Study on the contamination of heavy metals and their correlations in mollusks collected from coastal sites along the Chinese Bohai Sea

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Abstract

Mollusks living in seas can accumulate heavy metals, and may serve as excellent passive biomonitors. During a period of 1 year, bioaccumulation of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn was examined in nine kinds of mollusks (*Rapana venosa*, *Neverita didyma*, *Scapharca subcrenata*, *Mytilus edulis*, *Amusium*, *Crassostrea talienwhanensis*, *Meretrix meretrix*, *Ruditapes philippinarum*, and *Macra veneriformis*), which were collected at eight coastal sites along the Chinese Bohai Sea. Metal concentrations were directly determined by inductively coupled plasma mass spectrometer (ICP-MS). Two certified reference materials, dogfish muscle (DORM-2) and mussel (GBW 08571), were used to validate the methods, and the recoveries were within 83.72–112.30% of the certified values. Bioaccumulation of metals varied strongly among sampling sites and species. Statistical analysis (one-way ANOVA) indicated that different species examined showed different bioaccumulation of metals, and perhaps they could be used as potential biomonitors to investigate the contamination levels of heavy metals. Principal component analysis (PCA) and correlation analysis were used to study the relationships between these heavy metals. The results showed that, in nine mollusks' tissues, there are significant correlations between these elements in adjacent main group or subgroup in periodic table of elements.

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Keywords: Heavy metals; Mollusks; Principal component analysis; Correlation analysis; ANOVA; Bohai Sea

1. Introduction

Aquatic ecosystems may receive anthropogenic pollutants originating from various sources located at the catchment area or at distant places. Many pollutants, including heavy metals, are toxic to aquatic organisms and cause their lethal or sublethal deterioration.

Mollusks have long been regarded as promising bioindicators and biomonitoring subjects. They are abundant in many terrestrial and aquatic ecosystems, being easily available for collection. They are highly tolerant to many pollutants and exhibit high accumulation property, particularly for heavy metals. In previous works, directions for monitoring marine pollution (Pérez et al., 2004; Goldberg et al., 1978, Goldberg and Bertine, 2000;

Rainbow, 1995) and implications in estimation of metal bioavailability in Mussel Watch program have been recommended (Soto et al., 2000; Szefer et al., 2000). *Mytilus* spp. have been considered to be potential biomonitors of toxic metals in marine ecosystems (Fowler and Oregoni, 1976; Koide et al., 1982; Szefer et al., 1997, 1999a; Chase et al., 2001).

Various metal-accumulating bivalve and gastropod species show a high presence and abundance in coast sites and play an important role as biomonitors for trace metal pollution in global monitoring programs (Sericano, 2000). They assimilate trace metals from their food and from the ingestion of inorganic particulate material (Phillips, 1977; Struck et al., 1997). In recent years, researchers have focused their attention on the identification of other possible biomonitors for trace metal pollution, such as the gastropod mollusks *Monodonta turbinata* and *Patella cerulea* (Conti and Cecchetti, 2003).

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According to China Marine Environmental Quality Communique in 2001 (Zhan et al., 2002), most estuaries, bays, and seas abutting big cities had high contamination of heavy metals. Many marine mollusks have been annihilated and even the living marine mollusks in the coastal sea area abutting the pollution sources are not suitable for consumption. However, few data on heavy metal pollution in mollusks of Chinese seawater have been published, and the corresponding criteria about the marine environmental quality in coastal areas are absent.

In this study, nine easily obtained and delicious mollusk species collected from eight sites around the Bohai Sea (37°30′–41°00′N latitude, 117°30′–121°30′E longitude) were studied. The main objectives and questions of the study are:

1. To investigate heavy metal contamination in the moderately polluted seawater at eight locations with different urbanization degree. This investigation will help to evaluate the potential hazards to human health, and provides data for setting interrelated policies and codes in China.
2. To compare the bioaccumulations of different heavy metals in different mollusks, to identify the potential biomonitors indicative of the pollution levels of heavy metals at coast sites along the Bohai Sea, and to

statistically examine the correlations among metal concentrations in mollusks' tissue.

2. Materials and methods

2.1. Sampling sites study

The Bohai Sea is structured into the inner region and the Liaodong, Bohai, and Laizhou bays. The coastal line of the Bohai Sea has a length of nearly 3800 km. The Bohai Strait in the east is the only passage connecting the Bohai Sea to the outer Yellow Sea. The Bohai Sea is the biggest semi-enclosed sea in China and its water change is very slow. Once polluted, it needs a long period of time for water quality to resume. Fig. 1 shows the map of the Bohai Sea and eight sampling sites: Weihai, Penglai, Yangkou, Tianjin, Qinhuangdao, Huludao, Yingkou, and Dalian.

