Effects of sulfur on arsenic accumulation in seedlings of the mangrove Aegiceras corniculatum

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Abstract. Levels of arsenic (As) contamination are generally increasing in the sediments of mangrove forests, which represent some of the world’s most threatened marine habitats. The highly anaerobic soils of these important coastal ecosystems are sulfide-rich, yet the potential role of sulfur (S) in the accumulation of As within mangrove tissues is poorly understood. To investigate those dynamics, the present study evaluated the effect of supplemental S on the accumulation of As in mangrove (Aegiceras corniculatum L.) seedlings. We applied treatments in a $4 \times 4$ completely randomised factorial design that consisted of four S concentrations (0, 1, 2 and 4 g kg$^{-1}$, in the form of S monomer) combined with four As concentrations (0, 30, 60 and 150 mg kg$^{-1}$, in the form of Na$_2$HAsO$_4$·7H$_2$O). Three replicates of each treatment combination were conducted. The experiment demonstrated that the effect of S was inversely related to As accumulation in the seedlings; it enhanced As accumulation when applied at low concentrations, and decreased its accumulation when applied at high concentrations. Moreover, supplying S altered the relative concentration of the different forms of As in seedlings, namely As (V) and As (III), and significantly decreased their concentration in roots ($P<0.01$). Taken together, our results suggest that the addition of exiguous S can mitigate the toxicity of As to mangrove seedlings, which has implications for the remediation of polluted coastal areas that are vegetated with mangrove forests.

Additional keywords: arsenate, arsenite, contamination, phytoremediation, toxicology.

Received 2 June 2015, accepted 2 September 2015, published online dd mmm yyyy

Introduction

Mangrove ecosystems dominate most tropical and subtropical coastlines (Alongi 2002). The sediments of these forests are rich in both organic carbon and reduced sulfide, and they are highly anaerobic; they act as both a sink and source of heavy metals (e.g. arsenic, As) in coastal ecosystems (Kirby et al. 2002; Liu et al. 2006). Arsenic is a non-essential element for plants, and inorganic forms of As are generally highly phytotoxic (Zhao et al. 2010); inorganic arsenate, As (V), and arsenite, As (III), are the most dominant forms in mangrove ecosystems (Kirby et al. 2002), and they are highly toxic to plants (Abedin and Meharg 2002).

Plants typically develop resistance to As by restricting its uptake, or limiting its translocation, and by removing it via metabolism in plant tissues (Meharg and Hartley-Whitaker 2002). Recent studies have shown that the presence of sulfur (S) can decrease the uptake of As and its toxicity; this occurs when plants adjust S-containing metabolites such as root iron plaque (Hu et al. 2007), thiols (Srivastava and D’souza 2009) and antioxidants (Srivastava and D’souza 2010). Knowledge about the detailed effects of S on the accumulation of As in mangroves is limited. The present study aimed to assess the effect of S on the accumulation of As in seedlings of the mangrove Aegiceras corniculatum L. The accumulation of the two elements was detected in seedlings, and investigations were extended to measure the content of As (V) and As (III), so as to more broadly define the effect of S on As forms.

Materials and methods

Hypocotyls of the mangrove tree A. corniculatum were collected from the Jiulong River Mangrove Estuary (24°24’N, 117°55’E) of Fujian Province, China. A measure of ~2.5 kg of As-treated soil was added to each pot, and each treatment had three replicates. The seedlings were cultivated in a greenhouse at Xiamen University, under natural light conditions, and maintained for 1 year at a day/night temperature of 33°C/25°C and relative humidity of 65%/85%. Plants were watered twice a week to maintain soil moisture at 70–80% of the field water holding capacity throughout the experimental period. Properties of the soil used are listed in Table 1. Treatments were applied to the seedlings according to a $4 \times 4$ completely randomised factorial design, carried out in three replicates, with each consisting of four concentrations of S (0, 1, 2 and 4 g kg$^{-1}$, applied in the form S monomer), combined with four concentrations of As (0, 30, 60, 150 mg kg$^{-1}$, applied in the form Na$_2$HAsO$_4$·7H$_2$O). After
treatments, plants were harvested and rinsed thoroughly with deionised water.

The harvested plants were oven-dried at 105 °C for 15 min, and then at 70 °C until the samples reached a constant dry weight. After digestion by concentrated HNO₃ on a heating block at 180 °C for 1 h, the total amount of As in the plant tissues was determined using inductively coupled plasma mass spectroscopy (ICP-MS, model ELAN DRC-e, Perkin Elmer, Waltham, MA, USA). To measure the forms of As, constituent materials were extracted twice from the dried plants, using 50% methanol with the assistance of ultrasound. Then, contents of As (V) and As (III) in the supernatant were determined by atomic fluorescence spectroscopy (AFS, model SA-10, Titan, China). The content of S was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, model Optima 7000, Perkin Elmer) as described by Hu et al. (2007).

Data are presented as means ± s.d. (n = 3). Two-way ANOVA was used to assess differences among treatments with SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Tukey’s honest significant difference test (Tukey’s HSD) was performed to compare the difference between treatment combinations at 0.05 and 0.01 levels of statistical significance.

