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Seasonal bioconcentration of heavy metals in *Onchidium struma* (Gastropoda: Pulmonata) from Chongming Island, the Yangtze Estuary, China

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Abstract

The seasonal concentration changes of selected heavy metal Cd, Cr, Cu, Fe, Mn, Pb, and Zn in five tissues of marine gastropod *Onchidium struma* were studied in the Chongming Island, the Yangtze Estuary in April 2007, July 2006, September 2006, and November 2006, respectively. The results demonstrated that the bioconcentration factor of Cu (biomass/water) in all selected tissues was about 10^4 magnitudes, Fe and Cd were 10^3, Zn was 10^2, and Mn, Pb, and Cr were 10^1. Hepatopancreas was proven to be the dominant storage tissue of Cr, Cu, Mn, and Zn, whereas Fe and Pb were mainly stored in muscle and digenetic gland, and Cd was stored in vitelline gland and albumen gland. Additionally, it was found that Cu, Fe, Mn, and Zn were concentrated significantly by *O. struma* (whole-body) in summer or autumn, and Cd, Cr, and Pb increased slightly in spring and winter. Furthermore, the bioconcentration of Cr was nearly 2-fold higher and Zn was 1.6-fold higher in the water compared with the Water Quality Standard for Fisheries. With view of excessive amount of Pb, Cd, and Cu according to seafood standard, the consumption of *O. struma* might have the risk of health hazard.

Key words: *Onchidium struma*; heavy metal; bioconcentration; the Yangtze Estuary
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Introduction

Anthropogenic pollutants have contaminated many ecosystems, such as sea, estuaries, and rivers (Campbell and Evans, 1991; Blais and Kalff, 1993). Heavy metals are now recognized to be among the most relevant contaminants in the marine environment and their concentrations are elevating in some coastal waters (Ober *et al*., 1987; Schuhmacher *et al*., 1990; García *et al*., 2001). Since aquatic organisms living in polluted ecosystems often bioconcentrate metals into their tissues, it has been argued widely that these organisms can be used as biomonitors indicating the bioavailability of contaminants and the degree of pollution (Luten *et al*., 1986; López-Artiguez *et al*., 1989; Peerzada and Kozlik, 1992; Schuhmacher, 1996). With a wide geographical distribution, high abundance in the benthic environment, selective absorption of certain ions and sedentary nature, mollusks are considered suitable as biomonitors (Sawidis *et al*., 1995; Blackmore, 1999; Blackmore and Wang, 2003). Mollusks, therefore, are used in monitoring heavy metals and other pollutants in the estuarine waters and other marine environments (Bryan *et al*., 1980, 1985; Sally and Bobby, 1996).

Onchidium (Gastropoda: Pulmonata) is one of the most widespread mollusks across semitropical coast, especially throughout the Indo-Pacific estuaries. Previous studies on *Onchidium* have been mostly focused on hormonal control (Hanumante *et al*., 1979; Deshpande *et al*., 1980), metabolism regulation (Hanumante and Deshpande, 1980; Chew *et al*., 1999), and reproductive physiology (Deshpande and Nagabhushanam, 1983). As for *Onchidium struma*, reports have been mainly targeted on its ecological habit (Huang *et al*., 2004), embryonic and larval development (Wang *et al*., 2005), as well as reproductive system and gonadal development (Wang *et al*., 2006). However, few studies report the bioconcentration of heavy metals in *Onchidium* (*Onchidium struma*) and their toxic effects. Marine gastropod *O. struma*, used in this study, was collected from Chongming Island. And the selected sampling site is located in the northern branch of the Yangtze Estuary, which is potentially new water resource for the regions nearby especially for Shanghai. So the bioconcentration of heavy metals in this area is of great importance to its water quality as well as to the exploitation and development in those regions nearby (Lim *et al*., 1996). Furthermore, it was found that the abundance of *O. struma* in this area was higher than other areas in the Chongming Island. Additionally, *O. struma* is being consumed by local people and potentially cultured in a large-scale in this area (Huang *et al*., 2004). Moreover, it feeds on the humus of sediments in the estuary where various pollutants are enriched heavily.

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Hence, the consumption of *O. struma* probably constitutes a health hazard. Levels of heavy metals in the water and sediments as well as bioconcentration in *O. struma* from the Chongming Island are of profound interest not only because they can be used to document those geographic areas where metal pollution might be problematic, but also because they may transfer potential health hazards to consumers.

