Arsenic speciation in polychaetes (Annelida) and sediments from the intertidal mudflat of Sundarban mangrove wetland, India

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Abstract:

This paper documents the concentration of total arsenic and individual arsenic species in four soft-bottom benthic polychaetes (*Perenereis cultifera, Ganganereis sootai, Lumbrinereis notocirrata* and *Dendronereis arborifera*) along with host sediments from Sundarban mangrove wetland, India. An additional six sites were considered exclusively for surface sediments for this purpose. Polychaetes were collected along with the host sediments and measured for their total arsenic using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Arsenic concentrations in polychaete body tissues varied greatly, suggesting species-specific characteristics and inherent peculiarities in arsenic metabolism. Arsenic was generally present in polychaetes as arsenate (As^{V} ranges from 0.16 – 0.50 mg kg^{-1}) or arsenite (As^{III} ranges from 0.10 – 0.41 mg kg^{-1}) (30 to 53% as inorganic As) and dimethylarsinic acid (DMA^{V}; <1 to 25%). Arsenobetaine (AB; <16%), and PO_4-arsenoriboside (8 to 48%) were also detected as minor constituents, whilst monomethylarsonic acid (MA^{V}) was not detected in any of the polychaetes. The highest total As (14.7 mg kg^{-1} dry wt) was observed in the polychaete *D. arborifera* collected from the vicinity of a sewage outfall in which the majority of As was present as an uncharacterized compound (10.3 mg kg^{-1} dry wt) eluted prior to AB. Host sediments ranged from 2.5 to 10.4 mg kg^{-1} total As. This work supports the importance of speciation analysis of As, because of the ubiquitous occurrence of this metalloid in the environment, and its variable toxicity depending on chemical form. It is also the first work to report the composition of As species in polychaetes from the Indian Sundarban wetlands.

Keywords: Arsenic, Sundarban, polychaetes, arsenic speciation, sediment

Introduction

Arsenic (As) is a metalloid with a wide distribution in nature, being commonly found in aquatic environments as a result of agricultural and industrial practices (Wang et al., 2004). Marine samples have so far provided the greatest number of naturally occurring arsenic compounds with up to 50 compounds identified (Nischwitz and Pergantis, 2006), although recent work revealed that terrestrial organisms also contain many of these compounds (Geiszinger et al. 1998; Kuehnelt et al. 1997; Watts et al. 2008; Button et al. 2009). There are clear patterns of arsenic compounds among marine organisms (Francesconi & Edmonds, 1997). The general interest for arsenic in the marine environment is related to the potential toxicity of this element. Inorganic arsenate (As^{V}) and arsenite (As^{III}) are the most toxic forms, As^{V} replacing phosphate during the oxidative phosphorylation processes, with mitochondrial impairment and inhibition of glycolytic energy metabolism. Methylated compounds such as monomethylarsonic acid (MA^{V}), dimethylarsinic acid (DMA^{V}), trimethylarsine oxide (TMAO) and tetramethylarsonium (TETRA) are considered moderately toxic (Phillips, 1990), while more complex organoarsenic compounds like arsenobetaine (AB), arsenocholine (AC) and arsenoribosides are not harmful (Francesconi et al. 1998; Gebel, 2001).

Due to the wide environmental distribution of arsenic it is important to discriminate various chemical forms of this element which in many circumstances can be accumulated at moderately high levels. In aquatic organisms non-toxic organoarsenic compounds are generally the predominant form, which probably represent the final products of a detoxification process (Francesconi *et al*., 1998). Interesting and unusual results have been recently obtained for some polychaete species; *Arenicola marina* contains arsenic mainly in the inorganic forms (79%) (Geiszinger et al. 2002a), while a significant biomethylation capability (to TETRA) has been shown for *Nereis diversicolor* and *N. virens* (Geiszinger *et al*., 2002b). Benthic polychaete species exhibit varying efficiency in accumulating the inorganic and organic As compounds in their body tissues and this is attributed to the combined effects of their diverse feeding guilds and habitat types (Waring and Maher, 2005; Waring et al., 2005, 2006; Geiszinger et al., 2002).

Most marine animals contain arsenobetaine as their major arsenical, but they can also contain a number of other arsenic compounds, usually as minor or trace constituents. A large number of arsenic compounds, including As^{III}, As^{V}, MA^{V}, DMA^{V}, TMAO, TETRA, AB, AC, and a range of arsenic-containing ribosides, have been found in marine ecosystems (Francesconi and Edmonds, 1994; Waring *et al*. 2005). Inorganic arsenic (As^{III} and As^{V}) is often present in marine animals, although generally as a trace constituent depending upon the feeding guild (Waring *et al*. 2005).