Results and discussion

Seedling biomass differed among treatments with the varying combinations of As and S (Fig. 1). At a given S concentration, biomass decreased with increasing concentrations of As; it was strongly inhibited only by the highest concentration of As (150 mg kg⁻¹), indicating that A. corniculatum seedlings had a relatively high tolerance to As. At any given concentration of As (30, 60 and 150 mg kg⁻¹), plant biomass decreased as S concentration increased, whereas in the absence of As, plant biomass was significantly (P < 0.05) higher in seedlings treated with S than in those that received no S. These findings suggested that, in addition to maintaining plant growth, S may play a role in the mechanism of A. corniculatum for resistance to As. Our results are consistent with those of Fan et al. (2013), who showed that an excessive S supply reduced As accumulation in brown rice (Oryza sativa L.).
We found that As accumulated mainly in the roots of the seedlings, with the mean amount in roots measuring 157.89 times the amount in leaves (Fig. 2). This may have been due to inhibited transport of As from roots to leaves (Abedin and Meharg 2002). The total amount of As measured in both roots and leaves increased significantly when seedlings were supplied with increasing concentrations of As (0–150 mg kg\(^{-1}\); \(P < 0.01\)). In contrast, the total amount of As in both roots and leaves was higher at low concentrations of S (1 and 2 g kg\(^{-1}\)) than in non-S treatments, but lower at a high concentration of S (4 g kg\(^{-1}\)) than in non-S treatments; that is, there was a significant (\(P < 0.05\)) inverse relationship between S supply and As concentration. This pattern of enhanced As accumulation in the presence of low S concentrations has also been demonstrated in *Hydrilla verticillata* (Lf) Royle (Srivastava and D’souza 2009; Zhong et al. 2011).

In our study, the total amount of As (48.61–919.51 mg g\(^{-1}\) DW in roots and 0.96–3.05 mg g\(^{-1}\) DW in leaves) was even higher than what has been found in hyperaccumulator plant species (Zhao et al. 2009). This suggests the possibility of improving the phytoremediation efficiency of As by mangrove by supplying S, as has been indicated by results with other heavy metals (Liu et al. 2009).

Further, our results showed that As (V) was more abundant than As (III) in roots but not in leaves (Fig. 3a, b). The concentrations of As (V) and As (III) increased significantly (\(P < 0.01\)) in both roots and leaves with increasing concentrations of As, similar to the trend observed in total As. We found that S supply decreased the amounts of As (V) and As (III) in roots and leaves, with the decrease being significant (\(P < 0.01\)) in roots. Further, As (III) was not detected in the leaves of seedlings treated with low concentrations of As (from 0 to 30 mg kg\(^{-1}\); Fig. 3d). Our results indicated that the As (V) applied (as Na\(_2\)HAsO\(_4\)-7H\(_2\)O) caused an increase in the amount of As (III) in roots and leaves of seedlings. In particular, the leaves contained higher concentrations of As (III) than As (V), whereas the opposite pattern was observed in the roots, where As (V) concentrations were much higher. Just as Smith et al. (2008) observed, As (V) concentrations were reduced by the plant via transformation to As (III), and As (III)–S compound(s) hence accounted for the majority of As in the leaf and stem of *Raphanus sativus* L. This

![Fig. 3.](image-url)
suggests that soils and mangroves would be able to transform As (V) into As (III), similar to the results reported by others (Thomson et al. 2007; Maher et al. 2009). Our findings indicated that supplying S decreased the toxicity of As to A. conrniculatum seedlings by reducing the accumulation of the most two toxic As forms, As (V) and As (III).

We also investigated S accumulation in the seedlings, so as to assess the correlation between S and As concentrations. In the absence of a supply of S, the S concentration decreased significantly (P<0.05) in roots when the concentration of As increased, but the concentration of S in leaves increased when the concentration of As increased, except at 150 mg kg\(^{-1}\) (Fig. 4a, b). This suggested that As might hinder the uptake of S in roots, whereas the transport of S was not inhibited (Watanabe et al. 2014). In the present study, we found that the accumulation of S increased in both roots and leaves at a given concentration of As when exogenous S was supplied. Moreover, in roots there was a significant (P<0.05) negative correlation between the concentration of S and the concentrations of both As (V) and As (III). In a study on rice, Zhang et al. (2011) reported that S is an important element for the development of plant resistance to As (Rausch and Wachter 2005; Zhang et al. 2011). Moreover, S-mediated reduction of As toxicity involves efficient thiol metabolism, and the antioxidant defence system, in rice treated with high levels of S (Dixit et al. 2015). Taken together, our results suggested that the enhanced accumulation of S might play an important role in the resistance of A. conrniculatum seedlings to As, including the transformation of As forms and alteration of their relative concentrations.

In conclusion, supplying S at low concentrations enhanced As accumulation in A. conrniculatum seedlings, whereas higher concentrations decreased the accumulation. Therefore, there was a pronounced inverse relationship in the presence of these two elements. Further, S may alter the relative concentrations of the As forms As (V) and As (III), thus mitigating the toxicity of As to mangrove seedlings. These findings have potential value for use in studies of phytoremediation, on the basis of the tolerance of mangroves to increasing As concentration in sediments.

Acknowledgements

The authors thank Professor John Merefield for assistance with English grammar.

References


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Fig. 4. Effects of sulfur supply on sulfur (mg kg\(^{-1}\) DW) accumulation in (a) roots and (b) leaves of Aegiceras corniculatum L. seedlings exposed to arsenic. Error bars indicate standard deviation (n=3). Treatments labeled with different letters are significantly different from each other (P=0.05).