Numerous surveys for metal concentrations in the Yangtze Estuary have been undertaken in recent years (Zhang, 1999; Lin *et al*., 2002; Feng *et al*., 2004; Qiao *et al*., 2007; Quan *et al*., 2007). It should be taken into account that differences in the size, age, genetic difference, gonadal maturation, individual variability in metal uptake, induction of metal-binding proteins, sampling season and so on. These factors may also influence the results (Lytle and Lytle, 1990; Páez-Osuna and Marmolejo-Rivas, 1990). Therefore, differences coming from metal category (essential or non-essential), seasonal physicochemical conditions (water temperature, pH, salinity, and dissolved oxygen), species speciality (hibernation), individual parameters (body weight and length), population density, and tissue-specificity were taken into consideration in this study. Furthermore, by detecting the metal concentrations (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in the water, sediments and tissues of *O. struma* seasonally, this study will provide basic information on heavy metal bioavailability in the marine water, sediments and mollusk within selected territory to evaluate metal pollution and ecosystem quality together with providing references for the large-scale culture of *O. struma* in this area.

1 Materials and methods

1.1 Sample collection

Marine gastropod *O. struma* was collected from Beibao Harbor (Station O, Fig. 1) of Chongming Island, Shanghai, China (31°38′12.7″N, 121°41′03.4″E) in July 2006, September 2006, November 2006, and April 2007, respectively. Approximately 100 marine gastropods were collected from three sites (located in upper, middle, and low tidal flat respectively) in Station O and placed in polyethylene plastic barrels, ten of them were randomly selected at each site, dissected into hepatopancreas, muscle, albumen gland, vitelline gland and digenetic gland, and then kept frozen at –80°C after measuring the weight and length. Samples of water and sediments were also collected in the same time.

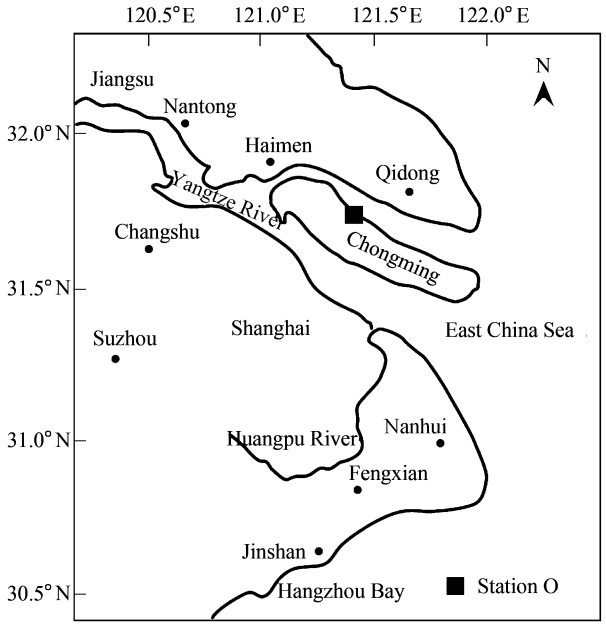

Fig. 1 Sampling location at Station O.

1.2 Sample treatment and analysis

Water and tissue treatment were carried out according to Water Quality-Determination of Selenium-Graphite Furnace Atomic Absorption Spectrometric Method (GB/T15505-1995), while sediment treatment was carried out according to Determination of Iron, Magnesium and Manganese in Foods (GB/T5009-1990). To determine the concentrations of heavy metals in the water, each 100 mL of water sample was acid-digested with 10 mL of 65% HNO_3 (analytical-reagent grade) and evaporated until the total volume reached to approximately 1 mL. Then the mixture was digested using 5 mL of diluted HNO_3 (1:49, *V/V*) for 12 h at room temperature. Five tissues including hepatopancreas, muscle, albumen gland, vitelline gland and digenetic gland of *O. struma* were analyzed to determine the concentrations of selected heavy metals. Tissular samples (1 g, wet weight) were heated at 100°C for 1.5 h to evaporate most water and improve the efficiency of the following steps (Tilstone and Macnair, 1997). Then all the samples were subjected to the carbolite at 700°C for 4 h until into ashes. The samples were then treated with 2 mL additional 65% HNO_3, digested for 12 h at room temperature. Approximately 1 g sediment (dry weight) was subjected to the carbolite at 700°C for 4 h into ashes. Charred sediment was treated with 5 mL mixed acid of HNO_3 and $HClO_4$ (analytical-reagent grade) with the ratio of 4:1 (*V/V*) and heated until completely bright. The dealt samples including water, tissues, and sediments were filtrated through microporus membrane of 220 nm in diameter and adjusted to 10 mL with Millipore ultrapure water.

