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Accumulation of Arsenic in Pacific Oysters, *Crassostrea gigas*, Collected from Aquaculture Sites in Western Taiwan

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ABSTRACT

Oyster, farmed inland on the west coast of Taiwan, can provide a good indication of pollution levels in aqua environments, which may be affected by upland watershed, industrial and municipal waste water discharge, precipitation and local runoff. Using a continuous flow-hydride generation-atomic absorption technique, this study measured arsenic concentrations in Pacific oysters (*Crassostrea gigas*) collected from several aquaculture sites along the west coast of Taiwan over different seasons. The arsenic concentrations ranged between 8.83 and 19.51 mg/kg dry wt. (mean 13.7 ± 2.2 mg/kg). Excluding Hsianshan, a suspected source of toxin, it was possible to create an exponential decay model between the arsenic content in oyster and accumulated precipitation. We found the lifetime cancer risk associated with the inorganic arsenic in oysters to be 4.2×10^{-6}, which is 4 times higher than what is considered acceptable by the U.S. Environmental Agency.

Key words: arsenic, oyster, aquaculture, cancer risk, Taiwan

INTRODUCTION

Oysters filter 4-40 L of water per hour per oyster[1], making them sentinel organisms able to indicate the level of environmental pollutants, and also able to remove the pollutants from the marine environments[2,3]. Oyster is an important inshore aquaculture product on the west coast of Taiwan[4]. However, with the growth of the population and increase in commercial activities in these areas over the last few decades, several incidents of oyster pollution were suspected in accordance with anthropogenic contamination[5]. The factors affecting the aquafarming environment include fresh water inflow from upland watersheds, industrial and municipal waste water discharge, precipitation and local runoff[6].

In marine organisms, there are many species of arsenic, with the inorganic arsenic species being more toxic than organic arsenic species[7]. The most abundant arsenic species present in marine fauna, especially in shellfish, is arsenobetaine[8,9], which is quickly excreted from human bodies and is considered to be harmless[10,11]. Since most of the arsenic in shellfish is of organic form, generally believed to be noncarcinogenic, the cancer risk assessment of arsenic in the shellfish should be based on concentrations of inorganic arsenic species, rather than the total amount of arsenic[12].

This study began with the accurate monitoring of the level of arsenic, in oysters from the west coast of Taiwan, on the effects of the temporal and spatial variability. The collected data were used to (1) assesses the influence of climate change on body burden of arsenic in oysters; and (2) estimate the target cancer risk and the weekly intake amount of arsenic that oysters can be tolerated by humans.

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MATERIALS AND METHODS

I. *Chemicals*

Nitric acid, sulfuric acid, potassium persulfate, hydrochloric acid, potassium iodide, sodium hydroxide, and sodium borohydride used were all reagent grade (Riedel-deHaën). Arsenic primary standard (1000 mg/L) was obtained from J.T. Backer. Reagent water with resistivity equaling or greater than 18 MΩ-cm was used for preparation of solutions. The calibration standards were prepared by adding 8 mL of concentrated HCl and 10 mL of 20% KI (w/v) solution, with adequate quantity of arsenic primary standard, and diluting to 50 mL. The concentrations of calibration standards were 0, 0.50, 1.00, 1.50, and 2.00 μg/ L, respectively.

II. *Method of Validation and Fresh Samples*

A retail dried oyster was purchased from Ilan (east coast of Taiwan) to use as a test sample to first establish the optimized analytical parameters. Three standard reference materials, DORM-2/NRCC (dogfish muscle), DOLT-2/NCRR (dogfish liver) and 1566b/NIST (oyster tissue), were used to validate the analytical procedure.

Fresh oyster (*Crassostrea gigas*) samples were collected from six aquaculture sites located on the west coast of Taiwan: Hsianshan, Wanggung, Taishi, Budai, Tainan, and Dunggung (Figure 1). Samples were collected at low

tide in March, May, August and November 2002. All samples were kept at 4˚C until they arrived at the laboratory, where they were immediately frozen at -20˚C. Laboratory samples were prepared by freeze-drying gross samples with a lyophilizer (Savant, Speedvac Plus SC110A) and by homogenization with agate motars and pestles.