2.2. Sampling and sample pretreatment

The sampling was carried out twice from July to August of 2002 and 2003. The collected mollusks were depurated in filtered seawater for approximately 24 h before being transported to the laboratory with ice freezing. The soft tissues of mollusks were excised by stainless steel scalpel

Fig. 1. Sampling sites along the Chinese Bohai Sea.

Table 1
Denomination and abbreviations of the selected mollusks used in this study

Species	Abbreviation	Denomination
<i>Rapana venosa</i>	Rap	Valenciennes, 1846
<i>Neverita didyma</i>	Nev	Röding, 1798
<i>Scapharca subcrenata</i>	Sca	Lischke, 1869
<i>Mytilus edulis</i>	Myt	Linnaeus, 1758
<i>Amusium</i>	Amu	Röding, 1798
<i>Crassostrea talienwhanensis</i>	Cra	Crosse, 1862
<i>Meretrix meretrix</i>	Mer	Linnaeus, 1758
<i>Ruditapes philippinarum</i>	Rud	Adams and Reeve, 1850
<i>Macra veneriformis</i>	Mac	Reeve, 1854

blades, and then thoroughly rinsed with Milli-Q water to remove extraneous impurities. After sufficient homogenization by a blender, the samples were kept at -18°C until analysis. Different species of mollusks (shown in Table 1) were identified according to the catalog of marine mollusks in reference books (Qi et al., 1989).

2.3. Chemical analysis

2.3.1. Instrument

Agilent 7500i inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies Co. Ltd., USA) was used for the determination of elements. The working parameters are listed below: RF power 1450 W; sampling depth 9.5 mm; carrier gas flow rate 0.85 l/min; makeup gas flow rate 0.3 l/min; spray chamber temperature 2°C ; sample uptake rate 0.4 ml/min. The orifice of nickel sampling cone and skimmer cone is 1.0 mm and 0.4 mm, respectively. Nitric acid and hydrogen peroxide were guarantee reagents (GR) and purchased from Merck and Beijing Chemical Reagents Company, respectively. The mixture of 1.0 $\mu\text{g}/\text{ml}$ Sc (45), Ge (72), and In (115) in 1% vol/vol nitric acid was used as calibration internal standard.

2.3.2. Sample analysis

Approximately 1g (wet weight) of soft tissues was weighed in a PTFE digestion container. Each sample was

added with 3 ml of concentrated nitric acid and left to predigest overnight at 40°C . After cooling, 2 ml of 30 % hydrogen peroxide was added. Thereafter the container was covered and placed in a high-pressure stainless steel bomb then put in an oven. The oven temperature was increased to 160°C and kept for 4 h. After cooling, the solution was diluted with Milli-Q water and transferred into PET bottle to 50 g. As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn were determined with ICP-MS. All analyses were repeated thrice by external calibration method. The accuracy of the method was validated by the determination of dogfish and mussel (GBW 08571) certified reference materials, and the results are reported in Table 2. The found values of all elements were in good agreement with the certified values, with the recoveries ranging from 83.72% to 112.30%, suggesting that the proposed method was feasible in the determination of heavy metals in biota samples. The detection limits of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn were 3.15, 2.70, 2.10, 30, 34.5, 3300, 0.70, 40.50, 3, 8.40, 2.10, and 210 ng/g, separately.

2.4. Statistical analysis

Multivariate analysis methods such as one-way analysis of variance (ANOVA), principal component analysis (PCA), and correlation analysis (bivariate correlations) have been used to extract information from the chemical analysis in order to find the relationships among these heavy metals in organisms (Foster, 1995; Szefer et al., 1998, 1999b, 2002; Holmes et al., 1998; Lucila Lares et al., 2002; Capelli et al., 2000; Kim, 1995). In this work, the results of chemical analyses were presented as mean $\pm$ S.E. One-way ANOVA was applied to the data. A value of $P < 0.05$ was considered to indicate a significant difference in all statistical analysis. Principal component analysis (PCA) was executed on the analytical data in order to obtain a visual representation of the main characteristics of relationship among these metal concentrations in the studied mollusks. The combined plot of scores and loadings allowed us to recognize groups of

Table 2
Comparison of measured and certified concentrations of heavy metals in two certified reference materials ($\mu\text{g}/\text{g}$, dry weight)