Millipore ultrapure water was used as control for each method mentioned above. To avoid metal contamination, all glassware were cleaned with HNO_3 and rinsed with ultrapure water. The concentrations of heavy metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in the treated samples including water, sediments, tissues and control groups were determined in triplicate respectively by IRIS Intrepid II XSP Spectrometer (ICP, Thermo Electron Corporation, USA). Unit for metal concentrations in the water is expressed as μg/L. The data of organism are defined as μg/g wet weight while those of sediments described as μg/g dry weight.

1.3 Statistical analysis

Statistical analysis was performed with SPSS v.11.0 software (SPSS Inc., Illinois, USA). One-way ANOVA of variance with the 95% confidence interval followed

by a Duncan's multiple-range test was adopted to check the differences between concentrations of elements in the water, sediments and organism in different seasons. Pearson bivariate correlation was used, whenever possible, to investigate whether positive or negative correlation existed between parameters of physicochemical, marine gastropod's parameters and metal concentrations in all samples, and to identify the key factors responsible for such seasonal and tissue-specific pattern. Before calculating bioconcentration factor (BCF, bioconcentration coefficient or enrichment ratio) of tissues in comparison with element concentrations in the water and sediments, dry weight was assumed to equal to 20% of wet weight, a conversion derived from the data of Williams and Robins (1982). The whole-body bioconcentration was represented as the sum of metal concentrations in five tissues. All data are expressed as the mean ± standard error (SE).

2 Results

Some physicochemical parameters in different seasons are presented in Table 1. In the selected location, there was no significant difference of the pH values observed between spring and autumn, however, the pH values were relatively higher in these seasons than in the other seasons. The salinity and dissolved oxygen were significantly low in summer and autumn. Pearson bivariate correlation showed that positive correlation existed among salinity, weight and length ($R_{\text{salinity-weight}} = 0.992$, $R_{\text{salinity-length}} = 0.992$, and $R_{\text{weight-length}} = 0.995$, $p < 0.001$). No significant correlation was found among other parameters. Based on our seasonal investigation, it was found that, as a hibernating and intertidal animal, the population density of *O. struma* changed significantly in all seasons. The population density in summer and autumn were larger than that in spring and winter. Additionally, Mn and Zn were bioconcentrated mostly in autumn (Table 3). Moreover, positive correlations were found between Mn and population destiny in the muscle together with between Zn and population destiny in the albumen gland ($R_{\text{Mn-population destiny}} = 0.970$, $R_{\text{Zn-population destiny}} = 0.999$, as shown in Table 5).

Concentrations of elements in the water, sediments and tissues of *O. struma* are presented in Table 2. They were significantly different in terms of tissues and seasons. The levels of metal concentrations (μg/L): Cd 1.60–2.69, Cr 187.54–206.07, Cu 6.44–7.49, Fe 1.05–1.73, Mn 54.20–62.10, Pb 29.86–38.90, and Zn 151.00–164.70, were detected in the water. It was found that Cd, Cr, and Pb were enriched mostly in winter while Cu, Fe, Mn, and Zn were enriched in autumn. However, less of metals were concentrated in spring except for Zn. In contrast, the metal concentrations (μg/g dw) of Cd 0.08–0.11, Cr 33.08–49.44, Cu 10.20–12.54, Fe 3.03–4.96, Mn 218.00–335.40, Pb 27.14–29.12, and Zn 101.64–139.50 were determined in the sediments. During one year, BCFs of sediment/water demonstrated the following order: Mn > Fe > Cu > Pb > Zn > Cr > Cd. Seasonally, however, Cd, Cr, and Zn were enriched mostly in autumn in sediments, Cu and Mn in summer while Fe and Pb in spring. But Cd, Cr, Pb, and Zn were concentrated slightly in winter, so were Cu, Fe, and Mn in autumn. Significant positive correlations were found among concentrations of Cr, Fe, and Pb in the water with the correlation coefficients $R_{\text{Cr-Pb}} = 0.996$ ($p < 0.01$), $R_{\text{Cr-Fe}} = 0.969$ ($p < 0.05$), and $R_{\text{Fe-Pb}} = 0.956$ ($p < 0.05$), respectively. In contrast, both positive and negative correlation were found in sediments, i.e., $R_{\text{Cr-Fe}} = -0.966$ ($p < 0.05$) and $R_{\text{Cr-Zn}} = 0.981$ ($p < 0.05$). No significant correlation was found among other parameters such as metal concentrations, physicochemical and marine gastropod's parameters.