III. *Analytical Procedure*

Dried and homogenized oyster samples, 0.1 g each, were placed into separate 125-mL conical flasks into which 20 mL of concentrated nitric acid had first been added. The flasks were placed on a hot plate until the solutions were evaporated to less than 1 mL. Then, 50 mL of reagent water, 4 mL of 2.5 N sulfuric acid, and 5 mL of 5% (w/v) potassium persulfate were added and digested. When the solution was nearly dry, 8 mL of concentrated hydrochloric acid and 10 mL of 20% (w/v) potassium iodide were added. Reagent water was used to increase the final volume to 50 mL. The solutions were then warmed in a 60˚C water bath for 1 hr to assure the arsenic was completely reduced to arsenite form. They were analyzed within 6 hr.

IV. *Measurement of Arsenic*

A continuous flow-hydride generation-atomic absorption spectrophotometer (CF-HG-AAS) was used to measure arsenic in the prepared samples. The continuous flow-hydride generator (Model HFS-2, Hitachi), equipped with a single peristaltic pump at a flow rate of 8.5 mL/min, delivered and mixed the sample solution with on-line hydrochloric acid (5%, v/v) and sodium borohydride (0.75%, w/v) in 0.04% NaOH (w/v). Arsine was separated from the liquid and carried to the AAS with the auxiliary argon gas at flow rate of 300 mL/min. Arsine was detected with an Hitachi Z-8200 atomic absorption spectrophotometer (AAS) with a flame heated quartz-tube atomizer (QTA) set at the selected parameters: wavelength (193.7 nm), band pass (1.3 nm), lamp current (10.0 mA), air flow (13.6 L/min), acetylene flow (1.4 L/min), burner height (10.0 mm) and time constant (2.0 sec).

RESULTS AND DISCUSSION

I. *Method Performance*

Repeatability of the analytical procedures was tested by taking replicate measurements of a retail dried oyster (n = 4), resulting in a relative standard deviation 4.1% (Table 1). Accuracy was tested by measuring the arsenic in three standard reference materials, DORM-2/NRCC, DOLT-2/NCRR and 1566b/NIST, resulting in 102.4, 101.2 and 104.7%, respectively. To assure us that the matrix effect was not affecting the analytical accuracy of our field samples, we routinely performed the spike recovery test, which ranged between 95.0 and 106.3% (Table 2).

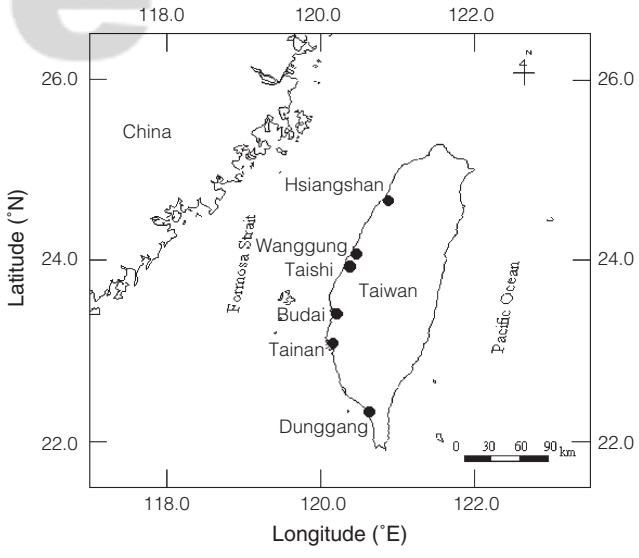

Figure 1. Map of the sampling sites in this study.

Table 1. Method repeatability and recoveries of arsenic in standard reference materials

Sample	As concentration (mg/kg)	Certified value (mg/kg)	Recovery
Retail dry Oysters	9.78(± 4.1%)[a]	NA[b]	NA
DORM-2	18.44(± 2.3%)	18.0 ± 6%	102.4%
DOLT-2	16.64(± 1.7%)	16.6 ± 7%	101.2%
1566 b	8.01(± 1.1%)	7.65 ± 8%	104.7%

[a]Relative standard deviation in parentheses (n = 4).
[b]NA: not available.

