5, 7 and 9 at 27°C and (4) similar as (3) at 37°C . All experiments were performed in triplicates. All flasks were incubated for 15 days on a rotary shaker (100-150 rpm). After incubation, the media were filtered through 0.45 mm filter paper. Filtrates were analyzed for arsonite and arsonate using hydride generation atomic absorption spectroscopy as described earlier.

All experimental data represent mean standard deviation ($\pm\text{SD}$) of three replicates calculated by Microsoft Excel spreadsheet software.

Results and Discussion

All 10 tests arsonic tolerant fungi were selected in arsonic tolerance assay. Either some stress or complete check in growth of arsonic tolerant fungi (Table 1) was recorded when grown in high arsonic-containing media (10 and 20 g l^{-1} for arsonate; 2 and 4 g l^{-1} for arsonite) as compared to the control (without arsonic). A marked change in the degree of arsonic-tolerance was observed at 4 g l^{-1} concentration of inorganic arsonite which completely inhibited the growth of 50% of arsonic tolerant fungi, named ATF-1, ATF-5, ATF-20, ATF-21 and ATF-16. High concentration of arsonic adversely affected the growth of 3 arsonic tolerant fungi (ATF-1, ATF-5 and ATF-20) in arsonate medium (20 g l^{-1}) and growth of 4 arsonic tolerant fungi (ATF-1, ATF-5, ATF-20 and ATF-21) in arsonite medium (2 g l^{-1}) during early incubation period (5 days). Later, the growth of these isolates enhanced significantly, which might be due to their habituation for the arsonic environment. In the present study, highest arsonic tolerance capacity (up to 20 g l^{-1} for arsonate and 4 g l^{-1} for arsonite) in fungi has been reported as compared to the previous record of 10 g l^{-1} (Shrivastava *et al.*, 2011).

The high arsonic concentration had little or no effect on the growth of some arsonic tolerant fungi while higher

Table 1: Arsonic tolerance assay of selected arsonic tolerant fungi

Sample Name	Arsonate (10 g l^{-1})			Arsonate (20 g l^{-1})			Arsonite (2 g l^{-1})			Arsonite (4 g l^{-1})		
	5 Days	10 Days	15 Days	5 Days	10 Days	15 Days	5 Days	10 Days	15 Days	5 Days	10 Days	15 Days
ATF-1	+	+	+	-	+	+	-	+	+	-	-	-
ATF-5	+	+	+	-	+	+	-	+	+	-	-	-
ATF-13	+	+	+	+	+	+	+	+	+	+	+	+
ATF-16	+	+	+	+	+	+	+	+	+	-	-	-
ATF-20	+	+	+	-	+	+	-	+	+	-	-	-
ATF-21	+	+	+	+	+	+	-	+	+	-	-	-
ATF-25	+	+	+	+	+	+	+	+	+	+	+	+
ATF-32	+	+	+	+	+	+	+	+	+	+	+	+
ATF-39	+	+	+	+	+	+	+	+	+	+	+	+
ATF-44	+	+	+	+	+	+	+	+	+	+	+	+

(+ = Growth; - = No Growth)

Table 2: Arsenate removal by selected arsenic tolerant fungi from broth culture medium after 15 days of incubation period at 27°C

Fungal isolate	Control (mg l ⁻¹)	Mean arsenate concentration in culture medium (±SD)		
		Remaining (mg l ⁻¹)	Arsenate removal	
			(mg l ⁻¹)	%
ATF-1	10	1.15±0.02	8.85±0.02	88.52±0.17
ATF-5	10	0.88±0.01	9.12±0.01	91.24±0.13
ATF-13	10	1.09±0.01	8.91±0.04	89.14±0.35
ATF-16	10	1.09±0.02	8.91±0.02	89.11±0.20
ATF-20	10	0.81±0.02	9.19±0.02	91.85±0.17
ATF-21	10	1.96±0.02	8.04±0.02	80.43±0.21
ATF-25	10	1.06±0.03	8.94±0.03	89.38±0.26
ATF-32	10	1.35±0.02	8.65±0.02	86.53±0.25
ATF-39	10	0.99±0.02	9.01±0.02	90.14±0.25
ATF-44	10	1.24±0.02	8.76±0.02	87.63±0.21

Table 3: Arsenite removal by selected arsenic tolerant fungi from broth culture medium after 15 days of incubation period at 27°C

