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Arsenic concentrations and speciation in a temperate mangrove ecosystem, NSW, Australia†

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Total arsenic concentrations and species were measured in the sediments, vegetation and tissues of marine animals from a temperate mangrove ecosystem. Mean arsenic concentrations ranged from 0.3 to 55 $\mu g\ g^{-1}$ dry mass. Epiphytic algae/fungi associated with mangrove fine roots had relatively higher arsenic concentrations ($12 \pm 3\ \mu g\ g^{-1}$) than mangrove leaves, bark or main roots (0.3–1.2 $\mu g\ g^{-1}$) and algae/fungi attached to main roots ($1.5 \pm 0.8\ \mu g\ g^{-1}$). The concentrations of arsenic in detritivores (8.5–55 $\mu g\ g^{-1}$) were significantly higher than in the major primary producers (0.3–1.5 $\mu g\ g^{-1}$), two herbivores (8 ± 1 and $14 \pm 2\ \mu g\ g^{-1}$) and omnivores (2–16.6 $\mu g\ g^{-1}$). Most marine animal tissues contained large percentages of arsenobetaine (28–81%). Glycerol arsenoribose was found in all tissues examined (1–23%) except oyster tissues. Relatively large concentrations of this arsenoriboside were found in the digestive tissues of two crab species (13–23%). Small amounts of trimethylarsoniopropionate (1–8%), tetramethylarsonium ion (1–7%), sulfate arsenoribose (2–13%) and trace amounts of arsenocholine (<1%), trimethylarsine oxide (<1%), dimethylarsinic acid (<2%), phosphate arsenoribose (<2%), arsenate (<1%), and sulfonate arsenoribose (<3%) were found in some tissues. Methylarsonic acid was not found in any tissues. Two unknown cationic arsenic compounds (1–2%) and three anionic arsenic compounds (1–17%) were present in some marine animal tissues. The arsenic concentrations and species found in animals could not be attributed to their position in the food web or feeding mode, but are likely to be related to their dietary intake of arsenic and their ability to assimilate, metabolize and retain arsenic species. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: arsenic; mangrove ecosystem; Australia; concentrations, speciation

INTRODUCTION

Marine organisms have been shown to accumulate high concentrations of arsenic.[1,2] Arsenic in marine organisms is not usually present as inorganic arsenic or simple methylated forms, but as a variety of organic arsenic species.[1,2] The main arsenic compounds that are present in marine organisms are arsenobetaine in animals[1,2] and arsenoribosides in macroalgae.[1–3] Small amounts of trimethylarsine oxide, tetramethylarsonium ion, phosphatidylarsenocholine, arsenocholine, arsenoribosides and trimethylarsoniopropionate are also found in marine animals.[1,2,4,5] The intermediate stages involved in the biosynthesis of these compounds are not known.

Mangrove forests in temperate waters occur at the upper tidal level on sheltered coastlines and along the margins of tidal creeks and inlets. They provide a habitat for estuarine and marine assemblages and are important nursery areas for fish, crabs and shrimps.[6] High concentrations of trace metals are known to accumulate in mangrove ecosystems, because mangrove vegetation trap suspended particulate matter and fine sediment.[7]

In this study we report total arsenic concentrations and species in organisms and sediments of a temperate mangrove ecosystem, and examine if arsenic concentrations and species can be related to trophic feeding position.

STUDY LOCATION

Samples were collected from Moona Moona Creek (35° 07′S,

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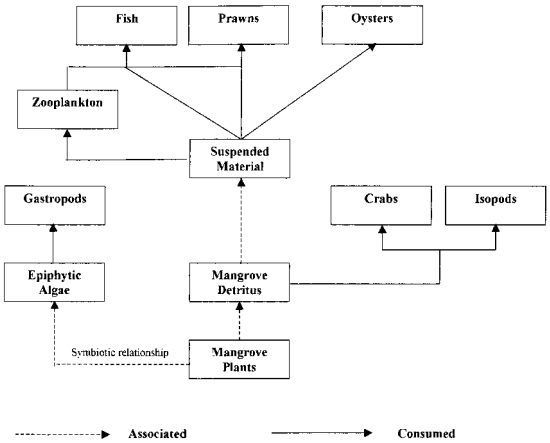

Figure 1. Temperate mangrove food web.[8–11]

150° 42′E) within the Jervis Bay area NSW, Australia. The catchment is forested with *Eucalyptus, Banksia* and *Causarina*, contains some grazing land, and is free from anthropogenic inputs of arsenic. The study site was located along the northern side of the estuary, approximately 1 km upstream from the mouth of the creek. Mangrove forests dominated by *Avicennia marina* line both sides of the creek.

SAMPLING

Samples were collected in March 1998 and 1999 based on the simplified food web shown in Fig. 1. Morphological characteristics, literature and expertise were used to partition the animals into their appropriate trophic groups. All samples were placed into acid-washed plastic bags, frozen and transported to the laboratory on ice.

Sediment cores

Sediment cores ($n = 8$) were collected from five 1 m^2 plots, which were 5 m apart at low tide, using a 5 cm diameter $\times$ 10 cm length of PVC pipe.

Epiphytic algae/fungi

Epiphytic algae/fungi ($n = 13$) were randomly scraped off *A. marina* root surfaces.

Mangrove litter

All vegetative matter (<5 cm in length) present within five 1 m^2 plots which were 5 m apart at low tide was collected and a composite sample of material from each quadrat analysed ($n = 5$).

Mangrove leaves

Leaves were picked at random from *A. marina* trees ($n = 16$).

