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# Species- and tissue-specific bioaccumulation of arsenicals in various aquatic organisms from a highly industrialized area in the Pohang City, Korea

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ABSTRACT

Contamination of water and sediment with arsenic (As) in a highly industrialized area of Pohang City, Korea was investigated, with emphasis on in situ bioaccumulation of arsenicals by various aquatic organisms. Species- and tissue-specific concentrations of arsenicals were determined by use of HPLC-ICP/MS and μ-X-ray absorption near-edge structure (μ-XANES). Concentrations of arsenic in aquatic organisms were strongly associated with corresponding water concentrations, which indicates point sources associated with land use and activities. Arsenobetaine was the most dominant form of arsenic found in fishes, bivalves, crabs, and shrimps, while $As^{III}$ was predominant in freshwater snails. The μ-XANES analysis provided additional information about the unidentified arsenicals such as As-thiol. Arsenicals were mainly localized in intestine of mullet and marsh clam. Distribution and bioaccumulation of arsenic were strongly correlated with salinity, which indicates that natural processes controlling biogeochemistry of arsenic would be important in estuarine lotic system.



## 1. Introduction

Both inorganic and organic forms of arsenic (As) can enter aquatic environments through either natural or human activities (Akter et al., 2005; Cullen and Reimer, 1989). The primary releases are due to human activities, such as mining, combustion of municipal solid waste, fossil fuels in coal- and oil-fired power plants, metal smelting, and direct use of As-containing herbicides by industry and agriculture (Cullen and Reimer, 1989; Nield et al., 2014; Sharma and Sohn, 2009; Zhang et al., 2002). Arsenic can occur in a number of inorganic compounds and minerals, depending on oxidation state and chemical–physical parameters of the environment such as redox potential (Eh), pH, and ionic strength (Akter et al., 2005; Sharma and Sohn, 2009). The form in which As exists dictates its fate in the environment, as well as bioavailability and toxicity (Bissen and Frimmel, 2003). Thus, determination of speciation of As is necessary when assessing risks it might pose (Saunders et al., 2011).

During the last few decades, the ability to separate, identify, and quantify the various forms of arsenicals has been facilitated by development and application of analytical instruments such as high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP/MS) (Branch et al., 1994; Hirata et al., 2006; Larsen et al., 1993). More than 50 inorganic and organic arsenicals have been identified in biota, including arsenobetaine (AB), which is mainly found in marine organisms (Saunders et al., 2011). In addition, X-ray absorption

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near-edge structure (XANES) analysis, which has been utilized to determine oxidation state and the local chemical environment of As in solid matrices could provide useful information about unidentified arsenicals. Furthermore, the combined use of HPLC-ICP/MS and XANES was a powerful technique for understanding speciation of As (Caumette et al., 2011, 2012a; Smith et al., 2005; Whaley-Martin et al., 2012a). Numerous studies have investigated speciation of As in aquatic organisms, which has provided abundant information on occurrence of arsenicals in aquatic environments and food that originates from it (Caumette et al., 2011; Koch et al., 2007; Lai et al., 2012; Whaley-Martin et al., 2012a; Williams et al., 2006).

Inorganic As is, in general, more mobile and toxic than are As–C bonds (organo-arsenicals) (Eisler, 1988). In surface water, As is commonly present as inorganic forms such as pentavalent arsenate ($As^{V}$, $AsO_4^{3-}$) and trivalent arsenite ($As^{III}$, $AsO_3^{3-}$). Inorganic As can be transformed into organic forms through bio-methylation by phytoplankton (Azizur Rahman et al., 2012). Organic compounds containing As can be transformed to inorganic As and methyl-As by microbial activity. Thus, microorganisms such as phytoplankton and bacteria play important roles in forms of As, distribution, and cycling in marine and freshwater environments (Azizur Rahman et al., 2012). More higher trophic level organisms such as fish, bivalves, crabs, shrimp, and gastropods can be exposed to As through waterborne (inorganic) and dietary (inorganic and organic) routes, biotransform, accumulate, and retain it inside their bodies (Azizur Rahman et al., 2012). Each organism seems to have its own metabolic pathways to convert organic and/or inorganic As and can excrete As through urine and feces or accumulate As in their body (ATSDR, 2007). Results of previous studies have suggested that arsenicals do not biomagnify along the aquatic food chain (ATSDR, 2007; Caumette et al., 2012b). To understand the biogeochemistry and potential risk of As in aquatic ecosystems, studies on in situ species-specific bioaccumulation and tissue distributions of arsenicals would be beneficial (Edmonds et al., 1993; Hirata and Toshimitsu, 2005; Maher et al., 1999; Schaeffer et al., 2006). However, there are few data from in situ studies of the characteristics of bioaccumulation of As in various aquatic organisms or its overall fate and distribution in water and sediments.

Distribution, fate, and bioactivity of As seem to be influenced by salinity in estuaries (Azizur Rahman et al., 2012). In general, As is nearly conservative during estuarine mixing and concentrations of As in water are directly proportional to salinity (Tremblay and Gobeil, 1990; Seyler and Martin, 1991). In estuaries where As concentrations are often greater, the interface between freshwater and saltwater has been found to be a zone of co-precipitation of Fe and As (Cullen and Reimer, 1989; Kossoff and Hudson-Edwards, 2012). In mining- or industry-affected estuaries, behavior and fate of As is often non-conservative and As is accumulated in bottom sediments rather than being moved out to the open ocean (Duan et al., 2010; Kossoff and Hudson-Edwards, 2012). Thus, due to its water chemistry (pH and ionic strength), bioavailability and bioaccumulation of As in estuaries might be influenced by salinity. However, the effects are not fully understood.

The present study investigated biogeochemistry of As to: i) determine current status and trends of concentrations of As in water, sediment, and biota and field-based bioaccumulation factor (BAF); ii) identify forms of As in aquatic organisms by combined HPLC-ICP/MS and μ-XANES analyses; iii) determine distribution of arsenicals among tissues of fish and bivalves; and iv) determine effects of salinity in estuarine lotic systems on the biogeochemistry of As.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Chemicals and reagents used in identification and quantification of target forms of As are presented in Table S1 of Supplemental Materials (S).

### 2.2. Study area, sample collection, and preparation

Pohang City is located on the east coast of South Korea, where the largest steel and iron making plants are situated at the center of the city (Fig. 1). Since these facilities are close to a residential area (<1 km), possible adverse effects of corresponding pollutants from the steel plant was of great public concern, particularly to residents in the area (Baek et al., 2010; Hong et al., 2014). Environmental samples were collected from around the steel and iron making plants (S1–S5), municipal areas (M1–M5), and from reference sites (R1 and R2) in June and August, 2010 and February, 2011 (12 sites for water, 8 sites for sediment, and 8 sites for aquatic organisms (see Figs. 1 and 2). Temperature, salinity, pH, and dissolved oxygen were measured in the field with a calibrated multiprobe (YSI 556 MPS, Yellow Springs, OH). Samples of water for the determination of total concentrations of As (dissolved phase), were filtered through a pre-washed 0.45 μm filter (Nuclepore, Whatman, Maidstone, UK) and acidified (pH < 2) with nitric acid. Sediments were freeze-dried, sieved (<2 mm), and stored at 4 °C prior to analysis. Biota samples were classified, pooled, and freeze-dried. Some samples of biota including fish and bivalves were further necropsied to allow tissue-specific distribution analysis and stored at −20 °C.

### 2.3. Quantification of total concentrations of As

Total concentrations of As in water were measured by use of Elan DRC II ICP/MS (PerkinElmer, Shelton, CT) as AsO at $m/z$ 91 in DRC mode by use of oxygen as the reaction cell gas. Operating conditions of the ICP/MS are presented in Table S2. Freeze-dried sediment and biota were digested with a mixture of concentrated nitric acid and hydrogen peroxide on a heating mantle (120 °C) for 4 h then evaporated to near dryness and diluted with 1% $HNO_3$, and kept frozen. Accuracy of determination of total As was assessed by use of certified reference material (CRM) MESS-3 (marine sediment, National Research Council (NRC), Canada). Recoveries ranged from 93 to 97% of the certified value of As ($n = 3$).

