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Arsenic and selected elements in marine angiosperms, south-east coast, NSW, Australia

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The leaves of four angiosperm species, the mangrove *Avicennia marina*, the samphire *Sarcocornia quinqueflora*, the seablight *Suaeda australis* and the seagrass *Posidonia australis*, were sampled from three locations from the south-east coast of NSW. Mean total arsenic concentrations (mean ± SD) in dry mass for all locations were *A. marina* (0.38 ± 0.18 to 1.2 ± 0.7 μg g^{-1}), *S. quinqueflora* (0.13 ± 0.06 to 0.46 ± 0.22 μg g^{-1}), *S. australis* (0.03 ± 0.06 to 0.05 ± 0.03 μg g^{-1}) and *P. australis* (0.34 ± 0.10 to 0.65 ± 0.26 μg g^{-1}). Arsenic concentrations were significantly different between species and locations but were consistently low compared with marine macroalgae species. Significant relationships were found between arsenic and iron concentrations for *A. marina*, *S. quinqueflora* and *P. australis* and a negative relationship between arsenic and zinc concentrations for *S. quinqueflora*. No relationship between arsenic and phosphorus concentrations was found in this study. Angiosperms contained predominantly inorganic arsenic in the water-extractable and residue fractions with minor concentrations of DMA in the water-soluble fraction. *P. australis* also contained dimethylated glycerol and phosphate arsenoriboses. The presence of arsenobetaine, arsenocholine, trimethylated glycerol arsonioribose and an unknown cation in *P. australis* is most likely due to the presence of epiphytes on fronds. There is no evidence to suggest that angiosperms produce arsenobetaine as arsenic is mostly present as inorganic arsenic. In conclusion, marine angiosperms only accumulate low arsenic concentrations and uptake appears to be dependent on iron uptake but not phosphorus uptake. Marine angiosperms mainly cycle inorganic arsenic with little biomethylation of arsenic occurring.

KEYWORDS: arsenic; marine angiosperms; concentrations; species; Australia

INTRODUCTION

Mangrove and salt marsh ecosystems interface between the terrestrial and marine environments and are prevalent on much of Australia's coastline.[1] Mangroves and salt marshes are widely recognized as having a major role in coastal defence and wildlife conservation and are a major source of organic matter and nutrients.[2] Mangroves also act as a major breeding area for several commercially important marine fauna.[3] Additionally, it acts as both a source and sink of sediments and contaminants such as metalloids and heavy metals both naturally occurring and from inputs associated with urbanization and industrial activity.[2,4–7]

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Flora within mangroves and salt marshes survive high salt concentrations, variable inundation by seawater, wave action and tidal movements, anoxic soils and water-logging.[8] Mangroves and salt marsh plants have a number of mechanisms to combat the extreme conditions experienced in this environment, including the formation of iron plaques at the root zone to reduce uptake of metals[9] and the exudation of metals via salt glands in leaves.[10] Additionally, the formation of metal–phytochelatin complexes that are sequestered into the vacuoles of plant cells[11,12] may also be important.

Elevated arsenic concentrations can occur in sediments surrounding mangroves and salt marsh plants even where no anthropogenic sources are present[5] and readily adsorb to organic matter, and to metal hydroxides such as iron, aluminium and manganese.[13] In anoxic sediments, As(V) is reduced to As(III) and is immobilized by binding to sulfides within the sediment.[14] To combat anoxic sediments, plants

direct oxygen to their roots, forming an oxic environment that may remobilize metals and increases their availability to plants.[15] In this oxic environment at the root zone, As(III) is oxidized to As(V) and, because of its chemical similarity to phosphate,[16] is thought to be taken up in a similar manner to phosphate.[17] All these conditions are likely to affect arsenic concentrations, the arsenic species present, and the relationship of arsenic to other trace elements in marine angiosperms.

In this paper we report total arsenic concentrations and arsenic species present in the dominant marine angiosperms found in mangrove, salt marsh and seagrass ecosystems in the south-east coastal area of NSW, Australia. Additionally, total concentrations of macro and micronutrient elements such as phosphorus, iron, zinc, copper, manganese, magnesium and molybdenum were also measured to address any interaction or relationship with arsenic concentrations. These elements are involved in photosynthetic and enzymatic processes in cells of photosynthetic organisms and may influence the transport and sequestering of arsenic into plants.

METHODS

Study location

Four marine angiosperm species, the mangrove *Avicennia marina*, two salt marsh species, *Sarcocornia quinqueflora* and *Suaeda australis*, and a seagrass species, *Posidonia australis*, were collected from a temperate region situated on the south-east coast of NSW, Australia (Fig. 1). These plants represent species that are commonly found in this region of Australia.

Study design

A nested sampling design (three locations, two sites within locations, four plant species and five replicates per species) was used that allowed variation between locations to be investigated. Samples were collected in March and April of 2004. Factors are location (fixed), sites (nested in location) and species (fixed).

Sample preparation

Five replicates of each species were collected, placed into clean zip-locked plastic bags, transported on ice and stored in a cool room. Sample preparation occurred as soon as possible once samples were returned to the laboratory to limit decomposition and changes to arsenic species. Leaves were rinsed with deionized water (18.2 mΩ, Millipore), to remove salt, and scraped with a stainless steel razor blade rinsed in ethanol to remove epiphytic growth, especially for *Posidonia australis*. Leaves were then placed in 2% v/v acid-washed 50 ml polypropylene vials and immediately frozen in preparation for freeze drying. Frozen leaf samples were freeze dried (Labconco) until dry (approximately 24–48 h). Freeze-dried samples were homogenized using a Retsch ZM100 mill (0.2 mm stainless steel mesh, Retsch) and stored in clean polyproplyene vials in a desiccator until analysed.

Sample analysis

Reagents and standards

Nitric acid (HNO_3; Aristar, BDH) was used for the determination of total arsenic concentrations. Ammonium dihydrogen phosphate (Suprapur, Merck) and pyridine (Extra Pure,

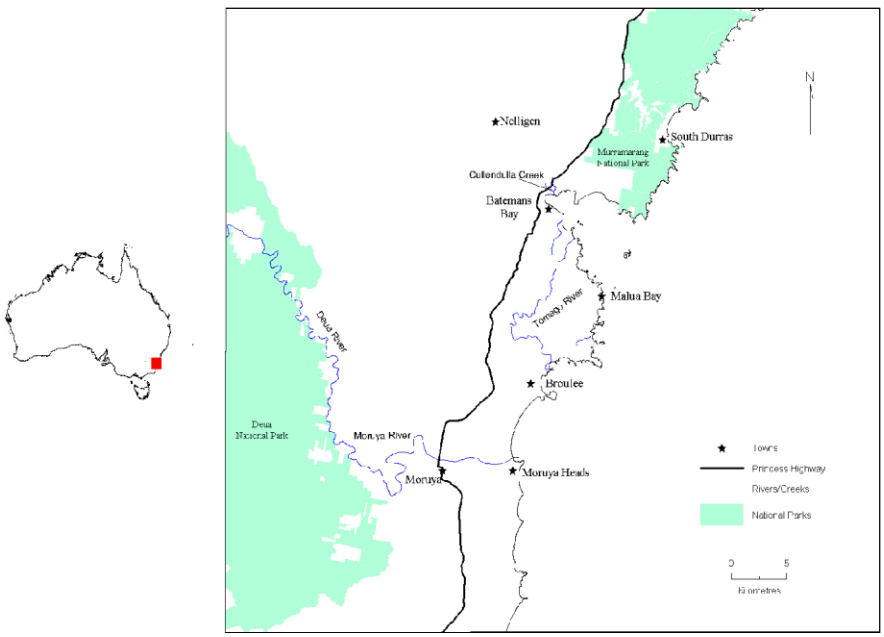

Figure 1. Sampling locations on the south-east coast, NSW, Australia: Moruya River, Tomago River and Cullendulla Creek. This figure is available in colour online at www.interscience.wiley.com/AOC.

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Merck) were used in the preparation of high-pressure liquid chromatography (HPLC) mobile phases. Formic acid (Extra Pure, Fluka) and ammonia solution (>99.9%, Aldrich) were used for the adjustment of mobile-phase pH. Methanol (HiPerSolv, BDH), acetone, (Unichrom, Ajax Laboratory Chemicals), chloroform (Laboratory Reagent, May and Baker) and deionized water (18.2 mΩ, Millipore) were used for the extraction of arsenic species. Hydrochloric acid (HCL; Trace Pur, Merck), L-cysteine (BioChemika, Fluka) and sodium tetrahydroborate ($NaBH_4$; Laboratory Chem Chemicals, APS) were used for the reduction and derivatization of arsenic species. The $NaBH_4$ solution was stabilized by the addition of 0.01 M sodium hydroxide (Pronalys, Selby-Biolab) in deionized water.