As shown in Table 3, BCFs of biomass/water from high to low were: Cu > Fe > Cd > Zn > Pb > Mn > Cr in general, of which Cu was mostly bioconcentrated by *O. struma* with its BCF magnitude reaching to 10^3–10^4, followed by Fe (10^3). The BCFs of Cd and Zn varied from 1.72×10^2 to 2.14×10^3 and 0.41 to 2.72×10^2, respectively. The BCF magnitude of Mn, Pb and Cr reached to 10^1. The BCFs of biomass/sediment had similar shift trend with the BCFs of biomass/water, however, the values of BCFs of biomass/sediment were 2–3 order lower. Cr, Cu, Mn, and Zn were mostly concentrated in the hepatopancreas, whereas Cd, Cu, Pb, and Zn were least bioconcentrated in the muscle. Interestingly, the enrichment of Fe in the muscle and digenetic gland was approximately 2-times larger than that in the hepatopancreas. BCF of Cd in the vitelline gland and albumen gland was 2.5 and 1.5 times higher than that in the hepatopancreas, respectively. BCF of Pb in the digenetic gland was nearly as 10 times as that in the other tissues. BCFs at different seasons showed that Cu, Fe, Mn and Zn were concentrated significantly in summer or autumn. But the bioconcentration of Cd, Cr and Pb in spring or winter increased slightly (Table 4).

Pearson bivariate correlations among parameters determined in different tissues are shown in Table 5. Results demonstrated that bioconcentration of metals in tissues was correlated with some physicochemical conditions and

Table 1 Physicochemical and marine gastropod's parameters of four seasons in Station O (mean ± SE).

Season	Water temperature (°C)	pH	Salinity (‰)	DO (mg/L)	Population density (gastropod/m^2)	Wet weight (g)	Body length (cm)
Spring, 2007	19.27 ± 0.17 b	8.29 ± 0.04 c	15.72 ± 0.16 d	6.55 ± 0.26 c	2.74 ± 0.21 b	6.97 ± 0.27 a	4.4 ± 0.2 a
Summer, 2006	30.89 ± 0.16 d	8.10 ± 0.04 b	8.85 ± 0.24 a	6.05 ± 0.22 a	3.39 ± 0.12 c	8.57 ± 0.30 c	4.8 ± 0.1 c
Autumn, 2006	21.77 ± 0.23 c	8.25 ± 0.19 c	9.44 ± 0.11 b	5.98 ± 0.36 a	3.57 ± 0.24 d	8.68 ± 0.49 c	4.8 ± 0.1 c
Winter, 2006	14.86 ± 0.09 a	7.96 ± 0.02 a	13.36 ± 0.29 c	6.12 ± 0.14 b	0.44 ± 0.07 a	7.54 ± 0.33 b	4.5 ± 0.1 b

Significant differences in the same column are shown as different small superscript letters (One-way ANOVA, LSD Duncan multiple comparison, $p < 0.05$).
DO: Dissolved oxygen.

Table 2 Metal concentrations in the water (μg/L), sediments (μg/g dry weight) and tissues of *Onchidium struma* (μg/g wet weight) of four seasons in Station O (mean ± SE)