Mangrove roots and bark

A. marina main roots ($n = 6$), fine roots ($n = 6$) and bark ($n = 6$) were sub-sampled at random from mangrove trees.

Isopods

Decaying mangrove wood was broken open and isopods ($n = 19$) collected using forceps.

Crabs

Two crab species, the Mangrove crab *Paragrapsus gaimardii* ($n = 13$) and the Soldier crab *Mictyris longicarpus* ($n = 29$), were collected randomly from crab burrows in the exposed sediment of the quadrats.

Plankton

At high tide, a 50 μm plankton trawl net was hauled along a 10 m transect three times on two occasions (night and day).

Gastropods

Two gastropod species, *Pyrazus ebeninius* ($n = 52$) and *Bembicium auratum* ($n = 56$), were collected randomly from mangrove roots.

Fish, prawns and shrimps

A 250 μm dip net was used to capture juvenile Silver Bream, *Acanthopagrus australis* ($n = 29$), prawns, *Panaeus spp.* ($n = 12$), and Palemonid shrimps ($n = 16$).

Oysters

Saccrostrea commercialis ($n = 16$) were collected from above and below the high tide mark on mangrove trunks.

SAMPLE PREPARATION AND ANALYSIS

Sample preparation

Sediments

Sediments were homogenized by thorough mixing with a motor-driven stirrer and 2 g sub-samples transferred to clean polyethylene vials and freeze dried for 48 h.

Biological tissues

Vegetative litter and mangrove leaves, bark and roots were scrubbed in deionized water and oven dried at 34 °C for 48 h before grinding and storage. Shells were removed from gastropods and carapaces removed from crabs. All tissues were freeze dried for 48 h, homogenized, ground and stored in clean polyethylene vials in a desiccator until analysed.

Sample digestion

Sediments

Sediment samples (0.1–0.2 g) were weighed into 50 ml polypropylene vials and 5 ml of Aristar nitric acid (BDH, Australia) added. Samples were digested using an MDS-2000 microwave oven (CEM, USA) at 110 °C for 40 min and,

Table 1. Arsenic recoveries from certified reference materials (mean ± standard error; $n = 8$)

	NIST 1646 Estuarine sediment	CRRC MESS-1 Marine sediment	NIST 1575 Pine leaves	NIST 1515 Apple leaves	NIST 1566 Oyster tissue	AGAL-2 Shark tissue
Recovered As (μg g^{-1} dry mass)	8.5 ± 0.3	8.4 ± 0.1	0.24 ± 0.02	0.04 ± 0.01	14.5 ± 0.2	25 ± 1
Certified value As (μg g^{-1} dry mass)	11.6 ± 1.3	10.6 ± 1.2	0.21 ± 0.04	0.038 ± 0.007	14.0 ± 1.2	23 ± 2
Recovery (%)	74 ± 3	79 ± 1	116 ± 9	105 ± 25	104 ± 2	108 ± 4

on cooling, diluted with deionized water (Milli-Q, Millipore, Australia) to 50 ml for total arsenic analysis.

Biological tissues

The freeze-dried and homogenized samples were digested with nitric acid using a low-volume microwave digestion procedure.[12] Approximately 0.07 g of freeze-dried tissue was weighed into a 7 ml Teflon polytetrafluoroacetate digestion bomb and 1.0 ml of concentrated nitric acid added (Aristar, BDH, Australia). The microwave time program consisted of three steps: 2 min at 600 W; 2 min at 0 W; 45 min at 450 W. After digestion, vessels were allowed to cool at room temperature and then diluted to 10.0 ml with deionized water. Digests were stored in polyethylene vials in a cool room until analysed for total arsenic.

Total arsenic analysis

Total arsenic concentrations were determined using an electrothermal atomic absorption spectrometer with Zeeman background correction (Perkin Elmer 5100, HGA-600) using a palladium–magnesium modifier (0.15 μmol palladium and 0.4 μmol Magnesium on the platform[13] or by inductively coupled plasma–mass spectrometry (ICP-MS).[14] The accuracy of the procedure was assessed by the analysis of six reference materials (Table 1). Arsenic concentrations measured in these reference materials were in agreement with the certified values, except for sediments. The nitric acid digestion procedure used does not recover arsenic bound within silicate phases.

Arsenic standards

Arsenate and arsenite were prepared by dissolving sodium arsenate heptahydrate (Sigma–Aldrich, Australia) and sodium arsenite (Sigma–Aldrich, Australia) respectively in deionized water (Milli-Q, Millipore, Australia). Dimethylarsinic acid (DMA) and methylarsonic acid (MA) were prepared by dissolution of sodium dimethylarsenic (Sigma–Aldrich, Australia) and disodium monomethylarsenic (Pfalz and Bauer, Germany) in deionized water (Milli-Q, Millipore, Australia).

Arsenobetaine (AsB), arsenocholine (AsC), trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TETRA) were kindly supplied by Dr Erik Larsen (National Food Agency, Institute of Food Chemistry and Nutrition, Denmark). The phosphate, sulfonate, sulfate and glycerol arsenoriboses were isolated and quantified from the marine macroalgae *Fucus* (IAEA 140/TM) and *Ecklonia radiata*. Confirmation of the four arsenoriboses was achieved by using liquid chromatography (LC)–MS–MS (Perkin Elmer SCIEX API 300). The four arsenoriboses were separated using a Hamilton PRP-X100 anion-exchange column (250 mm × 4.6 mm, 10 μm) (Phenomenex, USA) with an aqueous 20 mM ammonium carbonate buffer (pH 9.2). The *m/z* of 329, 483, 393, and 409 were selectively monitored to identify the glycerol, phosphate, sulfonate and sulfate arsenoriboses respectively. The concentrations of these arsenriboses measured in *Fucus* were 10.51 μg g^{-1}, 0.69 μg g^{-1}, 9.45 μg g^{-1}, 10.51 μg g^{-1} respectively, and 5.54 μg g^{-1} as arsenite/MA.