### 2.4. Characterization of species of As by HPLC-ICP/MS

Forms of As in biota were identified and quantified by use of previously described methods (Whaley-Martin et al., 2012a, 2012b) with some modifications. In brief, 0.1–0.5 g of freeze-dried and homogenized biota sample was weighed into 15 mL polypropylene centrifuge tubes, and 10 mL of 2% nitric acid solution ($HNO_3$, Sigma–Aldrich, Saint Louis, MI) added. Samples were sonicated for 30 min and placed on a water bath shaker for 4 h (60 °C, 120 rpm), then centrifuged for 15 min at 1000×g. All extracts were filtered through 0.22 μm membrane filters (13 mm, MCE filter, Jet Biofil, Guangzhou, China) and kept frozen until instrumental analysis. Six forms of As, including arsenocholine (AC, $C_5H_{14}AsO^+$), arsenobetaine (AB, $C_5H_{11}AsO_2$), monomethylarsonic acid (MMA, $CH_5AsO_3$), dimethylarsinic acid (DMA, $C_2H_7AsO_2$), $As^{III}$, and $As^{V}$ were separated and quantified by use of HPLC-ICP/MS (PerkinElmer Series 200 HPLC and ELAN DRC II ICP/MS System) with a Hamilton PRP X100 anion exchange column (250 × 4.1 mm, 10 μm particle, Reno, NV) with a gradient mobile phase (mobile phase A: 1 mM ammonium carbonate and mobile phase B: 12 mM ammonium phosphate + 12 mM ammonium nitrate, pH = 9.5, 1.5 mL $min^{-1}$). Injection volume of sample was 50 μL and flow rate of mobile phase was 1.5 mL $min^{-1}$. Detailed instrumental conditions for HPLC analysis are given in Table S2. The instrumental software used for the HPLC-ICP/MS was Chromera Chromatography Data System (Ver. 2.1, PerkinElmer).

### 2.5. Characterization of speciation of As by μ-XANES

The μ-XANES spectra of biota were collected at the Hard X-ray Micro-Analysis (HXMA) beamline at the Canadian Light Source (CLS) at the University of Saskatchewan in Saskatoon, SK, by use of published method (Smith et al., 2005) with some modifications. The μ-XANES analysis of the CLS has been validated previously and several papers on speciation of As have been published using the HXMA beamline (Button et al., 2011; Koch et al., 2011). Due to limited beam time, a subset of biota containing relatively greater concentrations of unidentified arsenicals, were measured by use of μ-XANES. Freeze-dried and homogenized biota samples were placed between two layers of Kapton tape and packed in a sample holder. Five to ten scans of each sample were collected and first three data were averaged as no damage to the sample by the beam was observed. XANES spectra of the K-edge (11,868 eV) of As were fit within −20 to + 40 eV to $E_0$ by use of Athena software (2001–2008 Bruce Ravel). The Si (111) double-crystal monochromator was calibrated using the first inflection point of the gold $L_{III}$ absorption edge (11,919.7 eV). A reference gold foil was measured simultaneously with samples. Several forms of As measured previously by other groups were identified and used for comparison (Smith et al., 2005).

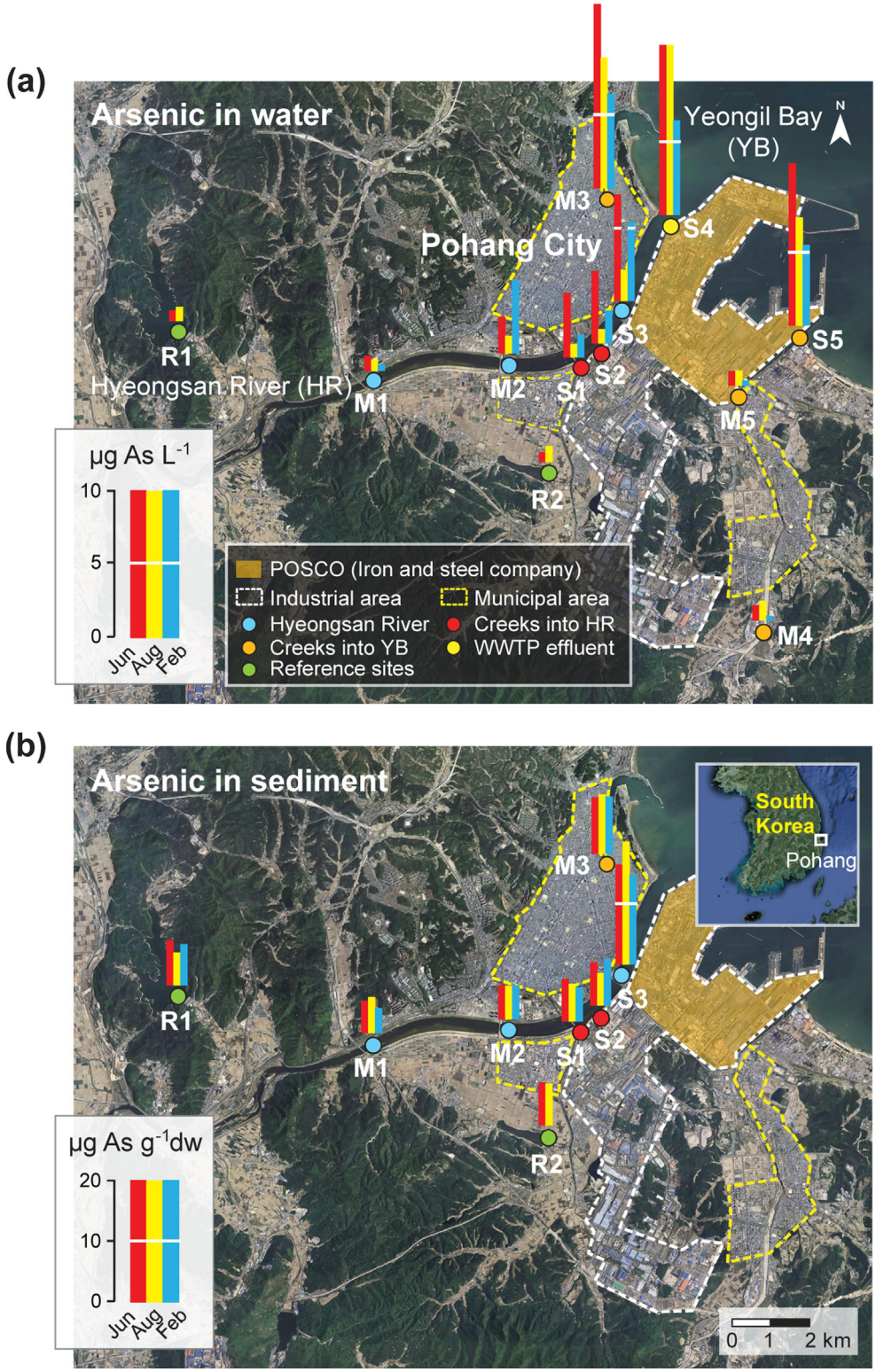


**Fig. 1.** Sampling sites of the Pohang study area, Korea, showing spatio-temporal distributions of total As in (a) water and (b) sediments in June and August of 2010 and February of 2011.