Season	Cd	Cr	Cu	Fe	Mn	Pb	Zn
Water							
Spring, 2007	1.60±0.12 a	187.54 ± 9.23 a	6.44 ± 0.21 a	1.05 ± 0.07 a	54.20 ± 2.12 a	29.86 ± 1.29 a	151.60 ± 9.24 a
Summer, 2006	1.95 ± 0.08 b	195.62 ± 12.21 b	6.51 ± 0.14 a	1.49 ± 0.12 b	56.10 ± 1.62 b	33.17 ± 1.38 b	162.10 ± 8.26 b
Autumn, 2006	2.15 ± 0.13 c	202.41 ± 15.32 c	7.49 ± 0.11 c	1.73 ± 0.10 c	62.10 ± 3.43 c	37.34 ± 2.04 c	164.70 ± 7.21 c
Winter, 2006	2.69 ± 0.22 d	206.07 ± 14.15 c	6.85 ± 0.09 b	1.73 ± 0.05 c	55.50 ± 2.62 a	38.90 ± 2.35 d	151.00 ± 9.25 a
Sediment							
Spring, 2007	0.08 ± 0.00 a	34.06 ± 1.02 b	11.61 ± 0.15 b	4.96 ± 0.21 d	322.80 ± 14.29 c	29.12 ± 0.48 c	107.70 ± 4.00 b
Summer, 2006	0.09 ± 0.00 a	47.76 ± 2.02 c	12.54 ± 0.39 c	3.57 ± 0.25 b	335.40 ± 18.34 d	28.47 ± 0.56 b	112.20 ± 6.42 c
Autumn, 2006	0.11 ± 0.01 b	49.44 ± 1.73 d	10.20 ± 0.41 a	3.03 ± 0.19 a	218.00 ± 16.24 a	27.14 ± 0.43 a	139.50 ± 7.41 d
Winter, 2006	0.08 ± 0.00 a	33.08 ± 1.63 a	11.38 ± 0.37 b	4.63 ± 0.20 c	304.40 ± 16.24 b	28.22 ± 0.41 b	101.64 ± 5.02 a
Hepatopancreas							
Spring, 2007	1.36 ± 0.01 a	16.12 ± 0.47 b	205.71 ± 10.00 a	8.61 ± 0.29 c	1.08 ± 0.04 a	1.23 ± 0.03 a	32.33 ± 0.68 b
Summer, 2006	1.75 ± 0.05 c	11.43 ± 0.82 a	375.84 ± 21.45 d	5.21 ± 0.15 a	5.01 ± 0.04 b	1.61 ± 0.05 c	56.87 ± 0.25 c
Autumn, 2006	2.23 ± 0.04 d	16.29 ± 1.40 c	348.33 ± 33.46 c	6.59 ± 0.54 b	6.97 ± 0.58 c	1.47 ± 0.11 b	62.81 ± 3.78 d
Winter, 2006	1.55 ± 0.01 b	16.32 ± 0.18 c	234.97 ± 7.27 b	10.28 ± 0.92 d	1.00 ± 0.08 a	1.95 ± 0.02 d	21.73 ± 0.57 a
Muscle							
Spring, 2007	0.50 ± 0.01 c	5.54 ± 0.97 a	53.20 ± 1.23 b	15.72 ± 0.22 b	2.27 ± 0.04 b	0.93 ± 0.06 a	6.94 ± 0.06 a
Summer, 2006	0.42 ± 0.00 b	6.72 ± 0.05 b	57.41 ± 0.24 c	17.01 ± 0.04 c	3.05 ± 0.01 c	0.97 ± 0.00 b	9.85 ± 0.06 c
Autumn, 2006	0.33 ± 0.01 a	6.91 ± 0.69 c	38.43 ± 0.34 a	15.03 ± 0.01 a	3.52 ± 0.02 d	0.99 ± 0.10 b	11.07 ± 0.01 d
Winter, 2006	0.40 ± 0.01 b	6.78 ± 0.28 b	51.66 ± 0.41 b	17.22 ± 0.25 c	1.01 ± 0.22 a	1.04 ± 0.03 c	8.01 ± 0.07 b
Albumen gland							
Spring, 2007	3.16 ± 0.29 d	5.46 ± 0.15 c	86.53 ± 4.83 c	8.27 ± 0.20 b	3.73 ± 0.71 d	0.78 ± 0.04 a	31.04 ± 0.35 b
Summer, 2006	2.15 ± 0.01 a	4.72 ± 0.25 a	52.68 ± 2.17 b	6.91 ± 0.02 a	2.92 ± 0.05 c	0.92 ± 0.03 c	34.89 ± 0.46 c
Autumn, 2006	2.67 ± 0.00 b	5.69 ± 0.30 d	85.54 ± 2.17 c	8.33 ± 0.25 c	0.86 ± 0.08 a	0.85 ± 0.06 b	36.45 ± 0.18 d
Winter, 2006	2.77 ± 0.01 c	5.18 ± 0.11 b	50.00 ± 3.46 a	8.31 ± 0.17 b,c	1.57 ± 0.17 b	0.98 ± 0.06 d	18.34 ± 0.10 a
Vitelline gland							
Spring, 2007	4.37 ± 0.02 b	1.45 ± 0.06 b	209.00 ± 1.04 b	1.75 ± 0.21 a	0.27 ± 0.04 a	1.09 ± 0.10 b	7.51 ± 0.22 b
Summer, 2006	4.62 ± 0.21 c	1.53 ± 0.13 c	221.56 ± 6.48 c	2.62 ± 0.32 c	0.85 ± 0.06 c	1.28 ± 0.04 c	30.15 ± 0.34 c
Autumn, 2006	4.19 ± 0.08 a	1.23 ± 0.17 a	255.66 ± 8.24 d	3.28 ± 0.67 d	0.49 ± 0.01 b	0.92 ± 0.16 a	35.16 ± 0.67 d
Winter, 2006	4.06 ± 0.05 a	1.67 ± 0.08 d	182.64 ± 13.86 a	2.00 ± 0.17 b	1.11 ± 0.16 d	0.97 ± 0.06 a	5.08 ± 0.21 a
Digenetic gland							
Spring, 2007	1.97 ± 0.04 c	5.07 ± 0.67 a	166.43 ± 2.71 d	10.48 ± 0.71 a	0.96 ± 0.04 d	0.41 ± 0.01 a	31.21 ± 0.79 c
Summer, 2006	1.47 ± 0.21 a	8.63 ± 0.62 c	145.48 ± 2.54 b	13.25 ± 0.79 b	0.85 ± 0.03 c	0.44 ± 0.05 a	26.24 ± 0.15 b
Autumn, 2006	1.72 ± 0.01 b	9.98 ± 0.64 d	148.78 ± 3.27 c	14.57 ± 0.36 c	0.49 ± 0.04 b	0.83 ± 0.12 c	19.39 ± 0.54 a
Winter, 2006	1.65 ± 0.04 b	6.45 ± 0.31 b	140.27 ± 6.78 a	14.41 ± 0.65 c	0.29 ± 0.02 a	0.55 ± 0.04 b	36.78 ± 0.91 d