Trimethylarsoniopropionate (TMAP), also known as AsB 2, was isolated from lobster hepatopancreas (TORT-2; NRCC, Canada) using a Supelcosil LC-SCX cation-exchange column (250 mm × 4.6 mm, 5 μm) (Supelco, USA) with a 20 mM pyridine–formic acid buffer at pH 2.6. The identity of this compound was confirmed by comparison with TMAP reported in dogfish muscle tissue (Dorm-2).[5]

Arsenic speciation analysis

Eight animal tissues and *A. marina* leaves were selected for arsenic speciation analysis to gain an understanding of the accumulation, distribution and cycling of arsenic compounds in the mangrove ecosystem.

Acetone extraction

Approximately 0.2–0.3 g of freeze-dried homogenized animal tissue was added to 50 ml polypropylene centrifuge tubes and 10 ml of acetone (HiPerSolv, BDH) added. The mixtures were shaken for 1 h and the supernatant removed after centrifuging at 3000 rpm for 15 min. The extraction procedure was repeated twice, with the supernatants combined for total arsenic analysis. After the final acetone extraction the residue pellet was dried under vacuum at room temperature (~25 °C).

Total arsenic concentration of the acetone supernatant was determined by weighing approximately 5 ml of the supernatant into 7 ml Teflon polytetrafluoroacetate digestion vessels (A. I. Scientific, Australia) and evaporating the acetone to dryness at room temperature (~25 °C). The residue was resuspended in 0.5 ml concentrated nitric acid (Aristar, BDH) and digested by the procedure previously

outlined for biological tissues. Extracts were diluted to 5 ml with deionized water (Milli-Q, Millipore, Australia) prior to analysis for total arsenic by ICP-MS.

Methanol–water extraction

Water-soluble arsenic compounds were extracted from animal tissues by microwave-assisted extraction with 50% methanol–water. Approximately 0.1–0.2 g of biological material was weighed into 50 ml polypropylene vials and 10 ml of 50% (v/v) methanol (HiPerSolv, BDH, Australia) –deionized water (Milli-Q, Millipore, Australia) added. Mixtures were loaded into the carousel of an MDS-2000 microwave oven (CEM, USA, 630 W) and heated to 70–75 °C for 5 min. The extracts were centrifuged at 3000 rpm for 15 min and the supernatants removed. The procedure was repeated twice, with the combined supernatant from all three 50% (v/v) methanol–water extractions used for arsenic speciation.

Total arsenic concentrations of the methanol–water fractions were determined by evaporating approximately 2 ml of the combined supernatant to dryness at 50 °C using an RVC 2-18 rotational vacuum concentrator (CHRIST, Quantum, Australia). The residue was resuspended in 0.5 ml of 10% nitric acid (Aristar, BDH, Australia) and diluted with deionized water (Milli-Q, Millipore, Australia) to 5 ml prior to arsenic determination by ICP-MS.

For speciation analysis, approximately 25–28 ml of combined methanol–water supernatant was evaporated to dryness at 50 °C using an RVC 2-18 rotational vacuum concentrator (CHRIST, Quantum, Australia). The residue was resuspended in deionized water (Milli-Q, Millipore, Australia) and filtered through a 0.45 μm Iso-Disc N-4-4 Nylon filter (Supelco, USA).

Acetic acid extraction

Arsenic compounds could not be extracted from mangrove leaf tissues with methanol–water and so were extracted with acetic acid. Approximately 0.1–0.2 g of biological material was weighed into 50 ml polypropylene vials and 20 ml of 1 M acetic acid (HiPerSolv, BDH, Australia) added. Mixtures were agitated mechanically for 2 h, centrifuged at 3000 rpm for 15 min and the supernatants removed. For speciation analysis, the supernatant was evaporated to dryness at 50 °C using an RVC 2-18 rotational vacuum concentrator (CHRIST, Quantum, Australia). The residue was resuspended in deionized water (Milli-Q, Millipore, Australia) and filtered through a 0.45 μm Iso-Disc N-4-4 Nylon filter (Supelco, USA).

Arsenic speciation

Aliquots of extracts (100 μl) were injected onto a high-performance liquid chromatograph (HPLC) consisting of a Perkin Elmer Series 200 mobile phase delivery and auto sampler system (Perkin Elmer, Australia). The elutant from HPLC columns was directed by polyether-ether-ketone capillary tubing into the cross-flow nebulizer of a Perkin Elmer Elan-6000 ICP-MS (Perkin Elmer SCIEX, Australia). Ion intensities were monitored at *m/z* 75, 77 and 82. The chromatography package Turbochrom (Perkin Elmer, Australia) was used to quantify arsenic compounds by peak area.