Fungal isolate	Control (mg l ⁻¹)	Mean arsenate concentration in culture medium (±SD)		
		Remaining (mg l ⁻¹)	Arsenate removal	
			(mg l ⁻¹)	%
ATF-1	10	1.16±0.02	8.85±0.02	88.45±0.20
ATF-5	10	1.61±0.01	8.39±0.01	83.90±0.07
ATF-13	10	1.73±0.01	8.27±0.01	82.66±0.08
ATF-16	10	1.35±0.01	8.65±0.01	86.35±0.08
ATF-20	10	1.42±0.01	8.58±0.01	85.81±0.06
ATF-21	10	1.03±0.02	8.97±0.02	89.70±0.16
ATF-25	10	1.47±0.01	8.53±0.01	85.27±0.07
ATF-32	10	1.89±0.03	8.11±0.03	81.15±0.32
ATF-39	10	1.66±0.02	8.34±0.02	83.43±0.20
ATF-44	10	1.32±0.02	8.68±0.02	87.05±0.19

concentration of arsenite (4 g l⁻¹) has caused complete growth retardation in others, as seen in 5 arsenic tolerant fungi (Table 1). These fungi might have adopted a mechanism to survive in arsenic-rich media either by arsenic intake, arsenic resistant gene expression or via arsenic adsorption protein of fungal cell walls. A few arsenic expressed genes have already been identified in fungi in an effort to develop a fungal biosensor for arsenic. Seven putative arsenic metabolisms and transport genes have been reported in *Aspergillus niger* (Choe *et al.*, 2012). Among the genes tested for ribonucleic acid expression in response to arsenate, 'acrA' gene encodes a putative plasma membrane arsenite efflux pump and display an over 200-fold increase in gene expression in response to arsenate. The function of *A. niger* protein in arsenic efflux by gene knockout confirmed that gene 'acrA' was located at the cell membrane. Cernasky *et al.* (2009) had reported 4 mg l⁻¹ trivalent and 17 mg l⁻¹ pentavalent forms of arsenic tolerant *A. niger*, *A. clavatus* and *Nassarius fischeri*. The growth of *Penicillium* sp. collected from arsenic-polluted soil was slightly affected by 1 g l⁻¹ arsenite or arsenate concentrations, but lower concentrations at 10 mg l⁻¹ and 100 mg l⁻¹ had no effect on

fungal growth (Visoottiviseth and Panviroj, 2001) which confirms the present findings. Su *et al.* (2011) also reported up to 50 mg l⁻¹ arsenate-resistant fungi named *Trichoderma asperellum* (SM-12F1), *Penicillium janthinellum* (SM-12F4) and *Fusarium oxysporum* (CZPage 8F1). A highly arsenic tolerant isolate of *A. flavus* (up to 2000 ppm), also tolerant to other heavy metals (Cd, Pb, Hg, Zn and Cr), was reported from waste dumping sites of Dhapa, Kolkata (Mukherjee *et al.*, 2013). Two fungi named *Trichoderma ghanense* and *Rhizopus microspores* (Oladiipo *et al.*, 2018) isolated from gemstone and gold mining sites in south-west Nigeria were also able to tolerate and grow in high arsenic-rich medium (125–500 mg kg⁻¹). Recently, *Talaromyces* sp. KM-31 isolated from soil from a mine site located near the city of Cheongyang, Republic of Korea was reported to tolerate arsenic concentrations up to 1000 mg l⁻¹ (Nam *et al.*, 2019).

The tolerance index for all arsenic tolerant fungi was calculated as a measure of arsenic tolerance capacity to select the highly arsenic tolerant fungi at two concentrations of arsenite and arsenate after 15 days of incubation period (Fig. 1). The

Fig. 1: Tolerance Index of selected arsenic tolerant fungi at different concentration of arsenic after 15 days of incubation period.

tolerance index value ranged between 0.1-1.0 and most of the isolates showed a higher tolerance index (>0.9). At 10 g l^{-1} and 20 g l^{-1} arsenate concentration, only one fungi, i.e., ATF-13 showed tolerance index <1 (1.01) as compared to others (0.92–0.99). At 2 g l^{-1} arsenite concentration, tolerance index value of all arsenic tolerant fungi measured between 0.96-0.99 but at 4 g l^{-1} arsenite concentration, only 5 arsenic tolerant fungi (ATF-32, ATF-39, ATF-13, ATF-44 and ATF-25) measured with high tolerance index (0.97, 0.96, 0.95, 0.93, 0.91 respectively). The tolerance index of the other 5 arsenic tolerant fungi ranged low (0.13 to 0.22). The criterion of tolerance index as a measure of arsenic tolerance capacity was also considered by Srivastava et al. (2011) to study toxicity and tolerance of 15 different fungal isolates where only 1 isolate i.e. sterile mycelium was reported with high tolerance index (0.956) while other fungi such as *Rhizopus* sp., *Neocosmospora* sp., *Penicillium* sp. and *Aspergillus* sp. were measured as 0.311 or below in at $10,000 \text{ mg l}^{-1}$ arsenate concentration. The 5 out of 10 arsenic tolerant fungi that have been measured with high tolerance index values in present studies were designated as highly arsenic tolerant fungi and later used for arsenic removal at different pH and temperatures.