Stock standard solutions (1000 mg l^{-1}) of arsenous acid (As^{3+}), arsenic acid (As^{5+}), methylarsonic acid (MA) and dimethylarsinic acid (DMA) were prepared by dissolving sodium arsenite, sodium arsenate heptahydrate (Ajax Laboratory Chemicals), disodium methyl arsonate and sodium dimethylarsenic (Alltech-Specialists), respectively, in 0.01 M HCL–deionized water. Synthetic arsenobetaine (AB; BCR-626, Institute for Reference Materials and Measurements) was diluted with deionized water to desired concentration. Arsenocholine (AC), trimethylarsine oxide (TMAO), tetramethylarsonium ion (TETRA) and glycerol trimethylated arsonioribose (TriMeOH-ribose) were kindly supplied by Professor Kevin Francesconi and Dr Walter Goessler (Institute of Analytical Chemistry, Karl-Franz-University, Graz, Austria). Glycerol arsenoribose, sulfonate arsenoribose and sulfate arsenoribose (OH-ribose, SO_3-ribose, OSO_3-ribose, respectively) were isolated in-house from the marine macro algae certified reference material *Fucus* 140 (IAEA). The phosphate arsenoribose (PO_4-ribose) was isolated in-house from the marine animal certified reference material oyster 1566a (NIST). The identity of these arsenoriboses was previously confirmed by high-performance liquid chromatography–mass spectrometry (HPLC-MS).[18] Trimethylarsoniopropionate (TMAP) was isolated in-house from the marine animal certified reference material, lobster hepatopancreas (TORT-2; NRC-CNRC).[19]

Total arsenic and element analysis

Digestion of samples for total arsenic concentrations was performed using a microwave digestion technique as outlined by Baldwin *et al*.[20] with modifications. Approximately 0.1 g of ground sample was accurately weighed and recorded into 7 ml Teflon polytetrafluroacetate digestion vessels (AI Scientific) and 1 ml of concentrated nitric acid added (Aristar, BDH). Digestion vessels with sample and acid were left in the fume cupboard for approximately 1 h prior to digestion. The microwave digestion (MDS 81D, CEM, Indian Trail) program cycle was run at 2 min 600 W, 2 min 0 W, 45 min 450 W for each set of samples with certified reference materials and blanks. Samples were allowed to cool after digestion for ~60 min then diluted to 10 ml with deionized water in 10 ml polyethylene vials. Certified reference material pine leaves (NIST 1575) and citrus leaves (NBS 1572) were treated in the same manner as samples. Total element concentrations in samples were analysed using a Perkin Elmer Elan 600 inductive coupled plasma-mass spectrometer (ICP-MS) with an AS-90 autosampler. Internal standards were added on-line to compensate for any acid side effects and instrument drift.[21] The potential interference to arsenic (m/z 75) from $^{40}Ar^{35}Cl^+$ was determined by measuring chloride at m/z 35, $^{35}Cl^{16}O^+$ at m/z 51. $^{35}Cl^{17}O^+$ at m/z 52 and $^{40}ArCl^+$ at m/z 77. Selenium was monitored at m/z 82 as a cross check for $^{40}Ar^{37}Cl^+$. Other elements were corrected for interferences as outlined in Maher *et al*.[21]

Calibration standards using the multi element calibration standard (Accu Trace, Calibration Standard 2, 10 mg l^{-1}) was used to produced standards (0, 1, 10, 100, 1000 μg l^{-1}). All calibration standards were prepared daily in 10% v/v HNO_3 to determine element concentrations of As, Fe, P, Cu, Mn, Zn, Co, Mo, Mg by ICP-MS.

Fractionation of arsenic and total arsenic analysis

A single sample of each species from each location was used for fractionation into lipid, water-soluble and residue fractions (a total of 12 samples). Samples were freeze dried and ground to a fine powder as outlined for sample preparation for total arsenic analysis.

Lipid extraction

Approximately 0.1 g of sample was weighed into a 50 ml polyethylene vial. The extraction process as carried out by Folch *et al*.[22] allows the separation of the lipid and water-soluble phases, with analysis of total arsenic for each phase used to determine the amount in each phase. To the 0.1 g sample, 5 ml of chloroform–methanol (2 : 1v/v) was added, vortexed for 30 s to assist mixing and placed on a rotary wheel for 4 h. Samples were then centrifuged for 10 min at 5000 rpm to separate sample and supernatant. The supernatant was pipetted into a 50 ml polypropylene vial. This procedure was repeated and the supernatant combined with the first extraction. To the supernatant, 4 ml of deionized water was added to assist in separating the lipid and water-soluble phases and left to stand overnight. The lipid and water-soluble phases were separated and placed in 10 ml centrifuge tubes and evaporated to dryness using a RVC 2–18 rotary vacuum concentrator (60 °C, 3000 rpm; Christ). Once dry, samples were stored in a freezer (−18 °C) until required for analysis. Prior to quantification, samples were re-suspended in 5 ml of 1% v/v HNO_3.

Water extraction

The residue from the chloroform–methanol–water extraction was freeze dried (~24 h). To this residue, 2 ml of hot water was added and it was placed in a hot water bath (100 °C) for 1 h, to remove any soluble arsenic species remaining after the previous extraction. The extracts were centrifuged at 5000 rpm for 10 min and the supernatant pipetted into 10 ml

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polyethylene tubes containing the methanol–water soluble material from the previous extraction. The supernatant was stored in a refrigerator until required for analysis. Prior to analysis, the supernatant was made up to 5 ml with 1% v/v HNO_3.

Residue

The remaining residue was freeze dried (~24 h). To digest the residue, 1 ml of 2% HNO_3 was added and the solution placed in a water bath for 2 h. The final extract was made up to 2 ml with deionized water, giving a final acid concentration of 1% v/v HNO_3.

Arsenic speciation

Acetone extraction

Samples were initially extracted with acetone prior to methanol–water extraction to remove components such as fats, that may interfere with chromatography. Approximately 0.2 g of homogenized freeze-dried whole tissue was added to 50 ml polypropylene vials and 10 ml of acetone added. The samples were then agitated on a mixing wheel for 1 h and the supernatant removed after centrifuging at 5000 rpm for 10 min. The extraction procedure was repeated twice more, with the supernatant removed after each centrifugation. After the final acetone extraction, the residue pellet was dried to a constant mass in a fume cabinet at room temperature (~25 °C). The acetone extract was discarded.

Methanol–water extraction

Water-soluble arsenic species were extracted from biological material by a microwave extraction procedure developed by Kirby and Maher.[19] Approximately 0.1 g of the acetone extracted pellet was weighed into 50 ml polypropylene vials and 10 ml of 50% v/v methanol-deionized water added. Mixtures were loaded into the carousel of an MDS-200 microwave oven (CEM) and heated to 75 °C for 10 min. The supernatants were removed after centrifuging at 5000 rpm for 10 min. The procedure was repeated twice and the supernatants combined. Thirty millilitre of the methanol–water supernatant was evaporated to dryness using an RVC 2-18 rotor vacuum concentrator (50 °C; Christ) and stored in a freezer (−18 °C) until speciation analysis.

Nitric acid extraction

The residue was extracted with 1 ml of 2% v/v HNO_3 at 70 °C for 2 hrs. The final extract was made up to 2 ml with deionized water.

Arsenic species measurement

Prior to chromatography, methanol–water extracts were re-suspended in 4 ml deionized water, while 1% v/v HNO_3 acid residue extracts were analysed with a 1 : 10 dilution. Total arsenic was determined by digesting 1 ml of the re-suspended methanol–water extract with 0.5 ml of concentrated HNO_3 and 0.5 ml of H_2O_2 in a water bath at 90 °C. Residue extracts were analysed without further treatment. The digest was diluted to 10 ml with deionized water and analysed for arsenic by ICP-MS as previously described. All extracts were filtered through a 0.20 μm RC syringe filter (Millipore). Aliquots of 20 or 40 μl were injected onto a high-pressure liquid chromatography (HPLC) system consisting of a Perkin Elmer Series 200 mobile phase delivery and an auto sampler system (Perkin Elmer). The eluant from HPLC columns was directed by PEEK (polyether-ether-ketone; i.d. 0.02 mm; Supelco) capillary tubing into a Ryton crossflow nebulizer of a Perkin Elmer Elan-6000 ICP-MS, which was used to monitor the signal intensity of arsenic at m/z 75. Potential polyatomic interferences were checked by monitoring for other ions as described for total arsenic analysis. The column conditions used for the separation of arsenic species are outlined in Table 1.

