Marine Pollution Bulletin 63 (2011) 124–134

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Stable isotope-guided analysis of biomagnification profiles of arsenic species in a tropical mangrove ecosystem

Nguyen Phuc Cam Tu [a], Tetsuro Agusa [b,c], Nguyen Ngoc Ha [b], Bui Cach Tuyen [d,1], Shinsuke Tanabe [b], Ichiro Takeuchi [a,*]

[a] Faculty of Agriculture, Ehime University, Tarumi 3-5-7, Matsuyama, Ehime 790-8566, Japan
[b] Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama, Ehime 790-8577, Japan
[c] Department of Legal Medicine, Shimane University Faculty of Medicine, Enya 89-1, Izumo, Shimane 693-8501, Japan
[d] Research Institute for Biotechnology and Environment (RIBE), Nong Lam University, Hochiminh City, Vietnam

ARTICLE INFO

Keywords:
Arsenic species
Arsenobetaine
Biomagnification
Stable isotope ratio
Tropical mangrove ecosystem
Vietnam

ABSTRACT

We performed stable carbon and nitrogen-guided analyses of biomagnification profiles of arsenic (As) species, including total As, lipid-soluble As, eight water-soluble As compounds (arsenobetaine (AB), arsenocholine (AC), tetramethylarsonium ion (TETRA), trimethylarsine oxide (TMAO), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenate (As[V]), and arsenite (As[III])), and non-extracted As in a tropical mangrove ecosystem in the Ba Ria Vung Tau, South Vietnam. Arsenobetaine was the predominant As species (65–96% of water-soluble As). Simple linear regression slopes of log-transformed concentrations of total As, As fractions or individual As compounds on stable nitrogen isotopic ratio ($\delta^{15}N$) values are regarded as indices of biomagnification. In this ecosystem, lipid-soluble As (slope, 0.130) and AB (slope, 0.108) were significantly biomagnified through the food web; total As and other water-soluble As compounds were not. To our knowledge, this is one of the first reports on biomagnification profiles of As compounds from a tropical mangrove ecosystem.

1. Introduction

In the last decade, arsenic (As), a metalloid released from both natural and anthropogenic sources, has been detected in various biota and environmental samples across South Vietnam (e.g., Ikemoto et al., 2008a; Tu et al., 2008a,b; Agusa et al., 2009). There is concern about sources and concentrations in food items that are frequently consumed by humans and wildlife. Arsenic ranks as the number one toxic agent in the Agency for Toxic Substances and Disease Registry Priority List of Hazardous Substances (ATSDR, 2007). Toxicity, bioavailability and trophic transfer of As depend on its chemical form (Neff, 1997). Generally, toxicity of inorganic As (arsenate, As[V] and arsenite, As[III]) is higher than that of methylated molecules, such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TETRA) (Gebel, 2001). Arsenobetaine (AB) and other organoarsenic compounds found in seafoods are not toxic, mutagenic, or carcinogenic when ingested in food by mammals (Neff, 1997). Marine algae are able to accumulate dissolved As[V] from seawater and then synthesize organoarsenic compounds including MMA, DMA and several As-containing ribosides (Francesconi and Edmonds, 1994). Arsenobetaine is the most abundant form of As in marine animals, including fish, crustaceans, and mollusk (Francesconi and Edmonds, 1993). The origin of AB in animals is likely to be the As-containing ribosides in algae (Francesconi and Edmonds, 1994). The sources of other As compounds found in the marine environment are still not clear.

The determination of As species in a variety of samples from different trophic levels might help reveal the biosynthetic pathway of As in marine environments and its transfer through food chains. To date, there have been few studies of the accumulation and distribution of As compounds in marine animals in relation to trophic status (Goessler et al., 1997; Kirby and Maher, 2002; Kirby et al., 2002; Foster et al., 2005, 2006, 2008; Khokiattiwong et al., 2009), and none has used stable isotope analysis to guide determinations of trophic level-dependent accumulation of As compounds in marine food webs.

In order to evaluate food web structure and energy pathways in aquatic ecosystems, stable isotope ratio analyses of bio-elements such as carbon and nitrogen have become increasingly useful for tracing contaminants in food webs. In general, the stable nitrogen isotope ratio ($\delta^{15}N$) of a predator is about 3–5‰ higher on average than that of its prey (Minagawa and Wada, 1984), allowing identi-

* Corresponding author. Address: Department of Life Environment Conservation, Faculty of Agriculture, Ehime University, Tarumi 3-5-7, Matsuyama, Ehime 790-8566, Japan. Tel./fax: +81 89 946 9899.
E-mail address: takeuchi@agr.ehime-u.ac.jp (I. Takeuchi).
[1] Present address: Vietnam Environmental Protection Administration, 67 Nguyen Du Street, Hai Ba Trung District, Hanoi, Vietnam.

0025-326X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2011.03.002

fications of species' trophic position in a food web. In contrast, the stable carbon isotope ratio ($\delta^{13}C$) increases only slightly with increasing trophic level (about 1‰ per trophic level). For this reason, $\delta^{13}C$ is used mostly to identify dietary carbon sources in a food web (DeNiro and Epstein, 1978; Peterson and Fry, 1987). Accordingly, simultaneous measurement of $\delta^{15}N$, $\delta^{13}C$ and contaminants in the same biota samples can be used to determine biomagnification profiles of contaminants through food webs. In particular, regression slopes of log transformed contaminant levels on $\delta^{15}N$ have been used for quantitative representation of trophic position in determinations of biomagnification rates of trace element and anthropogenic chemicals through food webs in South-East and East Asia (e.g., Ikemoto et al., 2008a,b; Murai et al., 2008; Matsuo et al., 2009; Takeuchi et al., 2009).