Metal	Dogfish muscle			Mussel		
	Certified	Found	Recovery (%)	Certified	Found	Recovery (%)
As	18.0 $\pm$ 1.1	17.13 $\pm$ 0.05	95.17	6.1 $\pm$ 0.6	6.85 $\pm$ 0.08	112.30
Cd	0.043 $\pm$ 0.008	0.036 $\pm$ 0.001	83.72	4.5 $\pm$ 0.3	4.265 $\pm$ 0.001	94.78
Co	0.182 $\pm$ 0.031	0.169 $\pm$ 0.001	92.86	0.94 $\pm$	0.854 $\pm$ 0.009	90.85
Cr	34.7 $\pm$ 5.5	31.14 $\pm$ 0.26	89.74	0.57 $\pm$ 0.04	0.63 $\pm$ 0.03	110.53
Cu	2.34 $\pm$ 0.16	2.40 $\pm$ 0.04	102.56	7.7 $\pm$ 0.5	7.35 $\pm$ 0.04	95.45
Fe	NC	144.7 $\pm$ 0.4	–	221 $\pm$ 7	219 $\pm$ 1	99.10
Mn	3.66 $\pm$ 0.34	3.49 $\pm$ 0.04	95.36	10.2 $\pm$ 0.9	8.7 $\pm$ 0.1	85.29
Ni	19.4 $\pm$ 3.1	16.6 $\pm$ 0.2	85.57	1.03 $\pm$ 0.07	1.05 $\pm$ 0.01	101.94
Pb	0.065 $\pm$ 0.007	ND	–	1.96 $\pm$ 0.05	1.91 $\pm$ 0.01	97.45
Se	1.40 $\pm$ 0.09	1.43 $\pm$ 0.03	102.14	3.65 $\pm$ 0.09	3.78 $\pm$ 0.10	103.56
Zn	25.6 $\pm$ 2.3	27.02 $\pm$ 0.18	105.55	138 $\pm$ 5	123.7 $\pm$ 0.6	89.64
Hg	4.640 $\pm$ 0.260	4.560 $\pm$ 0.460	98.28	0.067 $\pm$ 0.004	0.057 $\pm$ 0.007	85.07

ND: no detection; NC: no comparison.

samples with similar behavior and the existing correlation among the original variables. Usually the PCs are obtained by their eigenvalues >1. The correlation analysis was used by Pearson correlation. Correlation is significant at the 0.05 level (two-tailed). All statistical analyses were performed with SPSS 12.0 for windows release 12.0 (September 4, 2003, SPSS Inc., 1989–2003).

3. Results and discussion

3.1. Metal concentrations in mollusks

The ranges of measured metal concentrations in mollusks are listed in Table 3. It showed that all samples were contaminated with different levels of heavy metals. The results clearly indicated that the concentrations of some heavy metals, such as Cd, Cu, and Zn, in some mollusks exceeded the maximum permissible levels (MLPs) of 2 µg/g Cd, 10 µg/g Cu, and 100 µg/g Zn according to the codes of WHO (1982).

There are significant differences between mollusks from different locations within the same area. The levels of As and Se ranged from 0.53 µg/g to 19.01 µg/g and from 0.38 µg/g to 3.30 µg/g, respectively. The gastropods (Rap and Nev) loaded higher As and Se concentrations than bivalves. According to a previous report, As was generally lower than 0.5 µg/g in human's food but could reach 1–100 µg/g in halobios due to the processes of biotransformation and bioaccumulation (Larsen, 1995). Cd concentrations in mollusks varied from 0.14 µg/g to 52.05 µg/g. As a whole, Rap, Amu, Sca, and Mac manifested more Cd levels in the soft tissues. The exceptionally high Cd level in the tissue of Rap from Tianjin and Huludao was probably caused by the Cd pollution in the sea due to the drainage of wastewater and waste residue from the chemical industries (Qiao, 1991). Mac had the highest enrichment of Co, Fe, and Mn among these species and tissue concentrations ranged from 0.02 µg/g to 0.76 µg/g, from 5.45 µg/g to 760.80 µg/g, and from 0.74 µg/g to 58.51 µg/g, respectively. Cu concentration (0.98 µg/g–146.85 µg/g) and Zn concentration (5.09 µg/g–550.12 µg/g) in Rap and Cra were significantly higher than others, but there was no significant difference between the two species, which was similar to previous studies (Martincic et al., 1984; Han and Hung, 1990; Beliaeff et al., 1998). Cr contamination (0.07 µg/g–3.24 µg/g) in the Bohai Sea was not outstanding and the probable pollution sources might be its main use in alloys and the general chemical industries. The results showed that Mer and Rud have stronger enrichment of Ni than others. Pb contamination in the Bohai Sea was also well known because of the drainage of wastewater from zinc plant in Huludao. According to the investigation in 1988 (Qiao, 1991), the geometric mean of Pb concentrations in sediments presented to be 29.7 µg/g. Fortunately, Pb concentration in the selected mollusks was very low (0.02 µg/g–1.33 µg/g). However,

Table 3
Summary statistics for heavy metal concentrations (µg/g, wet weight) in the selected mollusks from the Bohai Sea