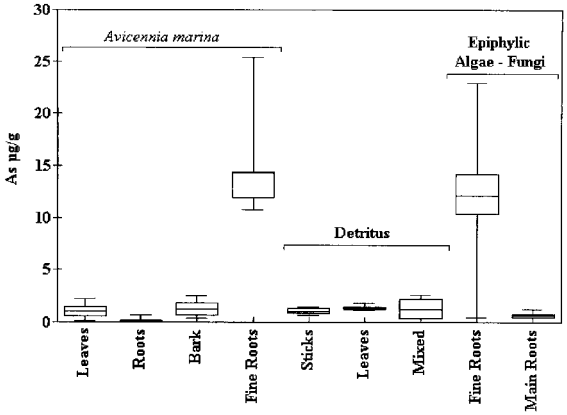

Figure 2. Arsenic concentrations in *A. marina* leaves, roots and detritus and associated epiphyte algae/fungi. The box represents the 25th and 75th percentiles, while the whiskers represent the 5th and 95th percentiles, and the line in the box is the mean.

A Hamilton PRP-X100 anion-exchange column (250 mm × 4.6 mm, 10 μm) (Phenomenex, USA) and an aqueous 20 mM $NH_4H_2PO_4$ (Suprapur, Merck) mobile phase at pH 5.6 (flow rate: 1.5 ml min^{-1}; temperature: 40 °C) was used for the identification of arsenate [arsenic(V)], DMAA, MAA, phosphate, sulfonate and sulfate arsenoriboses.

A Supelcosil LC-SCX cation-exchange column (250 mm × 4.6 mm, 5 μm) (Supelco, USA) and an aqueous 20 mM pyridine (Extra Pure, Merck) mobile phase adjusted to pH 2.6 and pH 2.2 with formic acid (flow rate: 1.5 ml min^{-1}; temperature: 40 °C) was used for the identification of AsB, TMAP, AsC, TMAO, TETRA and the glycerol arsenoribose. At pH 2.2 the AsB is incompletely separated from the glycerol ribose (AsB: k' 0.83; glycerol ribose: k' 0.78), but these compounds are well separated at pH 2.6 (AsB: k' 0.63; glycerol ribose: k' 0.83). However, at pH 2.6 TMAP shows poor separation from the broad peak for TMAO (TMAP: k' 1.53; TMAO: k' 1.70). At pH 2.2, TMAO (k' 1.28) elutes as a sharper resolved peak with good separation from TMAP (k' 1.5).

The accuracy of the speciation procedure was checked by the analysis of the certified reference material DORM-2 (NRC-CNRC, Canada). The concentrations (as arsenic) of AsB (16.8 ± 0.1 μg g^{-1}) and TETRA (0.24 ± 0.02 μg g^{-1}) were similar to the certified values (16.4 ± 1.1 μg g^{-1} and 0.248 ± 0.054 μg g^{-1} respectively). The concentrations (as arsenic) of the other constituents, AsC (0.023 ± 0.002 μg g^{-1}), TMAP (0.17 ± 0.01 μg g^{-1}), DMA (0.28 ± 0.01 μg g^{-1}) and TMAO (<0.003, μg g^{-1}) were similar to those reported for this material.[15]

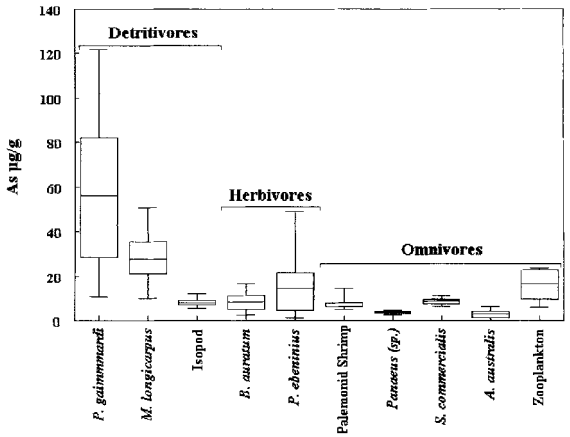

Figure 3. Arsenic concentrations in mangrove detritivores, herbivores and omnivores. The box represents the 25th and 75th percentiles, while the whiskers represent the 5th and 95th percentiles, and the line in the box is the mean.

Statistical analysis

One-way analysis of variance (ANOVA) was used to identify significant differences in arsenic concentrations between and within trophic groups, i.e. primary producers, herbivores, omnivores and detritivores. *Post hoc* analysis using Tukey–Kramer multiple comparisons tests was used to identify where significant differences occurred. Log arithmic transformation was used prior to ANOVA to satisfy the assumptions of normality and homogeneity of variance. All statistical procedures were performed using the SAS® statistical analysis package.

RESULTS

Total arsenic

Total arsenic concentrations in mangrove organisms are presented in Figs 2 and 3.

Table 2. Arsenic concentrations in acetone and methanol–water extracts of mangrove animal tissues

Species	Common name	Arsenic (µg g^{-1})	Extractable arsenic (%)	
			Acetone	Methanol–Water
B. auratum	Gastropod	17.7	0.09	76.4
P. ebeninius	Gastropod	14.2	0.03	80
M. longicarpus	Soldier crab			
muscle		19.9	0.05	85.8
visceral mass		21.5	0.09	77.0
P. gaimardii	Mangrove crab			
muscle		104	0.12	87.7
visceral mass		39.5	0.07	83.0
S. commercialis	Oyster	9.9	0.13	74.9
Panaeus spp.	Prawn	22.9	0.09	83.0

Table 3. Cationic arsenic species identified in methanol–water extracts of mangrove animal tissues

Species	Arsenic compounds (% of total arsenic)[a]							
	AsB	Glycerol ribose	TMAP	AsC	TETRA	TMAO	Unknown 1	Unknown 2
B. auratum	59	3	1	1	7	0	1	0
P. ebeninius	48	1	1	0	3	0	6	0
M. longicarpus								
muscle	56	4	8	0	1	0	0	0
visceral mass	28	23	4	0	0	0	1	0
P. gaimardii								
muscle	81	1	2	0	0	0	0	0
visceral mass	58	13	3	0	0	0	2	1
S. commercialis	57	0	0	1	0	1	0	0
Panaeus spp.	59	2	0	0	1	0	0	0

[a] See Table 2 for total arsenic concentrations.