Previous studies on the pollution status of Sundarban wetland revealed elevated concentrations of total trace elements (e.g. As, Cr, Ni) in surface and core sediments exceeding the Effects-Range Low (ER-L) values implying occasional or frequent biological effects (Sarkar et al., 2004; Chatterjee et al., 2007, 2009 a,b). Arsenic concentrations reported by Chatterjee et al. (2009b) were 3 to 18 mg kg^{-1}, exceeding the ERL of 8 mg kg^{-1}. Therefore, the speciation of arsenic in sediments is of great significance because it may provide information about the cycling of arsenic in the wetland ecosystem. However,

information regarding As speciation in Sundarban wetland is completely unexplored, largely due to the lack of locally available techniques. Marine organisms, including algae, bivalves, gastropods, crustaceans and fish normally contain the majority of As as non-toxic organic compounds, such as AB or arsenoribosides (Jankong et al. 2007; Nam et al. 2010). Elevated concentrations of arsenic have been described for polychaete species with varying As speciation profiles depending on their feeding guild, habitat or physiology (Waring et al. 2005). Hence the collaborative work has been undertaken to redress the relevant information of As and its species characteristics in benthic polychaetes (Annelida) along with the host sediments from Sundarban with the following objectives: (i) to quantitatively estimate the distribution of As with its speciation (ii) to evaluate factor(s) that affect spatial distribution of As and its species and (iii) to assess the ecological quality of this wetland. To the best of our knowledge, this is the first report on the characterization of As in sediments and biota in the Sundarban region, which will enhance the understanding of As toxicity and bioavailability in this wetland ecosystem.

Experimental

Sampling sites

The Indian Sundarban, formed at the estuarine phase of the Hugli (Ganges) river (area of ~ 9,600 km^2), is a tide-dominated mangrove wetland belonging to the low-lying humid and tropical coastal zone. This is one of the most dynamic, complex and vulnerable bioclimatic zones in a typical, tropical geographical location in the northeastern part of the Bay of Bengal. This has been acclaimed as UNESCO World Heritage Site for its capacity of sustaining an excellent biodiversity. The area is interspersed with a large number of islands and tidal channel systems through which semidiurnal tides of meso-macro-tidal amplitude interplay with moderate to strong wave effects. The wave and tide climate of this low-lying tropical coast primarily controls the sediment dispersal patterns.

This coastal environment suffers from environmental degradation due to rapid human settlement, tourism and port activities, and operation of excessive number of mechanized boats, deforestation and increasing agricultural and aquaculture practices. The ongoing degradation is also related to huge siltation, flooding, storm runoff, atmospheric deposition and other stresses resulting in changes in water quality, depletion of fishery resources, choking of river mouth and inlets, and overall loss of biodiversity (Sarkar and Bhattacharya 2003, 2007a). A significant ecological change is pronounced in this area due to reclamation of land, deforestation, huge discharges of untreated or semi-treated domestic and municipal wastes and effluents from multifarious industries such as tanneries, chemicals, paper and pulp, pharmaceuticals, (as shown in Fig. 1) as well as contaminated mud disposal from harbour dredging (Sarkar et al. 2007b). All of these factors impart a variable degree of anthropogenic stresses leading to elevated concentrations of both heavy metals and persistent organic pollutants (POPs).

Eleven sampling sites of distinctive geographic, geomorphic and sedimentological settings with variations of energy domains were selected covering both the eastern and western flank of Sundarban, namely, Lower Long Sand (S_1), Gangasagar (S_2), Mayagoyalinir Ghat (S_3),

Chemagari (S_4), Gushighata (S_5) and Lot 8 (S_6), Kakdwip (S_7), Gosaba (S_8), Canning (S_9), Dhamakhali (S_{10}) and Jharkhali (S_{11}). Sites S_1 to S_5 were sampled for both polychaete and sediment collection and sites S_6 to S_{11} were sampled only for sediments as they are not being inhabited by the polychaetes. The sites can be distinguished in the context of variable environmental and energy regimes of the wetland, which cover a wide range of substrate behavior, wave-tide climate, intensity of bioturbation (animal-sediment interaction), geomorphic-hydrodynamic regimes and distances from the sea. These contrasting habitats were selected in order to explore the behavioral sensitivity of polychaete communities to different environmental conditions. All sites were estuarine in nature except for S_1 and S_2. Site S_1 is an offshore island on the Bay of Bengal and S_2 represents beach sediment. Gangasagar (S_2) is the high energy mixing zone located at the confluence of the Hugli (Ganges) river estuary and the Bay of Bengal.

The variations of physical processes such as suspension-resuspension, lateral and vertical transport by biological activities (bioturbation), flocculation and deflocculation of mud clasts result in a spatial variation of the substratum behavior both on local and regional scales. The sites have diverse human interferences with a variable degree of exposure to heavy metal and trace organic contamination. Moreover, the sites can be differentiated in terms of river discharge, erosion and atmospheric deposition.

Polychaete and sediment collection

Surface sediment (up to 5 cm depth) was collected using a PVC spatula. Sediment samples were stored in labelled polyethylene bags stored in iceboxes and transported to the laboratory where they were frozen to -20^0C. Within two days, a portion of each sample was placed in a ventilated oven at low temperature (max. 45^0C). Dried samples were then disaggregated using an agate mortar and pestle, sieved through a 63 µm metallic sieve and stored in hermetic plastic bags until analysis. All visible marine organisms and coarse shell fragments, seagrass leaves and roots were removed manually immediately after collection. These were divided into two portions; (1) unsieved for sediment quality parameters (organic carbon, pH, % of silt, clay and sand), and (2) sieved for elemental analysis.

For polychaete collection, 20 congeneric species of uniform size were collected from each station, transported to the laboratory in acid-washed plastic containers, depurated for 2 to 3 days (defecation of sediments and any undigested materials), and dried to a constant weight at 45^0C. Polychaete species include: *P. cultifera* (herbivore), *G. sootai* (sediment ingester), *L. notocirrata* and *D. arborifera* (detritivores).

