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Toxicity, transformation and accumulation of inorganic arsenic species in a microalga *Scenedesmus* sp. isolated from soil

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Abstract Arsenic speciation and cycling in the natural environment are highly impacted via biological processes. Since arsenic is ubiquitous in the environment, microorganisms have developed resistance mechanisms and detoxification pathways to overcome the arsenic toxicity. This study has evaluated the toxicity, transformation and accumulation of arsenic in a soil microalga *Scenedesmus* sp. The alga showed high tolerance to arsenite. The 72-h 50 % growth inhibitory concentrations (IC_{50} values) of the alga exposed to arsenite and arsenate in low-phosphate growth medium were 196.5 and 20.6 mg L^{-1}, respectively. When treated with up to 7.5 mg L^{-1} arsenite, *Scenedesmus* sp. oxidised all arsenite to arsenate in solution. However, only 50 % of the total arsenic remained in the solution while the rest was accumulated in the cells. Thus, this alga has accumulated arsenic as much as 606 and 761 μg g^{-1} dry weight when exposed to 750 μg L^{-1} arsenite and arsenate, respectively, for 8 days. To our knowledge, this is the first report of biotransformation of arsenic by a soil alga. The ability of this alga to oxidise arsenite and accumulate arsenic could be used in bioremediation of arsenic from contaminated water and soil.

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Introduction

Humans have suffered due to arsenic more than any other element or toxic compound in history, and thus it is considered as the king of poisons (Nriagu et al. 2007). It is primarily released into the environment via natural activities such as volcanic emissions, weathering of arsenic-bearing minerals, etc. and anthropogenic activities such as mining, smelting, combustion of fossil fuels, etc. (Bhumbla and Keefer 1994). Soils contaminated with very high levels of arsenic (up to 3,000 mg kg^{-1} soil) are very common around cattle dip sites throughout Australia (van Zwieten et al. 1998; Edvantoro et al. 2003, 2004). Arsenic concentrations have been reported to range from <1 to 10 μg L^{-1} in freshwaters and up to 5,000 μg L^{-1} in groundwaters (Smedley and Kinniburgh 2002). The natural occurrence of arsenic in groundwater aquifers in many parts of the globe and its subsequent contamination of drinking water and food is now a major concern to human health in many countries, particularly in Bangladesh and Southeast India (Mandal and Suzuki 2002). Although the World Health Organisation (WHO) has established a guideline value of 10 μg L^{-1} for arsenic in drinking water (WHO 1993), many developing countries including Bangladesh adopted 50 μg L^{-1} as the guideline value for economic reasons (Ng and Moore 2005).

Arsenic exists in four oxidation states in nature: arsine (−III), elemental arsenic (0), arsenite(+III) and arsenate(V). Arsenite [As(III)] and arsenate [As(V)] are the predominant inorganic forms found in the environmental samples, and the first two occur rarely (Cullen and Reimer 1989).

Microorganisms play an important role in the biogeochemial cycling of metals in the environment. They have developed several strategies to detoxify metals and metalloids such as arsenic. The microbial processes in arsenic metabolism include cellular uptake, oxidation/reduction, chemical binding with metallothionein/glutathione and efflux to the outer environment or impound in the cell (Levy et al. 2005; Yin et al. 2012).

Most studies investigating microalgal transformation and accumulation have focused on freshwater algae, and little is known about soil algae. Algae are an important component of soil environment participating in nutrient cycling besides remediation of heavy metals through adsorption and biotransformation. *Scenedesmus* sp. is very common in both freshwater and soil environments. Few studies have been conducted on the response of freshwater *Scenedesmus* to arsenic, but no information is available on soil alga. Therefore, we investigated the tolerance, biotransformation and accumulation of arsenic in *Scenedesmus* sp.