Species	As		Cd		Co		Cr		Cu		Fe		Mn		Ni		Pb		Se		Zn		Hg		Number
	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max	Min–Max		
2002 Samples	Rap	0.83–19.01	0.15–52.05	0.03–0.39	0.28–0.88	5.52–79.80	18.92–196.20	1.22–15.64	0.09–0.66	0.10–0.75	0.38–3.30	8.65–430.95	0.02–0.31	37											
	Nev	3.03–6.31	0.34–1.17	0.07–0.13	0.30–0.59	5.74–23.89	18.92–71.70	2.04–19.14	0.16–0.46	0.08–0.24	0.89–1.98	18.52–38.45	0.03–0.10	47											
	Sca	0.53–2.15	1.73–7.44	0.08–0.26	0.26–1.36	1.25–2.95	54.25–116.25	2.26–4.92	0.16–0.74	0.13–0.38	0.74–1.16	12.18–28.03	0.01–0.10	50											
	Myt	1.08–2.01	0.34–1.96	0.06–0.09	0.27–0.86	1.16–2.52	20.83–73.40	2.14–3.77	0.18–0.25	0.16–0.60	0.61–0.79	11.87–22.42	0.01–0.19	63											
	Amu	0.66–1.49	2.36–6.77	0.04–0.09	0.37–0.52	1.44–4.25	22.80–71.70	1.38–11.55	0.45–1.19	0.11–0.40	0.44–0.85	18.73–36.19	0.02–0.03	38											
	Cra	1.28–2.06	0.51–3.29	0.03–0.10	0.58–0.87	25.24–109.60	31.73–232.55	5.83–16.14	0.11–0.44	0.21–0.62	0.53–0.72	115.95–379.55	0.02–0.06	28											
	Mer	1.06–2.46	0.18–0.81	0.15–0.31	0.19–0.61	1.06–2.13	21.83–207.70	1.85–6.39	0.87–2.15	0.06–0.26	0.37–0.87	11.31–60.20	0.01–0.09	62											
	Rud	1.36–10.26	0.14–0.63	0.14–0.41	0.30–3.24	1.28–4.37	47.77–372.70	2.39–9.22	0.70–2.97	0.13–0.34	0.52–1.00	9.95–20.06	0.01–0.04	220											
	Mac	0.86–4.80	0.14–6.94	0.09–0.76	0.43–1.27	1.28–5.92	46.45–464.55	1.67–48.55	0.18–0.88	0.15–0.60	0.51–1.81	5.09–24.38	0.01–0.08	123											
2003 Samples	Rap	2.90–13.99	0.85–10.38	0.02–0.07	0.17–0.58	1.95–119.60	10.21–278.84	1.08–5.40	0.06–0.67	0.03–0.16	1.12–1.93	32.92–217.78	0.02–0.10	56											
	Nev	2.06–6.35	0.29–0.84	0.05–0.10	0.22–0.94	5.98–85.91	16.91–213.52	8.95–21.17	0.31–0.73	0.02–0.27	0.99–1.77	35.34–55.38	0.03–0.05	65											
	Sca	0.84–2.55	1.40–12.84	0.05–0.10	0.13–0.44	1.08–4.45	52.54–253.40	3.64–13.76	0.14–0.35	0.08–0.45	0.46–0.96	11.56–30.59	0.01–0.03	82											
	Myt	0.92–4.97	0.17–1.29	0.04–0.10	0.18–0.67	0.98–5.40	34.10–402.58	2.91–18.69	0.17–0.54	0.09–0.59	0.46–0.97	9.76–42.17	0.01–0.03	119											
	Amu	0.79–1.67	2.84–6.53	0.04–0.06	0.07–0.31	1.65–4.80	5.45–129.80	3.98–25.67	0.71–0.98	0.04–0.21	0.44–0.63	33.37–60.50	0.02–0.03	27											
	Cra	1.04–2.16	0.94–32.18	0.03–0.11	0.21–0.66	34.69–146.85	75.02–401.90	10.22–24.79	0.17–0.52	0.05–0.52	0.30–1.50	141.86–550.12	0.01–0.04	31											
	Mer	0.90–1.78	0.17–0.70	0.07–0.25	0.17–0.30	1.69–33.83	20.18–321.16	0.74–20.79	0.94–1.67	0.06–1.33	0.28–0.83	22.21–53.97	0.02–0.02	89											
	Rud	1.98–2.85	0.14–0.29	0.11–0.17	0.16–0.35	1.44–3.56	61.35–302.89	4.17–16.11	0.68–1.19	0.08–0.28	0.51–0.85	10.84–32.38	0.02–0.04	331											
	Mac	0.63–1.37	0.13–1.16	0.14–0.26	0.19–1.36	1.25–19.43	183.80–760.80	17.88–58.51	0.27–0.87	0.17–1.02	0.34–0.61	5.69–10.23	0.02–0.03	201											