Significant differences among column in the same tissue are shown as different small superscript letters (One-way ANOVA, LSD Duncan multiple comparison, $p < 0.05$).

Table 3 BCFs of different tissues in *Onchidium struma* from summer 2006 to spring 2007 in Station O

Tissue	BCF	Cd	Cr	Cu	Fe	Mn	Pb	Zn
Hepatopancreas	B/W	840.21	76.01	42620.87	5362.02	59.87	44.81	272.34
	B/S	95.12	1.92	128.11	9.49	0.07	0.28	1.84
Muscle	B/W	172.98	25.05	6283.57	9302.52	30.03	22.44	41.58
	B/S	23.65	0.81	21.83	20.77	0.04	0.17	0.39
Albumen gland	B/W	1337.29	26.62	10062.08	5533.07	40.75	25.45	190.69
	B/S	152.86	0.66	30.54	10.18	0.04	0.16	1.30
Vitelline gland	B/W	2139.65	7.43	31820.87	1619.27	12.01	31.17	120.66
	B/S	243.50	0.19	95.98	3.25	0.01	0.19	0.80
Digenetic gland	B/W	849.62	37.93	22132.88	8906.25	11.49	344.15	182.26
	B/S	96.52	0.91	66.06	17.18	0.01	2.01	1.28

BCF: bioconcentration factor; B/W: biomass/water; B/S: biomass/sediment.

Table 4 BCFs of whole-body *Onchidium struma* in four seasons in Station O

Season	BCF	Cd	Cr	Cu	Fe	Mn	Pb	Zn
Spring, 2007	B/W	7100.00	179.43	111936.34	42695.24	153.32	148.69	719.20
	B/S	710.00	4.94	310.45	45.19	0.13	0.76	5.06
Summer, 2006	B/W	5338.46	168.79	131024.58	30201.34	226.02	157.37	974.71
	B/S	578.33	3.46	340.10	63.03	0.19	8.57	7.04
Autumn, 2006	B/W	5 043.26	167.35	112436.98	19810.98	147.54	111.65	940.60
	B/S	506.36	4.05	429.77	78.88	0.28	0.93	5.91
Winter, 2006	B/W	3877.32	176.62	96283.21	30184.97	89.73	141.13	595.63
	B/S	651.88	5.50	289.78	56.39	0.08	0.97	4.42

Table 5 Pearson bivariate correlation for parameters determined in different tissues of *Onchidium struma*