Reagents and Standards

All reagents used were analytical grade or better quality. All aqueous solutions were prepared using deionised water (18.2 MΩ Millipore, UK). Arsenic speciation standards and arsenoribosides are described in Watts *et al*. (2008) along with reagents for chromatographic separation for HPLC-ICP-MS. Phosphoric acid (Fisher Scientific, UK) and ascorbic acid (Sigma Aldrich, UK) were used for the extraction of As species from sediments.

Concentrated nitric, hydrochloric and hydrofluoric acids (Fisher Scientific, UK) were used for the dissolution of sediments and polychaetes for total arsenic analysis.

Total digestion and extraction of sediments

Dried sediments (0.25 g) were prepared for total elemental measurement by ICP-MS based on a mixed acid digestion approach ($HF/HNO_3/HClO_4$) as described in Watts et al. (2008), to produce a final solution of 5 % nitric acid for analysis by ICP-MS. Certified reference materials were included with the sediment digestion as a measure of quality control. These were NRCC BCSS-1 and NRCC MESS-2 Marine sediments, which gave good recoveries of 110 ± 12 % (n = 4) and 92 ± 9 % (n = 4), respectively (Table 2). Duplicate digestions were completed for each of the 11 sediment samples, giving mean percentage difference of 3 ± 8 % (n = 11; ± 1 SD). Duplicate percentage difference data was largely skewed by sediments from Site S_1 and S_5, with 18 and 20 %, respectively. For the other nine sediments, the mean percentage difference was 1 ± 2 %.

The extraction of arsenic species from the sediments used a modified method based on the use of phosphoric acid and ascorbic acid (Gallardo, et al. 2001; Garcia-Mayes, et al. 2002). Dried sediments (0.2 g) were weighed into 50 ml polyethylene centrifuge tubes and 10 ml of 1 M phosphoric acid and 0.5 M ascorbic acid added and then shaken for 4 hours at 200 rpm on an orbital shaker. The extracts were centrifuged at 2000 rpm for 15 minutes and the supernatant decanted into 15 ml polypropylene bottles and analyzed immediately for arsenic speciation, following an adequate dilution of at least x2, to minimize chromatographic distortion due to phosphoric acid. Extraction efficiency was monitored using NRCC BCSS-1 and NRCC MESS-2 Marine sediments, which provided mean recoveries for the extract of 55 ± 5 % (n = 17) and 74 ± 8 % (n = 8), respectively (Table 2).

Total digestion and extraction of polychaetes

For total As analysis, dried samples were pulverised and homogenised in a teflon mortar and digested using microwave assisted (CEM MARS5, CEM Corporation, UK) dissolution on 0.1 g of polychaete homogenate (dry weight). 10 ml of concentrated nitric acid and 100 µl of hydrofluoric acid was added and allowed to stand for 30 minutes. Following an initial heating programme (ramp to 100 ^{0}C over 5 min then hold for 5 min, ramp to 200 ^{0}C over 5 min and hold for 20 min) the vessels were allowed to cool (<50 ^{0}C) and then 1 ml of 30 % v/v H_2O_2 was added. The vessels were sealed and microwaved for a second cycle (same program). After cooling, the sample solutions were transferred to PTFA Savellex containers and evaporated to dryness on a hotplate (100 ^{0}C) to reduce the presence of organic compounds that could form possible polyatomic interferences by ICP-MS measurement. Samples were reconstituted by addition of 2 ml 50 % v/v nitric acid, heated at 50 ^{0}C for 30 min and then made up to 10 ml with deionised water. This final stage reduced the dilution of the acid content required for ICP-MS measurement (<2.5 % v/v). The method described is a routine procedure for the dissolution of biological samples. The method accuracy was measured using CRM 627 tuna fish tissue (BCR, Brussels). Mean total arsenic recoveries of 88.9 ± 0.01 % (n = 3; Table 3) were obtained, compared to the certified value. Duplicate samples provided a percentage difference of less than 1 %.

The extraction of arsenic species from the polychaetes followed the method described in Button *et al*. (2009). Homogenized dried polychaete powder (0.25 g) was weighed directly into 50 ml polyethylene centrifuge tubes. Homogenised polychaete powder (0.25 g) was weighed directly into 30 ml round-bottom Nalgene extraction vessels. 10 ml of methanol: water (1 : 1 v/v) was then added and the tubes shaken on an orbital shaker at 175 rpm for 4 h. The extracts were centrifuged at 3000 rpm for 10 min and the supernatant transferred to 10 ml polypropylene bottles. The methanol was evaporated off using a rotary evaporator before freeze drying. The freeze-dried residue was reconstituted in 10 ml of deionised water and analysed immediately. Extraction efficiency was monitored using the CRM 627 tuna fish tissue (BCR, Brussels). A mean recovery of 108 ± 10% (n = 3, Table 3) of the total arsenic measured in the extract was obtained, comparable with Button et al. (2009) 100 ± 6%. Duplicate samples provided a percentage difference of less than 1 %. Duplicate digestion and extraction was only possible on two of the polychaetes owing to the lack of sample material.

Instrumentation

Total arsenic analysis

Sample digests and sample extracts were analysed for arsenic concentrations using an Agilent 7500 ICP-MS. The standard operating conditions were as follows: RF power 1550 W; gas flow rates, coolant 15 l min^{-1}, auxiliary 0.8 l min^{-1}, nebulizer 0.85 l min-1, make-up gas 0.25 l min^{-1} and collision cell gas He 5.5 ml min^{-1}. An internal standard solution was added via a t-piece to the sample stream containing Ge, Rh, In, Te and Ir was used, giving approximate signal sensitivity of greater 200k cps.