low levels of Pb could not be ignored, as Pb and its salts were capable of damaging the nerve system (Chuang et al., 2004), hematosiis, and kidney in the human body. Hg pollution was mainly caused by the wastewater drainage of oil refining plant, chloroalkaline industry, and petrochemical industry. But it was exciting that Hg concentrations in the tissue of selected mollusks were relatively low, which did not exceed the MPLs of 0.5 µg/g according to the codes of WHO (1982). The concentrations ranged from 0.01 µg/g to 0.31 µg/g. Similar to As, Hg concentrations in gastropod species were generally higher than bivalves. The partial heavy metals contents in soft tissue of *Mytilus edulis* in the present study were further compared with those from other sites along the Chinese coastline. The metal contents at different sampling times and sites could be found in Table 4.

On the whole, Penglai and Huludao were the most seriously polluted cities by heavy metals among the selected. The source of the pollution in Penglai came from the local paper mills and indigenous method smelting gold. Cu, Zn, Cd, and Hg were the main heavy metal contaminations found in Huludao, where the largest zinc industry in Asia is located.

In order to compare the bioaccumulation of metals in mollusks of two batch samples, normalized data of original metal concentrations (enrichment factor (EF)) defined by Zonta et al. (1994) were used to plot a figure by using the following formula:

$$EF = \frac{C - C_{\min}}{C_{\max} - C_{\min}} \quad (1)$$

where C is the heavy metal concentration in mollusks, and $C_{\min}$ and $C_{\max}$ are the minimum and maximum concentrations. Fig. 2 is the EF plot, which showed that the bioaccumulation ability of bivalves and gastropods for heavy metals was species-dependent. Fig. 3 showed that the mean concentrations of heavy metals (mean ± S.E.) of the two batches of samples were similar, except Cr, Fe, Pb, and Hg. This illuminated that, in this area, the heavy metals pollution did not change significantly from July 2002 to August 2003.

3.2. One-way ANOVA analysis

Significant differences in heavy metal concentrations were tested with one-way ANOVA for each species and

for each metal for the two batches of samples. Table 5 shows the ANOVA results. Differences in heavy metal concentrations were found among the studied species. For example, Rap significantly accumulates As, Cu, Se, Zn, and Hg, while Mac accumulates Co, Fe, Mn, and Pb more efficiently than other mollusks. Similarly, one heavy metal can be accumulated by different mollusks. Cu can be significantly accumulated by Rap and Cra, Ni accumulated by Amu, Mer, and Rud, while Zn can be accumulated by Rap and Cra.

The results of this study indicate that mollusks selectively can accumulate certain elements. This selectivity is the most important criterion in choosing species as biomonitor (Duquesne et al., 2000). Based on Figs. 2 and 3, mollusks marked by an asterisk in Table 5 could be selected as potential biomonitors for certain metals to monitor the ocean's heavy metals pollution. Others, such as Rap, could be selected to monitor the pollution of As, Cd, Cu, Se, and Hg, and Mac selected for Co, Fe, Mn, Pb, etc. The metal selectivity of mollusks is investigated further in our laboratory.

3.3. Inter-elemental relationships in view of correlation and factor analysis

Results of correlation analyses (bivariate correlations with Pearson correlations coefficients) among these heavy metal concentrations were listed in Table 6 ($P < 0.05$ or $P < 0.01$). The correlations of samples collected in 2002 (2002 samples) and the samples collected in 2003 (2003 samples) were not completely same. For example, in 2002 samples, As was significantly correlated with Cd, Se, and Zn ($P < 0.01$), while in 2003 samples, As was correlated with Cu, Se, and Hg ($P < 0.01$). But on the whole, there were some correlations among concentrations of metals found in both sets of samples (Cr–Mn–Fe, Zn–Cd, As–Se, Pb–Co, etc.).

Tessier et al. (1985) pointed out that, under the same environmental conditions, the following equation can be used to interpret the significant correlations between any two heavy metals:

$$C_{Me} = [Me] \sum \beta_i [L]^i \quad (2)$$

where C_{Me} represents the total concentration of the complex metal; β is the stability constant; $[Me]$ represents the

Table 4

Concentrations of heavy metals (µg/g, wet weight) in soft tissue of *Mytilus edulis* from different sites along the Chinese coastline

Region	Cd	Co	Cu	Ni	Pb	Zn	Reference
Fuzhou	0.35–0.70		3.23–3.65		1.76–2.45	21.01–24.60	Gao and You (1992)
Bohai and Huanghai Sea	0.22–3.50		0.48–2.30		0.05–0.81	7.20–89.8	He (1996)
Dalian Bay	0.34		0.58		0.06	34.49	Xue et al. (1994)
Dalian Bay ^a	0.42	0.22	4.54	0.86	0.59	50.60	Liu et al. (1995)
Xinghai Bay ^a	0.20	0.69	2.32	0.18	0.64	49.60	Liu et al. (1995)

^a The values have been converted to wet weight contents (calculated as 80% water content). The further comparison to other regions of the concentrations of heavy metals in *M. edulis* can be seen in the work by Szefer et al. (1999a,b, 2002, 2004).