II. *Arsenic in C. gigas*

The average size, weight and water content of the sampled oysters are shown in Table 2. The water content ranged between 81.2 and 95.8% (w/w), averaging 84.7 ± 4.2%. Arsenic concentrations in the oysters were based on the dry weight, the highest arsenic value being observed in oysters obtained from Tainan in May 2002 (19.51 mg/kg), and the lowest from those obtained from Dunggang on August 2002 (8.83 mg/kg). The average overall concentration of arsenic in the oyster samples was 13.7 ± 2.2 mg/kg. In other countries, the arsenic concentrations in oysters have been reported to be as low as 5.69 mg/ kg at Venice of Italy[13] and as high as 26.7mg/kg at Arcachon Bay in France[14]. On the west coast of Taiwan, the arsenic concentrations in oysters have been reported in range between 0.025 and 29.3 mg/kg by Han *et al*.[13] Mean values of arsenic concentrations presented in this study was in accordance with those reported by previous studies, though the variance in this study was lower than that reported by Han *et al*.[15]

Individual averages of each sampling site were ranked as follows: Hsianshan (15.18 ± 1.12) > Taishi (13.72 ± 1.88) > Wanggang (13.21 ± 1.24) > Budai (12.63 ± 1.96) > Dunggang (12.45 ± 2.56). Data from Tainan was excluded because only two observations were available. Analysis of variance (ANOVA) of data from the five sets of data (exclud-

ing Tainan) resulted in an experimental $F_0 = 1.53$. Since $F_{0.10}(4,15) = 2.36$ and $F_{0.05}(4,15) = 3.06$, both 90% or 95% confidence levels revealed that the difference between the arsenic values from different sites was insignificant.

Temporal averages of each sampling time were ranked as follows: March (14.48 ± 0.82) > May (14.36 ± 2.51) > November (13.47 ± 1.28) > August (11.58 ± 2.49). Because environmental factors influence arsenic accumulation in oysters[16], we interpreted seasonal variation using the accumulated precipitation that took place during the two-month period prior to the months that the samples were collected. As shown in Figure 2, arsenic concentrations

Table 2. Data regarding samples, their environment, and concentrations of arsenic, 2002

Site	Sampling month	Accumulated precipitation[a] (mm)	Information of samples[b]				As information[c]	
			Length (cm)	Width (cm)	Weight (g)	Water content (%, w/w)	Original conc. (mg/kg dry wt.)	Spike recovery (%)
Hsianshan	March	78.5 (Jan+Feb)	3.6(0.5)	2.3 (0.5)	8.9 (1.1)	94.8 (1.5)	14.90	—
	May	149.9 (Mar+Apr)	4.7 (0.9)	2.9 (0.6)	9.3 (0.8)	88.6 (1.9)	16.92	—
	August	406.3 (Jun+Jul)	5.1 (1.2)	3.0 (0.5)	11.7 (3.0)	84.0 (2.2)	15.77	—
	November	182.9 (Sept+Oct)	4.6 (1.5)	3.0 (0.5)	10.9 (1.2)	89.8 (2.0)	14.36	106.3
Wanggung	March	10.5 (Jan+Feb)	4.5 (0.7)	2.5 (0.5)	7.3 (3.2)	96.9 (5.2)	14.75	—
	May	5.0 (Mar+Apr)	4.5 (0.6)	2.8 (0.7)	10.4 (1.2)	85.9 (1.4)	13.90	—
	August	331.0 (Jun+Jul)	3.6 (0.8)	2.5 (0.5)	7.4 (1.5)	84.3 (2.1)	11.94	—
	November	41.0 (Sept+Oct)	3.7 (0.6)	2.7 (0.5)	7.9 (0.8)	86.9 (1.0)	12.76	101.1
Taishi	March	29.5 (Jan+Feb)	5.1 (0.7)	3.4 (0.6)	11.8 (1.6)	89.5 (4.3)	15.95	—
	May	20.5 (Mar+Apr)	4.5 (0.5)	2.7 (0.4)	8.7 (2.8)	86.4 (2.3)	12.71	—
	August	761.5 (Jun+Jul)	4.5 (0.8)	2.8 (0.6)	7.4 (1.5)	87.2 (2.1)	11.77	—
	November	153.5 (Sept+Oct)	5.0 (1.5)	3.0 (0.8)	10.5 (1.9)	87.3 (1.9)	14.63	95.0
Budai	March	34.0 (Jan+Feb)	4.7 (1.1)	2.8 (0.4)	11.0 (3.0)	95.6 (2.3)	13.71	—
	May	4.6 (Mar+Apr)	4.7 (0.9)	3.3 (0.4)	12.6 (2.1)	88.2 (2.9)	15.25	—
	August	767.7 (Jun+Jul)	4.2 (0.7)	2.9 (0.5)	7.3 (1.4)	87.3 (1.8)	11.28	—
	November	172.9 (Sept+Oct)	4.7 (1.2)	3.3 (0.9)	11.4 (2.6)	90.1 (2.9)	11.24	100.9
Tainan	May	0.8 (Mar+Apr)	5.9 (1.3)	2.7 (0.6)	9.6 (1.6)	81.2 (2.4)	19.51	—
	November	23.0 (Sept+Oct)	3.8 (0.7)	2.9 (0.3)	5.7 (1.5)	82.3 (1.6)	14.19	102.1
Dunggang	March	23.0 (Jan+Feb)	5.2 (0.9)	2.8 (0.7)	6.9 (2.2)	95.8 (2.8)	14.34	—
	May	17.5 (Mar+Apr)	4.7 (1.1)	3.0 (0.5)	10.4 (1.4)	88.8 (2.0)	13.75	—
	August	480.0 (Jun+Jul)	5.5 (0.8)	3.2 (0.5)	8.9 (1.7)	85.8 (1.5)	8.83	—
	November	32.0 (Sept+Oct)	4.7 (1.5)	3.0 (0.4)	7.4 (1.7)	91.1 (3.0)	13.65	98.5

