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Speciation of Arsenic in Environmental Samples of the Nha Trang Harbor, Vietnam, Using HPLC Coupled HG-AAS

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Abstract A coupled High Performance Liquid Chromatography – Hydride Generation-Atomic Absorption Spectroscopy system was used to determine the speciation of Arsenic in samples from the Nha Trang Harbor, Vietnam. Concentrations of Arsenic in seawater, pore water, suspended solid, and sediment were 4.12–9.81 μg/L, 13.10–24.32 μg/L, 1.87–6.42 μg/g, and 3.37–9.06 μg/g, respectively. Extraction using H_3PO_4 + $NH_2OH{\cdot}HCl$ and ultrasonic digestion was optimized to yield a 76–85% of total Arsenic. Arsenic (III) was the most abundant species in suspended solids and sediments whereas Arsenic (V) represented for 30–50% of Arsenic (III) concentration. Monomethylarsonic acid and Dimethylarsinic acid species were undetectable.

Keywords Arsenic speciation · Vietnam · HPLC · AAS

As a consequence of both natural causes and anthropogenic impacts, arsenic (As) contamination has been reported in many countries including Vietnam (Berg et al. 2007). The environmental risks of excessive Arsenic (As) depends to a great extent on the chemical forms and their respective mobility: It is therefore necessary that As speciation analyses in solid matrices are conducted in order to identify the species susceptible to be mobilized under various environmental conditions (Rochette et al. 1998). In Vietnam, despite a known As contamination problem, no complete and standardized experimental protocol for As speciation analysis exists. The purpose of this study was to optimize the extraction procedure and analytical equipment (a high performance liquid chromatography (HPLC) coupled with a hydride generation – atomic absorption spectrometry [HG-AAS]) for As speciation analysis on samples taken from around the Nha Trang City (South Central Coast of Vietnam) where As enrichment is probable due to anthropogenic activities.

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Materials and Methods

An arsenite standard solution was prepared by dissolving 17.34 mg $NaAsO_2$ (Fluka Chemie, Switzerland) in 10 mL of 5 g/L NaOH. Arsenate standard solution was prepared by dissolving 41.65 mg of $Na_2HAsO_4{\cdot}7H_2O$ (Fluka Chemie, Switzerland) in 10 mL of 0.5 M HCl. A standard solution of monomethylarsonic acid (MMA) was prepared by dissolving 21.61 mg $CH_3NaHAsO_3$ (AccuStandard, USA) in 10 mL of water. Standard solution of dimethylarsinic acid (DMA) was prepared by dissolving 28.57 mg $(CH_3)_2AsNaO_2{\cdot}3H_2O$ (Fluka Chemie, Switzerland) in 10 mL of water. The analysis was taken on a HPLC system coupled to a Solaar M6 HG-AAS (Thermo Elemental, UK). The HPLC system consists of a Series 2500 Gradient HPLC Pump (Lab Alliance, USA), a Rheodyne Model 9725 Sample Injector (Upchurch Scientific, USA), and a separation column. Two columns were used: a PRP-X100 (Hamilton, USA) and a reversed-phase C18 (Phenomenex, USA).

Samples were collected from three sites: Tran Phu Bridge, Binh Tan Bridge, and Hon Do Island (Fig. 1).

Fig. 1 Map of the study area (*stars* indicate sampling sites)