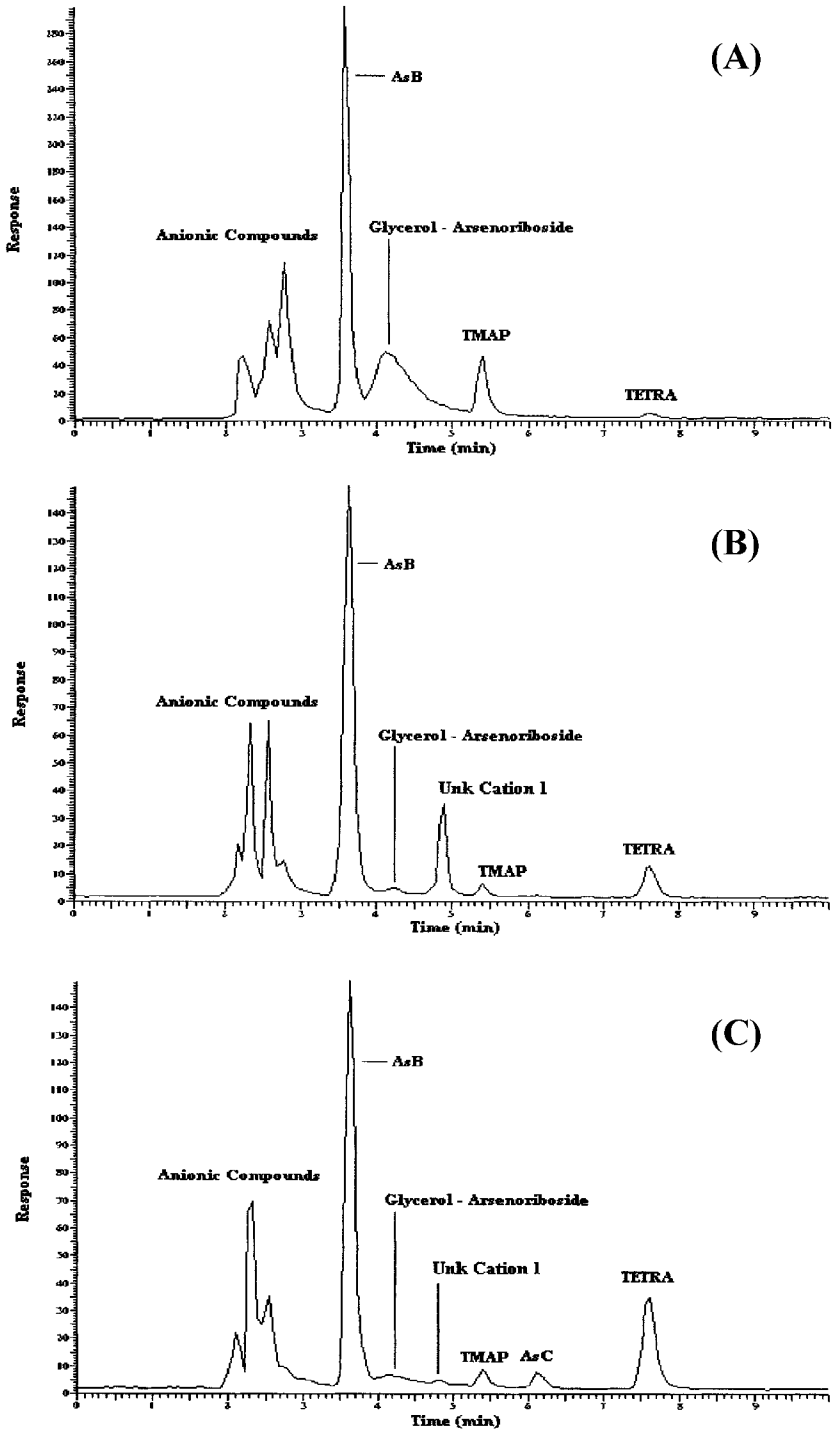

Figure 4. Cationic arsenic species identified in three mangrove animal tissues using a Supelco LC-SCX cation exchange column with a 20 mM pyridine–formic acid mobile phase (pH 2.6; flow rate: 1.5 ml min^{-1}; temperature: 40°C). (A) *M. longicarpus* (visceral mass), (B) *P. ebeninius* (whole tissues), (C) *B. auratum* (whole tissues).

Sediments

Sediments taken from the mangrove system had a mean arsenic concentration of 1.4 ± 0.1 μg g^{-1}.

Biological tissues

Mean arsenic concentrations ranged from 0.33 ± 0.16 μg g^{-1} in mangrove roots, to 56 ± 11 μg g^{-1} in crab tissues (Figs 2 and 3).