Arsenic species were separated and quantified using HPLC-ICP-MS. Arsenic anions were analysed using PRP-X100 and arsenic cations were analysed using a Supelcosil LC-SCX at pH 2.6 and pH 3 (Table 1). Residues were analysed using HPLC with hydride generation ICP-MS.[23] External calibration curves for quantification of arsenic species were prepared by diluting As(III) for anionic species and AB for cationic species to 0, 0.5, 1, 10 and 100 $\mu g\,l^{-1}$ daily. Peak area responses relative to AB and As(III) have been reported previously.[24] The purity of arsenic species was periodically determined by HPLC-ICP-MS.

The chromatography package Total Chrom (Perkin Elmer) was used to quantify arsenic species by peak areas. Arsenic

Table 1. HPLC column specifications for arsenic species analysis

Column	Hamilton PRP-X100 (PEEK)	Supelcosil LC-SCX	Supelcosil LC-SCX
Size	250 × 4.6 mm	250 × 4.6 mm	250 × 4.6 mm
Particle size	10 μm	10 μm	10 μm
Buffer	20 mM $NH_4H_2PO_4$, 1% CH_3OH	20 mM pyridine	20 mM pyridine
pH	5.6	2.6	3
Flow rate	1.5 ml min^{-1}	1.5 ml min^{-1}	1.5 ml min^{-1}
Temperature	40 °C	40 °C	40 °C
Arsenic species	As(V), DMA, MA, PO_4, SO_3 and OSO_3 arsenoribosides	DMAE, glycerol trimethyl arsonioribose, TETRA, AC and TMAP	AB and OH arsenoribosides

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species were identified by spiking with known standards and comparisons of retention times.

Statistical analysis

Nested analysis and single-factor ANOVA (factor-location) was used to determine significant differences in total arsenic concentrations between locations ($p = 0.05$) on normalized data (SPSS 12.0). Cluster analysis and principle component analysis (PCA) by Primer 5; PRIMER-E[25] was used to classify groups of plant species with similar element concentrations or similar phosphorus or arsenic species.

Quality assurance

Total arsenic and other elements

Certified reference material pine needles (NIST1575) and citrus leaves (NBS1572) were used to verify experimental values were within the range of certified material. Certified values and measured values are listed in Table 2.

Arsenic speciation

The accuracy of the arsenic speciation procedure was determined by the analysis of the certified reference material, DORM-2. The concentrations (mean ± SD) of AB (16.3 ± 0.5 μg g^{-1}) and TETRA (0.241 ± 0.005 μg g^{-1}) measured in DORM-2 tissues were similar to certified values (AB, 16.4 ± 1.1 μg g^{-1}; TETRA, 0.248 ± 0.054 μg g^{-1}).

RESULTS

Total arsenic, macro and micronutrients

Arsenic

Mean concentrations and standard deviations for arsenic, major and minor elements in angiosperms are presented in Table 3. Arsenic concentrations were significantly higher

Table 2. Comparison of measured and certified concentrations of two certified reference materials

Certified reference material	Element	Certified μg g^{-1}	Measured μg g^{-1}
Pine needles (NIST 1575)	As	0.21 ± 0.04	0.18 ± 0.04
	Fe	200 ± 10	178 ± 6
	Cu	3.0 ± 0.3	1.75 ± 0.09
	Mn	675 ± 15	645 ± 4
	Zn	N/A	61 ± 2
	Mg	N/A	1084 ± 24
Citrus leaves (NBS 1572)	As	3.1 ± 0.3	3.1 ± 0.2
	Fe	90 ± 10	75 ± 8
	Cu	16.5 ± 1.0	10.1 ± 0.1
	Mn	23 ± 2	16.5 ± 0.1
	Zn	29 ± 2	27 ± 4
	Mg	N/A	5320 ± 70

N/A, not available.

Table 3. Total arsenic and micro and macro nutrient concentrations in four marine angiosperm species leaf tissues from three locations on the south-east coast, NSW, Australia

Location	Species	Element concentrations μg g^{-1} ± SD								
		As	Fe	P	Co	Cu	Mn	Zn	Mo	Mg
Moruya River	*Avicennia marina*	0.58 ± 0.23	134 ± 42	2007 ± 272	5.00 ± 0.05	11 ± 4	23 ± 6	18 ± 6	0.13 ± 0.17	8604 ± 3365
	Suaeda australis	0.05 ± 0.03	183 ± 54	1822 ± 771	7 ± 1	12 ± 2	46 ± 23	41 ± 9	0.64 ± 0.82	13392 ± 2692
	Sarcocornia quinqueflora	0.13 ± 0.06	223 ± 82	840 ± 168	5.3 ± 0.2	13 ± 3	27 ± 25	22 ± 7	n.q.	7811 ± 1113
	Posidonia australis	0.36 ± 0.10	21 ± 2	125 ± 38	0.77 ± 0.03	0.74 ± 0.02	13 ± 2	1.37 ± 0.14	0.19 ± 0.04	653 ± 108
Tomago River	*Avicennia marina*	1.2 ± 0.7	165 ± 62	1718 ± 629	4.94 ± 0.04	9 ± 2	26 ± 9	14 ± 6	n.q.	9640 ± 3556
	Suaeda australis	0.03 ± 0.06	144 ± 27	2600 ± 643	6.0 ± 0.2	11 ± 2	42 ± 24	45 ± 12	0.53 ± 0.66	19416 ± 3460
	Sarcocornia quinqueflora	0.46 ± 0.22	300 ± 157	625 ± 115	4.98 ± 0.05	7.61 ± 0.66	31 ± 10	11 ± 2	n.q.	7293 ± 904
	Posidonia australis	0.34 ± 0.10	15 ± 4	189 ± 20	0.73 ± 0.03	0.75 ± 0.03	7 ± 2	1.80 ± 0.18	0.16 ± 0.10	540 ± 37
Cullendulla Creek	*Avicennia marina*	0.38 ± 0.18	103 ± 17	1505 ± 436	4.98 ± 0.03	14 ± 23	18 ± 5	17 ± 16	n.q.	7533 ± 1831
	Suaeda australis	0.03 ± 0.05	135 ± 9	1878 ± 431	5.9 ± 0.5	6.88 ± 0.83	30 ± 19	39 ± 25	n.q.	18655 ± 2663
	Sarcocornia quinqueflora	0.17 ± 0.11	161 ± 36	811 ± 113	4.96 ± 0.05	6.48 ± 0.66	26 ± 8	19 ± 5	n.q.	6483 ± 1025
	Posidonia australis	0.65 ± 0.26	26 ± 5	129 ± 27	0.78 ± 0.28	0.76 ± 0.02	13 ± 2	1.6 ± 0.1	0.13 ± 0.08	543 ± 83

n.q., not quantified < 0.01 μg g^{-1} Mo.
Elements are chosen based on involvement in photosynthetic and enzymatic processes in cells of photosynthetic organisms.

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Table 4. Significant differences in arsenic concentration of leaf tissues from four marine angiosperm species from three locations on the south-east coast, NSW, Australia

Species	MR	TR	CD
Avicennia marina	*a*	*b*	*a*
Suaeda australis	*a*	*a*	*a*
Sarcocornia quinqueflora	*a*	*b*	*a*
Posidonia australis	*a*	*a*	*b*

MR, Moruya River; TR, Tomago River; CD, Cullendulla Creek. $b > a$.

