Ecotoxicology and Environmental Safety 109 (2014) 1–9

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Trace elements in major marketed marine bivalves from six northern coastal cities of China: Concentrations and risk assessment for human health

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ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form
22 July 2014
Accepted 23 July 2014
Available online 15 August 2014

Keywords:
Trace elements
Bivalves
Contamination
Bioaccumulation
Health risk assessment

ABSTRACT

One hundred and fifty nine samples of nine edible bivalve species (*Argopecten irradians*, *Chlamys farreri*, *Crassostrea virginica*, *Lasaea nipponica*, *Meretrix meretrix*, *Mytilus edulis*, *Ruditapes philippinarum*, *Scapharca subcrenata* and *Sinonovacula constricta*) were randomly collected from eight local seafood markets in six big cities (Dalian, Qingdao, Rizhao, Weifang, Weihai and Yantai) in the northern coastal areas of China for the investigation of trace element contamination. As, Cd, Cr, Cu, Hg, Pb and Zn were quantified. The risk of these trace elements to humans through bivalve consumption was then assessed. Results indicated that the concentrations of most of the studied trace element varied significantly with species: the average concentration of Cu in *C. virginica* was an order of magnitude higher than that in the remaining species; the average concentration of Zn was also highest in *C. virginica*; the average concentration of As, Cd and Pb was highest in *R. philippinarum*, *C. farreri* and *A. irradians*, respectively. Spatial differences in the concentrations of elements were generally less than those of interspecies, yet some elements such as Cr and Hg in the samples from different cities showed a significant difference in concentrations for some bivalve species. Trace element concentrations in edible tissues followed the order of Zn > Cu > As > Cd > Cr > Pb > Hg generally. Statistical analysis (one-way ANOVA) indicated that different species examined showed different bioaccumulation of trace elements. There were significant correlations between the concentrations of some elements. The calculated hazard quotients indicated in general that there was no obvious health risk from the intake of trace elements through bivalve consumption. But care must be taken considering the increasing amount of seafood consumption.

1. Introduction

The Earth system is currently operating in a no-analogue state, and human activities are significantly altering the environment on a global scale (Steffen et al., 2004). Massive amounts of inorganic/organic substances are discharged into the environment each year, transforming into pollutants and usually having negative effects. Trace elements are natural and fundamental components of the geosphere and biosphere. Many trace elements are essential to maintain the metabolism of organisms. However, they can be poisonous if their concentrations exceed certain limits. Once entering the environment, these elements cannot be degraded or destroyed but tend to bioaccumulate and increase in the concentrations over time, pass along the food chain and directly/indirectly influence human health. Many environmental quality guidelines, within which trace elements such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb) and zinc (Zn) are usually used as criteria, have been developed to deal with environmental concerns as well as to respond to regulatory programs (e.g. Rawson and Burton, 2002).

Fishery products are a very valuable source of protein and can provide essential micronutrients for human beings. The Food and Agriculture Organization of the United Nations (FAO) reported that fisheries and aquaculture supplied the world with about 128 million tons of food for people in 2010 (FAO (Food and Agricultural Organization of the United Nations), 2012). The share of aquaculture in the world's total fishery yield has been increasing significantly in recent years. China has been the world's leading aquaculture producer for decades and the aquaculture production accounted for 82 percent of the world's marine farmed

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http://dx.doi.org/10.1016/j.ecoenv.2014.07.023
0147-6513/

fishery yield in 2010 (FAO (Food and Agricultural Organization of the United Nations), 2012). Marine aquaculture production accounted for 39 percent of the total aquaculture production of China in 2010, about 70 percent of this was comprised of bivalves such as clams, oysters, scallops, mussels and cockles. As the country's first- and fourth-biggest marine aquaculture producers, respectively, Shandong and Liaoning Provinces together contributed 42.4 percent of the marine aquaculture production in China in 2011 according to the report from the BFMAC (Bureau of Fisheries of the Ministry of Agriculture of China) (2012).

The coastal zone is the main area for marine bivalve farming; meanwhile it traps a majority of pollutants in the process of transporting pollutants from land to sea. Most marine bivalves bury themselves in sediment on the seabed, some lie on the sea floor or attach themselves to rocks or other hard surfaces and a few can swim a short distance. Their living habits make them good indicators of environmental quality on a local/regional scale.

The entire coast of the Bohai Sea and the coast of the Yellow Sea in Shandong and Liaoning Provinces are located in the Bohai Sea Economic Rim (Fig. 1), one of the three most densely populated and industrialized zones in China (Gao et al., 2014). Extraordinarily rapid industrial and agricultural development in the northern coastal areas of China has resulted in a significant anthropogenic increase in the ambient levels of pollution and environmental damage in the marine ecosystem (Australian Agency for International Development, 1996; Jacinto, 1997; Xu et al., 2010; Zhang et al., 2010; Gao et al., 2014). Previous reports indicated that the edible marine bivalve species sampled from the Bohai Sea had been contaminated with trace elements to different degrees (Liang et al., 2004; Wang et al., 2005; Du et al., 2009). However, the purpose of these studies was merely monitoring the environmental quality, and there was a lack of risk assessment concerning human health from seafood consumption.

In this study, the edible tissues of nine most marketed marine bivalve species collected from six northern big coastal cities of China, namely Dalian, Qingdao, Rizhao, Weifang, Weihai and Yantai, were analyzed for the determination of As, Cd, Cr, Cu, Hg, Pb and Zn. The sampled cities were all located in the Bohai Sea Economic Rim of China. The main objectives of this study were to examine whether the widely consumed bivalve species were contaminated with trace elements, and to test whether there were any spatial or interspecific variations in the concentrations of trace elements. Health risk assessment was then conducted to evaluate whether these bivalves posed any potential risk to human beings as a result of consumption. From a public health perspective, another aim of this study was to provide consumers with better knowledge of contamination problems associated with seafood consumption. We also tried to compare the trace element results with those obtained from other studied coastal areas.