[a]Two months accumulated precipitation prior to the sampling month; source from Central Weather Bureau, Taiwan (http://www.cwb.gov.tw/V4/index.htm).

[b]Mean and standard deviation in parenthesis, n = 20. (Courtesy of Professor S.M. Liu, National Taiwan Ocean University, Taiwan)

[c]Spike arsenic standard 10 mg/kg (spike 1 mL of 1 mg/L of arsenic standard, and test sample weight 0.1 g).

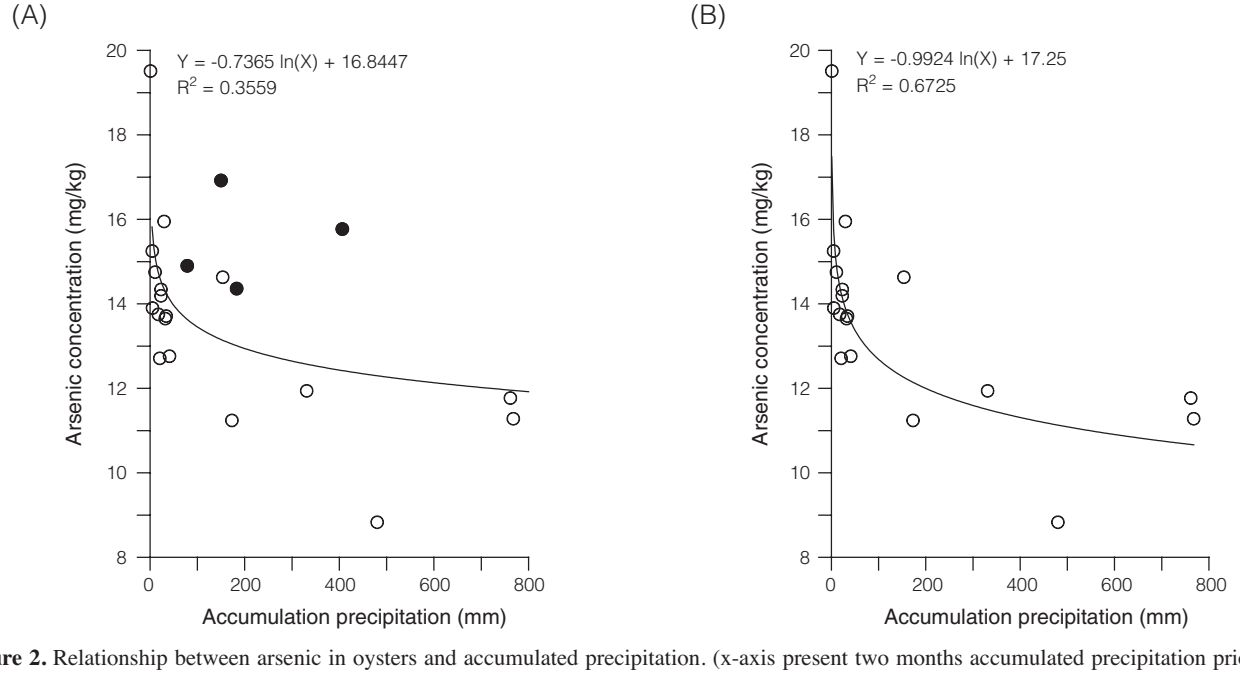

Figure 2. Relationship between arsenic in oysters and accumulated precipitation. (x-axis present two months accumulated precipitation prior to the sampling month; ○: Hsianshan's samples, ●: other sites' samples).