Tissue	Pearson correlation coefficient (R)	p
Hepatopancreas	$R_{\text{Pb-pH}} = -0.969$	$p < 0.05$
	$R_{\text{Cu-length}} = 0.989$	
	$R_{\text{Cu-salinity}} = 0.986$	
	$R_{\text{Cu-weight}} = 0.972$	
	$R_{\text{Mn-Zn}} = 0.967$	
Muscle	$R_{\text{Cr-DO}} = -0.986$	$p < 0.05$
	$R_{\text{Zn-weight}} = 0.975$	
	$R_{\text{Zn-length}} = 0.962$	
	$R_{\text{Mn-population destiny}} = 0.970$	
Albumen gland	$R_{\text{Zn-population destiny}} = 0.999$	$p < 0.01$
Vitelline gland	$R_{\text{Zn-length}} = 0.957$	$p < 0.05$
Digenetic gland	$R_{\text{Cr-weight}} = 0.980$	$p < 0.05$
	$R_{\text{Cr-length}} = 0.966$	

marine gastropod's parameters, e.g., Pb was significantly correlated with pH, and so was Cu with length, salinity and weight in the hepatopancreas respectively. In the muscle, Cr was negatively correlated with dissolved oxygen, however, positive correlation were found between Mn and population destiny, so was between Zn and length or weight; Zn was positively correlated with population destiny in the albumen gland and so was length with Zn in the vitelline gland; Cr was positively correlated with length and weight in the digenetic gland.

3 Discussion

Marine organisms have been used widely as monitors of metal concentration, for example, decapods can concentrate non-essential metals (e.g., Ca and Pb), while barnacles are net concentrators of Zn (Rainbow, 1997). Among heavy metals determined in this study, as an indispensable role in a large number of enzymes, Cu is a cofactor for regulating the activity of copper-dependent enzymes (Lehtonen and Leiniö, 2003) and also an essential component required for synthesis of hemocyanin (Méndez *et al*., 2001). Fe is also an indispensable part for many proteins such as ferritin that is the main iron storage protein in cells (Goralska *et al*., 2000). As an essential trace element, Zn is known to act as an enzyme cofactor in over 200 enzymes with important biological functions regulating many physiological processes including DNA synthesis, behavioral response and reproduction (Vallee and Auld, 1990). Mn is required by a number of enzymes such as Mn-SOD and plays essential roles in some metabolic pathways such as tricarboxylic acid cycle (Jakubovics and Jenkinson, 2001). As toxicants, Cr, Cd, and Pb enable to cause some toxic effects on marine organisms if excessively concentrated (ATSDR, 2005). Our results indicated that nearly every selected tissue of *O. struma* had high bioconcentration levels of essential elements Cu, Fe, and Zn (except for Mn) along with comparatively low levels of non-essential metals Cr and Pb (with exception of Cd). The reasons why there existed comparatively higher Cd but low Mn bioconcentration in the selected tissues of *O. struma* were unknown. Additionally, as for correlations between metal bioconcentration and other parameters, whether they might be taken into account in the estimation of metal bioconcentration requires further argumentation.

Levels and bioconcentration of heavy metals in marine organisms usually fluctuate as the changes of some seasonal factors such as seasonal dietary and temperature (Stewart *et al*., 1994). The results of metal bioconcentration in whole-body *O. struma* showed that most of essential elements Cu, Fe, Mn, and Zn were bioconcentrated selectively in summer or autumn except for non-essential metals Cd, Cr, and Pb. In addition, there existed significant change of population density of *O. struma* among seasons that could be primarily attributed to its special living habit, especially in term of hibernation. *O. struma* comes out only when the temperature reaches to 20–28°C and humidity is nearly 100% (Huang *et al*., 2004).

Metal bioconcentration, however, is likely to vary within tissues such as two Brazilian crabs *Ucides cordatus* and *Callinectes danae* that showed significantly higher Cd, Hg, Pb, and Zn levels in the hepatopancreas than in the other tissues. This suggests that different body compartment probably exhibits specific metal bioconcentration pathway and mechanism. The results of metal bioconcentration in five tissues of *O. struma* demonstrated that hepatopancreas had high bioconcentration levels of Cr, Cu, Mn, and Zn, muscle enriched Fe mostly, and Pb was enriched mostly in the digenetic gland as well as Cd in the vitelline gland. These results could be linked to metal sequestration and detoxification by metallothioneins in the hepatopancreas. In general, heavy metals undergo detoxification in the hepatopancreas and are subsequently excreted as granules from the epithelium of the hepatopancreas. As the main tissue for metal detoxification, the bioconcentration in the hepatopancreas could be the reflection of whole-body metal enrichment (MacFarlane and Burchett, 2000). Additionally, there are no molting and tissular turnover in the hepatopancreas. The hepatopancreas is, therefore, often adopted for metal analysis to provide a time-integrated estimation of metal contamination (Ahearn *et al*., 2004).