### 2.6. Quality assurance and quality control

Calibration standards of 1, 5, 10, 50, 100, and 500 ng $g^{-1}$ of six As compounds were used. Method detection limits (MDL) for six arsenicals were calculated as the blank + 3.707 × SD (standard deviation, $n = 7$). MDLs ranged from 0.02 to 0.06 μg $g^{-1}$ dw for arsenicals in biota (Table S3). Stabilities of forms of As during extraction were determined according to the same method. Degradation or interconversion of arsenicals did not significantly occur and recoveries ranged from 75 to 97% ($n = 5$) (Table S3). Accuracy for As speciation was assessed using two CRMs, DORM-3 (fish protein, NRC) and TORT-2 (lobster hepatopancreas, NRC). These

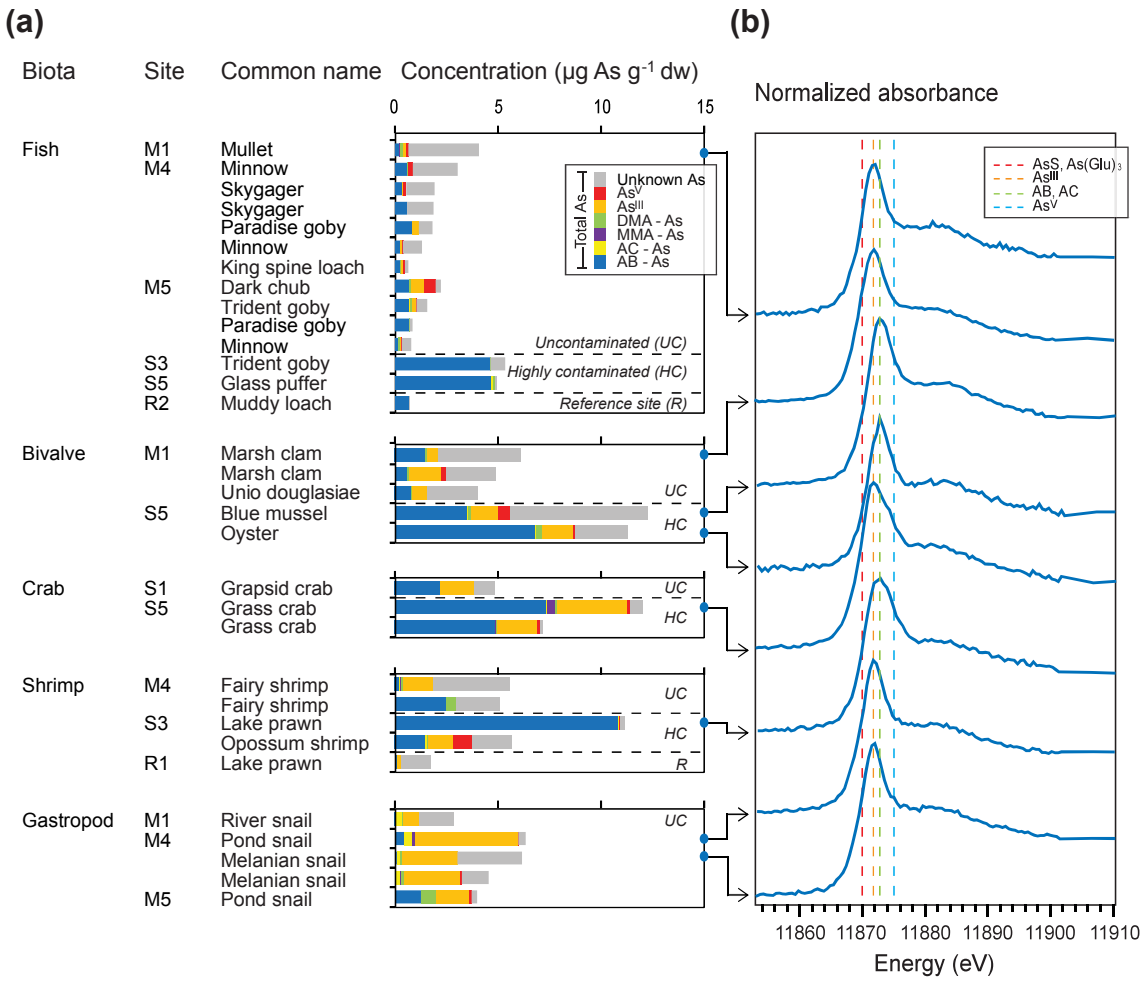


**Fig. 2.** Results of (a) HPLC-ICP/MS analysis and (b) μ-XANES analysis of arsenicals and in various aquatic organisms including fish, bivalves, crab, shrimp, and gastropods collected from the Pohang area (UC: uncontaminated; HC: highly contaminated; and R: reference site). The dotted lines in μ-XANES spectra indicate the various arsenicals reported previously (Smith et al., 2005). White line energies of various arsenicals were shown in Table S4.

materials were analyzed by the described methods and results obtained for the CRM consistent with previously reported values (Leufroy et al., 2011; Wahlen et al., 2004). A mid concentration of calibration standard and procedural and instrumental blanks were analyzed after every 10 samples as a check for drift in instrumental sensitivity and background contamination and/or carryover. Concentrations of all analytes in certified, CRMs were within 10% of the standard concentration and concentrations in all blank were found to be less than the corresponding MDLs.

## 3. Results and discussion

### 3.1. Concentrations of As in water and sediment

Concentrations of As in water ranged from <DL to 13 μg As $L^{-1}$ (dissolved phase) (Table 1 and Fig. 1). During the three surveys, the

**Table 1**
Concentrations of As in water and sediment and site categorization according to the environmental quality guideline.

| Sampling site | Surrounding activity | Salinity | | | Total As | | | | | | | | Comparison to WQG[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Water (μg As $L^{-1}$) | | | | Sediment (μg As $g^{-1}$ dw) | | | | |
| | | Jun. | Aug. | Feb. | Jun. | Aug. | Feb. | Mean | Jun. | Aug. | Feb. | Mean | |
| S1 | Industrial area | 8.0 | 0.3 | 6.5 | 4.5 | 0.92 | 1.6 | 2.3 | 7.0 | 6.2 | 5.7 | 6.3 | |
| S2 | | 12 | 0.5 | 10 | 5.0 | 1.0 | 2.3 | 2.8 | 7.2 | 5.4 | 7.8 | 6.8 | |
| S3 | | 20 | 3.4 | 27 | 7.4 | 2.2 | 5.5 | 5.0 | 17 | 21 | 15 | 18 | >WQG |
| S4 | | 30 | 26 | 33 | 12 | 12 | 6.6 | 10 | nc[b] | nc | nc | | >WQG |
| S5 | | 30 | 20 | 28 | 11 | 7.5 | 5.6 | 8.0 | nc | nc | nc | | >WQG |
| M1 | Municipal area | <0.1 | 0.1 | 0.9 | 1.0 | 0.82 | 0.44 | 0.75 | 5.3 | 6.0 | 4.2 | 5.2 | |
| M2 | | 6.0 | 1.2 | 23 | 2.6 | 1.2 | 5.1 | 3.0 | 5.7 | 5.6 | 5.6 | 5.6 | |
| M3 | | 28 | 23 | 32 | 13 | 9.1 | 6.6 | 9.6 | 9.4 | 9.8 | 9.5 | 9.6 | >WQG |
| M4 | | <0.1 | 0.2 | 0.5 | 1.0 | 1.4 | 0.29 | 0.90 | nc | nc | nc | | |
| M5 | | <0.1 | 0.2 | 0.4 | 1.0 | 1.0 | 0.36 | 0.82 | nc | nc | nc | | |
| R1 | Reference sites | <0.1 | 0.1 | <0.1 | 0.76 | 0.99 | –[c] | 0.88 | 7.5 | 5.4 | 6.9 | 6.6 | |
| R2 | | <0.1 | 0.3 | nc | 0.68 | 1.1 | nc | 0.89 | 7.1 | 7.0 | nc | 7.1 | |

[a] Comparison to WQG of As in water: 5 μg As $L^{-1}$ (freshwater aquatic life) (CCME, 2001).
[b] nc: Not collected.
[c] –: Less than MDL.

greatest, arithmetic, mean concentration of As was found at location S4 (WWTP effluent) where concentrations ranged from 6.6 to 12 μg As $L^{-1}$, followed by M3 (municipal area, 6.6–13 μg As $L^{-1}$) and S5 (industrial area, 5.6–11 μg As $L^{-1}$). In sediment, the greatest concentrations of As were found at S3 (industrial area, 15–21 μg As $g^{-1}$ dw) and M3 (9.5–9.8 μg As $g^{-1}$ dw). Concentrations of As in some waters of the Pohang area were close to or exceeded (S3, S4, S5, and M3) existing water quality guideline (CCME, 2001) based on adverse effects on aquatic organisms (Table 1). In general, concentrations and spatial distributions of As in water and sediment of the Pohang area were affected by point sources in the surrounding industrial and municipal areas.