The growth curve of all 10 arsenic tolerant fungi was constructed as per their fresh biomass weight up to 15 days both in arsenite and arsenate media separately and a control (without arsenic) was also maintained. There was little effect of 10 mg l^{-1} concentrations of either arsenite or arsenate on the growth of these fungi as compared to the control. Fig. 2 (A–J) shows the growth curves of each arsenic tolerant fungi in arsenic-containing media. Most of these fungi have attained a stationary phase between 11-15 days with a log phase of 5-10 days, hence; 7-day-old actively growing culture was used in all experiments. Srivastava et al. (2011) have recorded maximum fungal biomass in control (without arsenic) followed by arsenate and arsenite (10 mg l^{-1} each) and confirmed that a lower concentration of arsenic

does not affect the biological activities and metabolic processes much. On the basis of per fold increase in total fungal biomass as compared to the inoculum, maximum fold increase was recorded in ATF-1 (8.95 times) in arsenate and in ATF-5 (8.63 times) in arsenite medium. The minimum fold increase was measured in ATF-21 in all cases, i.e., arsenite (3.80 times), arsenate (4.71 times) and control (5.14 times). Visoottivisacth and Panviroj (2001) obtained a highly arsenic-tolerant fungal isolate (RRMT2-40I) that was also able to grow faster in arsenite (10 mg l^{-1}) and arsenate (100 mg l^{-1}) supplemented agar media. The growth inhibited at $1,000 \text{ mg l}^{-1}$, which was the highest concentration for both arsenite and arsenate with most noticeable at day 10.

All ten fungi were tested for their ability to remove arsenic from aqueous culture medium under laboratory conditions. All test fungi were able to remove high arsenic contents (more than 80%) from the liquid medium. Among them, 6 fungi could remove $> 85\%$ of arsenate (Table 2) and 9 fungi were able to remove $> 85\%$ of arsenite (Table 3) from the liquid media with the highest removal of arsenate by ATF-20 (91.85%) and that of arsenite (89.7%) by ATF-21. Two arsenic resistant isolates of *Aspergillus*, namely *A. flavus* and *A. niger* were isolated from arsenic-polluted sites of Kolkata. They were capable of removing 50-76% arsenic from different arsenic enriched media (Mukherjee et al., 2013) as compared to 10.92 to 65.81% removal by the biomass of 10 fungal isolates (Srivastava et al., 2011). Iron oxide treated fungal biomass (Pokhrel and Viraraghavan, 2006) was reported to remove 95% arsenate and 75% arsenite. The autoclaved fungal mats pre-treated with FeCl_3 could remove 100% of arsenite and iron after 30 min contact time and 77% of arsenate after 90 min contact time (Murugesan et al., 2006). The optimum adsorbent dose was obtained as $1.0 \text{ g } 50 \text{ ml}^{-1}$ of water sample. Arsenite resistant isolate, *Stenotrophomonas* sp. removed 79.6% arsenic from the culture broth (Shardendu et al., 2017). The fungal isolates obtained in this study could remove both arsenite and

Fig. 2: Growth curve of selected arsenic tolerant fungi in arsenite and arsenate rich media as compared to control (without arsenic).

Fig. 3: Effect on pH (%) of culture medium (supplemented with 10 mg l⁻¹ of arsenic) by selected arsenic tolerant fungi during 15 days of incubation period.

arsenate at the rate of more than 85% from the liquid medium and, hence, are expected to remove even more arsenic if utilized with a suitable carrier, however, it needs more testing and confirmation. Bhar *et al.* (2003) reported increased removal efficiency by *Aspergillus*, *Rhizopus* and *Penicillium* species with an increase in culturing time interval (9th day). Rudakiya *et al.* (2018) reported that a fungus named *Phanerochaete chrysosporium* absorbed 29.95 mg l⁻¹ of arsenic within 1 hr at 30°C and 120 rpm. *Talaromyces* sp. KM-31 isolated from soil from a mine site located near the city of Cheongyang, Republic of Korea had effectively removed arsenic (>70%) from aqueous medium (Nam *et al.*, 2019). Arsenic toxicity in groundwater is an alarming global issue. The problem is intensifying day by day and may become chronic if urgent necessary actions are not taken (Mukherjee *et al.*, 2013). The metabolic potential of microorganisms to clean up contaminated environments is viewed as the most suitable strategy for bioremediation of arsenic (Bundschuh *et al.*, 2015). The earlier reports have suggested that fungi can remove arsenate or arsenite when grown in liquid medium and supports the present findings. Hence, among microorganisms, fungi could be the most powerful candidates in resolving this issue and can be utilized for arsenic bioremediation from groundwater in the future.

