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Manganese-mediated immobilization of arsenic by calcifying macro-algae, *Chara braunii*

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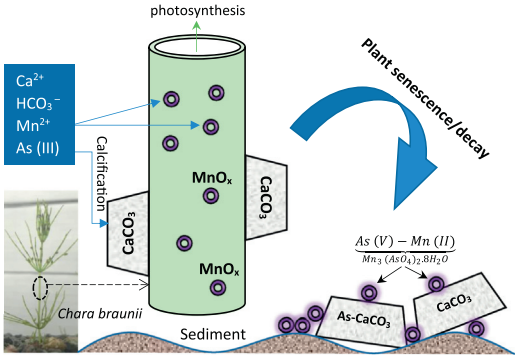
HIGHLIGHTS

- Charophyte biomineralization and its implication in removing As in water was studied.
- Calcifying *C. braunii* produced craterlike deposits of MnO_x in media containing Mn.
- Up to 55.8% of As accumulated by the plants exposed to 0.5 mg L^{-1} As was Ca/Mn-bound.
- *C. braunii* has the ability to immobilize and preserve As in aquatic environments.

GRAPHICAL ABSTRACT



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ABSTRACT

The restoration capability of charophyte *Chara braunii* was studied in arsenic-polluted water in the context of biogenic calcium and manganese depositions on the plant. In addition to calcite encrustation, formation of craterlike shape deposits of manganese oxides (MnO_x) with diameters of 5–10 μm was detected on the cell walls of the plants grown in Mn-rich media. Relative proportions of arsenic taken up by the plant biomass to those incorporated into the calcium and manganese biominerals were determined using a modified sequential chemical extraction method. The mean total arsenic recovery from water reached its highest value at 375 mg kg^{-1} in treatment with HCO_3^- and high concentrations of Ca and Mn (40 and 2 mg L^{-1}, respectively). The percentage of arsenic associated with the manganese deposits in the plants exposed to 0.5 mg L^{-1} As(III) increased from 16.3% to 51.7% of the total arsenic accumulation at low and high Mn levels (<0.05 and 2 mg L^{-1}, respectively), that accounted for the highest Mn-bound arsenic contribution. Surface oxidation of As(III) by MnO_x and subsequent precipitation-adsorption of the formed As(V) onto the evolving structure of MnO_x could be a plausible mechanism for arsenic removal. The presence, and in some cases dominance of arsenic bound to the biogenic Ca and Mn deposits on the studied aquatic plant may contribute to preservation of arsenic in sediments in a less bioavailable form upon its senescence and decomposition.

1. Introduction

Arsenic's natural occurrence and anthropogenic release into the environment are widely known to cause adverse effects on human and environmental health (Mandal and Suzuki, 2002). For instance, arsenic mobilization from sediments and its availability to biota is an issue of concern to the health of aquatic ecosystems (Ahmann et al., 1997).

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Charophytes are aquatic macrophytes known as indicators of water quality, and their presence is important for improving and stabilizing water clarity in different types of water bodies (Lambert and Davy, 2011). *Chara*, genera of charophytes, are able to form abundant calcite encrustation through a mechanism of utilizing bicarbonates as a carbon source for photosynthesis (Kufel et al., 2016), which is accompanied by phycoremediation of various pollutants in water, such as heavy metals, radionuclides, and some nutrients (e.g. phosphorus) in natural and constructed wetlands (Gomes and Asaeda, 2009; Atapaththu et al., 2016). Factors affecting the growth of charophytes for massive occurrence in lakes are light and hard water with relatively high alkalinity. High shade tolerance and higher affinity to HCO_3^- of most of the Characeae family have distinguished them from vascular macrophytes (Kufel and Kufel, 2002). This gives a competitive advantage to the charophytes (selected for this study) over the vascular aquatic plants.

One of the disadvantages of phytoremediation methods for removing toxic elements, such as heavy metals from aquatic systems is release of the accumulated materials to water bodies after the plants senescence and decomposition (Gomes and Asaeda, 2013). However, the portion of heavy metals co-precipitated during the calcite encrustation process in calcifying charophytes is less mobile and may not leach back easily to water following the decay of the dead plants (Asaeda and Zaman, 2013). As such, calcite encrustation in these aquatic plants can potentially provide a long-term storage for some pollutants in sediments. Studies on calcification mechanism of charophytes and their role in nutrient cycling in lakes are available in the literature (McConnaughey, 1991; Siong and Asaeda, 2006; Kufel et al., 2013); however, efficiency of charophytes in trapping heavy metals and some metalloids along the formation of calcium and manganese biominerals on the plants requires attention. In this study, *Chara braunii* (Charophyta), a fresh water calcareous alga common to rice fields in Japan and one of the ecorticate species of *Chara* known to form calcite deposits on the surface of the plant's internodal cells in hard water (Okazaki and Tokita, 1988), was chosen to assess its capability to remove arsenic from aqueous solutions.