Fig. 2. The enrichment factor (EF) of heavy metals in mollusks. X axis: species; Y axis: heavy metal; Z axis: EF. (a1) As, Cd, Co, Cr, Cu, and Fe of 2002 samples; (a2) Mn, Ni, Pb, Se, Zn, and Hg of 2002 samples; (b1) As, Cd, Co, Cr, Cu, and Fe of 2003 samples; (b2) Mn, Ni, Pb, Se, Zn, and Hg of 2003 samples. WH: Weihai; PL: Penglai; YAK: Yangkou; TJ: Tianjin; QHD: Qinhuangdao; HLD: Hutliao; YIK: Yingkou; DL: Dalian.

Fig. 3. Mean concentrations of heavy metals ($\mu\text{g/g}$) in the studied mollusks.

concentration of one specific metal ion; and $[L]$ represents the concentration of one specific ligand. Since the stability constant (β) that appeared in Eq. (2) is invariable, the metal ion concentration (i.e., metal input) and quantities of ligand (i.e., tissues of mollusks) are two main factors determining the concentrations of the complex metals. Accordingly, significant correlations between some heavy metals simply imply that the variations in C_{Me} with different mollusks of coastal sites along the Bohai Sea comply well with the input of heavy metals discharging into the waters or sediment and β (which is related with the property of heavy metal).

In order to reduce relatively large numbers of variables to a smaller number of orthogonal factors, the original data obtained in the present study were processed by multivariate statistical techniques and principal component

analysis (PCA), and the results are shown in 3-D loading plots (Fig. 4). In the present study, the first three factors described 68.06% and 67.38% of the total variance (concentrations of metal of 2002 samples and 2003 samples), respectively. On the whole, PCA revealed the presence of three groups of metals in two batches of samples. The first comprised As, Se, and Hg; the second comprised Cd, Cu, and Zn; and the third comprised Co, Cr, Cu, Fe, (Pb), Mn, and Ni.

V.M. Goldschmidt (Zhang et al., 1994) classified elements into several groups: elements that have iron affinity, such as Fe, Co, Ni, and Pb, which accumulate in centrosphere; elements that have sulfur affinity, such as Cu, Zn, Cd, Hg, As, and Se, which accumulate in sulfide; elements that have stone affinity, such as Mn, which

Table 5
Results of the one-way ANOVA of multiple comparisons for metal concentrations

