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Toxicity of arsenic species to three freshwater organisms and biotransformation of inorganic arsenic by freshwater phytoplankton (*Chlorella* sp. CE-35)

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ABSTRACT

In the environment, arsenic (As) exists in a number of chemical species, and arsenite (As^{III}) and arsenate (As^{V}) dominate in freshwater systems. Toxicity of As species to aquatic organisms is complicated by their interaction with chemicals in water such as phosphate that can influence the bioavailability and uptake of As^{V}. In the present study, the toxicities of As^{III}, As^{V} and dimethylarsinic acid (DMA) to three freshwater organisms representing three phylogenetic groups: a phytoplankton (*Chlorella* sp. strain CE-35), a floating macrophyte (*Lemna disperma*) and a cladoceran grazer (*Ceriodaphnia* cf. *dubia*), were determined using acute and growth inhibition bioassays (EC_{50}) at a range of total phosphate (TP) concentrations in OECD medium. The EC_{50} values of As^{III}, As^{V} and DMA were 27 ± 10, 1.15 ± 0.04 and 19 ± 3 mg L^{-1} for *Chlorella* sp. CE-35; 0.57 ± 0.16, 2.3 ± 0.2 and 56 ± 15 mg L^{-1} for *L. disperma*, and 1.58 ± 0.05, 1.72 ± 0.01 and 5.9 ± 0.1 mg L^{-1} for *C.* cf. *dubia*, respectively. The results showed that As^{III} was more toxic than As^{V} to *L. disperma*; however, As^{V} was more toxic than As^{III} to *Chlorella* sp. CE-35. The toxicities of As^{III} and As^{V} to *C.* cf. *dubia* were statistically similar ($p > 0.05$). DMA was less toxic than iAs species to *L. disperma* and *C.* cf. *dubia*, but more toxic than As^{III} to *Chlorella* sp. CE-35. The toxicity of As^{V} to *Chlorella* sp. CE-35 and *L. disperma* decreased with increasing TP concentrations in the growth medium. Phosphate concentrations did not influence the toxicity of As^{III} to either organism. *Chlorella* sp. CE-35 showed the ability to reduce As^{V} to As^{III}, indicating a substantial influence of phytoplankton on As biogeochemistry in freshwater aquatic systems.

1. Introduction

Arsenic (As), a potentially toxic environmental contaminant, occurs naturally in geological substrata; however, human activities can also contribute to its release in the environment (Sharma and Sohn, 2009). Due to direct inputs from natural and anthropogenic sources and their relatively high concentrations, As in freshwater environments is a serious problem in many areas of the world (Villaescusa and Bollinger, 2008). Arsenic concentrations in freshwater can range from less than 0.5 μg L^{-1} to more than 5000 μg L^{-1} depending on the source of contamination (Rahman and Hasegawa, 2012).

Arsenic exists mainly in four oxidation states (+3, +5, 0, −3) of which pentavalent arsenate (As^{V}) and trivalent arsenite (As^{III}) are the most common species in freshwater systems (Rahman and Hasegawa, 2012; Sharma and Sohn, 2009). Along with these, but found at far lower concentrations, are their methylated derivatives; dimethylarsinic acid (DMA) and monomethylarsonic acid (MA) (Rahman and Hasegawa, 2012). Numerous studies have shown that the chemical speciation of As, which can be affected by the physicochemical properties of water, determines its

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toxicity to organisms (e.g. Duester et al., 2011; Howard et al., 1995; Levy et al., 2005; Sharma and Sohn, 2009; Zhang et al., 2009). Arsenite tends to dominate in anoxic water, while As^V is more prevalent in oxic waters (Rahman and Hasegawa, 2012). pH is also known to affect As speciation and solubility of other anions such as Fe-hydroxides, which can influence the bioavailability and toxicity of As in aquatic systems (Masscheleyn et al., 1991). The forms of As determine their toxicity to organisms by their mode of actions. Although inorganic As (iAs) forms are generally more toxic to organisms than organoarsenicals, trivalent methylarsenicals (monomethylarsenite; MA^{III} and dimethylarsenite; DMA^{III}) are more toxic than iAs (Rahman and Hasegawa, 2012). Arsenate inhibits oxidative phosphorylation (Oremland and Stolz, 2003) and As^{III} toxicity results in impairment of the function of proteins (Delnomdedieu et al., 1994; Scott et al., 1993). The toxicological modes of action of DMA and MA are less understood (Oremland, 2003).

Microorganisms such as phytoplankton have the ability to biotransform toxic iAs species to less toxic organoarsenicals (Oremland and Stolz, 2003), which has been considered to be a detoxification mechanism by some researchers (Levy et al., 2005; Maeda et al., 1992). Biotransformation of iAs also results in the production of MA^{III} and DMA^{III}, which are more toxic than iAs (Dopp et al., 2010; Mass et al., 2001; Petrick et al., 2000), and therefore, biotransformation of iAs should not be generalised as a detoxification process for microorganisms. The reduction As^V to As^{III} can be a detoxification mechanism for the microorganisms since As^{III} is easier to excrete out of cells. Biomethylation of iAs can also be a detoxification mechanism if the microorganisms have the ability to transform iAs predominantly to less toxic pentavalent methylarsenicals (MA^V, DMA^V, and $TMAO^V$) (Rahman and Hassler, 2014).