Materials and methods

Scenedesmus sp. isolated from soil was maintained axenically in the Phycology Laboratory of Centre for Environmental Risk Assessment and Remediation, University of South Australia. The culture was checked for any contamination periodically by microscopic analysis and by streaking on Bold's basal medium (BBM) and bacteriological agar plates. Stock culture was maintained in Erlenmeyer flasks containing BBM plugged with sterile cotton and kept under continuous illumination (200 μmol photons $m^{-2}s^{-1}$ PPED) at 25±2 °C on an orbital shaker (Megharaj et al. 1986).

Arsenic toxicity analysis

The toxicity of As(III) as $NaAsO_2$ and As(V) as $Na_2HAsO_4{\cdot}7H_2O$ to *Scenedesmus* sp. was determined in terms of growth inhibition compared to control. Cells in the exponential growth phase (5 d old) were used as inoculum in bioassays after centrifugation at 3,000 rpm for 15 min and washing three times with Milli-Q water to remove residual contents of the culture medium. Aliquots from arsenic stock solutions were dispensed into sterile culture flasks containing modified BBM (low phosphate) to reach the final desired concentrations. Each test included ten arsenic concentrations and a control. Arsenic treatments [both As(III) and As (V)] ranged from 3.75 to 375 mg L^{-1}. All the flasks were inoculated with exponentially growing alga to an initial cell density in the flask of 10^5 cells mL^{-1}. The flasks were then placed randomly on an orbital shaker set at 120 rpm and incubated in a temperature-controlled (25 °C) room under continuous illumination (200 μmol photons $m^{-2}s^{-1}$ PPED) provided by cool white fluorescent lamps. Aliquots were taken from the treatment flasks at different time intervals up to 96 h to measure the cell growth. The growth was measured by direct microscopic cell counts with a Neubauer hemocytometer and the cell density expressed in cells mL^{-1} medium. All the assays were conducted in triplicate. The 50 % inhibitory concentration (IC_{50}) was defined as the initial As(III) concentration resulting in a 50 % decrease in growth when compared with control culture containing no As(III). It was determined by applying a nonlinear regression model with a four-parameter logistic curve, using the software SigmaPlot.

The growth rate of algal culture was determined according to Wong and Cheng (1991) using the equation $k = (\ln X_1 - \ln X_0)/(T_1 - T_0)$, where k is the specific growth rate, X_0 is the initial (T_0) absorbance at 650 nm and X_1 is the absorbance at time T_1 (48 h after incubation).

Arsenic biotransformation study

Algal cells with cell density of 10^5 cells mL^{-1} were exposed to 0.75, 3.5 and 7.5 mg L^{-1} of both As(III) and As(V) in the low-phosphate BBM medium. To examine the influence of phosphate, an additional experiment was conducted with two different phosphate concentrations (low 2 mg L^{-1} and high 10 mg L^{-1}) in growth medium where arsenic concentration remained same (0.75 mg L^{-1}). After 8 days of incubation at the above-mentioned growth conditions, the medium solution was collected to determine the arsenic biotransformation by *Scenedesmus* sp.

Bioaccumulation of arsenic in *Scenedesmus*

Cells grown in the above-mentioned experiment (low phosphate, 0.75 mg L^{-1} arsenic) were harvested after 8 d of incubation by centrifugation at 4,000 rpm. Cells were rinsed with de-ionised water followed by phosphate buffer wash to remove the externally bound arsenic to the cells. The total arsenic in cells was determined. The bioconcentration factor (BCF) is calculated as the ratio of arsenic concentration in the algal biomass (dry) to the initial arsenic concentration in feed solution as described by Zayed et al. (1998).

Arsenic analysis

To determine the concentration of total arsenic in algal cells and arsenic species in solution, inductively coupled plasma emission mass spectrometer (ICP-MS) and liquid chromatograph coupled with inductively coupled plasma emission mass spectrometer were used (Chen et al. 2008). Intracellular arsenic was determined following the digestion of filtered algal biomass with 2 mL concentrated HNO_3.

Standard reference materials (SRMs) from the National Institute of Standards and Technology, USA such as SRM 1640 (trace elements in natural water), SRM 1568a (rice flour) and SRM 1573a (tomato leaves) were analysed to verify the analytical results for arsenic as reported earlier (Rahman et al. 2011).