As(III) is present in neutral form at the pH ranges of natural waters, and there are no electrostatic interactions between arsenite species and mineral surfaces. As a result, sorption and biosorption techniques are not much effective for As(III) removal from aqueous solutions, and there is a high demand for developing efficient methods for As(III) remediation (Vaclavikova et al., 2008). As(III) compared to As(V) is more difficult to be removed from water; it is more mobile and soluble in water and also more toxic (Ahmann et al., 1997; Vaclavikova et al., 2008). The present study was focused on As(III) detoxification capacity of the charophyte *C. braunii*.

The promising applications of biogenic manganese oxides in natural ecosystems as well as advanced water treatment as oxidants, adsorbents, and catalysts for bioremediation of organic and inorganic contaminants have gained interest among researchers (Spiro et al., 2010; Hennebel et al., 2009). However, the role of biogenic manganese oxides induced by Mn-enriched media in removal of heavy metals and metalloids is so far unclear. Wang et al. (2017) tested the removal of organic compound bisphenol-A by biogenic manganese oxides generated by a green alga in the presence of manganese. This study aims to: (i) report the appearance of two distinct types of biogenic minerals on the surface of *C. braunii* in the presence of Ca, Mn, and bicarbonates in the culture medium; (ii) assess the phytoremediation capability of the plant for removal of arsenic from aqueous solutions; and (iii) discern the relative proportions of arsenic stored in the plant biomass to those incorporated into each of the biominerals in the plant.

2. Material and methods

2.1. Plant culture

Chara braunii were collected from a rice field in Ibaraki prefecture in Japan and cultured in tanks filled with tap water and substrate comprised of 99% (dry wt.) commercial river sand (90% <1 mm) on a thin layer of humus (~1% dry wt.) purchased separately from the local market: DIY, DoIt Japan. Experimental plants were obtained from the culture tanks after 10 days of acclimatization. Ten apical tips with 3 internodes of 2–3 cm tall were clipped and planted in each 1-l glass beaker containing the substrate. All beakers were placed in a temperature-controlled incubator at 20 ± 2 °C with 12 h: 12 h light: dark photoperiod. The plants grew at photosynthetic photon flux density of ~90–125 $\mu mol \cdot m^{-2} \cdot s^{-1}$ maintained by fluorescent lamps. The pH of the solutions was retained at ~8.0 ± 0.1 using 0.1 N NaOH or HCl. Wang et al. (2013) showed that additional HCO_3^- improved the calcification of *Chara* plants. In this work, the influence of adding 1 mM $NaHCO_3$ to the culture media on biomineralization of *C. braunii* was also investigated.

The effects of Ca and Mn in water on removal of arsenic by *C. braunii* were determined by treatments designed in triplicate in the conditions summarized in Table 1. The desired concentrations of Ca, Mn, and As(III) were achieved by adding $CaCl_2.2H_2O$, $MnSO_4.4H_2O$, and As_2O_3 (Wako Pure Chemical Industries, Ltd., Japan), respectively, to the culture media. Non-toxic Mn concentrations under 5 mg L^{-1}, the tolerance capability of charophytes to manganese (Schöler et al., 2014), were utilized.

2.2. Elongation and chlorophyll fluorescence measurements

The plants shoot lengths (mm) were recorded weekly and the shoot elongation (%) and cumulative growth rate (mm/day) were calculated respectively relative to the initial lengths: $(L_t - L_0) / L_0 \times 100$, and $(L_t - L_0) / t$; where L_t is the average shoot length in the *t*th day.

Chlorophyll fluorescence parameters were measured by a Handy FluoroCam program (FC1000-H, Photon Systems Instruments, Czech Republic) connected to an Olympus auto imager (Olympus Tokyo) following 20 min of dark adaptation at the end of the fourth week. The maximum photosynthetic efficiency of PSII (F_v/F_m) was obtained by the following equation (DeEll and Toivonen, 2012): $F_v/F_m = (F_m - F_0) / F_m$; where F_m, F_0 and F_v are the maximum, minimum, and variable fluorescence in dark-adapted plants, respectively.

2.3. SEM-EDX analysis

A Hitachi S-3400N Scanning Electron Microscope (SEM) in conjunction with Energy Dispersive X-ray (EDX) elemental mapping was employed to study the surface of the plant intermodal cells. The SEM operated in vacuum mode (30 Pa) at −120 °C without any sample coating. The EDX spectroscopy provided some quantitative and qualitative information about the elements presented on the surface layers of the observed deposits. SEM-EDX sample preparation method was similar to that of described by Asaeda et al. (2014).

2.4. Plant total As, Ca, and Mn contents

The tested plants were collected at the end of the fourth week of the experiment and dried at 70 ± 5 °C. To determine the total amounts of As, Ca, and Mn (mg g^{-1}) in each plant, a digestion procedure using HNO_3, H_2O_2, and HCl (USEPA, 1996) was employed. The digested solutions were diluted and filtered through 0.45 μm filter nylon membrane, and their elemental contents measured by an ICP-AES (Optima 5300 DV; mixed ICP standard from Wako Pure Chemical Industries, Ltd., Japan). The dry plant samples were weighed to an accuracy of 0.01 mg in 3 replicates.

2.5. Arsenic sequential fractionation

To quantify the apportioned arsenic among the different parts of the plant, a modified chemical extraction method adapted from Tessier