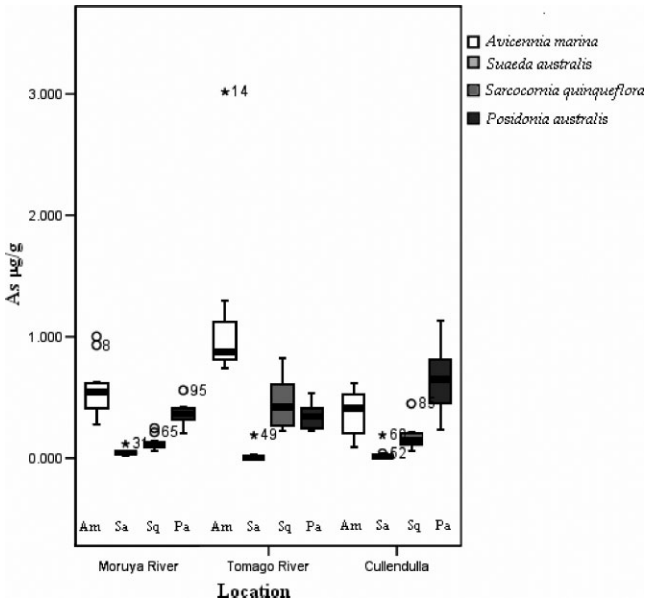

Figure 2. Total arsenic concentrations in four angiosperm species leaf tissues, south-east coast, NSW, Australia. *Avicennia marina*, *Suaeda australis*, *Sarcocornia quinqueflora* and *Posidonia australis* by location. Box whisker plot: box, 25–75% of values; line, median; whiskers represent 5 and 95% confidence intervals.

at Tomago River for *A. marina* and *S. quinqueflora. P. australis* had significantly higher arsenic concentrations at Cullendulla Creek and *S. australis* had comparable concentrations across all locations (Table 4, Fig. 2). Differences within sites for total arsenic concentrations were not significant at the 95% confidence interval (Table 5). Data was pooled and a single-factor ANOVA was performed to test for significant differences in arsenic concentrations between locations and plant species. Significant differences in arsenic concentrations between locations were found for *A. marina, S. quinqueflora* and *P. australis* (Table 6) as well as significant differences between species in the order highest to lowest arsenic concentrations *A. marina* > *P. australis* > *S. quinqueflora* > *S. australis*.

Table 5. Nested analysis of significant differences in arsenic concentrations of leaf tissues from four marine angiosperm species from two sites at three locations on the south-east coast, NSW, Australia

Source	d.f.	Mean square	*F*	*p*
Location	2	0.541	9.047	0.000
Site	1	0.084	1.396	NS
Species	3	2.409	40.258	0.000
Location × site	2	0.106	1.766	NS
Location × species	6	0.560	9.351	0.000
Site × species	3	0.043	0.711	NS
Location × site × species	6	0.034	0.565	NS

$R^2 = 0.682$ (adjusted $R^2 = 0.605$); significance, $p < 0.05$.

Table 6. Significant differences in arsenic concentrations between locations (site data pooled) of leaf tissues from four marine angiosperm species south-east coast, NSW, Australia

Species	Variable	d.f.	MS	*F*	*p*
Avicennia marina	Loc	2	1.594	8.104	0.002
Suaeda australis	Loc	2	0.001	0.458	NS
Sarcocornia quinqueflora	Loc	2	0.321	26.682	<0.001
Posidonia australis	Loc	2	0.303	10.748	<0.001

Significance, $p < 0.05$.

Major and minor elements and their relationships to arsenic

Macro and micronutrients are not the focus of this study and are analysed to determine relationships with arsenic concentrations in marine angiosperms. Principle component analysis (PCA) confirms three groups of angiosperms (Table 7, Fig. 3). Group 1 contains *S. australis* which has higher zinc concentrations than the other plant species. Group 2 contains *S. quinqueflora* and *A. marina* with similar zinc concentrations. *A. marina is* separated from *S. quinqueflora* by having higher arsenic concentrations. Group 3 contains *P. australis*, which has lower zinc concentrations than the other plant species (Fig. 4). The third PCA axis (not shown) also shows that iron and phosphorus concentrations in the two salt marsh plants also influence groupings (Table 7). Cluster analysis groups similar species regardless of the location sampled (Fig. 4).

As arsenic, zinc, iron and phosphorus concentrations in the angiosperms were identified as influencing groupings by PCA, regression analysis was performed to determine any relationships between these elements and arsenic [Fig. 5(a–d)]. Significant relationships between arsenic and iron concentrations were identified for *A. marina* ($r^2 = 0.15$, $p < 0.05$), *S. quinqueflora* ($r^2 = 0.70$, $p < 0.001$) and *P. australis* ($r^2 = 0.32$, $p < 0.01$). A significant negative relationship was found between arsenic and zinc concentrations for

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Table 7. Principle component analysis of elements for leaf tissues from four marine angiosperm species from three locations on the south-east coast, NSW, Australia

Axis	Eigenvalues	Percentage variation	Cum% variation	Variable	Axis 1 (PC1)	Axis 2 (PC 2)	Axis 3 (PC3)
1	4.68	66.9	66.9	As	**0.212**	**−0.765**	0.434
2	1.05	15.0	82.0	Fe	−0.296	−0.480	**−0.649**
3	0.75	10.8	92.7	P	−0.401	−0.100	**0.538**
				Cu	−0.374	−0.317	−0.049
				Mn	−0.428	−0.036	−0.162
				Zn	**−0.441**	**0.252**	0.116
				Mg	−0.434	0.098	0.242

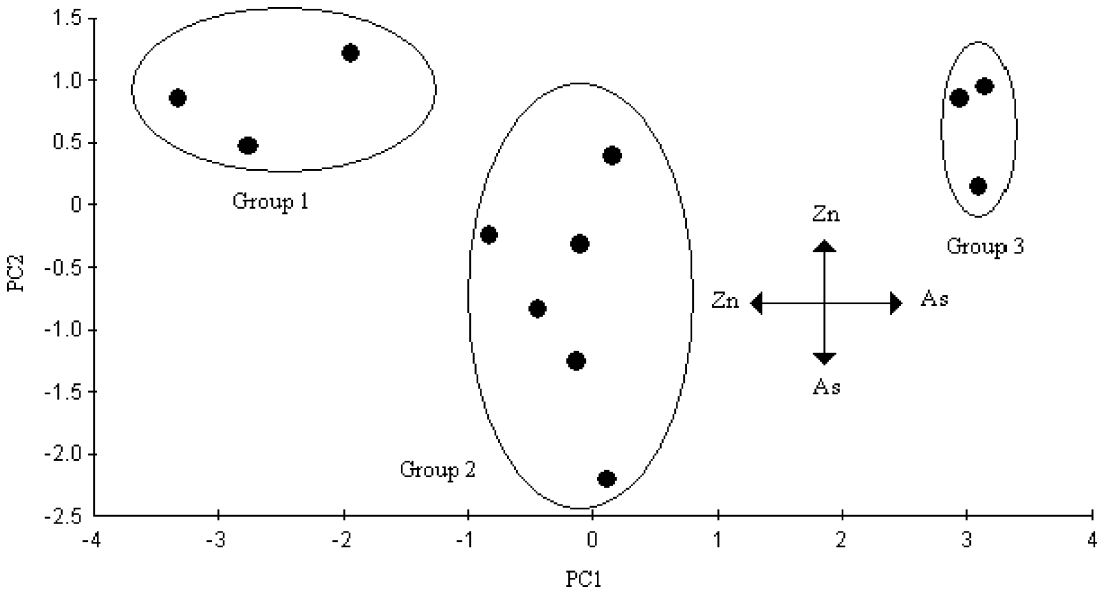

Figure 3. Principle component analysis of elements in leaf tissues from four angiosperm species, south-east coast, NSW, Australia. Group 1: *Suaeda australis*; group 2: *Avicennia marina, Sarcocornia quinqueflora*; group 3: *Posidonia australis.* Arrows indicate factors contributing to the pattern in two-dimensional space.

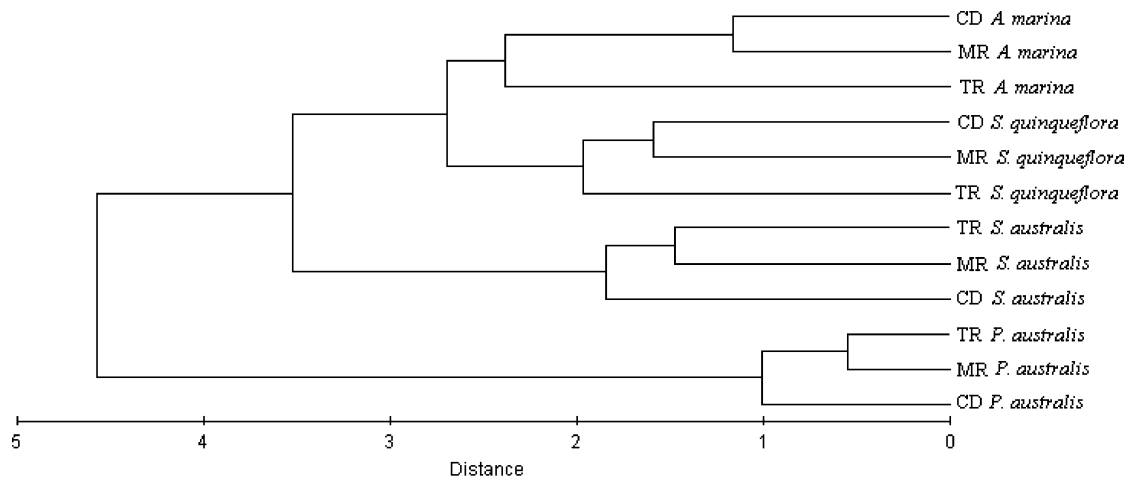

Figure 4. Cluster plot of elements in *Avicennia marina, Suaeda australis, Sarcocornia quinqueflora* and *Posidonia australis* leaf tissues from south-east coast, NSW, Australia.