The first two sites, in salinity gradient zone, are frequently subject to solid and liquid wastes, whereas the last site, situated 1 km offshore, is considered to be unimpacted by solid and liquid waste. The sites are typical of the different environmental conditions found in coastal area of Vietnam. At each site, water and sediment were sampled during three consecutive days around 3 pm when tidal flow was at its slowest. The sampling places were selected in shallow water (about 1 m depth) for easy sediment sampling. A diagram of the sample treatment steps prior to analysis is shown in Fig. 2. In detail, in the Speciation Extraction, about 0.1 g of sample was precisely weighed and mixed with 10 ml of extracting solution. The mixture was then shaken for 1 h in an ultrasound bath (Transsonic T 420 Lab-line, USA) and stabilized overnight. The 1 h extraction time was chosen based on our previous tests that showed that extraction efficiency did not significantly change with extraction times longer than 1 h. The supernatant was then separated by centrifugation followed by filtration (0.45 µm poresize Whatman membrane). The process was repeated three times for a complete extraction of exchangeable As. Supernatant collected from this triple extraction was then kept at 4°C for As speciation analysis. Selection of extracting solution is discussed in detail in next section. In the Sediment Pretreatment step, the frozen sediment sample was lyophilized in a Liobeta 35 lyophilyzer (Telstar, Spain) and then sieved with a 0.05 mm sized sieve. In the Microwave Digestion step, about 1 g of sample was precisely weighed and digested in 5 mL concentrated HNO_3 using a MARS 240/50 microwave oven (CEM, USA). The digested solution was then diluted with deionized water to 50 mL volume, filtered through 0.45 µm Whatman membrane, and kept at 4°C for the total As analysis.

Results and Discussion

The characteristics of the developed method were checked and found satisfactory for trace metal analysis (e.g. limit of detection was as low as 1 µg/L and relative standard deviation was lower than 4%). Comparison of arsenic in reference marine sediment was determined and found to agree well with reference values (reproducibility higher than 90%), as shown in Table 1.

It is vital that the separation procedure preserves species in their original forms, especially since many Arsenic species are very unstable. Therefore, the choice of an

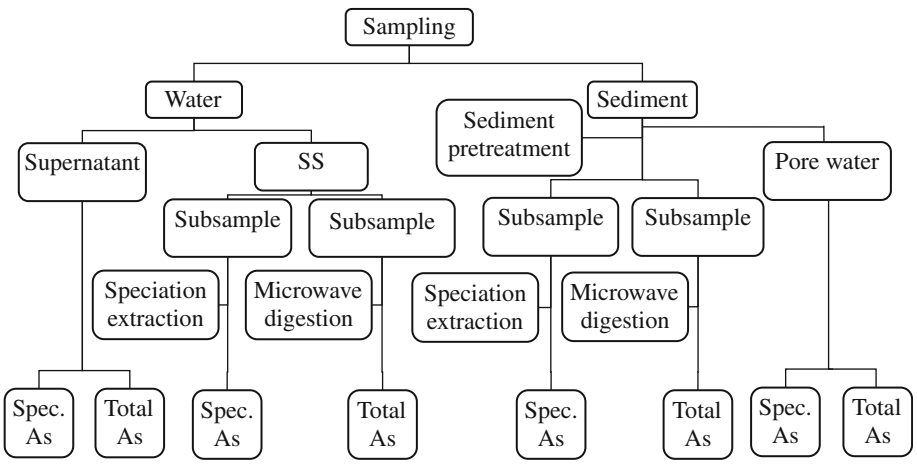

Fig. 2 Sample treatment scheme

Table 1 Total Arsenic concentration (μg/g) determined in reference marine and artificial samples

Sample	Certified	Measured
MESS-2	20.7 ± 0.8	19.85 ± 1.5
MESS-3	21.2 ± 1.1	20.75 ± 1.8