Most studies investigating As biotransformation have focused on marine environments (Duncan et al., 2013; Foster et al., 2008; Maher et al., 2009), where As concentration is very low and may not be a problem, except for areas with low phosphate concentrations (Rahman and Hassler, 2014). However, As biotransformation in freshwater systems, where its concentration is much higher than marine systems (Rahman and Hasegawa, 2012), has received little attention. Due to the differences in the biogeochemistry and physicochemistry of marine and freshwater systems (Neff, 1997), as well as differences in the biology of marine and freshwater phytoplankton, toxicities of As species to and the biotransformation ability of freshwater phytoplankton may differ with that of marine phytoplankton.

Numerous studies report freshwater macrophytes that accumulate As and suggest that these plants can be used for remediation of contaminated water (Rahman and Hasegawa, 2011); however, little is known about the toxicity of As species to freshwater plants. Phytoplankton are important components of freshwater ecosystems as primary producers and the source of energy for higher trophic levels (Stockner and Porter, 1988). The influence of aquatic organisms on As speciation and biogeochemistry in freshwater systems has recently been studied (Hasegawa, 1996; Hasegawa et al., 2009; Rahman and Hasegawa, 2012). To fully understand the influence of phytoplankton on As biogeochemistry and trophic transfer in a freshwater system, it is important to determine the toxicity of As species to freshwater phytoplankton and their ability to biotransform As, particularly iAs, which are the predominant forms in oxic aquatic systems. The toxicity of As species to several groups of primary consumers in freshwater ecosystems also needs to be determined in order to understand trophic transfer of As through the food chain and the toxic effects of As on freshwater organisms.

This study addresses the influence of As chemical speciation on its toxicity to as well as its biotransformation by freshwaters organisms. The objectives of the present study were to – (i) determine the toxicities of iAs species (As^{III} and As^V) and DMA to three freshwater organisms representing three phylogenetic groups: a phytoplankton (*Chlorella* sp. strain CE-35), a macrophyte (*Lemna disperma L.*) and a cladoceran grazer (*C.* cf. *dubia*); (ii) determine the influence of phosphate, a chemical analogue of arsenate, on the toxicities of As^{III} and As^V to *Chlorella* sp. CE-35 and *L. disperma*; and (iii) determine the ability of *Chlorella* sp. CE-35 to biotransform As species.

2. Materials and methods

2.1. *Arsenic species*

The As species used in this study were sodium arsenate dibasic heptahydrate (As^V; $Na_2HAsO_4 \cdot 7H_2O$, purity 98–102%, Sigma-Aldrich, USA), sodium meta arsenite (As^{III}; $NaAsO_2$, purity 90%, Sigma-Aldrich, USA), and dimethylarsinic acid ($(CH_3)_2AsOOH$, purity 98%, Sigma-Aldrich, USA). All arsenic compounds were of ACS reagent grade.

2.2. *Stock culture of the organisms*

2.2.1. *Macrophyte (L. disperma L.)*

L. disperma L. was obtained from the University of Technology, Sydney and cultured axenically in the laboratory. Cultures were grown in sterile 500 mL Erlenmeyer flasks containing approximately 200 mL of standard, filtered (0.45 µm pore size cellulose acetate membrane, Millipore) OECD media (OECD, 2006) (Table 1) with pH adjusted to 7.5 ± 0.1 using HCl (37%, ACS grade, Sigma-Aldrich, USA) or NaOH (97%, ACS grade, Sigma-Aldrich, USA). The cultures were maintained in a dedicated, constant temperature room (24 ± 2 °C) on a 12:12 light:dark cycle and 70–80 µmol photons $m^{-2}\,s^{-1}$ under daylight-type fluorescent lights.

2.2.2. *Phytoplankton (Chlorella sp. CE-35)*

Eleven strains of phytoplankton were obtained from the Commonwealth Science and Industrial Research Organisation (CSIRO), Lucas Heights, NSW, and

Table 1
Composition of OECD media for *Lemna disperma* and *Chlorella* sp. strain CE-35 toxicity bioassays.

Chemicals	Concentration ($mg\ L^{-1}$)
For macrophyte (*Lemna disperma*)	
$NaNO_3$	85
KH_2PO_4	13.4
$MgSO_4 \cdot 7H_2O$	75
$CaCl_2 \cdot 2H_2O$	36
Na_2CO_3	20
H_3BO_3	1.0
$MnCl_2 \cdot 4H_2O$	0.2
$Na_2MoO_4 \cdot 2H_2O$	0.01
$ZnSO_4 \cdot 7H_2O$	0.05
$CuSO_4 \cdot 5H_2O$	0.005
$Co(NO_3)_2 \cdot 6H_2O$	0.01
$FeCl_3 \cdot 6H_2O$	0.84
$Na_2\text{-}EDTA \cdot 2H_2O$	1.4
MOPS	490
For phytoplankton (*Chlorella* sp.)	
$NaHCO_3$	50
NH_4Cl	15
$MgCl_2 \cdot 6H_2O$	12
$CalCl_2 \cdot 2H_2O$	18
$MgSO_4 \cdot 7H_2O$	15
KH_2PO_4	1.6
$FeCl_3 \cdot 6H_2O$	0.064
$Na_2EDTA \cdot 2H_2O$	0.100
H_2BO_3	0.185
$MnCl_2$	0.415
$ZnCl_2$	0.003
$CoCl_2 \cdot 6H_2O$	0.0015
$CuCl_2 \cdot 2H_2O$	0.00001
$Na_2MoO_4 \cdot 2H_2O$	0.007