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decreased with increases in accumulated precipitation. The highest concentration of arsenic was found in the samples collected from Tainan in May, which had a low level of accumulated precipitation (0.8 mm), while the lowest concentration of arsenic was found in samples collected from Dunggang in August, which had a high level of accumulated precipitation (480 mm).

The growth rate of the oysters has been found to be positively correlated with both temperature and abundance of phytoplankton[17], and the geographic patterns of temperature and precipitation patterns tend to differentiate the contaminant body burdens and population health of the oyster in adjacent bays[18,19]. In the present study, oysters had lower concentrations of arsenic during the wet season and higher concentrations in the dry season. Looking at the data together, the arsenic concentrations in the oysters and the two-month accumulated precipitation did not seem to be well correlated with the exponential decay regression (R^2 = 0.3559) (Figure 2-A). However, Figure 2 (A) shows that precipitation was not found to be relevant to arsenic accumulation in Hsianshan. If data from Hsianshan were excluded, we found an acceptable coefficient of determination (R^2 = 0.6725), from which could be interpreted that arsenic accumulation was in general affected by climate. With the arsenic concentration in Hsianshan being high (15.77 mg/kg) in August and the accumulated precipitation also high (406.3 mm), consequently, we suspected there was an anthropogenic source of arsenic.

III. *The Health Risk Assessment*

The model for estimating target cancer risks for lifetime cancer risks (U.S. EPA 1996)[20] is:

$$TR = \frac{EFr \times EDtot \times SFI \times MCS \times CPSo}{BWa \times ATc} \times 10^{-3}$$

where TR: target cancer risk; EFr: exposure frequency (350 days/year); EDtot: exposure duration, total (30 years); SFI: seafood ingestion (g/day); MCS: metal concentration in edible portion of seafood (μg/g); CPSo: carcinogenic potency slope, oral (risk per mg/kg/day); BWa: body weight, adult (65 kg); ATc: averaging time, carcinogens (25,550 days).

Because organic arsenic is generally believed to be noncarcinogenic[21], carcinogenic effects are usually estimated based on the concentrations of inorganic arsenic species, with the CPSo value of inorganic arsenic being 1.5 mg/kg/day as suggested by U.S. EPA[20]. It has been reported that most of the arsenic in seafood is organic[22,23]. The predominant arsenic species found in oysters on the west coast of Taiwan is arsenobetaine, making up an average of 50.4% of the total arsenic[24]. Based on dry weight measurement, inorganic arsenic makes up only 1.0%, the overall mean being 0.15 mg/kg[24]. Table 2 shows the average water content of our sample oysters to be 84% (w/w), which made the inorganic arsenic concentration come to 0.024 μg/g based on fresh weight. Therefore, the value 0.024 μg/g was considered as an MCS in the estimation of the TR value in this study. More than 90% of the residents in Taipei, Taiwan, consume less than 18.6 g/day of seafood[15]. This value was selected as SFI in this study. By substituting these values, the target cancer risks (TR) was 4.2×10^{-6}, which is 4 times higher than what would be considered acceptable by the U.S. EPA. The provisional maximum tolerable daily intake of inorganic arsenic defined by World Health Organization is 2 μg/kg (WHO, 1983)[25], thus the tolerable weekly intake by a 60 kg adult is 840 μg. On account of inorganic arsenic in this study was 0.024 μg/g based on fresh weight, the weekly tolerable intake of oysters was estimated to be 35 kg.

CONCLUSIONS

Using a validated method, this study investigates temporally and spatially the concentrations of arsenic in oysters. While the difference between sampling sites was found to be insignificant by ANOVA analysis, when outlying data from Hsianshan was excluded, the arsenic concentrations were found to be high in the dry season and low during the wet season. Target risk and tolerable intake of the arsenic in oysters were also established in present study.

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