appropriate extractant is of the utmost importance as sediments in the area have been altered by various anthropogenic impacts and it is not easy to predict a priori the species present. We tested a range of solvents in order to determine which solvent was the best for arsenic extraction to samples in the area. These extractants were chosen based on their known ability to remove arsenic associated with different sediment phases. Sodium hydroxide (NaOH) and hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) have been used to remove arsenic associated with amorphous and well-crystallized Fe–Mn oxides, phosphoric acid (H_3PO_4) or phosphoric acid (H_3PO_4)/methanol (CH_3OH) (1:1, v/v) have been used to remove arsenic associated with organics, hydrochloric acid (HCl) have been used to remove arsenic from carbonates, and ascorbic acid ($C_6H_8O_6$) have been used to determine metals bound to Fe–Mn oxides (Ellwood and Maher 2003). We used a concentration of 0.5 M for all extractions so that the results could be compared on an equimolar basis. The test showed extraction yield of mixture of H_3PO_4/$NH_2OH{\cdot}HCl$ was highest among the tested extractants. Compared with total Arsenic concentration in the sediment, yields of up to 85% were obtained using this mixture as opposed to less than 50% by others extractants. Hydroxylamine hydrochloride is used to preserve the unstable As (III) and As (V) species. In addition, an experiment aimed at determining the extracting efficiency of the mixture at different H_3PO_4 concentrations (0.1–0.7 M) was performed for sediment collected at the Binh Tan Bridge. This test revealed that maximum efficiency was reached at concentration of equal or higher than 0.5 M. Higher H_3PO_4 concentrations were not investigated because the dilution required to preserve peak integrity would have diluted arsenic signals below the limit of detection of the system used. A mixture of 0.5 M H_3PO_4/0.1 M $NH_2OH.HCl$ (1:1, v/v) was then used for further analysis. One important point that can be concluded from the test is that Arsenic was most bound to organic and Fe–Mn oxide forms in the examined samples. Indeed, as sediment in the area is impacted by municipal waste that contains a large fraction of degradable organic matter, it is probable that Arsenic would be bound with organic matter or would co-precipitate with Iron and Manganese oxides/hydroxides. The optimized procedure was then used to analyze the samples collected from the three study sites. Typical chromatograms are presented in Fig. 3 and results are shown in Table 2.

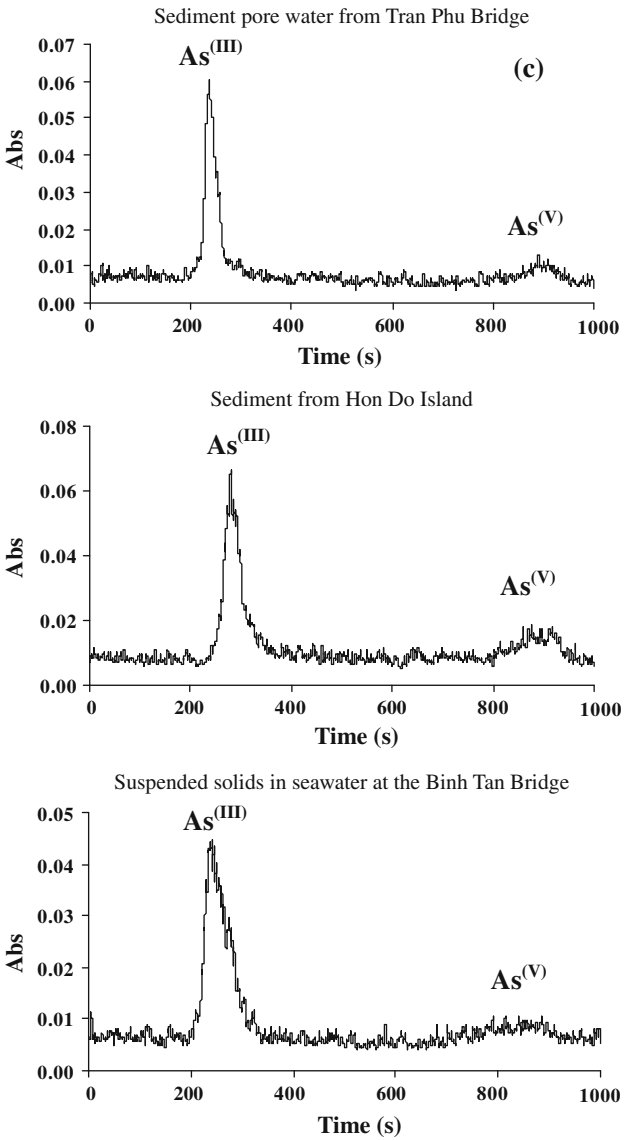

Fig. 3 Chromatograms obtained by HPLC-HG-AAS of samples collected from the Nha Trang Harbor