Moreover, it is important to detect the heavy metal concentrations in marine organisms, which are of great value to determine whether these aquatic organisms may constitute a health hazard for consumers. Among selected metals, Pb, Cd, and Cr were ranked top 2, 8, and 18 in the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) Priority List of Hazardous Substances regulated by Agency for Toxic Substances and Disease Registry (ATSDR, 2005). In addition, although Cu and Zn are known to play important roles in metabolism, they may also be potential pollutants if concentrated in mollusks that might be consumed by humans. The current seafood standard regulated by the Food Standards Australia New Zealand (FSANZ, 2002) specifies the maximum allowable limits of Cu (100 mg/kg wet weight), Pb (1.5 mg/kg wet weight), and Zn (150 mg/kg wet weight) in mollusks, but no upper limits for Cd, Cr, Fe, and Mn are specified inside. The Hygienic Standard for Fresh and Frozen Marine Products of Animal Origin (GB 2733-2005) in China only specifies the maximum levels of Cd (0.5 mg/kg wet weight) and Pb (0.5 mg/kg wet weight). Based on the standard of FSANZ, therefore, Cu in the hepatopan-

creas, vitelline gland, and digenetic gland of *O. struma* had exceeded the upper limit. Pb also had exceeded according to GB 2733-2005, and its standard is 3-fold lower in GB 2733-2005 than in FSANZ. Furthermore, Cd in the tissues of *O. struma* with exception of muscle had exceeded its standard in the GB 2733-2005. Hence, with view of its excessive amount of Pb, Cd, and Cu, we suggest here that the marine gastropod *O. struma* consumed by human constitutes a health hazard. According to the Water Quality Standard for Fisheries in China (GB 11607-1989), the concentration of Cr and Zn in the examined location of the Yangtze Estuary was 2-fold and 1.5-fold higher than the standard (100 μg/L), respectively. The concentrations of other metals were within the standard. These excessive heavy metals in the investigated estuary may be caused by agricultural and industrial wastewater coming from upstream areas together with municipal wastewater from the cities nearby (Chen and Lin, 2001).

Related studies have previously centered on elucidating the heavy metal bioconcentration in mollusks collected from estuaries. Blackmore (1998) detected heavy metal bioconcentrations in the mussels collected from four coastal sites in Hong Kong and found that the green mussels *Perna viridis* showed ranking of sites which reflects the degree of industrialization and population in each site, however, the blue mussels *Mytilus edulis* showed no significantly different bioconcentrations with regard to sampling sites. The bivalve *Crassostrea talienwhanensis* from Bohai Bay possessed high bioconcentration of essential metals Cu and Zn which was mainly caused by the wastewater and waste residue drainage from the chemical industries nearby (Liang *et al*., 2004). Moreover, *Corbicula fluminea* from the Yangtze Estuary also had high enrichment of Cu and Zn but comparatively low enrichment of Cr and Pb (Li *et al*., 2006). Furthermore, Blasco and Puppo (1999) examined Cd, Cu, and Pb in different tissues of the clam *Ruditapes philippinarum* and observed the highest Cd and Cu level in the digestive gland together with Pb in the gills; whereas, the lowest levels of studied metals were detected in the muscular tissues of the foot. In our study, marine gastropod *O. struma* possessed high bioconcentration of Cu but low bioconcentration of Cr, Mn, Pb and Zn. And hepatopancreas was the main storage tissue for metal bioconcentration. As there are many factors (including physicochemical as well as biotic factors) known to influence the bioconcentration of heavy metals, mechanisms about heavy metal bioconcentration in mollusks need further studies.

4 Conclusions

In conclusion, the results in the present study demonstrated that *O. struma* could selectively bioconcentrate heavy metal, i.e., high enrichment of Cu, median enrichment of Cd and Fe and comparatively low enrichment of Cr, Mn, Pb, and Zn. The differences of bioconcentration in different tissues and seasons indicated that hepatopancreas was the main metal-concentration tissue while summer or autumn is the main season of metal-bioconcentration. As the concentrations of Pb, Cd and Cu in *O. struma* were above standard level, there exists some consumption hazards. Also, the concentration of Cr and Zn in the water of the selected area exceeded the Water Quality Standard for Fisheries.

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