To address seasonal variation, concentrations of As in water and sediment were measured three times during the year. As expected, concentrations of As in water varies among seasons, with greater concentrations in June and lesser concentrations in February and August (Fig. 1a). Approximately 75 mm of rainfall was recorded in Pohang during the sampling period (5 day) of August, thus drastic decreases in waterborne As during this time might be due to dilution. Another possible explanation for seasonal variation in waterborne concentrations of As could be differences in atmospheric deposition. For example, southwesterly winds prevailed in winter (Baek et al., 2010; Fang et al., 2012) could facilitate atmospheric transport and dispersion of As from the industrial area to the residential city area (Nield et al., 2014; Pacyna, 1987). There was no apparent seasonal variation in concentrations of As in sediments (Fig. 1b), which suggests that sedimentary distribution of As would be less influenced by short-term weather conditions. Overall, concentrations of As in Pohang area were greater than the background for the region and most likely affected by direct input from the surrounding industrial complex and city as well as atmospheric deposition.

### 3.2. Concentrations of As in biota

Arsenic was detected in all samples of fishes, bivalves, crabs, shrimps, and gastropods with varying concentrations between species (Table 2). The greatest mean concentration of As was found in crab, followed by bivalves, shrimp, and gastropods and finally in fishes with lesser concentrations. Concentrations of As in biota were dependent on corresponding waterborne concentrations of As, particularly for fish and filter-feeder organisms such as bivalves and shrimps (Table 1 and Fig. S1). For example, concentrations of As in the trident goby (*Tridentiger brevispinis*) were 1.6 and 5.4 μg As $g^{-1}$ dw in individuals collected from locations M5 and S3 (As in water > WQG), respectively. Also, concentrations of As in lake prawn (*Palaemon paucidens*) collected from R1 (reference site) and S3 were 1.7 and 11 μg As $g^{-1}$ dw, which were consistent with concentrations of As in water. Concentrations of As in bivalves were generally proportional to concentrations of As in water (Fig. S1). Such relationships or variations cross the target organisms could be due to similarity and/or difference in: i) natural habitat condition; ii) food sources and feeding guild; and iii) rates of uptake and

**Table 2**
Results for HPLC-ICP/MS analysis of arsenicals in various aquatic organisms collected from more contaminated sites, uncontaminated sites, and reference sites of the Pohang area, Korea.

| Sampling site | Biota | Common name | Scientific name | *n* | Total As (μg As $g^{-1}$ dw) | Inorganic As (μg As $g^{-1}$ dw) | | Organic As (μg As $g^{-1}$ dw) | | | | EE[a] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $As^{III}$ | $As^{V}$ | AB | AC | MMA | DMA | |
| S1 | Crab | Grapsid crab | *Helice tridens tridens* | 15 | 4.8 | 1.6 | – | 2.2 | 0.019 | 0.015 | 0.010 | 80 |
| S3 | Fish | Trident goby | *Tridentiger obscurus* | 3 | 5.4 | –[b] | – | 4.6 | 0.015 | – | – | 87 |
| | Shrimp | Lake prawn | *Palaemon paucidens* | 27 | 11 | – | 0.010 | 11 | 0.014 | 0.023 | 0.014 | 98 |
| | | Opossum shrimp | *Mysidacea* | 3 | 5.7 | 1.2 | 0.93 | 1.5 | 0.044 | – | 0.073 | 66 |
| S5 | Fish | Glass puffer | *Fugu niphobles* | 3 | 4.9 | – | – | 4.7 | 0.097 | – | 0.095 | 99 |
| | Bivalve | Blue mussel | *Mytilus edulis* | 10 | 12 | 1.3 | 0.57 | 3.5 | 0.043 | – | 0.14 | 46 |
| | | Oyster | *Crassostrea gigas* | 43 | 11 | 1.5 | 0.084 | 6.8 | 0.049 | – | 0.30 | 77 |
| | Crab | Grass crab | *Hemigrapsus penicillatus* | 5 | 12 | 3.4 | 0.16 | 7.4 | 0.016 | 0.42 | 0.082 | 95 |
| | | Grass crab | *Hemigrapsus penicillatus* | 14 | 7.1 | 1.9 | 0.16 | 4.9 | 0.017 | 0.050 | 0.039 | 99 |
| M1 | Fish | Mullet | *Mugil cephalus* | 10 | 4.1 | 0.15 | 0.11 | 0.20 | 0.017 | 0.021 | 0.15 | 16 |
| | Bivalve | Marsh clam | *Corbicula fluminea* | 13 | 6.1 | 0.51 | – | 1.5 | – | – | 0.13 | 34 |
| | | Marsh clam | *Corbicula fluminea* | 25 | 4.9 | 1.6 | 0.21 | 0.59 | – | – | 0.10 | 51 |
| | | Unio douglasiae | *Unio douglasiae* | 3 | 4.0 | 0.73 | – | 0.81 | – | – | 0.025 | 39 |
| | Gastropod | River snail | *Cipangopaludina chinensis* | 3 | 2.8 | 0.77 | – | 0.10 | 0.30 | – | 0.028 | 42 |
| M4 | Fish | Minnow | *Moroco oxycephalus* | 3 | 3.1 | – | 0.20 | 0.63 | – | – | 0.037 | 29 |
| | | Skygager | *Erythroculter erythropterus* | 72 | 1.9 | 0.070 | 0.11 | 0.36 | – | – | – | 28 |
| | | Skygager | *Erythroculter erythropterus* | 22 | 1.9 | – | – | 0.59 | – | – | – | 31 |
| | | Paradise goby | *Rhinogobius giurinus* | 3 | 1.8 | 0.30 | – | 0.86 | – | – | – | 63 |
| | | Minnow | *Moroco oxycephalus* | 9 | 1.3 | 0.077 | 0.041 | 0.26 | – | – | – | 29 |
| | | Southern king spine loach | *Iksookimia hugowolfeldi* | 3 | 0.64 | 0.087 | 0.079 | 0.26 | – | – | 0.062 | 76 |
| | Shrimp | Fairy shrimp | *Neocaridina denticulata* | 41 | 5.6 | 1.4 | – | 0.24 | 0.010 | 0.052 | 0.12 | 33 |
| | | Fairy shrimp | *Neocaridina denticulata* | 5 | 5.1 | – | – | 2.5 | – | – | 0.48 | 59 |
| | Gastropod | Pond snail | *Radix auricularia coreana* | 6 | 6.4 | 5.0 | 0.012 | 0.44 | 0.40 | 0.13 | 0.021 | 94 |
| | | Melanian snail | *Semisulcospira libertina* | 106 | 6.2 | 2.7 | – | 0.082 | 0.17 | 0.024 | 0.059 | 49 |
| | | Melanian snail | *Semisulcospira libertina* | 31 | 4.6 | 2.7 | 0.059 | 0.062 | 0.21 | 0.032 | 0.14 | 71 |
| M5 | Fish | Dark chub | *Zacco temmincki* | 7 | 2.2 | 0.66 | 0.53 | 0.68 | 0.009 | – | 0.091 | 88 |
| | | Trident goby | *Tridentiger brevispinis* | 3 | 1.6 | 0.22 | – | 0.72 | – | – | 0.099 | 67 |
| | | Paradise goby | *Rhinogobius giurinus* | 2 | 0.85 | – | – | 0.71 | – | – | – | 84 |
| | | Minnow | *Rhynchocypris oxycephalus* | 17 | 0.78 | 0.078 | 0.010 | 0.18 | – | – | 0.065 | 42 |
| | Gastropod | Pond snail | *Radix auricularia coreana* | 5 | 4.0 | 1.6 | 0.087 | 1.3 | – | – | 0.75 | 93 |
| R1 | Shrimp | Lake prawn | *Palaemon paucidens* | 21 | 1.7 | 0.19 | – | 0.11 | – | – | – | 17 |
| R2 | Fish | Muddy loach | *Misgurnus mizolepis* | 3 | 0.69 | – | – | 0.69 | – | – | – | 99 |

[a] Extraction efficiency (%) = Sum of identified As / Total As × 100.
[b] –: Less than MDL.

excretion of As among aquatic organisms (Katagi, 2010; Williams et al., 2006).