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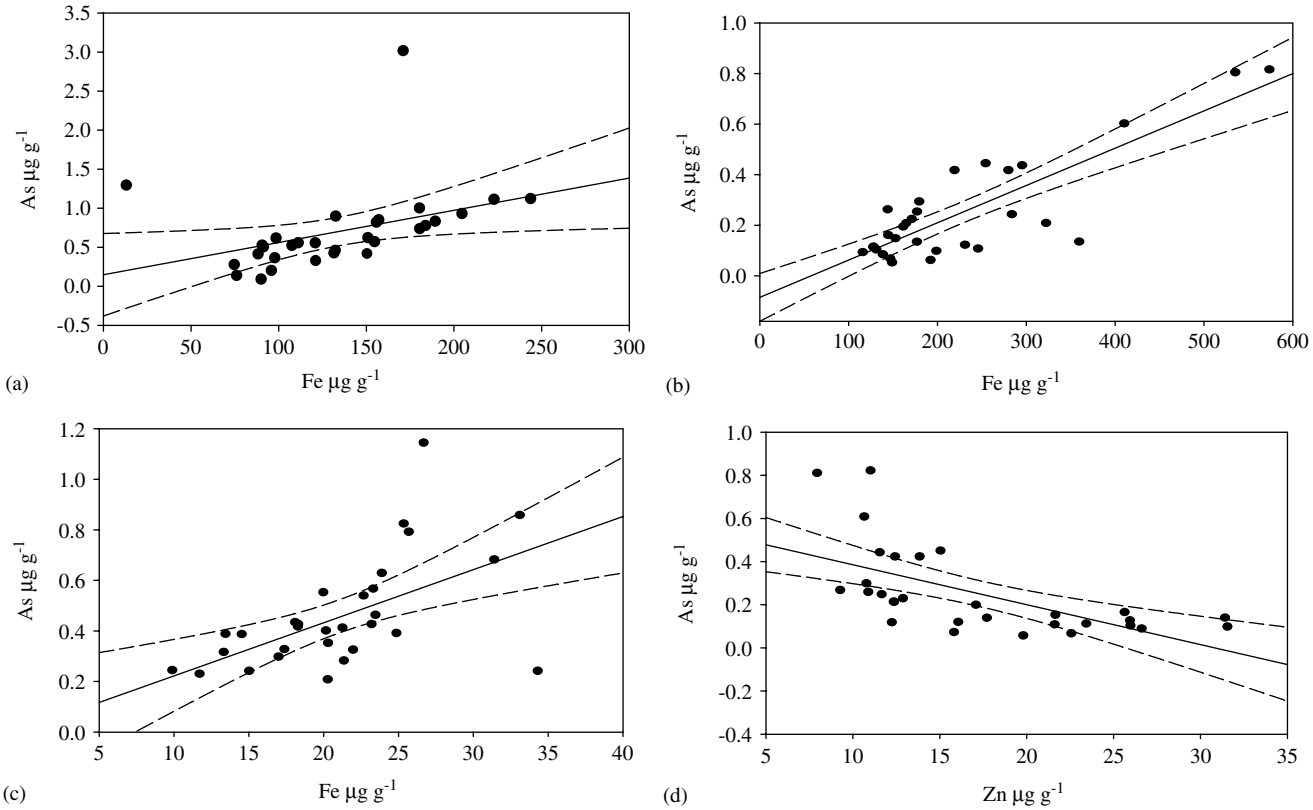

Figure 5. Regressions analysis for arsenic, macro and micronutrients concentrations in leaf tissues from four angiosperms, south-east coast, NSW, Australia, identified by principle component analysis. (a) As vs Fe for *Avicennia marina*; (b) As vs Fe for *Sarcocornia quinqueflora*; (c) As vs Fe for *Posidonia australis*; and (d) As vs Zn for *Sarcocornia quinqueflora*. Dashed lines represent 95% confidence limits.

Table 8. Fractionation of arsenic in four marine angiosperm species leaf tissues from three locations on the south-east coast, NSW, Australia

Species	Sample	Total As, µg g^{-1}	Lipid soluble, %	Water soluble, %	Residue, %
Avicennia marina	MR	1.00	<1	22	77
	TR	1.12	<1	22	77
	CD	0.56	<1	17	83
Suaeda australis	MR	0.04	<1	33	67
	TR	0.002	21	39	41
	CD	0.014	<1	45	55
Sarcocornia quinqueflora	MR	0.25	<1	36	64
	TR	0.30	12	39	49
	CD	0.20	<1	43	56
Posidonia australis	MR	0.40	21	16	63
	TR	0.23	31	20	48
	CD	0.67	20	13	67

MR, Moruya River; TR, Tomago River; CD, Cullendulla Creek.

S. quinqueflora ($r^2 = 0.37$, $p < 0.001$). No significant relationships between arsenic and phosphorus concentrations were found.

Fractionation of arsenic

The percentages of arsenic associated with lipid-soluble, water-soluble and residue fractions of angiosperms are

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presented in Table 8. In all plant species, most arsenic was in the residue fraction (41–83%). Negligible arsenic concentrations (<1%) were found in most of the lipid fractions of *A. marina*, *S. australis* and *S. quinqueflora*, except for the two salt marsh plant species collected from Tomago River (12–21%). At all locations, *P. australis* contained a higher percentage of arsenic in the lipid fraction (20–31%). The water-soluble fraction contained 13–45% of arsenic with *S. australis* and *S. quinqueflora* containing more arsenic in this fraction (33–45%) compared to the other species (13–22%).

Arsenic species

Arsenic species concentrations in water-soluble and residue fractions of angiosperms are presented in Table 9. Arsenic species were not analysed in the lipid fraction as at the time of this study we had no reliable procedures to do so. PCA analysis of water-soluble and residue arsenic species (Table 10) showed the dominant species was inorganic arsenic (group 1, Fig. 6). The water-soluble extracts contained mainly As(V) and As(III) with ng g^{-1} concentrations of DMA [Table 10, Figs 7(a)–10(a)]. *S. quinqueflora* at Moruya River contained proportionally higher DMA concentrations relative to inorganic arsenic (group 2, Fig. 6), but DMA concentrations were still low in this species. *P. australis* contained AB in all three samples tested and at Tomago River also contained nanogram per gram concentrations of OH-ribose, PO_4-ribose, TriMeOH-ribose and AC [group 3, Figs 6 and 10(b)]. An unknown cation was detected in *P. australis* at Cullendulla Creek. SO_3-ribose and OSO_3-ribose were not detected in any of the plant species. Residues of all plants only contained As(III) and As(V) [Figs 7(b), 8(b), 9(b) and 10(c)].

DISCUSSION

Total arsenic

Arsenic concentrations in *A. marina*, *S. quinqueflora* and *S. australis* (Table 3) are similar to arsenic values previously reported for these plant species from this region[26,27] and similar to arsenic concentrations measured in other wetland plants from non-contaminated environments.[28] Total arsenic concentrations in *P. australis* are at the lower range of concentrations found in other studies.[29,30]

Arsenic concentrations in angiosperms (0.03–1.2 μg g^{-1}, Table 3) are low compared with other marine photosynthetic organisms such as macroalgae.[31] Although significant differences in arsenic concentration were found between angiosperm species (Table 6), differences are small and considerable variability is evident. *A. marina* and *S. quinqueflora* have significantly higher arsenic concentrations at Tomago River than other locations (Table 4, Fig. 2). Sediment arsenic concentrations at this location are similar to other locations[26] and cannot be used to explain the higher arsenic concentrations in plants at this location. *S. australis* did not show any differences in arsenic concentrations between locations but high variability and very low arsenic concentrations in this plant may have masked any location effects.