Results

Toxicity of arsenic to *Scenedesmus*

The effect of As(III) and As(V) towards the growth of *Scenedesmus* sp. was investigated. Growth of the alga was inhibited by As(V) even at a very low concentration in low-phosphate BBM. In contrast, As(III) up to 75 mg L^{-1} did not inhibit the algal growth (Fig. 1). The lower concentration of As(V), even though initially inhibitory to the algal growth, started increasing after 72 h finally reaching on par with control by 96 h. The specific growth rate of the alga was similar to the control up to 75 mg L^{-1} As(III) which was reduced to 60 % at 225 mg L^{-1} and no growth at 375 mg L^{-1} (Fig. 2). On the other hand, the specific growth rate of the alga was reduced by 25 % when the cells were grown with only 3.75 mg L^{-1} of As(V). The 72-h IC_{50} values for As(III) and As(V) were 196.5±15.2 and 20.6± 3.5 mg L^{-1}, respectively.

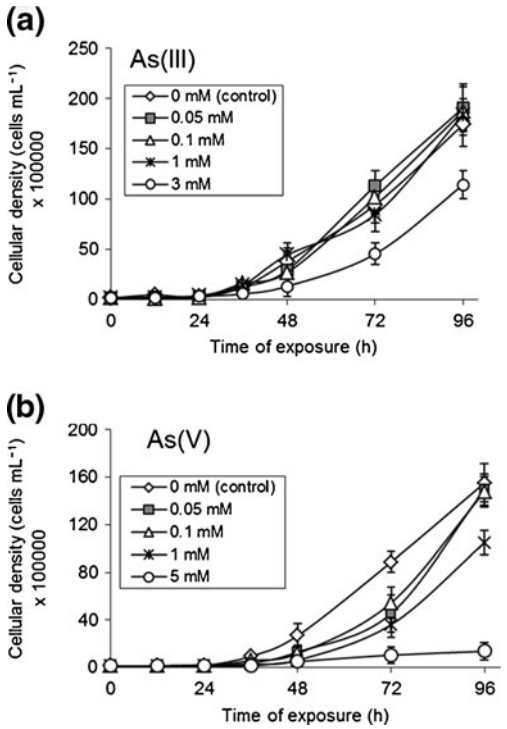

Fig. 1 Growth of *Scenedesmus* sp. in low-phosphate medium spiked with different concentrations of (**a**) As(III) and (**b**) As(V). All data are means ± SE (n=3)

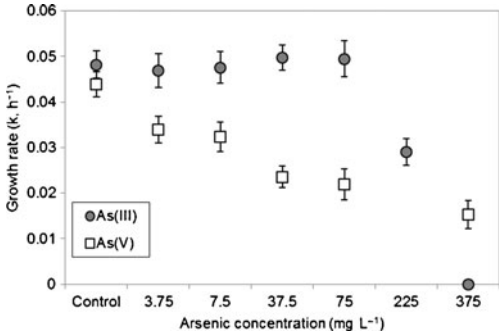

Fig. 2 Effect of arsenic on 48-h growth rate of *Scenedesmus* sp. All data are means ± SE (n=3)

Arsenic biotransformation by *Scenedesmus* sp

Cells were incubated with three different concentrations of both As(III) and As(V) in a low-phosphate BBM. After 8 d of incubation, the total arsenic remained in the solution was about 50 % of the total initial concentration (Table 1). In the case of As(III) treatment, all the remaining arsenic was found in the form of As(V) at all three concentrations. No As(V) was detected in the abiotic controls (no algae), indicating that the presence of As(V) in As(III) treatment was due to the biological oxidation. The unaccounted portion of total arsenic in the solution would have been taken up by algal cells and accumulated in the cell.