The results in Table 2 show that As contents for water and sediments at the Binh Tan Bridge are marginally higher than at other sites, and that the main species are the most toxic As (III) and As (V). Such high As content is potentially due to the fact that, the Binh Tan Bridge area, is adjacent to the wastewater outlet of the Phuoc Dong shrimp hatching site, a large shrimp aquaculture area near the city. In addition, the area is also a fishing port where the majority of the fishing boats of the city tie-up. Several activities such as the fisheries and waste dumping also result in pollution of the area with fish related waste. Our previous study showed elevated As concentrations in marine products from the area which is in agreement with other reports worldwide (Nguyen and Le 2007; Van Hulle et al. 2002). During decomposition, organic As fraction of

Table 2 Arsenic content in seawater, pore water, suspended solids, and sediments of samples collected in the Nha Trang Harbor

Samples	Total As		Recovery (%)	Species					
	μg/L	μg/g		Total As		As (III)		As (V)	
				μg/L	μg/g	μg/L	μg/g	μg/L	μg/g
SS at BT		6.42	85		5.48		4.61		0.87
SS at TP		5.38	81		4.37		3.88		0.49
SS at HD		1.87	78		1.45		1.33		0.12
Sea water at BT	9.81		86	8.41		6.24		2.17	
Sea water at TP	8.10		81	6.59		4.99		1.60	
Sea water at HD	4.12		85	3.50		2.65		0.85	
Pore water at BT	24.32		90	21.94		17.83		4.11	
Pore water at TP	21.20		81	17.21		13.69		3.52	
Pore water at HD	13.10		83	10.93		8.41		2.52	
Sediment at BT		9.06	89		8.08		5.23		2.85
Sediment at TP		7.45	89		6.67		4.16		2.51
Sediment at HD		3.37	87		2.94		2.10		0.84

BT binh tan bridge, *TP* tran phu bridge, *HD* hon do island

seafood and aquacultural waste are transformed into the dissolved forms in seawater as well as enriched in the near shore sediment.

Comparison between results found in this study and other reports shows that Arsenic concentrations was slightly elevated in water of Nha Trang Harbor. Average values found in the Harbor are 7.3 μg/L, 19.5 μg/L, and 6.7 μg/g in surface water, pore water, and sediment, respectively. Corresponding values reported worldwide for uncontaminated sites are approximately 1 μg/L, 24 μg/L, and 10 μg/kg (Francesconi and Edmonds 1998; Ellwood and Maher 2003). We concluded in a former paper that municipal waste containing large portion of seafood and marine products was the cause of Arsenic concentration in sea water (Nguyen and Le 2007). The lack of difference in Arsenic concentration in other phases (sediment and pore water) as compared to non-contaminated sites implies that there is no historic arsenic contamination in the Nha Trang City area. Nevertheless, it is interesting to note that in the sediment the As (III) dominates instead of As (V), as usually found in other places. This dominance may well be due to the anoxia present in this system which favors the formation of As (III) (Ellwood and Maher 2003). These results also show that the sampling and treatment method performed here retains the Arsenic species in their original state until analysis as inappropriate sampling and treatment would have converted As (III) to As (V).

Comparison of the separation technique for identification of minor As species used in this study and that of other published research was made. Here we clearly identified two principal peaks representing As (III) and As (V) (Fig. 3). In similar work, Ellwood and Maher (2003) using extraction conditions of 20 mM $NH_4H_2PO_4$ at pH = 9.2 found two additional peaks of Assugar 1a and As-sugar 1b using HG-ICP-MS detector. Since their sugar peaks were found close to the As (III) peak, it is probable that the separation procedure, and in particular, the pH manipulation, applied in our study was unable to separate As-sugar species from the As (III). Another explanation could be an inferior detection limit of HG-AAS detector used in this study as compared to that of the HG-ICP-MS. Unfortunately, as no reference Arsenic sample including As-sugar species was available, we were unable to test this assumptions.

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