Field-based bioaccumulation factors (BAF) values for As could be calculated for target aquatic organisms (divided into freshwater and marine) based on concentrations of As in water and biota (Table 3). Overall, field-based BAFs for As were slightly greater in freshwater organisms than those in marine organisms. Among freshwater organisms, gastropods (freshwater snail, *Radix auricularia coreana*, *Semisulcospira libertine*, *and Cipangopaludina chinensis*) exhibited the greatest log BAFs, ranging from 2.8 to 3.7, followed by shrimp, bivalve, and fish. For marine organisms, BAFs of crab and bivalves were greater than those of fishes and shrimp. The BAFs of As obtained from this study were generally comparable to the values reported by the previous field studies (Chen et al., 2000; Giusti and Zhang, 2002; Mason et al., 2000; Mitra et al., 2012; Wagemann et al., 1978) (Table 3). Values of BAF for As in aquatic organisms measured in the present study suggested that lower trophic level organisms had greater BAFs than did higher trophic level organisms and As seemed not to biomagnify in either freshwater or marine food webs. Overall, the field-based BAF values obtained from this study exhibited a wide range due to species-specific characteristics and field variances. Despite these limitations, the BAF values of As in freshwater and marine organisms are valuable to better understand bioavailability and biogeochemistry of As.

### 3.3. Speciation of As in various aquatic organisms

In order to identify the composition of arsenicals in aquatic organisms, six forms of arsenicals, AC, AB, MMA, DMA, $As^{III}$, and $As^{V}$ were quantified by use of HPLC-ICP/MS (Table 2 and Fig. 2a). Arsenobetaine was the predominant form of As in fishes, followed by unidentified forms of As. Inorganic arsenicals such as $As^{III}$ and $As^{V}$ and organic arsenicals such as MMA, DMA, and AC accounted for a relatively small portion of the total concentration of As in fishes. Overall, about 60% of arsenicals in fishes were unidentified forms other than the six forms quantified in this study. Lesser concentrations of As in tissues of fishes, compared to other organisms implied that arsenicals seemed to be rapidly transformed and excreted from bodies of fishes, which is consistent with the known lack of bioaccumulation of As and/or simply less uptake (Azizur Rahman et al., 2012; Neff, 1997; Williams et al., 2006). Concentrations of AB in fishes were greater in samples collected from sites S3 and S5 where concentrations of As were greater in water and sediment, while unidentified species of As accounted for the majority of the total concentration of As in most fishes from uncontaminated sites (<WQG) (Fig. 2a). The AB in fishes might be accumulated by dietary exposure which comes from degradation of arsenosugar in zooplankton (Azizur Rahman et al., 2012; Caumette et al., 2012b; Wrobel et al., 2002).

Both AB and $As^{III}$ were predominant forms of As in bivalves, and unidentified As accounted for a substantial proportion of the total (~50%). In crab and shrimp, AB and $As^{III}$ were also predominant, accounting for 88% and 64% of the total concentration of As, respectively. Greater concentrations of arsenicals were accumulated in bivalve, crab, and shrimp than in fishes. A large portion of As in bivalves, crab, and shrimp collected from more contaminated sites such as S3 and S5 was AB. Speciation of As in bivalves observed during this study was similar to the results of previous studies (Kohlmeyer et al., 2002; Larsen et al., 1997; Whaley-Martin et al., 2012a). Compositions of As in gastropods were apparently different from those of other taxonomic groups. For example, $As^{III}$ was the predominant form of As in gastropods (freshwater snail) (Fig. 2a). This result was consistent with the results of previous studies that found tetramethyl-arsonium oxide (Tetra), $As^{III}$, and arsenosugars to be the most abundant forms of As in freshwater snails, while AB

**Table 3**
Field-based bioaccumulation factor (BAF) of As in various aquatic organisms (freshwater and marine) obtained from this study and previous studies.

| Region | Marine/ Freshwater | Aquatic organisms | Number of pooled samples | Bioaccumulation factor (Log BAF)[a] | | References |
|---|---|---|---|---|---|---|
| | | | | Min. – Max. | Mean ± SD | |
| Pohang City, South Korea | Freshwater | Fish[b] | 12 | 2.2–3.4 | 2.6 ± 0.4 | This study |
| | | Bivalve[c] | 3 | 2.9–3.3 | 3.1 ± 0.2 | |
| | | Shrimp[d] | 3 | 2.5–3.5 | 3.1 ± 0.5 | |
| | | Gastropod[e] | 5 | 2.8–3.7 | 3.3 ± 0.4 | |
| | Marine | Fish[f] | 2 | 2.2–2.3 | 2.3 | |
| | | Bivalve[g] | 2 | 2.5–2.5 | 2.5 | |
| | | Crab[h] | 3 | 2.4–2.9 | 2.6 ± 0.3 | |
| | | Shrimp[i] | 2 | 2.1–2.3 | 2.2 | |
| Streams, western Maryland, USA | Freshwater | Fish | 9 | 2.4–2.9 | 2.6 ± 0.2 | Mason et al., 2000 |
| Manchar Lake, Pakistan | Freshwater | Fish | 10 | 0.69–1.6 | 1.1 ± 0.3 | Shah et al., 2009 |
| Venetian Lagoon, Murano, Italy | Marine | Mussel[j] | 4 | 2.8–3.1 | 3.0 ± 0.1 | Giusti and Zhang, 2002 |
| Lakes in the Yellowknife, Canada | Freshwater | Gastropod | 4 | 2.2–2.4 | 2.3 ± 0.1 | Wagemann et al., 1978 |
| | | Amphipoda | 4 | 2.0–2.3 | 2.3 ± 0.1 | |
| Lakes, northeastern USA | Freshwater | Small plankton (45–202 μm)[k] | 16 | 2.3–4.2 | 3.1 ± 0.6 | Chen et al., 2000 |
| | | Macrozooplankton (>202 μm)[k] | 15 | 1.9–3.6 | 2.6 ± 0.5 | |
| Sang-E-Noghreh area, Iran | Freshwater | Algae | 3 | 2.9–4.1 | 3.6 ± 0.7 | Mitra et al., 2012 |

[a] Log BAF = log (concentration of total As in biota (mg As $kg^{-1}$ wet weight)/concentration of total As in water (mg As $L^{-1}$)).
[b] *Erythroculter erythropterus*, *Iksookimia hugowolfeldi*, *Moroco oxycephalus*, *Misgurnus mizolepis*, *Rhinogobius giurinus*, *Rhynchocypris oxycephalus*, *Tridentiger brevispinis*, and *Zacco temmincki*.
[c] *Corbicula fluminea* and *Unio douglasiae*.
[d] *Neocaridina denticulata* and *Palaemon paucidens*.
[e] *Cipangopaludina chinensis malleata*, *Radix auricularia coreana*, and *Semisulcospira libertine*.
[f] *Fugu niphobles* and *Tridentiger obscurus*.
[g] *Crassostrea gigas* and *Mytilus edulis*.
[h] *Helice tridens tridens* and *Hemigrapsus penicillatus*.
[i] *Mysidacea* and *Palaemon paucidens*.
[j] Converted to wet weight assuming 80% water content.
[k] Converted to wet weight assuming 90% water content.

was a minor constituent (Lai et al., 2012). Metabolic pathways for detoxification and elimination of arsenicals would be different between freshwater snail and other aquatic organisms. In general, concentrations of organic arsenicals in biota, except for gastropods, were directly proportional to the total concentration of As (Fig. S2).