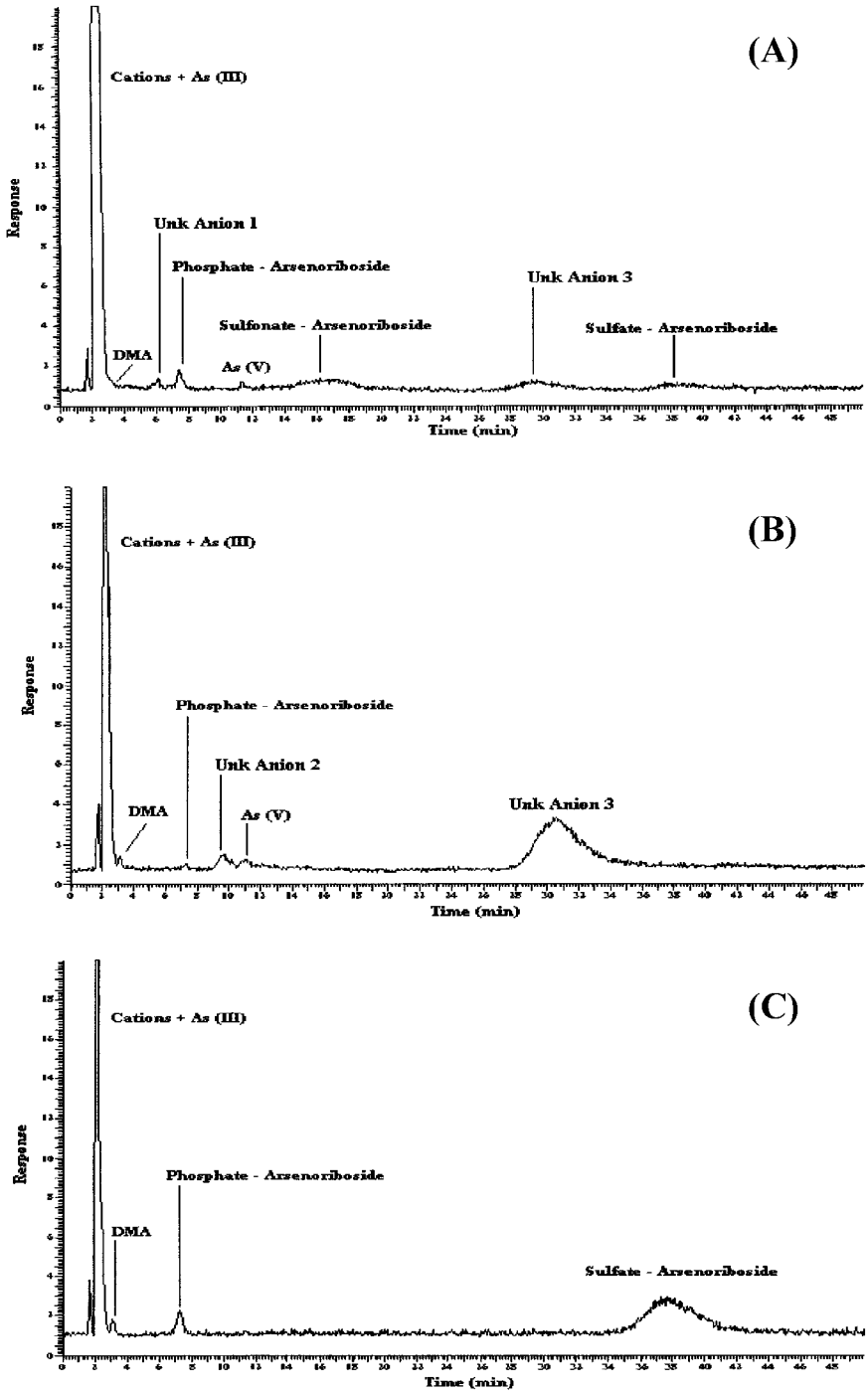

Figure 5. Anionic arsenic species identified in three mangrove animal tissues using a Hamilton PRP X-100 anion exchange column with a 20 mM ammonium phosphate mobile phase (pH 5.6; flow rate: 1.5 ml min^{-1}; temperature: 40 °C). (A) *P. gaimardii* (visceral mass), (B) *M. longicarpus* (whole tissues), (C) *Panaeus* spp. (whole tissues).

Significant differences in arsenic concentrations ($F = 28.16$; d.f. 8, 62; $P < 0.01$) were found between the primary producers (Fig. 2), with the fine mangrove roots (14 ± 2 μg g^{-1}) and epiphytic algae/fungi (12 ± 3 μg g^{-1}) attached to fine roots having significantly higher arsenic concentrations than other organisms (Fig. 2). Mangrove leaves, mangrove bark and epiphyte algae/fungi attached to main mangrove roots had similar arsenic concentrations (1.2 ± 0.2 μg g^{-1},

1.2 ± 0.3 μg g^{-1} and 1.5 ± 0.8 μg g^{-1} respectively). The main mangrove roots had the lowest arsenic concentration (0.33 ± 0.16 μg g^{-1}). Detritus consisting of mangrove leaves and sticks had similar arsenic concentrations (1.3 ± 0.5 μg g^{-1}) to the fresh leaves, roots and bark.