Arsenic speciation

A quaternary pump (GP50-2 HPLC Pump and an AS-50 autosampler (Dionex, USA)) was directly coupled to an ICP-MS (Agilent 7500) for the measurement of arsenic species as described in Watts et al. (2008) and O'Reilly et al. (2010). An analytical column comprising of a PRP-X100 anion exchange column (250 x 4 mm, 10 µm) and a guard column of the same material (Hamilton, USA) was connected directly to the ICP nebulizer using PEEK tubing. The two instruments were automated to provide reproducible sample injections of 100 µl. were achieved through the coupling of the two instruments. Chromatographic conditions utilised a gradient program using 4 and 60 mM NH_4NO_3 as described in Watts *et al*. (2008) and O'Reilly *et al* (2010).The HPLC-ICP-MS was operated in single ion monitoring mode at signal m/z 75, with a dwell time of 100 ms. The quantitative analysis of peak areas from the resultant chromatograms was performed using Agilent ICP-MS Chromatographic Data Analysis Software version B.03.06 (Agilent Technologies, UK). A series of blank solutions (de-ionised water) and calibration standards (1 to 50 µg l^{-1} As) for each of the five arsenic species were utilised within each analytical run (Fig. 1). The ICP-MS limit of detection for total As was 0.01 µg l^{-1}, whilst the LOD for each of the five arsenic species in solution by this method, expressed as the mean blank signal ± 3SD was As^{III}: 0.12 µg/l, As^{V}: 0.10 µg/l, MA^{V}: 0.12 µg/l, DMA^{V}: 0.15 µg/l and AB: 0.20 µg/l. Individual LODs for

concentrations of As in the solid are given separately in Tables 2 and 3 for sediments and polychaetes, respectively, calculated through 3SD of the extract blanks for each analytical batch and accounting for dilution factors. Isolated arsenosugar standards were utilized for the identification of arsenosugars by retention time matching. The calibration curve of MA was used for the quantification of the phosphate, sulfonate and sulfate arsenosugars. MA was used as an appropriate calibrant for these three arsenosugars, since it eluted within the same eluent concentration of 4 mM NH_3NO_3. Madsen et al. (2000) also used MA as a calibrant for the quantification of arsenosugars. Calibration for arsenic speciation on the sediments was performed by standard addition of the arsenic species to the NRCC BCSS-1 Marine sediment. Whilst inorganic arsenic could be determined as As^{III} and As^{V} during the extraction and analytical methods, sediment speciation data will be referred to as inorganic As (iAs), since sediment samples were dried using available drying ovens rather than by freeze drying, hence the lack of control on interconversion from As^{III} to As^{V} at the first stage of sample processing (Ellwood and Maher, 2003). Sediment quality characteristics pH, organic carbon and textural properties) were worked out by standard methods and the detailed methodology was described in previous work (Chatterjee et al., 2007).

3. Results and Discussion

3.1 Sediment geochemistry

Geochemical characteristics of sediments vary among them as depicted in Table 1. Values of pH ranged towards basic (8.0 – 8.6). Organic carbon content (C_{org}) values were below 1% in all the stations. The prevalent low C_{org} values are the result of sedimentation and mixing processes at the sediment-water interface where both the rate of delivery as well as the degradation by microbial-mediated processes can be high (Canuel and Martens, 1993). Very low content of organic carbon in intertidal sediments of Sundarban was also recorded by previous workers and this is related to the poor absorption capacity of organic compounds to negatively-charged quartz grains, which predominate in this estuarine environment (Sarkar et al., 2004; Chatterjee et al., 2007, Dominguez et al., 2010). Regarding textural composition, the five stations also exhibited wide variations, from silty clay to sandy and these differences may be attributed to vigorous estuarine mixing, suspension-resuspension and flocculation-deflocculation processes. The prevalent differences in the textural properties in terms of grain size, permeability of substratum and hydrodynamic conditions, all of which are considered a 'superparameters' for the distribution of polychaetes at five stations. These variations may also influence the As accumulation in the sediments (Table 2).

3.2. Arsenic in sediment from Sundarban

Arsenic concentrations in sediments (<1 mm) from Sundarban were 2.6 to 10.4 mg kg^{-1} dry wt. **The prevalent non-uniformity of As levels may be related to the particular hydrological characteristics of Sundarban wetland which are severely influenced by southeast monsoon and the meso-macrotidal regime (Bhattacharya and Sarkar, 2003).** Arsenic may enter the estuary through two potential sources, mainly by (i) anthropogenic sources (such as effluents from multifarious industries located in upper stretch of Hugli estuary as referred earlier as well as from erosion of agricultural land in upstream region irrigated with arsenic-containing shallow waters (Islama et al., 2012) followed by (ii) geogenic