In contrast to As(III) treatment, total remaining arsenic in As(V) treatment was found unchanged at higher concentrations (Table 1). However, at lower concentration (0.75 mg L^{-1}), about 75 % of the remaining arsenic was present as As(III). When the phosphate concentration was increased from 2 to 10 mg L^{-1}, As(V) reduction decreased from 75 to <25 % (Table 2). The result clearly indicates that the phosphate concentration in the medium has a great influence on the As(V) uptake and reduction by the alga.

Table 1 Arsenic transformation by *Scenedesmus* sp. in a low-phosphate (2 mg L^{-1}) medium

Initial arsenic concentration (mg L^{-1})		Arsenic remaining in medium after 8 d (mg L^{-1})			Column recovery (%)
		As(III)	As(V)	Total	
As(III)	0.75	nd	0.46±0.13	0.46±0.13	98±3
	3.75	nd	1.72±0.21	1.72±0.21	92±7
	7.5	nd	3.49±0.35	3.49±0.35	102±1
As(V)	0.75	0.29±0.09	0.09±0.02	0.38±0.09	96±3
	3.75	nd	1.75±0.18	1.75±0.18	94±4
	7.5	nd	3.78±0.45	3.78±0.45	104±2

Values indicate the means ± SE (n=3)

nd not detected

Table 2 Arsenic(V) reduction by *Scenedesmus* sp. in the medium with low phosphate (2 mg L^{-1}) and high phosphate (10 mg L^{-1})

	Arsenic remaining in medium after 8 d (mg L^{-1})			Column recovery (%)
	As(III)	As(V)	Total	
Low phosphate	0.29±0.09	0.09±0.02	0.38±0.09	96±5
High phosphate	0.08±0.02	0.24±0.05	0.32±0.06	101±3

Initial As(V) concentration was 0.75 mgL^{-1}. Values indicate the means ± SE (n=3)

Arsenic accumulation in *Scenedesmus* cells

Accumulation of arsenic in the algal cells was observed when exposed to both As(III) and As(V). Thus, the arsenic accumulation in the alga was greater with 761.6 μg g^{-1} dry weight (DW) when exposed to As(V) compared to 606.2 μg g^{-1} DW with AS(III) (Table 3). The algae showed high ability to accumulate arsenic as demonstrated by its BCF values of 808 and 1,015 for As(III) and As(V), respectively.

Discussion

The results of this study demonstrated that the toxicity of arsenic to *Scenedesmus* sp. depends on chemical species of arsenic in the test medium. The As(V) was more toxic with an IC_{50} value of 20.6±3.5 mg L^{-1} compared to As(III) with an IC_{50} value of 196.5±15.2 mg L^{-1} in a low-phosphate medium. Similarly, a higher toxicity of As(V) than As(III) was reported in a freshwater algae *Monoraphidium arcuatum* (Levy et al. 2005). In contrast, Karadjova et al. (2008) reported As(III) as more toxic than As(V) to a marine green alga, *Chlorella salina*. Although different experimental conditions reported in literature including phosphate concentrations and incubation periods make the comparison difficult, it was observed that the microalgae can exhibit differential sensitivity to arsenic. In a 72-h growth-inhibition test with the freshwater alga *M. arcuatum*, Levy et al. (2005) found IC_{50} values of 14.5 and 0.25 mg L^{-1} for As(III) and As(V), respectively. Contrary to this, a *Chlorella vulgaris*, isolated from arsenic-contaminated freshwater was more sensitive to As(III) than As(V) (Maeda et al. 1985). The growth of this alga was increased in medium containing 2,000 mg L^{-1} As (V) while >10 mg L^{-1} AS(III) inhibited the growth. Vocke et al. (1980) found *Scenedesmus obliquus* as very sensitive to As(V) with an IC_{50} value of 0.04 mg L^{-1}. Thus, when compared to other algae, the *Scenedesmus* sp. from this study has higher tolerance to arsenic.