	As		Cd		Co		Cr		Cu		Fe		Mn			
2002 Samples	Rap*	Significance	Rap*	Significance	Mac*	Significance	Rud	Significance	Rap*	Significance	Cra*	Significance	Mac*	Significance	Mac*	Significance
	Nev	0.050	Nev	0.002	Rap	0.000	Rap	0.045	Nev	0.003	Rap	0.180	Rap	0.000	Rap	0.000
	Sca	0.000	Sca	0.039	Nev	0.000	Nev	0.013	Sca	0.000	Nev	0.000	Nev	0.000	Nev	0.001
	Myt	0.000	Myt	0.005	Sca	0.000	Sca	0.062	Myt	0.000	Sca	0.000	Sca	0.000	Sca	0.000
	Amu	0.000	Amu	0.047	Myt	0.000	Myt	0.054	Amu	0.000	Myt	0.000	Myt	0.000	Myt	0.000
	Cra	0.000	Cra	0.009	Amu	0.000	Amu	0.029	Cra	0.180	Amu	0.000	Amu	0.000	Amu	0.000
	Mer	0.000	Mer	0.001	Cra	0.000	Cra	0.260	Mer	0.000	Mer	0.000	Cra	0.001	Cra	0.014
	Rud	0.007	Rud	0.002	Mer	0.008	Mer	0.008	Rud	0.000	Rud	0.000	Mer	0.000	Mer	0.000
	Mac	0.000	Mac	0.004	Rud	0.023	Mac	0.688	Mac	0.000	Mac	0.000	Rud	0.008	Rud	0.000
2003 Samples	Rap	Significance	Cra	Significance	Mac	Significance	Mac*	Significance	Rap	Significance	Cra	Significance	Mac	Significance	Mac	Significance
	Nev	0.000	Rap	0.434	Rap	0.000	Rap	0.140	Nev	0.041	Rap	0.062	Rap	0.000	Rap	0.000
	Sca	0.000	Nev	0.018	Nev	0.000	Nev	0.399	Sca	0.000	Nev	0.000	Nev	0.000	Nev	0.000
	Myt	0.000	Sca	0.216	Sca	0.000	Sca	0.028	Myt	0.000	Sca	0.000	Sca	0.000	Sca	0.000
	Amu	0.000	Myt	0.022	Myt	0.000	Myt	0.225	Amu	0.000	Myt	0.000	Myt	0.004	Myt	0.000
	Cra	0.000	Amu	0.411	Amu	0.000	Amu	0.033	Cra	0.062	Amu	0.000	Amu	0.000	Amu	0.000
	Mer	0.000	Mer	0.015	Cra	0.000	Cra	0.198	Mer	0.002	Mer	0.000	Cra	0.010	Cra	0.000
	Rud	0.000	Rud	0.010	Mer	0.012	Mer	0.993	Rud	0.000	Rud	0.000	Mer	0.001	Mer	0.000
	Mac	0.000	Mac	0.013	Rud	0.000	Rud	0.050	Mac	0.001	Mac	0.000	Rud	0.001	Rud	0.000
	Ni						Pb		Se		Zn		Hg			
2002 Samples	Amu	Significance	Mer*	Significance	Rud*	Significance	Mac*	Significance	Rap*	Significance	Rap*	Significance	Cra*	Significance	Rap*	Significance
	Rap	0.012	Rap	0.000	Rap	0.000	Rap	0.607	Nev	0.088	Nev	0.001	Rap	0.439	Nev	0.208
	Nev	0.011	Nev	0.000	Nev	0.000	Nev	0.001	Sca	0.005	Sca	0.001	Nev	0.000	Sca	0.079
	Sca	0.014	Sca	0.000	Sca	0.000	Sca	0.037	Myt	0.001	Myt	0.002	Sca	0.000	Myt	0.219
	Myt	0.007	Myt	0.000	Myt	0.000	Myt	0.162	Amu	0.000	Amu	0.004	Myt	0.000	Amu	0.021
	Cra	0.010	Amu	0.039	Amu	0.008	Amu	0.014	Cra	0.000	Cra	0.439	Amu	0.001	Cra	0.028
	Mer	0.039	Cra	0.000	Cra	0.000	Cra	0.587	Mer	0.000	Mer	0.001	Mer	0.000	Mer	0.010
	Rud	0.008	Rud	0.424	Mer	0.424	Mer	0.002	Rud	0.000	Rud	0.001	Rud	0.000	Rud	0.012
	Mac	0.147	Mac	0.000	Mac	0.000	Rud	0.035	Mac	0.001	Mac	0.001	Mac	0.000	Mac	0.024
2003 Samples	Amu	Significance	Mer	Significance	Rud	Significance	Mer	Significance	Rap	Significance	Rap	Significance	Cra	Significance	Rap	Significance
	Rap	0.000	Rap	0.000	Rap	0.000	Rap	0.003	Nev	0.201	Nev	0.054	Rap	0.000	Nev	0.654
	Nev	0.000	Nev	0.000	Nev	0.000	Nev	0.006	Sca	0.000	Sca	0.009	Nev	0.000	Sca	0.001
	Sca	0.000	Sca	0.000	Sca	0.000	Sca	0.025	Myt	0.000	Myt	0.009	Sca	0.000	Myt	0.003
	Myt	0.000	Myt	0.000	Myt	0.000	Myt	0.109	Amu	0.000	Amu	0.068	Myt	0.000	Amu	0.004
	Cra	0.000	Amu	0.000	Amu	0.403	Amu	0.004	Cra	0.000	Cra	0.000	Amu	0.000	Cra	0.012
	Mer	0.000	Cra	0.000	Cra	0.000	Cra	0.102	Mer	0.000	Mer	0.030	Mer	0.000	Mer	0.001
	Rud	0.403	Rud	0.002	Mer	0.002	Rud	0.012	Rud	0.000	Rud	0.005	Rud	0.000	Rud	0.002
	Mac	0.001	Mac	0.000	Mac	0.000	Mac	0.530	Mac	0.000	Mac	0.002	Mac	0.000	Mac	0.001

The mean difference of bold is significant at the 0.05 level.