Metalloids and metals in general are found in low concentrations in angiosperms even in contaminated environments with the soil matrix considered to be the most important factor influencing the mobility and bioavailability of arsenic and other metals to plants.[7,32] As discussed in the next section, plant uptake of arsenic and other elements may be controlled by iron plaque on root surfaces rather than the soil matrix.[33–36] Thus indiscriminate accumulation of arsenic is not occurring. As well, leaf drop and leaf salt excretion (plants that excrete salt have been shown to have enhanced excretion of metals)[10,15,37] may also be important mechanisms in removing arsenic from plants such that plant arsenic concentrations remain low.

Arsenic and macro and micronutrients

This study does not attempt to discuss individual macro and micronutrient concentrations but analyses possible relationships between these elements with arsenic. Generally, higher iron concentrations were found in *S. quinqueflora* compared with other angiosperm species examined, with higher zinc concentrations in *S. australis*. Significant relationships between arsenic and iron concentration were found in the leaves of *A. marina*, *S. quinqueflora* and *P. australis* [Fig. 5(a–c)], suggesting that iron may be influencing the uptake of arsenic. Foster *et al*.[26] found a strong relationship between arsenic and iron concentrations for *S. quinqueflora* in leaves and roots but not in the leaves of *S. australis*. It is likely that iron deposits on angiosperm roots influences the availability and hence the concentrations of arsenic and other elements found in the entire plant and may promote or inhibit the uptake of some elements.[33,35,36] Doyle and Otte,[35] found that arsenic and iron concentrations were significantly higher in the rhizosphere soil around plants roots compared with bulk soil and that iron oxides were accumulating arsenic and zinc in the oxidized root zone. Additionally, arsenic and zinc concentrations appear to co-vary with iron concentrations in plaque material. However, St-Cry and Campbell[36] found that zinc concentrations in two freshwater wetland angiosperms were inversely related to iron concentrations in root plaque, implying that iron is regulating zinc bioavailability. A negative trend between zinc and iron concentrations in *S. quinqueflora* was evident, resulting in a significant negative correlation between zinc and arsenic in this plant [Fig. 5(d)].

Unlike other studies,[26,29] no significant relationship between arsenic and phosphorus concentrations was found in any of the plant species examined. Arsenic and phosphorus are chemically similar and arsenic enters plants using similar biochemical pathways to phosphorus.[17] It is thought that higher phosphorus concentrations inhibit arsenic uptake in plants, reducing the effect of arsenic on phosphorylation reactions.[32] Foster *et al*.[26] found a weak relationship between arsenic and phosphorus concentrations in the halophyte species *S. quinqueflora* and *S. australis*, but could not conclude that phosphorus inhibits arsenic uptake. Cai *et al*.[29] found

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Table 9. Arsenic species concentrations in leaf tissues from four marine angiosperm species from three locations on the south-east coast, NSW, Australia. Percentages denote the percentage of arsenic species, characterized from the total eluted from the column

		Water-soluble										Residue	
									Dimethyl arsenoribosides				
Species	Sample	Total As	Inorg As	DMA	MA	AB	AC	Tri-MeOH	−OH	$-PO_4$	Unk Cation	Total As	Inorg As
Avicennia marina	MR	0.24	0.27 (93)	0.02 (7)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.74	0.20
	TR	0.54	0.45 (96)	0.02 (4)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.10	0.08
	CD	0.48	0.33 (97)	0.01 (3)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.09	0.08
Suaeda australis	MR	0.11	0.02 (73)	0.01 (27)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.07	0.07
	TR	0.11	0.02 (100)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.13	0.16
	CD	0.78	0.01 (100)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.07	0.06
Sarcocornia quinqueflora	MR	0.16	0.01 (20)	0.04 (80)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.04	0.05
	TR	0.12	0.04 (80)	0.01 (20)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.13	0.16
	CD	0.56	0.06 (100)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	0.21	0.26
Posidonia australis	MR	0.44	0.12 (71)	0.01 (6)	n.q.	0.04 (24)	n.q.	n.q.	n.q.	n.q.	n.q.	0.10	0.08
	TR	0.62	0.09 (29)	0.02 (6)	n.q.	0.05 (16)	0.05 (16)	0.04 (13)	0.03 (10)	0.03 (10)	n.q.	0.25	0.25
	CD	3.51	0.99 (85)	0.03 (3)	n.q.	0.04 (3)	n.q.	n.q.	n.q.	n.q.	0.10 (9)	0.89	0.99

MR, Moruya River; TR, Tomago River; CD, Cullendulla Creek.
n.q., not quantifiable $<0.005\ \mu g\ g^{-1}$ for organoarsenic species and $0.0003\ \mu g\ g^{-1}$ for inorganic arsenic.

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Table 10. Principle component analysis of arsenic species in water-soluble and residue fractions of leaf tissues from four marine angiosperm species from the south-east coast, NSW, Australia

Axis	Eigenvalues	Percentage variation	Cum% variation	Variable	Axis 1 (PC1)	Axis 2 (PC 2)	Axis 3 (PC3)
1	4.84	60.5	60.5	Inorg As	**0.326**	**−0.558**	0.056
2	1.55	19.4	79.9	DMA	−0.069	**0.790**	**0.071**
3	1.02	12.7	92.7	AB	−0.312	−0.044	−0.173
				AC	**−0.445**	−0.125	−0.030
				Tri Me-OH ribose	**−0.445**	−0.125	−0.030
				Di Me-OH-ribose	**−0.445**	−0.125	0.030
				PO_4 ribose	**−0.445**	−0.125	0.030
				Unk Cation	0.013	0.017	**−0.979**

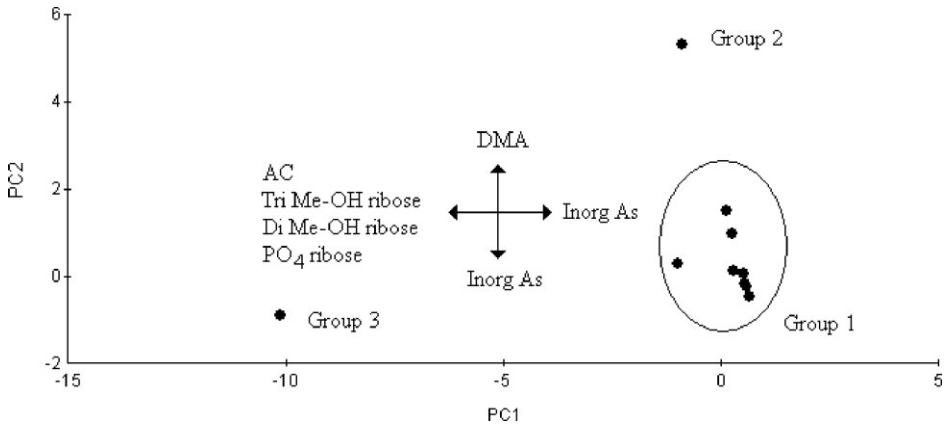

Figure 6. Principle component analysis of arsenic species in water-soluble and residue fractions of four angiosperm species leaf tissues, south-east coast, NSW, Australia. Group 1: *Avicennia marina, Suaeda australis, Sarcocornia quinqueflora, Posidonia australis*; water-soluble and residue arsenic species. Group 2: *Sarcocornia quinqueflora* Moruya River, water-soluble arsenic species. Group 3: *Posidonia australis* Tomago river, water-soluble arsenic species. Arrows indicate factors contributing to the pattern in two-dimensional space.

a positive relationship between arsenic and phosphorus concentrations in the seagrass, *Thalassia testudinum*, in one estuary, but a negative relationship between arsenic and phosphorus concentrations when all sites in the study were examined. It is likely that the low and small ranges of arsenic concentrations measured in this study make it difficult to establish any distinct relationships with phosphorus, if indeed any relationships exist.

Fractionation of arsenic

Generally, low arsenic concentrations were found in the lipid fractions of *A. marina, S. quinqueflora* and *S. australis*. The proportion of arsenic in the lipid fraction was higher in *P. australis*, although it is a higher proportion of a low arsenic concentration. Arsenic concentrations have not generally been measured in the lipid component of terrestrial plants and have not been considered a major pathway of arsenic metabolism in angiosperms. Angiosperms have been shown to contain a low proportion and a limited number of polar lipids.[38] Arsenic lipids have been found to be associated with polar phospholipids in macro algae[39,40] and the absence of polar lipids in angiosperms may explain the low proportions of arsenic found in the lipid fractions of angiosperms in this study.