A significant change in the pH of the culture medium was recorded in arsenic removal assay (Fig. 3). In 7 out of 10 arsenic tolerant fungi, the pH of the medium decreased by 72.23% to 79.17% in arsenate media and by 56.95% to 77.78% in arsenite media. The pH was increased in other 3 fungi, i.e., ATF-1, ATF-5 and ATF-25 in arsenite (1.38%–22.22%) and arsenate media (4.10% – 8.33%) both. The media pH increased by 1.38% to 27.77% in control of all fungal isolates. Many fungi secrete ammonia and produce lipase (Rapp, 1995) which may be responsible for increasing the pH of the medium. At high pH,

fungal cell surface becomes more negatively charged and favors the binding of oppositely charged metal ions. The yeast extract peptone medium used in the study contained peptone and might have produced lipase. In general, reduction in media pH is correlated with arsenic removal by different fungal isolates and might be the reason for high arsenic removal from the liquid medium by fungi (Das *et al.*, 2007). In the present study, the maximum arsenic removal (91.68%) was recorded with an average removal of 87% with decreasing pH values in most of the cases. The interaction between the metal ions and functional groups of the fungal biomass depends on the nature of the adsorbent as well as on solution chemistry of the adsorbate, which in turn depends on the pH. Many fungi are able to withstand wide pH ranges (Shrivastava *et al.*, 2011); able to remove inorganic metal compounds via production of organic acids (White *et al.*, 1997) or may release hydrogen ions in the growing medium, which is responsible for decreasing the pH of media (Kartal *et al.*, 2006).

The five highly arsenic tolerant fungi (to arsenate and arsenite both) named ATF-13, ATF-25, ATF-32, ATF-39, ATF-44 that have exhibited high tolerance index value, were tested for arsenic removal assay (Fig. 4) at different pH (pH 4, 5, 7 and 9) and temperature (27°C and 37°C). No growth was recorded at 37°C in all the 5 fungal isolates at all pH ranges tested in the present study. As a result, the concentration of arsenite/arsenate in liquid media (10 mg l⁻¹) at 37°C did not decrease after 5 days of incubation period at any pH value. At pH 7 and at 27°C, all highly arsenic tolerant fungi exhibited enhanced arsenite removal efficiency in the order of ATF-32 (88.46%), ATF-13 (87.81%), ATF-25 (86.28%), ATF-39 (85.39%) and ATF-44 (82.98%). Similar results were also obtained in the case of arsenate at pH 7 where four fungal isolates, i.e., ATF-32, ATF-25, ATF-39 and ATF-44 exhibited enhanced arsenate removal in the order of 90.54%,

Fig. 4 (A-B): Effect of different pH (4, 5, 7 and 9) at 27°C temperature on arsenic removal by five highly arsenic tolerant fungi.

88.31%, 87.73% and 86.10%, respectively.

ATF-13, i.e. *Penicillium polonicum* appeared to remove highest arsenite (89.92%) at pH 9 followed by ATF-32 (88.04%), ATF-25 (87.73%), ATF-39 (87.45%), and least removal by ATF-44 (85.08%). At pH 4, ATF-32 exhibited highest arsenic removal efficiency (89.93% in arsenate and 87.99% in arsenite at 27°C) whereas at pH 5 (Fig. 4) these fungi have also shown high arsenic removal efficiency (89.56% in arsenate and 88.11% in arsenite). The ATF-32 has removed highest arsenic (both arsenite and arsenate) at all pH ranges tasted excepted arsenate at pH 9, where it was second highest (88.04%). The fungal isolates *Penicillium citrinum* (ATF-44) was uniformly least arsenite as well as arsenate removing species, except at pH 9 where removal was highest

(85.08%). Visoottiviseth and Panviroj (2001) also reported that *Penicillium* sp. (RRMT2-40I) showed best growth at pH 5.0 or 7.0 at 27°C, reaching stationary growth phase within 4 days. At 27°C, the highest percent removal of arsenite was found at pH 5.0, followed by pH 7.0 while the lowest As percent removal was reported at pH 9.0. The percent removal of arsenate decreased with increasing pH in a similar manner with arsenite. The peak pH values of 4 and 9 were not found suitable for arsenic removal in laboratory conditions.

Arsonic contamination is a major problem of groundwater sources and is responsible for causing serious health hazards to the people. This study suggests that some fungi that inhabit arsenic-contaminated water were capable of removing high arsenic

contents biologically from the liquid medium under laboratory conditions hence may serve as potential agents in developing future strategies to evolve a biological-based arsenic removal system.

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