Table 6
Correlation matrixes of the concentrations of heavy metals in mollusks

	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn	Hg
As	1	0.076	−0.280*	−0.033	0.368	−0.197	−0.270*	−0.223	−0.248	0.705	0.121	0.71
Cd	0.750	1	−0.241	−0.014	0.486	0.021	−0.185	−0.178	0.013	0.319*	0.659	0.134
Co	−0.171	−0.164	1	0.265*	−0.255	0.589	0.565	0.456	0.396	−0.268*	−0.269*	−0.227
Cr	−0.087	−0.055	0.285*	1	0.114	0.627	0.270*	0.344	0.789	0.047	0.022	−0.004
Cu	0.227	0.201	−0.265*	−0.076	1	0.122	0.037	−0.270*	0.111	0.443	0.816	0.361
Fe	−0.180	−0.073	0.768	0.454	−0.068	1	0.613	0.131	0.726	−0.201	0.051	−0.169
Mn	−0.212	−0.144	0.714	0.330*	0.021	0.744	1	−0.062	0.323*	−0.254	0.024	−0.122
Ni	−0.154	−0.170	0.476	0.187	−0.312*	0.133	0.005	1	0.351	−0.197	−0.246	−0.23
Pb	0.030	0.206	0.438	0.324*	0.189	0.562	0.490	−0.130	1	−0.161	0.043	−0.208
Se	0.758	0.679	−0.251	−0.058	0.322*	−0.228	−0.179	−0.288*	0.003	1	0.299*	0.577
Zn	0.544	0.671	−0.236	−0.030	0.561	−0.069	−0.066	−0.248	0.326*	0.463	1	0.210
Hg	0.231	0.209	−0.147	−0.124	0.379	−0.177	−0.084	−0.237	0.266*	0.324*	0.385	1

Bold: Correlation is significant at the 0.01 level (two-tailed).

Upper triangular matrix is the correlation matrix of heavy metals of 2002 samples.

Lower triangular matrix is the correlation matrix of heavy metals of 2003 samples.

* Correlation is significant at the 0.05 level (two-tailed).

accumulates in silicate. Elements belonging to the same group have similar chemical properties. Based on his doctrine, the results of correlation analysis, PCA, as well

as the positions of 12 elements in the periodic table of elements, we divided these elements into four groups:

- A: Fe, Co, and Ni, which belong to the eighth group (iron affinity);
- B: Cr and Mn, which belong to the sixth and seventh subgroups, respectively (sulfur affinity);
- C: Cu, which belongs to the first subgroup; Zn, Cd, and Hg, which belong to the second subgroup (stone affinity);
- D: the fourth main group element Pb, the fifth main group element As, and the sixth main group element Se (stone affinity).

We found that there were significant correlations of elements in adjacent main group or adjacent subgroup in periodic table of elements. For example, there were significant correlations among tissue concentrations of: Cr (B)–Mn (B)–Fe (A)–Co (A)–Ni (A). Relatively strong associations were also observed among Cu (C)–Zn (C)–Cd (C)–Hg (C); As (D)–Se (D). In addition, Pb (D) was significant correlated with Cr (B), Mn (B), Fe (A), and Co (A). A–A correlation, B–B correlation, C–C correlation,

Fig. 4. Configurations of the studied heavy metals obtained by principal component analysis (PC1 vs. PC2 vs. PC3) of: (a) 2002 sample; (b) 2003 sample.

Table 7
Correlation data between elements during two times of sampling

Class	Sample 1		Sample 2	
	Correlation number	Percentage	Correlation number	Percentage
A–A	11	54	21	46
B–B	3		1	
C–C	10		12	
D–D	3		7	
C–D	27		32	
A–B	19	19	19	12
A–C	4	17	23	27
A–D	13		20	
B–C	4	10	11	15
B–D	6		12	
Σ	100	100	158	100

D–D correlation, and C–D correlation all belong to the same kind of correlations. The elements that belong to different groups have some crossover (e.g., Fe has sulfur affinity as well as stone affinity, Se has iron affinity as well as sulfur affinity). The correlations between A group and B, C, and D groups, and correlations between B and C, and D groups belong to different kinds of correlations.

Further, we studied the relationships of any two heavy metals in different mollusks (i.e., Rap: $C_{As} = C_{Cd} + b$, where C is tissue concentration of heavy metal and b is intercept of regression equation). Table 7 shows the percentages of correlations that are significant. Correlations existing in the same groups accounted for about half of the total. This also complied the rule: there were significant correlations of elements in the adjacent main group or adjacent subgroup in the periodic table of elements.

4. Conclusion

Bioaccumulations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn in two gastropods and seven bivalves were evaluated. The species examined showed a great ability to accumulate metals from marine water. The studied mollusks possessed high potential as biomonitors for trace metals in the Bohai Sea, according to the results of one-way ANOVA.

Although the species we studied in the present work and suggested as potential biomonitors present numerous advantages, further studies are necessary to confirm the accumulation patterns observed in this work.

In summary, there are statistically significant correlations among measured concentrations of some metals in mollusks (Cr (B)–Mn (B)–Fe (A)–Co (A)–Ni (A); Cu (C)–Zn (C)–Cd (C)–Hg (C); As (D)–Se (D), etc.), especially those in adjacent main groups or adjacent subgroups in the periodic table of elements.

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