processes (transported by Ganges River from weathering of bed rocks in the Himalayan). These values were not particularly high compared to highly contaminated sites cited in the literature and only three of the eleven sites exceeded the ERL of 8 mg kg^{-1}. However, in comparison, Maher et al. (2011) referred to literature concentrations of 1 to 4 mg kg^{-1} As in marine sediments and 1 to 2 mg kg^{-1} As in lake sediments from Australia (NSW). Whalley et al. (1999) reported As concentrations of 0.15 to 135 mg kg^{-1} dry wt in North Sea sediments, Rattanachongkiat et al. (2004) 7 to 269 mg kg^{-1} dry wt in sediments from Thailand and Meador *et al*. (2004) 1.7 to 2.3 mg As kg^{-1} and 3.9 to 10.4 mg kg^{-1} dry wt in Alaskan and Californian sediments, respectively. The highest value of total As was recorded at Chemagari (S_4) (Table 1) which may be related to the location of this station at the mouth of the Baratala River estuary (Fig. 1), infested with dense mangrove plants such as *Avicennia alba, A. marina, Nypa fruticans, Rhizophora sp*. The prevalent As enrichment can be attributed to As solubilisation, especially through diagenetic processes in organic-rich mangrove sediments (Shumilin et al., 2009). Again, Gomez-Ariza *et al*. (2000) reported that 60 to 70 % of arsenic is bound to Fe-Mn oxide phase in intertidal sediments. Iron, a good co-precipitator of As, has been known to be removed from the water column at river mouths as iron-oxide-organic matter colloids with increasing salinity of the river water (Boyle *et al*., 1977).

Inorganic arsenic (iAs) (arsenate As^{III} and arsenite As^{V}) usually predominate in the abiotic matrices, while methylated and more complex organo-arsenic compounds, are generally found in tissues of living organisms, thus probably representing the final products of detoxification processes (Phillips, 1990; Fattorini et al., 2008). The present findings also endorse the phenomenon where iAs exclusively predominate in the sediments (86 to 100%), with only a trace amount of DMA and MA in some of the samples, suggesting a relatively low biotransformation due to microorganism activity.

The speciation results corroborated the high As levels in sediments previously recorded from intertidal mudflats of Sundarban (Chatterjee *et al*., 2009b), mainly derived from untreated or semi-treated municipal and industrial discharges from multifarious industries located upstream of the Hugli (Ganges) River (Fig. 1) as endorsed by previous workers (Luoma and Cloern, 1982; Moore and Ramamoorthy, 1984). Moreover, being situated at the confluence of the Bay of Bengal, the Sundarban wetlands receive significant deposition of sediments from the Ganges-Brahmputra-Meghna river system (Kuehl et al., 1989) containing several trace elements, including As at elevated concentrations (Swaine, 2000). Input may also derive from various anthropogenic sources including use of pesticides, herbicides and fertilizers through adjacent agriculture, as well as fossil fuel burning.

A strong association of Fe and Mn in Sundarban sediments was previously recorded by Chatterjee et al. (2009b) and the precipitated Fe in the form of oxyhydroxides has the affinity to scavenge other metals including As as they pass through the water to the sediments (Waldichuk, 1985). Only limited information is available on arsenic compounds in marine sediments, the major compounds in the pore water of marine sediments are usually As^{III} and As^{V}, although methylated arsenicals (Reimer & Thompson, 1988) and arsenoribosides (Ellwood and Maher, 2003) have also been reported as minor constituents in sediments.

3.3. Arsenic in Polychaetes from Sundarban wetland

Polychaetes contained measurable concentrations of As in their body tissues from 0.98 to 11.62 mg kg^{-1} and arsenic species varied between the proportion of inorganic and organic forms (Table 3). The observed variations might be due to habitat and feeding differences of the polychaetes (as referred in Table 1) as well as physiological adaptation to ecological niches that probably controls bioaccumulation (Waring and Maher, 2005). The elevated level of As in detritivore *D. arborifera* may be involved in the deterrence of predators (Gibbs et al., 1983). Recoveries of total As in the extract were 42 to 81% and column recoveries were 61 to 98% (sum of As species / extract As total x 100), comparable to reported literature (Maher et al. 2011). Total As recorded in the present study is at the lower range for reported marine or estuarine organisms. For example, Meador et al. (2004) found 10 to 100 mg kg^{-1} dw and 2 to 1500 mg kg^{-1} dw in Alaskan and Californian polychaetes. A slightly positive correlation was observed between polychaete total As tissue content and sediment C_{org}, pH, sand and silt content ($r = 0.21, 0.43, 0.15. 0.38$, respectively; $P<0.05$), although no correlation was apparent with clay content.

The complex interaction between host sediment and polychaete can vary due to the inherent characteristics of the individual species such as, uptake and regulation of trace metals, digestive tract biochemistry, and physiological state and feeding preferences. Moreover, pH, organic carbon content (C_{org}), food availability and competition of other trace metals are also equally involved as extrinsic factors (Depledge and Rainbow, 1990). The bioaccumulation process in this macrozoobenthos is controlled by combinations of intrinsic and extrinsic, biological, physical and chemical factors (Depledge and Rainbow, 1990; Lee and Lee, 2005; Wang et al., 1999a; Wang and Fisher, 1999). The values for the accumulation factor (AF) (Total concentration in polychaete body tissues / Total concentration in host sediments) were generally low and did not exhibit bioaccumulation of As in the polychaetes from the host sediment, with the exception of *D. arborifera* from S_5 (Table 4) with an AF of 3.0 (range 0.2 to 3.0). This may be due to the fact that the studied sediment-feeding polychaete species (*D. arborifera*) swallow particulate organic matter, mud, sand, or plant materials, obtaining nutrients from their contents and/or the fine layer of algae and/or microbes coating each particle (Hutchings, 1984; Maher et al. 2011). This detrital organic material will often contain As-rich macroalgae (Waring *et al*. 2005).