There was a significant difference in accumulated arsenic concentrations ($F = 89.47$; d.f. 2, 289; $P < 0.01$) between various feeding groups (Fig. 3). Tukey's *post hoc* analysis indicated that arsenic concentrations were significantly higher in detritivores than the other feeding groups, with other feeding groups not being significantly different. Detritivores showed significant differences in arsenic concentrations within their feeding group ($F = 53.33$; d.f. 2, 69; $P < 0.01$), with both species of crab, *P. gaimardii* (56 ± 11 μg g^{-1}) and *M. longicarpus* (28 ± 3 μg g^{-1}), accumulating higher arsenic concentrations than the isopods (8.5 ± 0.7 μg g^{-1}). For the omnivore species, there was a significant difference in arsenic concentrations within the trophic group ($F = 26.20$; d.f. 4, 109; $P < 0.0001$), with zooplankton (16 ± 2 μg g^{-1}), Palemonid shrimps (7.7 ± 0.8 μg g^{-1}) and the oyster *S. commercialis* (8.6 ± 0.5 μg g^{-1}) having higher mean concentrations than *A. australis* (2.8 ± 0.8 μg g^{-1}) and the Panaeid prawns (3.6 ± 0.2 μg g^{-1}). The herbivore gastropods *B. auratum* (9 ± 2 μg g^{-1}) and *P. ebeninius* (14 ± 2 μg g^{-1}) had similar arsenic concentrations.

Examination of food links based on major food type showed that mean arsenic concentrations increased between food sources such as algae (1.5 ± 0.8 μg g^{-1}) and the herbivore gastropods (9 ± 2 and 14 ± 2 μg g^{-1}), between mangrove litter (1.2 ± 0.5 μg g^{-1}) and crabs (28 ± 3 and 56 ± 10 μg g^{-1}) and between mangrove litter and isopods (8.5 ± 0.7 μg g^{-1}). However, arsenic concentrations were lower in *A. australis* (2.8 ± 0.8 μg g^{-1}), prawns (3.6 ± 0.2 μg g^{-1}) and shrimps (7.7 ± 0.8 μg g^{-1}) than the zooplankton (16.6 ± 0.2 μg g^{-1}) these species may consume.

Arsenic speciation

The amounts of arsenic extracted by acetone and methanol–water are shown in Table 2. The tissue concentrations of arsenic species are presented in Tables 3 and 4. Typical HPLC–ICP-MS chromatograms for extracts of muscle and visceral tissues are shown in Figs 4 and 5 to illustrate the presence of unknown arsenic species. Most tissues contained large percentages of AsB (28–81%). Glycerol arsenoribose was found in all tissues examined (1–23%) except oysters. Relatively large concentrations of this arsenoriboside were found in the digestive tissues of the two crab species (13–23%). Small amounts of TMAP (1–8%), TETRA (1–7%), sulfate arsenoribose (2–13%) and trace amounts of AsC (<1%), TMAO (<1%), DMA (<2%), phosphate arsenoribose (<2%), arsenate (<1%), and sulfonate arsenoribose (<3%) were found in some tissues. MA was not found in any tissues. Two unknown cationic arsenic compounds (1–2%) and three anionic arsenic compounds (1–17%) were identified in some tissues. Extraction of mangrove leaves with 1 M acetic acid tentatively identified the presence of the glycerol arsenoribose. Other anionic arsenic species could not be identified using the Hamilton PRP X-100 column because of the high chloride concentrations.

DISCUSSION

Total arsenic

We are unaware of any studies that have reported arsenic concentrations in mangrove sediments and its associated ecosystem. The sediment arsenic concentrations measured in this study are at the lower end of published data on arsenic in marine sediments[1,16] and indicate no anthropogenic sources of arsenic in the mangrove area studied.

The mangrove trees main roots, leaves and bark have low mean arsenic concentrations (0.3–1.2 μg g^{-1}); this is consistent with other studies that have shown that trace metals in mangrove vegetation are normally low.[17–19] Arsenic concentrations measured in mangrove fine roots (14 ± 5 μg g^{-1}) that are in contact with sediment were relatively high. The high arsenic concentrations associated with fine roots, which have a high surface area, may result from oxygenation of sediments that surround the roots[18] and the subsequent release of arsenic from insoluble sediment arsenic sulfides. Alternatively, the high arsenic concentrations in fine roots may be attributed to attached epiphytic algae/fungi that contained high arsenic concentrations (12 ± 3 μg g^{-1}). Lacerda *et al.*[20] suggested that deposition of iron hydroxides ('iron plaque') occurs on mangrove root surfaces due to pumping of oxygen to avoid root anoxia. It is possible that the high concentrations of arsenic measured in fine roots and associated epiphytic algae/fungi are due to this plaque and that arsenic is not incorporated into tissues. Although thorough washing of these tissues with deionized water was used to remove adhering sediment particles, our procedures would not have removed this iron plaque. The epiphytic material scrapped from the main mangrove roots (not in contact with sediments) was low in arsenic concentration (1.5 ± 0.8 μg g^{-1}), supporting the hypothesis that fine roots and attached epiphytic algae/fungi are being exposed to sediment arsenic or coated with iron plaque containing arsenic. The herbivore gastropods only graze on the algae/fungi associated with the main roots and, therefore, will not be exposed to high arsenic concentrations associated with fine roots.

Mangrove litter, which consisted of mangrove leaves, stems, bark, algae and other unidentifiable material, contained a mean arsenic concentration of 1.3 ± 0.2 μg g^{-1}, which was only slightly higher than the mean arsenic concentration of the parent material (1.2 ± 0.2 μg g^{-1}). This indicates that the epiphytic algae/fungi associated with fine roots are not large contributors to detritus material.