Table 3 Arsenic accumulation in *Scenedesmus* sp

Arsenic in algal cell (μg g^{-1} DW)		BCF
As(III)	606.2±32.2	808
As(V)	761.6±45.5	1015

Values indicate the means ± SE (n=3)

Qin et al. (2009) assessed the biotransformation of arsenic by a thermo acidophilic alga *Cyanidioschyzon* sp. The alga was exposed to 20 μM (equivalent to 1.5 mg L^{-1}) of As (III) which initially oxidised As(III) to As(V), then reduced As(V) to As(III) followed by methylation to trimethylarsine oxide and dimethylarsenate [DMA(V)] after 8 d of incubation. Yin et al. (2012) also reported oxidation of As(III) in the culture medium by a cyanobacterium *Synechocystis* sp. This alga was treated with 2.67 μM (equivalent to 0.2 mg L^{-1}) of As(III), and after 72 h of incubation, the amount of As(V) was accounted for 83 % of the residual arsenic concentration in the medium. In our study, all the remaining arsenic in the growth medium was found in As(V) form when the cells were exposed to different concentrations of As(III) (0.75 to 7.5 mg L^{-1}). In contrast to the algae, As (III)-oxidising bacteria were found to be more efficient in oxidation of As(III) to As(V) without any intracellular accumulation (Weeger et al. 1999; Campos et al. 2009; Bahar et al. 2012). There are several mechanisms reported to be involved in the living organisms that can oxidise As(III) to As(V). In the case of aerobic As(III)-oxidising bacteria, the cellular oxidation is catabolised by a membrane-bound enzyme, arsenite oxidase (Ellis et al. 2001; Bahar et al. 2012). Partial reduction of As(V) to As(III) by the *Scenedesmus* sp. was also observed at low-phosphate growth medium. However, higher phosphate concentration in the medium significantly reduced the As(V) reduction rate ($p \leq 0.05$). Since phosphate is an essential nutrient and a chemical analog of As(V), it is likely that As(V) competes with phosphate when phosphate is present at a lower level; thereby, As(V) enters the cell and undergoes reduction and subsequent extrusion to outside the cell as a detoxification mechanism.

Metal uptake and accumulation in algae is regarded as a two-step process. Firstly, the metal ions are adsorbed to the cell surface by interaction between metal-functional groups. Secondly, the metal ions penetrate the cell membrane and enter the cells (Wang and Chen 2006). Arsenic accumulation in alga varies among different species depending on external arsenic concentration. The removal of As(III) was found to be around 70 % by *Scenedesmus abundans* when exposed to 5 mg L^{-1} As(III) (Jahan et al. 2006). Also, a cyanobacterium *Synechocystis* sp. accumulated 0.9 and 1.0 mg arsenic kg^{-1} algal dry biomass when exposed to both As(V) and As(III), respectively, at 37.5 mg L^{-1} (Yin et al. 2012). *C. vulgaris* accumulated 610 mg kg^{-1} arsenic in 1 d when exposed to only 30 μg L^{-1} arsenic (Suhendrayatna et al. 1999). Similarly, in this study, *Scenedesmus* sp.

exposed to As(III) and AS(V) each at 750 μg L^{-1} has accumulated 606 and 761 μg arsenic g^{-1} algal dry mass, respectively. This study has demonstrated the great potential of this alga to accumulate arsenic. According to Zhu et al. (1999), an organism exhibiting BCF for any metal greater than 1,000 is considered to be a hyperaccumulator. From this standpoint, the *Scenedesmus* sp. in this study with a BCF of 808 and 1,014 for As(III) and As(V), respectively, can be considered as a good arsenic accumulator.

The results of this study demonstrate that the *Scenedesmus* sp. has the ability to oxidise the toxic As(III) to less toxic As (V) in solution coupled with high cellular accumulation, irrespective of arsenic species. Though the presence of phosphate at very low concentration (2.0-mg L^{-1} medium) favoured the reduction of As(V) to As(III), increasing concentration of phosphate (>2.0 mg L^{-1}) inhibited the reduction. Thus, the ability of this alga to transform and accumulate arsenic could be gainfully employed in bioremediation of arsenic-contaminated environment.

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