D. arborifera was collected from the site S_5 in the vicinity of a sewage outfall in which the polychaetes exhibited nearly 90 % (11.62 mg kg^{-1}) of total As as an uncharacterized As species, which eluted prior to AB and requires further characterization. Almost 1 mg kg^{-1} (8 %) was exhibited as a PO_4-arsenoriboside, with other As species present as minor constituents. The proportion of As in polychaetes comprised of the following: As^{III}; 1 to 26 %, As^{V}; 1 to 43 %, PO_4-arsenoriboside; 8 to 48 %, DMA; <1 to 25 %, AB <1 to 16 %, although the latter was mainly present at <0.1 mg kg^{-1}. The median ∑iAs (38 %) and ∑organo-As (62 %) proportions are representative of uptake from anaerobic muddy sediments, with *D. cultifera* (S_1 and S_3) exhibiting a greater proportion of As as iAs. *G. sootai* (sediment

ingester), *L. notorciratta* and *D. arborifera* (detritivores) exhibited organo-As greater than 60 % compared to less than 50 % for *D. cultifera* (herbivore). The unusually high proportion of iAs (both arsenite and arsenate) was also reported by Casado-Martinez et al. (2010) in the deposit-feeding polychaete *Arenicola marina* defined using radiotracer techniques. Recently, Casado-Martinez et al. (2012) revealed that arsenic bioaccumulation in *A. marina* was stored in the cytosol as heat stable protein (~ 50%) including metallothioneins, possibly as As (III)–thiol complexes. The presence of a high percentage (56 to 79%) of iAs in body tissues in *P. cultifera*, suggests they have developed a physiological resistance to these or metabolically process it (i.e. compartmentalize it) to reduce its toxicity (Waring *et al*., 2005). However, the proportion should be viewed in context with the low concentrations of total As found in this study.

The pattern of arsenic compounds in the studied polychaetes has several unusual features. First, AB is present as only a minor constituent (median 2%). Marine animals examined to date usually contain AB as their main compound (Francesconi & Edmonds, 1997), and there have been very few reported exceptions, for example, the sea squirt *Halocynthia roretzi* (Shiomi *et al*. 1983). Waring *et al*. (2005) reported *Notomastus estuarius* to contain only 9% AB, 30% As^{III}, 8% As^{V}, 30% arsenoribosides and 4% as an unknown anionic species. Most polychaetes accumulate AB, with the exception of deposit feeders inhabiting estuarine mud habitats, in which significant proportions of As may be present as iAs and arsenoribosides that may be metabolized differently in higher organisms compared to AB.

Organo-As compounds were reported to be accumulated by detritivore polychaetes as in S_5, whereby bioaccumulation for iAs is limited, particularly in relation to salinity, whereby As^{V} is less bioavailable to marine than to estuarine animals. Organo-As compounds in marine organisms are often thought to represent non-toxic end products in a scheme for detoxifying harmful inorganic arsenic (Edmonds and Francesconi, 1987). Little of the organoAs accumulated by humans from seafood is converted to toxic As^{III} and therefore marine As represents a low risk to humans (Maher et al., 2011). In contrast, As^{V} is the most toxic of the arsenic species found in environmental samples (Neff, 1997); its presence as a significant arsenic species in polychaetes suggests that these organisms may have a particular resistance to As^{V}, or metabolises it in a unique manner. The third point of interest is the presence of DMA and PO_4-arsenoriboside as the most dominant organoarsenicals. The higher concentration of DMA in *G. sootai* (at S_2) (0.39 mg kg^{-1}) and *L. notocirrata* (at S_4) (0.41 mg kg^{-1}) might be related to degradation products of AB in sediments (Hanaoka et al., 1992a; 1992b; 1996). The PO_4-arsenoriboside contained almost 1 mg kg^{-1} in each of these polychaetes, representing 21 and <1 % of total As, respectively. The fourth point is the presence of an unknown compound in all polychaete tissues in negligible amounts in each polychaete, with the exception of S_5 (*D. arborifera*), which might be derived from the host sediments. The chemical form of this arsenic compound is currently under investigation following fresh collection of sample material.

P. cultifera (at S_3) exhibited a higher proportion of AB (0.24 mg kg^{-1} dw, 16%) compared to the other polychaetes where the concentration of AB in sediments were ≤0.01 mg kg^{-1}. It is unlikely that these polychaete species accumulated AB directly from interstitial water and/or sediments since AB has been reported to be readily degraded to DMA, trimethylarsine oxide

(TMAO) and inorganic arsenic under both anaerobic and aerobic redox conditions in marine sediments (Hanaoka et al., 1992a,b). AB is probably accumulated by the polychaetes through organic food sources containing AB within sediments. Methylarsenate (MA) was absent from the majority of sediment samples except for Gosaba (S_8) (0.02 mg kg^{-1}), Dhamakhali (S_{10}) and Jharkhali (S_{11}) (0.01 mg kg^{-1}), whilst MA was completely absent in all polychaetes.