Differences in feeding habits and physiology or proximity to sediments are usually evoked to explain differences in

Table 4. Anionic arsenic species identified in methanol–water extracts of mangrove animal tissues

	Arsenic compounds (% of total arsenic)[a]								
Species	DMA	MA	Unknown 1	Phosphate ribose	Unknown 2	As(V)	Sulfonate ribose	Unknown 3	Sulfate ribose
B. auratum	0	0	1	0	0	0	0	2	0
P. ebeninius	0	0	1	1	0	1	0	4	13
M. longicarpus									
muscle	0	0	0	0	1	1	0	17	0
visceral mass	1	0	0	1	0	1	3	12	4
P. gaimardii									
muscle	0	0	0	0	0	0	0	0	0
visceral mass	0	0	1	1	0	0	3	4	2
S. commercialis	2	0	3	2	0	0	0	3	0
Panaeus spp.	0	0	0	1	0	0	0	0	13

[a] See Table 2 for total arsenic concentrations.

trace metal concentrations measured in aquatic organisms.[21–24]

The ranges of arsenic concentrations in animal tissues illustrated in this study are similar to those reported for other marine organisms.[1,2] In the system studied, detritivores were found to have higher arsenic concentrations than other trophic feeding groups (Fig. 3); however, this trend was only in the two crab species (*P. gaimardii* and *M. longicarpus*) and not reflected in the isopods. The higher concentrations of arsenic found in crab tissues relative to other marine animals is consistent with other studies.[1,25,26] Further research is required to establish if relatively high concentrations of arsenic are a general characteristic of detritivores, or a specific characteristic of crabs.

In the mangrove ecosystem, food sources (algae/fungi, vegetative litter, isopods, zooplankton, etc.) have significantly different arsenic concentrations (Figs 2 and 3). Many omnivorous species display a mixed feeding mode, and diet may change seasonally, tidally or with the age of an organism.[27] For several food links the arsenic concentrations in consumers was higher than their primary food source, indicating that, in some cases, bioaccumulation of arsenic is occurring from food sources. However, some food sources, such as zooplankton, have higher arsenic concentrations than potential consumers, such as fish, prawns and shrimps; this indicates poor assimilation of arsenic or regulation by these consumers, or that zooplankton are only a small proportion of their diet owing to the transient nature of some omnivorous species. Arsenic concentrations are often lower in predators than their prey.[16] Although the herbivorous gastropods, and the detritivorous isopod and crabs have food sources with similar arsenic concentrations ($\sim$1 μg g^{-1}), these animals accumulate vastly different arsenic concentrations (8–55 μg g^{-1}). Thus, the availability of arsenic in food sources is probably overridden by behavioural and physiological differences that control the assimilation and retention of arsenic by organisms.

Speciation

Similar to other published results,[28] only small or negligible amounts of inorganic arsenic are present in marine animal tissues (0–1%, Table 4) relative to total arsenic concentrations. The amounts of simple methylated arsenic species (DMA, MA; Table 4) were also small or negligible (0–2%). These arsenic species are usually found in relatively high concentrations in sediment-dwelling animals ingesting inorganic arsenic[29–30] and are thought to be produced in the gut by bacterial methylation. The mangrove organisms in this study do not ingest sediment in search of their food, which may account for the low concentrations of these arsenic species. TMAO can be produced by the breakdown of AsB by bacteria.[31] The nearly complete absence of TMAO indicates that mangrove animals are not degrading ingested AsB, or, if they are, that the TMAO is not being retained or is metabolized to other arsenic species.

Tetramethylarsonium ion was found in the two gastropod species (3–7%; Table 3). Gastropods and other molluscs have been shown to accumulate high concentrations of TETRA.[32,33] This may be due to the further methylation of TMAO, but the pathway for its formation is not known.

Arsenic riboses were found in all mangrove animals, but their distribution between animals was very different (Tables 3 and 4). The presence of small amounts of arsenic riboses in *B. auratum* is consistent with that found previously for rocky intertidal herbivorous gastropods.[34] The visceral mass of the two crab species contained appreciable quantities of the glycerol arsenoribose, but little of this arsenoriboside was accumulated in their muscle tissues. These crab species mainly consume mangrove leaves that contain this arsenoribose, which accounts for this arsenic species being

found in their digestive tissues. The other animals (except oysters) also graze on mangrove leaves and detritus, so they may be directly ingesting glycerol arsenoribose or ingesting bacteria or meiofauna that are also grazing on this material. The source of the sulfate arsenoribose found in *P. ebeninius* and *Panaeus spp* is unknown. This arsenoribose may have also been present in mangrove leaves, but we were unable to confirm its presence because of salt interfering with the anion chromatography of mangrove leaf extracts. At present we are developing a procedure to remove chloride ions to allow anion chromatography of *A. marina* leaf extracts.

In all animal tissues the major arsenic species identified was AsB (Table 3). Arsenobetaine is not likely to be synthesized by animals,[29,35,36] nor is it derived from water[33] or sediment sources;[31,38–40] therefore, it must be obtained from their food.[41] Also, no conclusive evidence has been produced that marine animals can convert arsenoribosides from food sources to AsB. Thus, the pathway of formation of AsB in mangrove animals still needs to be established. Mangrove animals will also ingest bacteria as part of their food, and, as bacteria are known to accumulate AsB,[42,43] this may be a source of this compound.

At this time we are unable to characterize fully the arsenic species present in mangrove litter (leaves, roots, branches, etc.), although our results indicate the presence of glycerol arsenoriboside. The findings of this study indicate that mangrove animals are primarily cycling complex organic arsenic species, such as AsB and arsenoriboses, obtained from their food. Inorganic and simple methylated arsenic species are almost absent and are not being cycled, probably because mangrove animals examined in this study are not exposed to inorganic arsenic through their food. Further understanding of the cycling of arsenic in mangrove systems awaits the elucidation of unknown arsenic species in mangrove biota.

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