The objective of this study was to assess stable isotope-guided trophic transfers of As compounds in a tropical mangrove ecosystem; we analyzed total As, lipid-soluble As, eight water-soluble As compounds (AB, arsenocholine (AC), TETRA, TMAO, DMA, MMA, As[V], and As[III]), and non-extracted As in a wide range of species collected from an integrated shrimp mangrove farming system (ISMFS) in Ba Ria Vung Tau Province (BRVT), South Vietnam.

2. Materials and methods

2.1. Study area and sampling

The ISMFS is a form of extensive aquaculture that relies on trapping wild seed during high tides; no provisions are made for supplementary feeding of shrimp (Binh et al., 1997). All samples in this study were collected from a 12 ha ISMFS in the BRVT (10°31.310′N; 107°03.438′E) (Fig. 1) from 9 to 11 March 2007. The study site was located approximately 70 km southeast of Hochiminh City and about 30 km from the town of Vung Tau, BRVT.

Barramundi seabass *Lates calcarifer* were caught by angling on 9 and 10 March 2007. On the night of 10 March 2007, we collected the other fish, crustaceans and octopus using a bag net mounted on the water gate. On 11 March 2007, suspended particulate matter (SPM) sample was collected with a North Pacific plankton net (NORPAC) (0.1 mm mesh size) deployed horizontally at the sluice gate. SPM was washed from the sides of the net into acid-washed plastic bottles with a small volume of pond water. In the laboratory, samples collected with the plankton net were kept in a refrigerator for two to four hours to allow settling. After settling, a brown layer that sank close to the bottom of the bottle was designated SPM. In total, five species of crustaceans, six species of fish, and one species of cephalopod and SPM were collected. These samples were kept frozen at <−20 °C until dissection and chemical analysis.

2.2. Stable isotope analysis

In order to avoid contamination by digestive tract contents, we used muscle tissues of crustaceans, cephalopod, and fish as the standard for stable isotope samples. All samples prepared for $\delta^{13}C$ and $\delta^{15}N$ analyses were rinsed in distilled water, dried in an oven for 24 h at 60 °C, pulverized to a fine powder, and treated with a 2:1 chloroform–methanol solution for 24 h to remove lipids. Samples were then dried under ambient room conditions. SPM was soaked in 0.1 N HCl to remove carbonates, rinsed in distilled water and then dried. Homogenized samples of 1.0 mg were loaded into tin cups, after which, stable carbon and nitrogen isotopes were measured using a gas chromatograph–combustion–isotope ratio mass spectrometer (PDZ Europa Ltd., ANCA-SL). Stable isotope abundances were expressed in the δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X(‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

where X is ^{13}C or ^{15}N, and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The $R_{standard}$ values were based on PeeDee Belemnite for $\delta^{13}C$ and atmospheric nitrogen (N_2) for $\delta^{15}N$. Replicate measurements of the internal laboratory standard (histidine) indicated measurement errors of ±0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ determinations.

2.3. Analysis for total As, As fractions and As compounds

For total As, As fraction and As compound analyses, whole homogenized samples were weighed, deep-frozen and lyophilized for 24 h. They were weighed again to determine water contents, and then ground to a fine powder using a mortar and pestle. The average moisture contents were 84.1% in SPM, 69.1 ± 5.1% in crustaceans, 76.8 ± 3.5% in cephalopod, and 70.3 ± 4.4% in fish.

We used previously described procedures for analyzing total As, fractions and compounds of As (Kubota et al., 2002; Agusa et al., 2008) with several considerable modifications. To analyze total As, approximately 0.2 g of freeze-dried samples were digested with nitric acid in Teflon vials in a microwave oven (Ethos D, Milestone S.r.l., Sorisole, BG, Italy) under controlled pressure conditions; there were seven digestion steps: 2, 3, 5, 5, 5, and 10 min under 250, 0, 250, 400, 500 and 400 W, respectively, and ventilation for 5 min. The resulting clear solutions were made up to exactly 50 ml with Milli-Q water. Concentration of total As was determined with an inductively coupled plasma-mass spectrometer

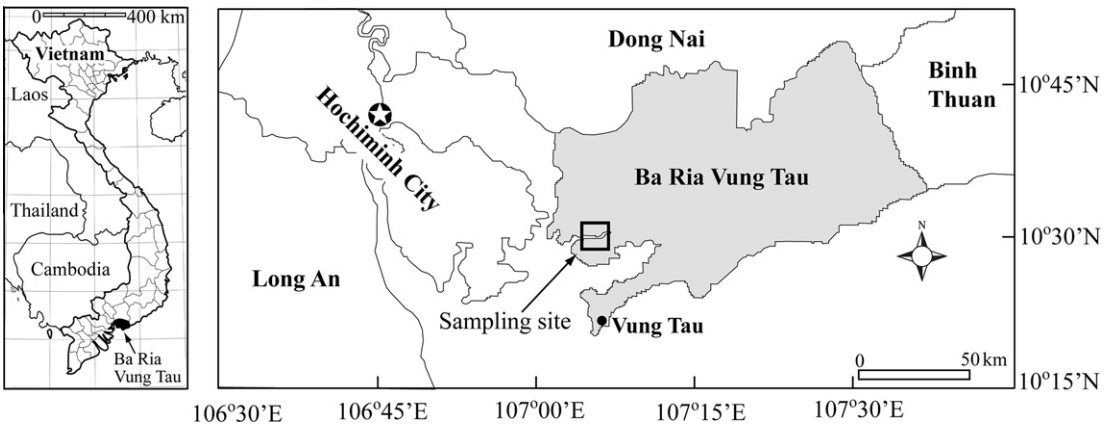

Fig. 1. Map of the study area showing the location of sampling sites in the integrated shrimp mangrove farming system at Ba Ria Vung Tau, South Vietnam.