To further examine the unidentified arsenicals, μ-XANES was performed for selected samples of several taxa. The μ-XANES analysis of aquatic organisms detected the presence of several other forms of As (Fig. 2b). The μ-XANES white line energy peak at ~11,872 eV, indicated that $As^{III}$, AB, AC, Tetra, and other ABs (C2-AB or C3-AB) occurred in mullet and marsh clams collected from location M1 (Smith et al., 2005). $As^{III}$ and AC occurred at lesser proportions in corresponding samples analyzed by HPLC-ICP/MS analysis (Fig. 2a). Thus, unidentified As in mullet and marsh clam was likely due to the presence of organic arsenicals such as Tetra and ABs. A peak of 11,873 eV, which corresponded to AB, AC, and arsenosugars, was observed in blue mussel and oyster from site S5 (Smith et al., 2005). Based on the results of HPLC-ICP/MS analysis, the unidentified As in blue mussel and oyster are also likely to be arsenosugars. A peak at ~11,872 eV, which corresponds to $As^{III}$, was observed in freshwater snails such as pond snail (*Radix auricularia coreana*) and melanian snail (*Semisulcospira libertina*) from site M4, which was consistent with the results of the HPLC-ICP/MS analysis.

### 3.4. Tissue-specific distributions of arsenicals in fish and bivalves

Tissue-specific distributions of arsenicals in a fish (mullet, *Mugil cephalus*) and the bivalve (marsh clam, *Corbicula fluminea*) were determined (Fig. 3). Results of HPLC-ICP/MS analysis revealed that greater concentrations of As in intestine of fish and various arsenicals such as $As^{V}$, $As^{III}$, MMA, DMA, and AB were also present (Fig. 3a). Unidentified arsenicals comprised relatively large proportions of total concentrations of As, and μ-XANES analysis with a peak at 11,870 eV that indicated the presence of As-thiol (Smith et al., 2005; Whaley-Martin et al., 2012a). Binding of inorganic As to thiol groups of proteins such as metallothioneines is one of the mechanisms for toxic effects and this interaction could also be related to detoxification processes (Kitchin and Wallace, 2008; Langdon et al., 2003). Metallothioneines (cyteine-rich proteins) can be induced when intracellular concentrations of metal and/or metalloids exceed a threshold. Consequently, As-thiol complexes could be transformed to the AB via subsequent methylation (Button et al., 2009; Whaley-Martin et al., 2012a). This result indicated that arsenicals could be accumulated selectively, localized, and/or biotransformed in the intestine.

In the case of the marsh clam, there was a trend in tissue-specific distribution which was similar to that of mullet (Fig. 3b). However, the greater concentrations of inorganic As and AB were found in the body. Lesser amounts of unidentified As were observed in bivalves and total concentrations of As did not significantly differ between intestine and other organs of marsh clam. Collectively these results suggested that arsenicals are less metabolized in the bivalve (marsh clam) than in the fish (mullet). In general, aquatic organisms are exposed to As directly from the water and via the diet then either being accumulated or biotransformed to less-toxic forms in the intestine (Azizur Rahman et al., 2012; Zhang et al., 2012). Consequently, As can be excreted from the body mostly in urine or feces and retained in the body (Neff, 1997; Reimer et al., 2010).

### 3.5. Effects of salinity on As distribution and bioaccumulation in estuaries

In estuaries, salinity might be one key factor to influence the distribution and bioaccumulation of As in aquatic organisms (Azizur Rahman et al., 2012; Tremblay and Gobeil, 1990). Results of the present study indicated that concentrations of As in water, sediments, and biota tended to increase with increasing salinity

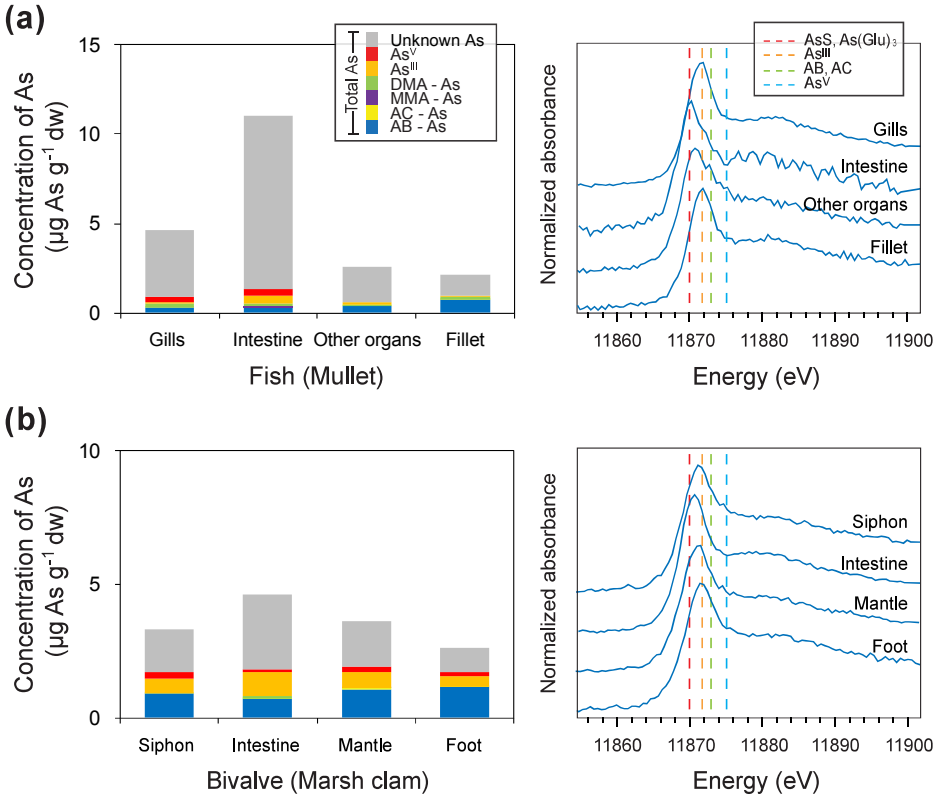


**Fig. 3.** Organ-specific distributions of arsenicals in (a) fish (mullet, *Mugil cephalus*) and (b) bivalve (marsh clam, *Corbicula fluminea*) identified by HPLC-ICP/MS and μ-XANES analyses.