The proportion of arsenic in the water-soluble fractions were also relatively low and not consistent between angiosperms (Table 8). Methanol–water extraction of arsenic in terrestrial plants has not proved as effective as for marine organisms. Zheng *et al*.[41] used mechanical agitation and methanol–water (9 : 1 v/v) for the extraction of arsenic from two species of submerged and terrestrial plants with resulting low arsenic recoveries (6–16%). Heitkemper *et al*.[42] extracted rice using methanol, water and/or mixtures of methanol-water in varying concentrations with the assistance of accelerated solvent extraction at room temperature and found for the SRM 1568a (rice flour) that all the extractants used gave good results (76–105%); however, real-world samples only yielded between 24 and 36%.

The residue fractions contained most arsenic and this indicates a high proportion of arsenic bound to structural cellular components such as thio-complexes of which arsenite in particular has a high affinity.[12,43] Plants that are tolerant

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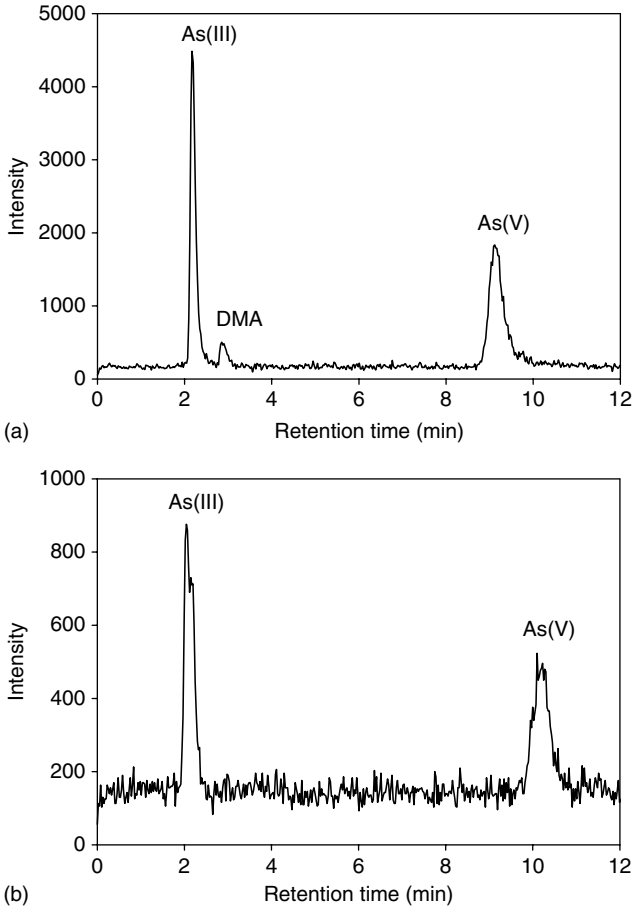

Figure 7. Arsenic species in *Avicennia marina* leaf tissue. (a) Water-soluble anions analysed by Hamilton PRP-X100 anion exchange column; (b) residue fraction determined by anion exchange HPLC-HG-ICPMS.

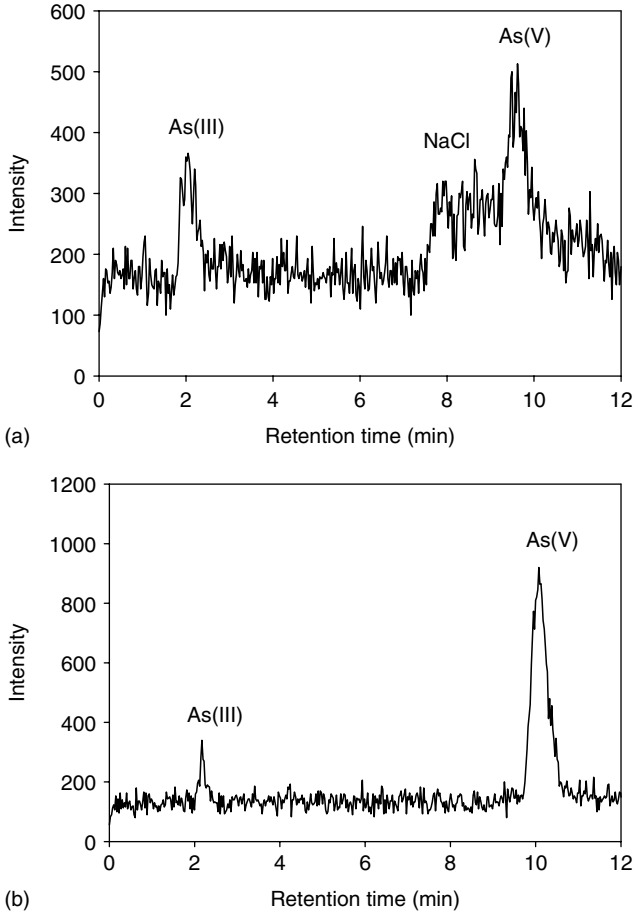

Figure 8. Arsenic species in *Sarcocornia quinqueflora* leaf tissue. (a) Water-soluble anions analysed by Hamilton PRP-X100 anion exchange column; (b) residue determined by anion exchange HPLC-HG-ICPMS.

of high levels of metals typically store toxic elements outside the endodermis or in cell walls to prevent metals being transported to rhizomes and photosynthetic tissues.[44,45] This would be an efficient mechanism for plants, such as marine angiosperms, to prevent inorganic arsenic interfering in phosphate metabolism associated with photosynthesis.

Arsenic species

The main arsenic species found in the water-soluble fractions of the angiosperms, based on current analytical evidence, were As(III) and As(V) with traces of DMA (Table 9, Figs 6–10). This is consistent with previous studies that have found that arsenic species in vascular plants are mostly inorganic with low amounts or traces of simple methylated arsenic species.[26,46–48] In submerged plants, Zheng[47] found that As(V) predominates and storage of As(III) may not apply for submerged plants. Although this was for a freshwater plant species, there are similarities to *P. australis* in this study, which had relatively low As(III) concentrations [Fig. 10(c)]. Dimethylated arsenosugars such as OH-ribose have been previously found in halophytes,[26] but were not detected in halophytes species examined in this study.

In contrast, *P. australis* contained a small proportion of some organic arsenic species including AB, AC, OH-ribose, PO_4-ribose, TriMeOH-ribose and, in one sample, an unknown cation most likely dimethylarsinoylethanol (Table 9). Seagrasses fronds often have attached epiphytes including algae, fungi and bacteria.[49–52] Although fronds were thoroughly cleaned and examined for epiphytes, it is extremely difficult to remove microscopic epiphytes. Arsenobetaine, AC and TriMeOH-riboses are not normally found in plant species and are probably present because of epiphytic growth on the seagrass fronds. Submerged aquatic plants *Lemna minor, Myriophyllum* sp. and *Sparganium augustifolium* have been shown to contain OH-ribose and PO_4-ribose as well as MA and inorganic arsenic.[32,53] However, whether submerged aquatic plants produce arsenoribosides or are present due to algal epiphytes needs to be resolved.

Terrestrial plants species have been shown to produce phytochelatins in response to metals and metalloids.[11,12,54]

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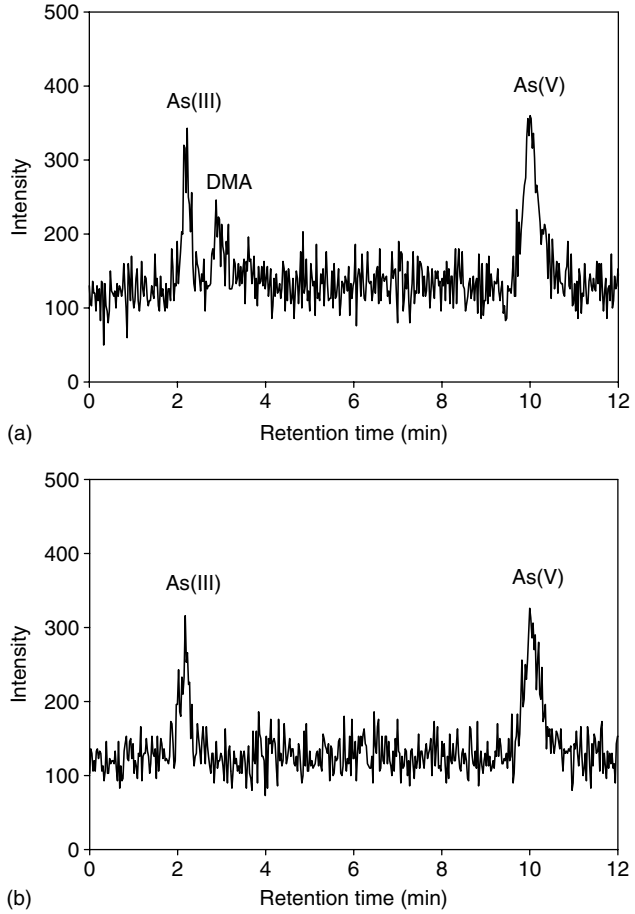

Figure 9. Arsenic species in *Suaeda australis* leaf tissue. (a) Water-soluble anions analysed by Hamilton PRP-X100 anion exchange column; (b) residue determined by HPLC-HG-ICPMS.