DMA is a relatively toxic organo-As compound for marine organisms (Fattorini et al. 2004; 2005). Notably, DMA in sediments was either absent or inconsistently present in low concentrations, whereas this was almost consistently present in all polychaete body tissues. The maximum DMA concentration in sediments was recorded at site S_{10} (0.06 mg kg^{-1}). However, the DMA is consistently present as the most important intermediates and degradation products in all polychaetes and the maximum concentrations were observed in *L. notocirrata* (0.41 mg kg^{-1} dw) and *G. sootai* (0.39 mg kg^{-1} dw) at sites S_4 and S_2 respectively confirming a rapid transformation of arsenic in these polychaetes as reported by Fattorini et al. (2005). The presence of DMA might suggest both the degradation of more complex arsenic compounds (i.e., arsenosugars) accumulated from phytoplankton algae or the methylation of inorganic arsenic usually present in abiotic matrices such as seawater and sediments (Notti et al., 2007). It was reported that the polychaete species *S. spallanzanii* from the coastal regions of Australia can produce DMA by methylating iAs or demethylating more complex arseno-compounds. Results of feeding experiments supported the species-specific function of arsenic making unpalatable to predators the more vulnerable tissues (Fattorini et al. 2004; Notti et al., 2007). A similar defensive role can be proposed for the marked accumulation of vanadium in branchial crowns of *P. littoralis* which were also vigorously rejected by fish after tasting these tissues. Hence the unusual prevalence of DMA could be anticipated with a possible antipredatory role for these benthic polychaetes, but further investigation is still required for confirmation.

CONCLUSION

The results demonstrate the capacity of the four benthic polychaete species to accumulate the As and its different species which might be ascribed to species-specific characteristics and inherent peculiarities in arsenic metabolism (Fattorini et al., 2005). The unusual prevalence of As^{III} and other less innocuous compounds (such as DMA and As-PO_4) was encountered in the sediment ingester *G. sootai* and detritivore *L. notocirrata* which is the most interesting aspect of the work. This indicates that these species have a certain ability of inorganic arsenic methylation, which may contribute to the detoxification process and thus needs further research. In contrast, arsenic has been accumulated as As^{V} as the most dominant form in the herbivore *P. cultifer*a with a small quantity of DMA*, suggesting* a limited biomethylation capacity in this species as asserted by Geiszinger et al., (2002a) in the deposit feeding polychaete *Arenicola marina*.

A detailed account of additional benthic polychaete species in terms of their habitat type, food preferences, physiology and exposure to arsenic species are essentially required for the assessment of arsenic uptake pathways and bioaccumulation through the food chain. This

would help to make a reference dataset of species-specific natural background concentrations of arsenic in polychaetes in Sundarban wetland environment.

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Tables and Figures

Table 1: Physiochemical properties of host sediment at five sampling sites along with the description of the studied polychaete species encountered in Sundarban wetland

Site	pH	C_{org}	Sand %	Silt %	Clay %	Polychaete species; Family	Feeding guild
S1	8.6	0.15	99.7	0.3	0	*Perinereis cultifera*, Nereididae	Herbivore, omnivorous, predator, scavenger
S2	8.2	0.2	99.3	0.7	0	*Ganganereis sootai,* Nereididae	Sediment ingester,
S3	8.3	0.53	2.6	34.2	63.2	*Perinereis cultifera,* Nereididae	Herbivore, omnivorous, predator, scavenger
S4	8.1	0.55	22.8	67.7	9.5	*Lumbrinereis notocirrata,* Lumbrineridae	Detritivore
S5	8	0.57	9.4	69.8	20.8	*Dendronereis arborifera,* Nereididae	Errant polychaete dominated in the mangrove habitats; Detritivore

Table 2. Concentrations (mg kg^{-1}) of As species [AB, As^{III}, DMA, MA, As^{V}, total As digest and extract, with % recoveries] in host sediments of polychaete species (S_1-S_5) and additional 6 stations (S_6-S_{11}).

Sampling Site	AB	i As [a]	DMA	MA	Sum As	Digest	Extract	Extract Recovery	Recovery HPLC %	Total Inorganic %
LLSand (S_1)	0.0	5.17	nd	nd	5.18	9.4	6.95	74	75	99.8
Gangasagar (S_2)	nd	3.92	nd	nd	3.92	7.25	5.05	70	78	100
M G Ghat (S_3)	nd	4.75	nd	nd	4.76	9.85	6.25	64	76	99.8
Chemagari (S_4)	nd	5.84	nd	nd	5.84	10.4	7.40	71	79	100
Gushighata (S_5)	nd	3.32	0.02	nd	3.34	4.85	4.35	90	77	99.4
Lot 8 (S_6)	nd	2.4	0.04	nd	2.79	2.55	1.95	77	144	86.0
Kakdwip (S_7)	nd	3.31	nd	nd	3.31	6	4.35	72	76	100
Gosaba (S_8)	nd	3.93	0.04	0.02	4	6.95	5.05	73	79	98.3
Canning (S_9)	nd	3.73	0.05	nd	3.78	6.45	4.95	77	76	98.6
Dhamakhali (S_{10})	nd	3.65	0.06	0.01	3.72	6.05	4.65	77	81	98.1
Jharkhali (S_{11})	nd	4.05	0.02	0.01	4.06	6.4	5.00	79	81	99.8
BCSS-1	nd	4.8	nd	nd	4.8	7.86	5.42	55 ± 5	87 ± 10	100
MESS-2	nd	3.26	nd	nd	18.17	25.64	20.35	74 ± 8	88 ± 15	100

iAs [a] = inorganic arsenic as sum of As(III) and As(V): sample drying did not allow to discriminate between arsenite and arsenate due to oxidation

nd – not detected, LOD (mg kg-1) AB: 0.02; iAs: 0.01; DMA: 0.015; MA: 0.01

Table 3: Concentrations (mg kg^{-1}) of As species (AB, As^{III}, DMA, MA, As^{V}, total As digest and extract, with % recoveries) in four polychaete species.