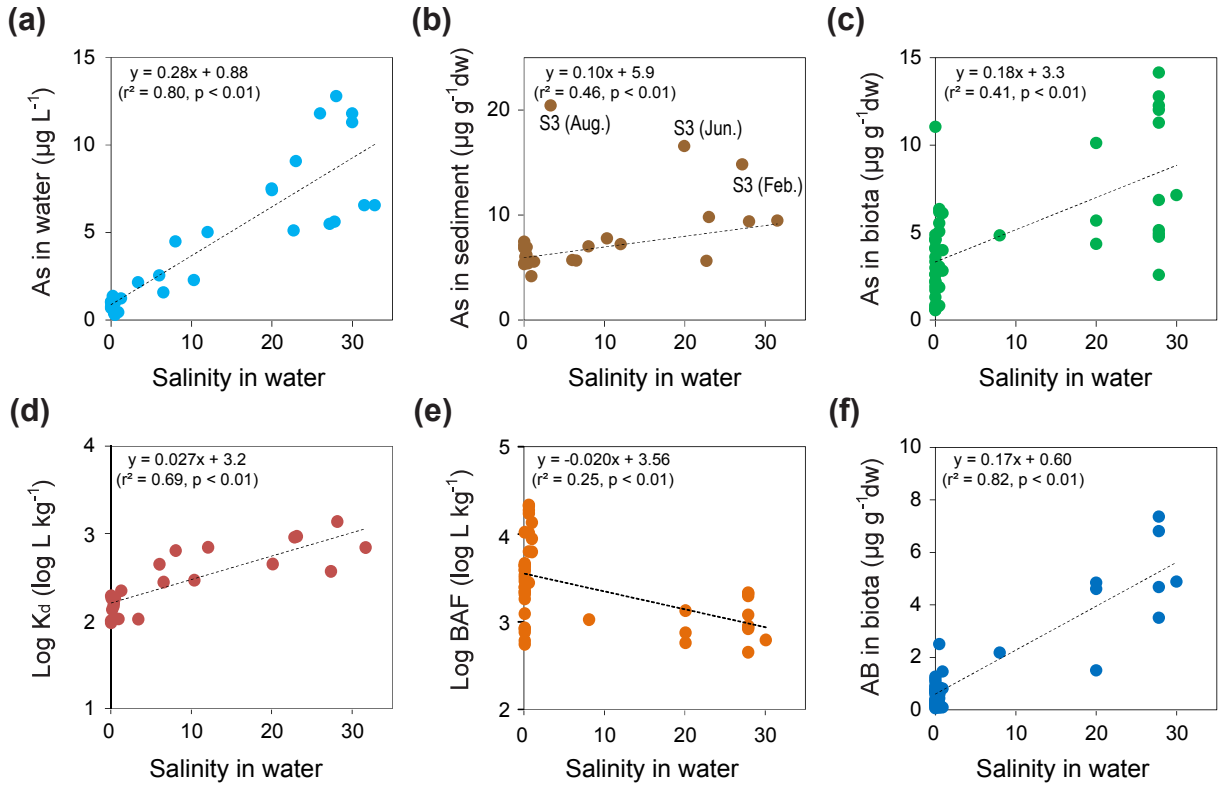


**Fig. 4.** Effects of salinity on As distribution and bioaccumulation. Scatter plots of (a) salinity vs. As in water, (b) salinity vs. As in sediment, (c) salinity vs. As in biota, (d) salinity vs. log $K_d$ (water-sediment distribution coefficient), (e) salinity vs. log BAF (bioaccumulation factor), and (f) salinity vs. AB in biota obtained from this study.

(Fig. 4a–c). Distribution of As in water along salinity gradient in the study area indicated that was due to simple mixing of local freshwater and seawater from land to ocean (Duan et al., 2010; Kossoff and Hudson-Edwards, 2012) (Fig. 4a). Distributions of As in sediments were slightly correlated with salinity (except for site S3) which seemed to be the results of scavenging of As from water column to sediments due to its adsorption onto hydrous iron oxides that precipitated (Fauser et al., 2013; Sánchez-Rodas et al., 2005; Zhao et al., 2013) (Fig. 4b). Concentrations of As in biota were also associated (but not significant) with salinity (Fig. 4c).

The water-sediment distribution coefficient (log $K_d$) of As generally increased as a function of salinity (Fig. 4d), which suggested that elevated log $K_d$ values could be explained by adsorption mechanisms including: i) "cation bridge" (i.e., divalent cations such as $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, etc. could form a bridge between anionic As and sedimentary organic matter) (Dang et al., 2014), ii) coprecipitation with metal-ion precipitates, and iii) anion exchange (Chan, 1999). Field-based BAFs of As were slightly inversely proportional to salinity, which indicates that As seemed to be more bioaccessible in freshwater organisms than in marine organisms (Fig. 4e). Among the target arsenicals, AB was the predominant organic form found in most of aquatic organisms, except for gastropods, especially enriched in regions with greater As concentrations in water and sediment. Meanwhile, salinity-dependent variation in As accumulation was inferred from the fact that AB contributed a greater proportion of total As in marine organisms from higher salinity environments (Fig. 4f and Fig. S3). AB might act as a cellular osmolyte, due to its structural similarity to glycine betaine which has been suggested to improve maintenance of ionic and osmotic homeostasis during seawater adaptation (Amlund and Berntssen, 2004; Caumette et al., 2012b; Whaley-Martin et al., 2012a). At this time the exact pathway in biogeochemistry of As in a salinity gradient system could be somehow limited, however, the results clearly indicated that the sallinity would be one particular concern governing the distribution and bioaccumulation of As in aquatic lotic systems.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2014.05.004.

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<Supplemental Materials>

# Species- and tissue-specific bioaccumulation of arsenicals in various aquatic organisms from a highly industrialized area in the Pohang City, Korea

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**Supplementary Tables**

Table S1. Information of target chemicals and reagents used in this study.

| Chemicals | Abbreviation | Concentration | Company |
|---|---|---|---|
| *Target compounds* | | | |
| Arsenite | $As^{III}$ | 1000 ppm | Inorganic Ventures Inc., Lakewood, NJ |
| Arsenate | $As^{V}$ | 1000 ppm | Inorganic Ventures Inc., Lakewood, NJ |
| Arsenobetaine | AB | > 95.0% | Sigma-Aldrich, St. Louis, MO |
| Arsenocholine bromide | AC | > 99% | Wako, Osaka, Japan |
| Disodium methyl arsenate | MMA | ~ 100% | Sigma-Aldrich, St. Louis, MO |
| Sodium cacodylate trihydrate | DMA | > 98% | Sigma-Aldrich, St. Louis, MO |
| *HPLC Mobile Phase* | | | |
| Ammonium carbonate | A | 1 mM | Fluka, Buchs, Switzerland |
| Ammonium phosphate | B | 12 mM | Fluka, Buchs, Switzerland |
| Ammonium nitrate | B | 12 mM | Sigma-Aldrich, St. Louis, MO |
| *Standard Reference Materials* | | | |
| Marine sediments | MESS-3 | | NRC, National Research Council, Canada |
| Lobster Tissue | TORT-2 | | NRC, National Research Council, Canada |
| Fish Protein | DORM-3 | | NRC, National Research Council, Canada |

Table S2. Instrumental conditions of HPLC and ICP/MS.

| | |
|---|---|
| HPLC system | PerkinElmer 200 |
| Column | Hamilton PRP X-100 (25 cm, 10 μm, 4.1 mm), Anion exchange column |
| Separation scheme | Gradient: A 100% (0-2 min) → B 100% (2-16 min) → A 100% (16-20 min) |
| Mobile phase A | 1 mM $(NH_4)_2CO_3$ |
| Mobile phase B | 12 mM $NH_4H_2PO_4$ + 12 mM $NH_4NO_3$ (pH 9.5) |
| pH adjustment | $NH_4OH$ |
| Flow rate | 1.5 mL $min^{-1}$ |
| Injection volume | 50 μL |
| ICP/MS system | ELAN DRC II |
| Nebulizer | Quartz concentric |
| Spray chamber | Quartz cyclonic |
| RF power | 1500 W |
| Analytes | AsO (m/z = 91), As (m/z = 75) |
| Reaction gas | $O_2$ = 0.6 mL $min^{-1}$ |
| RPq | 0.5 |
| Dwell time | 250 ms |

Table S3. Summary of quality assurance and quality control.

| Arsenicals | Stability test (Recovery) ($n$ = 5, %) | SRM test | | | | Method detection limits ($n$ = 7, μg $g^{-1}$ dw) |
|---|---|---|---|---|---|---|
| | | DORM-3 | | TORT-2 | | |
| | | Reference values[a] (μg $g^{-1}$ dw) | This study ($n$ = 3, μg $g^{-1}$ dw) | Reference values (μg $g^{-1}$ dw) | This study ($n$ = 3, μg $g^{-1}$ dw) | |
| AC | 78 ± 4 | < 0.080 | < DL | 0.043 | 0.29 ± 0.072 | 0.02 |
| AB | 92 ± 2 | 4.69 | 4.3 ± 0.32 | 14.25 | 14.0 ± 0.89 | 0.02 |
| DMA | 75 ± 6 | 0.459 | 0.47 ± 0.029 | 0.84 | 1.2 ± 0.066 | 0.02 |
| $As^{III}$ | 97 ± 7 | 0.085 | 0.13 ± 0.014 | - | < DL | 0.02 |
| MMA | 82 ± 9 | 0.091 | 0.064 ± 0.013 | 0.093 | 0.06 ± 0.002 | 0.03 |
| $As^{V}$ | 95 ± 5 | 0.243 | 0.31 ± 0.015 | 0.0928 | 0.89 ± 0.037 | 0.06 |

[a]Reference values of As species in DORM-3 and TORT-2 were presented in previous articles (Leufroy et al., 2011; Wahlen et al., 2004).