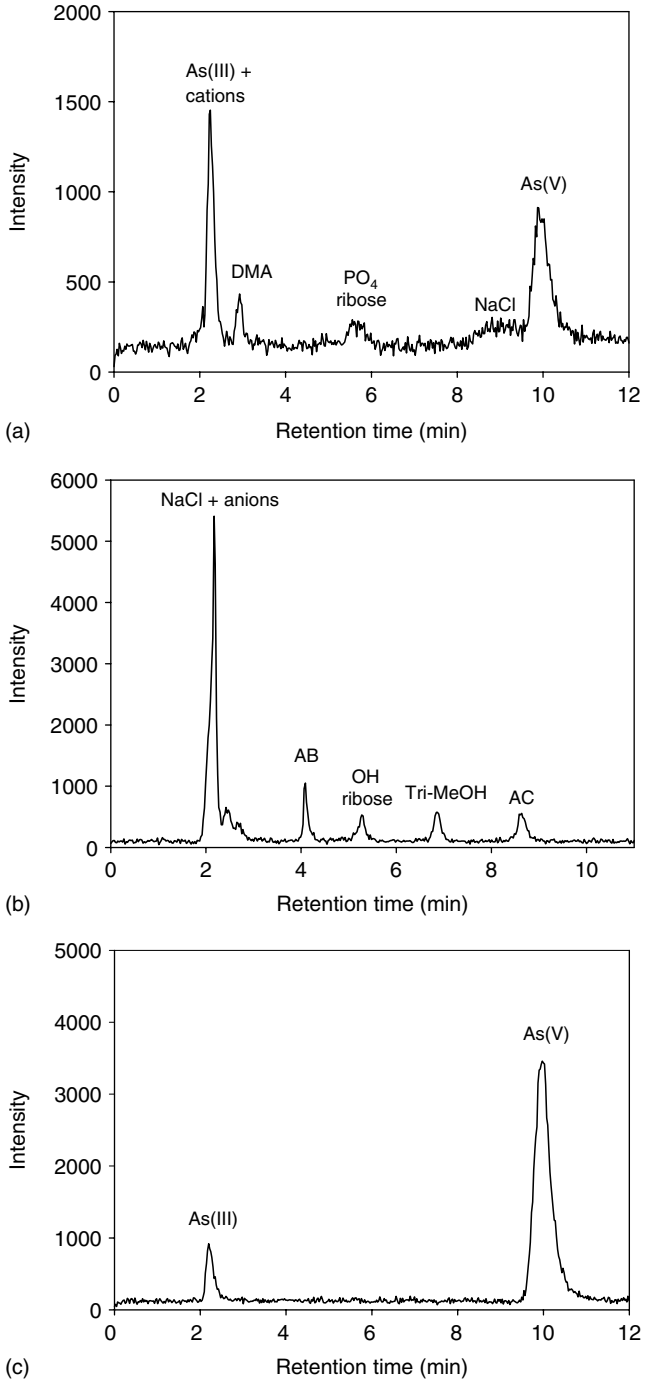

Figure 10. Arsenic species in *Posidonia australis* leaf tissue. (a) Water-soluble anions analysed using Hamilton PRP-X100 anion exchange column; (b) water-soluble cations analysed using Supelcosil SCX cation exchange column; (c) residue determined by anion exchange HPLC-HG-ICPMS.

Phytochelatins are polypeptides [γ-glutamine-cysteine]$_n$–glycine ($n = 2$–11) and have a high affinity for As(III), although As(V) also induces phytochelatin synthesis.[12] Methylation and formation of organoarsenic species does not occur in higher-order plants.[55] Given the high proportion of inorganic arsenic present in the angiosperm species in the current study, As–phytochelatin complexes may be a major pathway of sequestering arsenic in marine angiosperms; however, arsenic may only be binding non-specifically to cellular components.

Arsenic cycling in marine angiosperms

Based on our results and other information reported in the literature a summary of the uptake, metabolism, sequestering and excretion of arsenic in marine angiosperms is given below.

Uptake

Arsenic in the form of As(V) is reduced to As(III) in anoxic sediments that are prevalent in mangrove and salt marsh ecosystems and form insoluble arsenic sulfide species.[14] In anoxic soils, plants direct oxygen to the root zone where As(III) is oxidized to As(V) in this oxic zone around the rhizosphere. Iron is thought to provide a protective mechanism for plants by the formation of iron plaques on the root zone,[15] but due to adsorption of As(V) by iron oxides

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can also assist in the accumulation and transfer of arsenic towards the root zone. As(V) is then taken up by the plant via the roots[17] during the transport of other essential elements, possibly via the active phosphate pathway.[43] Based on the results reported in this paper, arsenic uptake appears to be dependent on iron uptake but not phosphorus uptake.

Metabolism

Within the plant, as As(V) interferes with oxidative phosphorylation, it is likely to be reduced to As(III), as occurs for other higher plants. There is little evidence to suggest that angiosperms use the formation of simple methylated compounds such as DMA and MA, and arsenoribosides as a major mechanism of processing inorganic arsenic, as only traces of MA and DMA and no arsenoribosides were detected in mangrove and salt marsh plant species. The very small amounts of OH-ribose and PO_4-ribose measured in the seagrass may be associated with algal epiphytes rather than intracellular arsenic of the seagrass.

Sequestration

The majority of arsenic in angiosperms was in the form of As(V) and As(III) complexed with water-insoluble constituents of the cells. The presence of a higher proportion of inorganic arsenic in the insoluble residue suggests that As(III) and As(V) are complexed with cellular components such as polypeptides or form arsenic–phytochelatins, as found for higher-order plants in previous studies.[11,12,56] However, freeze drying and the extraction procedures used in this study are likely to disrupt arsenic-phytochelatin complexes.[54,56] Thus sample preparation techniques would have to be carefully optimized to investigate the presence of arsenite/arsenate complexes such as arsenic–phytochelatins.

Homeostasis

Terrestrial plants use nitrogen and sulfur compounds such as glycine betaine, proline and dimethylsulfoniopropionate to maintain osmotic balance in changing saline environments.[57–59] Arsenic also forms inert compounds such as AB and TMAP that are analogues of the nitrogen and sulfur compounds glycine betaine and dimethylsulfoniopropionate, respectively, and these also could be used as osmotic regulators. As there was little evidence of the presence of these arsenic species, it is unlikely that higher order plants use arsenic to maintain their osmotic balance within the cell. Additionally, higher order plants maintain their rigid external structure by the use of cellulose and lignin[60,61] compared with macroalgae that primarily contain cellulose and mucilaginous polysaccharides to maintain cell wall structure.[62] Thus the formation of arsenic riboses would not be expected.

Excretion

A. marina can excrete significant quantities of metals such as Cu and Zn from leaf surfaces in the form of salt crystals,[15] so it is possible that arsenic may also be excreted from this plant in a similar manner. This could explain the low arsenic concentrations measured in this species and other marine angiosperms in this study. Additionally, leaf drop is an effective mechanism to remove metals from plants, especially for those that do not contain salt glands.[10,37] Older parts of the plant that have accumulated trace elements, such as arsenic, may be shed by the plant and arsenic returned to the sediments via leaf detritus. These may be important mechanisms in assisting marine angiosperms to maintain low arsenic concentrations.

In conclusion, marine angiosperms only accumulate low arsenic concentrations. Arsenic uptake may be dependent on iron uptake but does not appear to be dependent on phosphorus uptake. The importance of root iron plaque formation, excretion of arsenic by leaves and leaf drop in controlling arsenic uptake and accumulation needs to be determined. Marine angiosperms mainly accumulate and cycle inorganic arsenic. Further research should also focus on investigating the presence of arsenite/arsenate complexes such as arsenic–phytochelatins in angiosperms.

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