Stations & Polychaete species	AB	As^{III}	DMA	As-PO_4	As^{V}	Unknown	Sum As	Extract As	Digest As	Extract Recovery %	HPLC Recovery %
S_1/ *P. cultifera*	0.02	0.10	0.17	0.17	0.42	0.10	0.98	1.19	2.8	42	82
S_2 / *G. sootai*	0.06	0.41	0.39	0.44	0.19	0.09	1.58	2.55	3.8	67	61
S_3 /*P. cultifera*	0.24	0.31	0.22	0.19	0.50	0.08	1.53	1.93	2.0	96	80
S_4/*L. notocirrata*	0.02	0.26	0.41	0.92	0.31	0.01	1.93	3.17	4.3	74	61
S_5/ *D. arborifera*	0.03	0.14	0.05	0.97	0.16	10.27	11.62	11.91	14.7	81	98
CRM627	2.94	0.04	0.11	0.01	0.03	nd	3.12	4.62	4.26	108	68

nd – not detected, LOD (mg kg^{-1}) AB: 0.008; As^{III}: 0.005; DMA: 0.006; As-PO_4: 0.005; As^{V}: 0.004

Table 4: Concentration (mg kg^{-1}) of polychaetes versus host sediment and accumulation factor (AF).

	Polychaetes	Sediments	AF
S_1 / *P. cultifera*	2.8	9.4	0.3
S_2 / *G. sootai*	3.8	7.3	0.5
S_3 / *P. cultifera*	2.0	9.9	0.2
S_4 / *L. notocirrata*	4.3	10.4	0.4
S_5 / *D. arborifera*	14.7	4.9	3.0

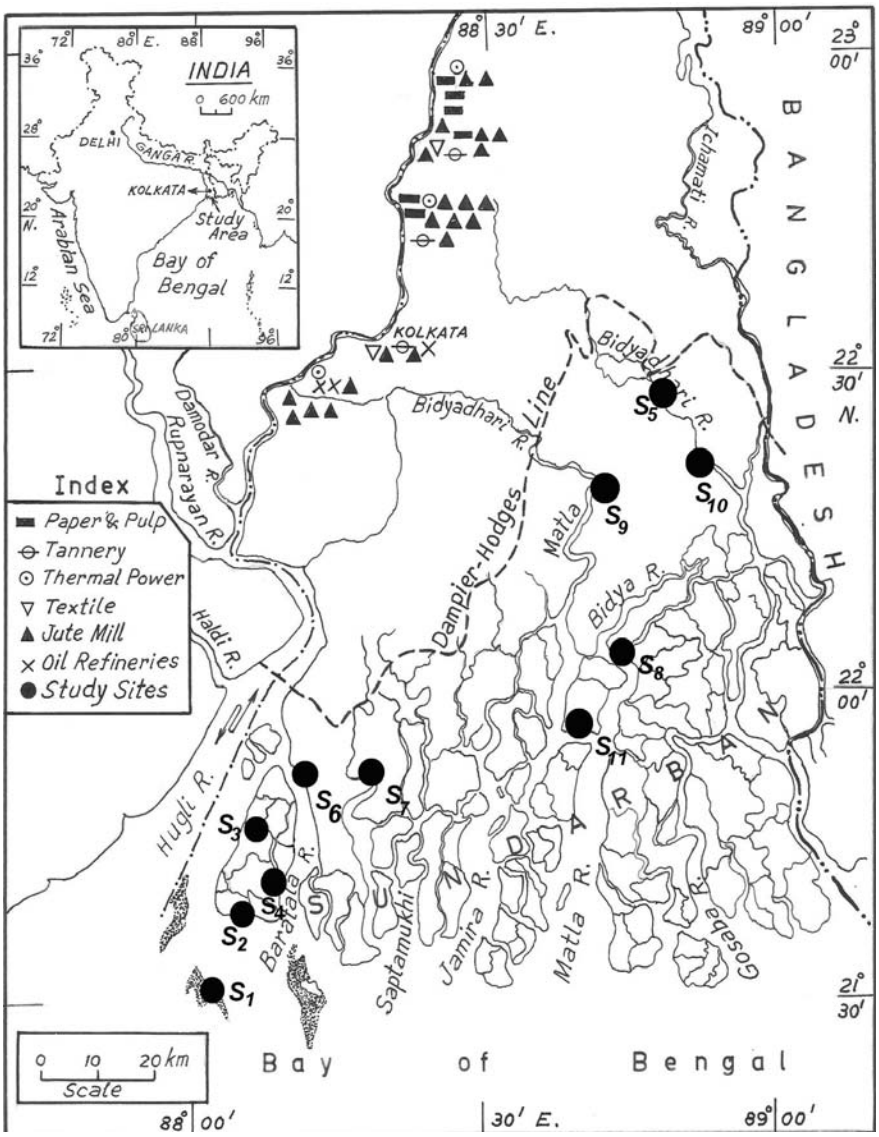

Fig 1: Map of Sundarban wetland and adjoining river network showing the location of sampling stations (S_1 – S_{11}).