Table S4. Summary of white line energies for arsenicals reported in previous literatures (Smith et al., 2005).

| Arsenicals | White line energy (eV) |
|---|---|
| AsS, $As(Glu)_3$ | 11870.0 |
| $As^{III}$ | 11871.7 |
| AB, AC, Tetra, C2-AB, C3-AB | 11872.6 |
| Arsenosugars | 11873.3 |
| MMA | 11874.1 |
| DMA, $As^{V}$ | 11875.3 |

**References**

Leufroy, A., Noël, L., Dufailly, V., Beauchemin, D., Guérin, T., 2011. Determination of seven arsenic species in seafood by ion exchange chromatography coupled to inductively coupled plasma-mass spectrometry following microwave assisted extraction: Method validation and occurrence data. Talanta 83, 770-779.

Smith, P.G., Koch, I., Gordon, R.A., Mandoli, D.F., Champman, B.D., Reimer, K.J., 2005. X-ray absorption near-edge structure analysis of arsenic species for application to biological environmental samples. Environ. Sci. Technol. 39, 248-254.

Wahlen, R., McSheehy, S., Scriver, C., Mester, Z., 2004. Arsenic speciation in marine certified reference materials Part 2. The quantification of water-soluble arsenic species by high-performance liquid chromatography-inductively coupled plasma mass spectrometry. J. Anal. At. Spectrom. 19, 876-882.

**Supplementary Figures**

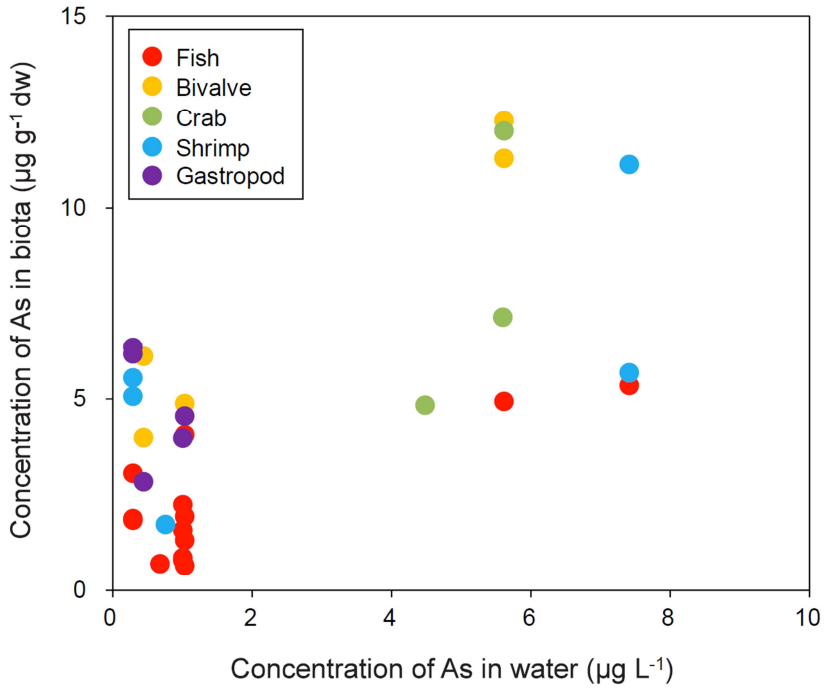


Fig. S1. Relationship between concentration of total arsenic in water and biota samples from the Pohang area, Korea.

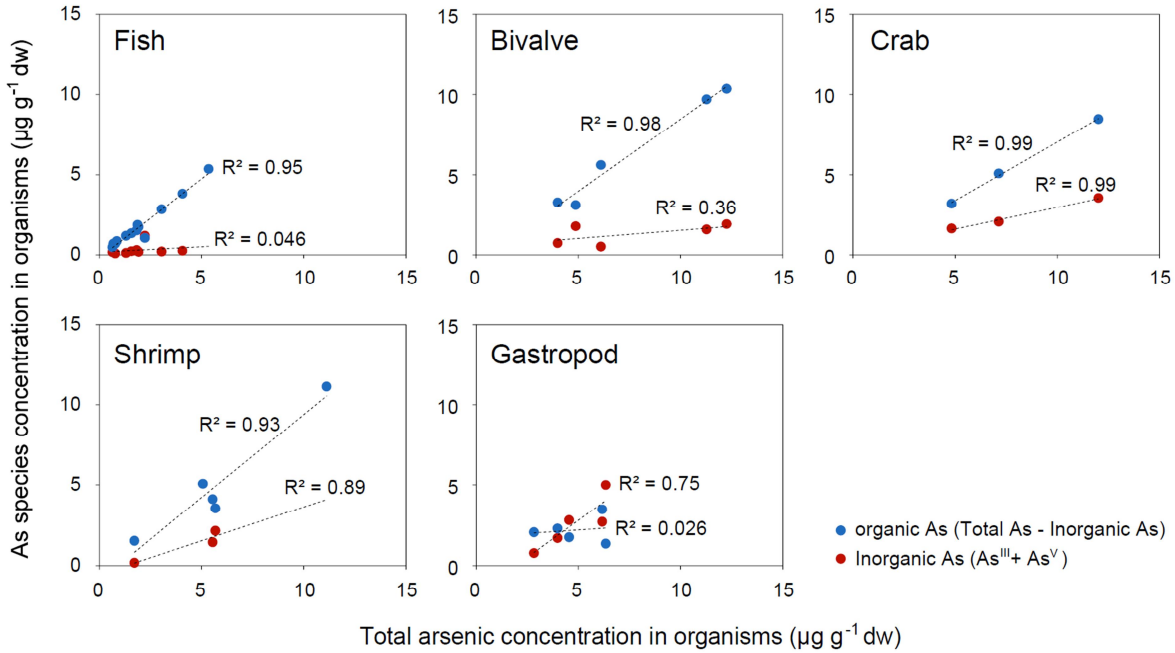


Fig. S2. Relationship between concentration of total arsenic and arsenic species in biota samples from the Pohang area, Korea.

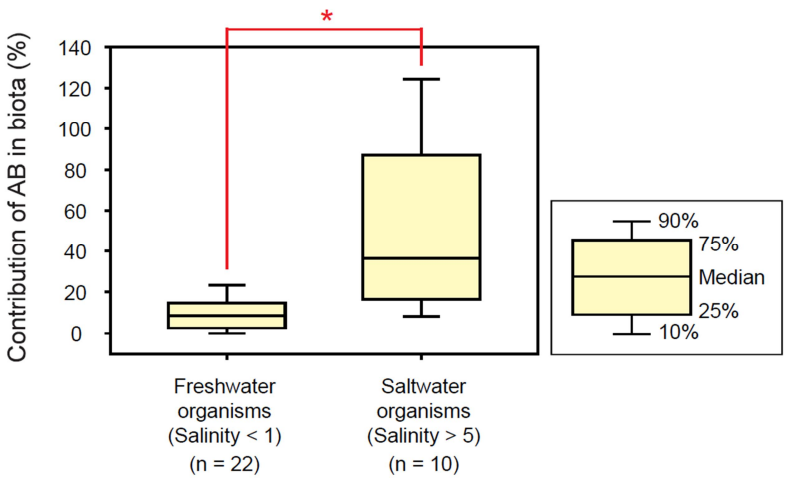


Fig. S3. Boxplot for the contribution of arsenobetaine in aquatic organisms from freshwater (S < 1) and saltwater (S > 5) in the Pohang area, showing comparison between two groups by the Welch-Aspin test (Asterisk (*) indicates significance at the level $p < 0.001$).