2. Materials and methods

2.1. Sampling

One hundred and fifty nine samples of nine marine bivalve species of similar sizes were randomly collected from eight large seafood markets in six big cities (Dalian, Qingdao, Rizhao, Weifang, Weihai and Yantai) in the northern coastal areas of China during 6th–27th November 2011 (Fig. 1). To ensure randomness and sufficient representativeness of sampling, 3–4 samples for each species from each city and 10–50 individuals with similar body lengths for each sample were collected. The samples were purchased with their usual conditions in markets and transferred to the laboratory in a cooler box with ice packs. The sampled bivalve species are identified as follows: *Argopecten irradians*, *Chlamys farreri*, *Crassostrea virginica*, *Lasaea nipponica*, *Meretrix meretrix*, *Mytilus edulis*, *Ruditapes philippinarum*, *Scapharca subcrenata* and *Sinonovacula constricta*.

2.2. Sample analysis

In the laboratory, the edible tissues of the collected bivalves were then dissected, rinsed with deionized water (18.2 MΩ-cm) three times to remove extraneous impurities, and any excess rinsing water was drained off and vaporized. The wet weights of all edible bivalve tissues were first recorded, and the tissues were then freeze dried until constant weights, after which their dry weights were recorded. The percentages of water were calculated and used to convert the trace element concentrations of the samples from a dry weight basis to a wet weight basis. The dried samples were homogenized and ground with a ceramic mortar and stored in small polyethylene zipper bags at −20 °C until further analysis.

For each sample, ~0.3 g of dried and ground soft tissues was weighed and added to a polytetrafluoroethylene (PTFE) digestion container with known weight. Each sample was added with 10 ml of concentrated nitric acid and left to predigest overnight at 80 °C. After cooling, the container was covered and placed in a high-pressure stainless steel bomb and then put in an oven. Thereafter the oven temperature was increased to 160 °C and kept for 8 h till clarification. After cooling to the room temperature, the solution was diluted with deionized water to the final weight of 50 g and then transferred into the polyethylene terephthalate (PET) bottle. As, Cd, Cr, Cu, Pb, and Zn were determined with the PerkinElmer ELAN DRC II

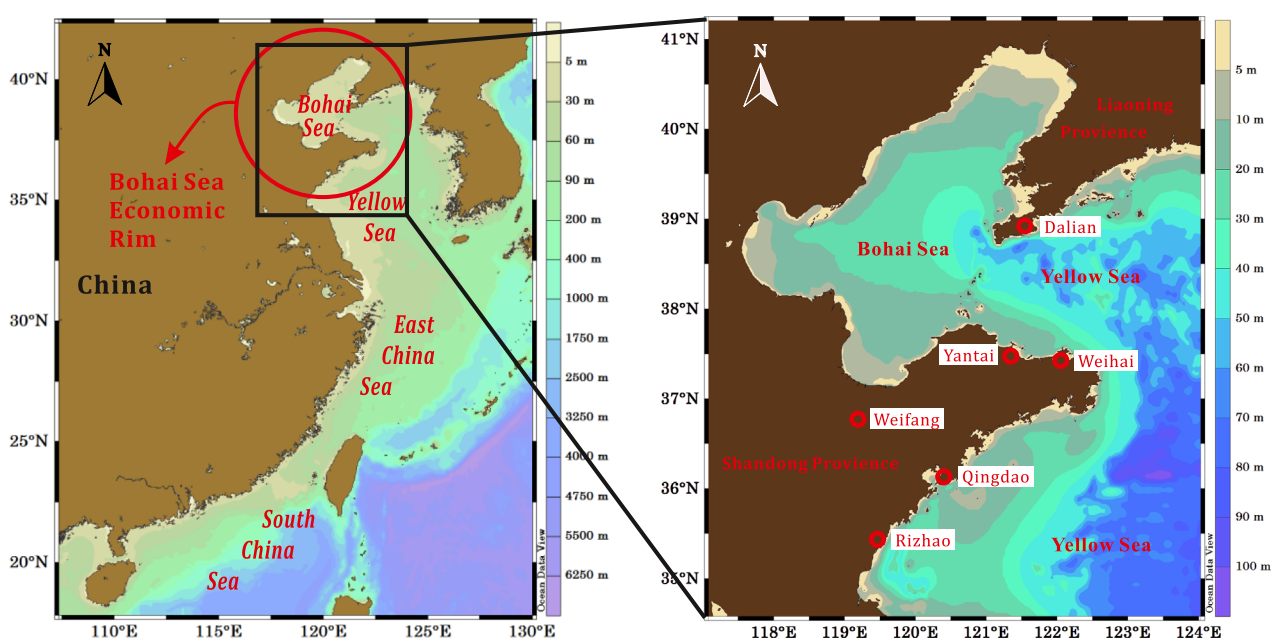

Fig. 1. Sampled cities along the northern coast of China.

inductively coupled plasma-mass spectrometer. The working parameters are listed below: RF power 1100 W; sampling depth 7 mm; carrier gas flow rate 0.98 l/min; makeup gas flow rate 0.1 l/min; spray chamber temperature 2 °C; sample uptake rate 0.4 ml/min. The openings of the nickel sampling cone and skimmer cone were 1.0 and 0.4 mm, respectively. Hg was specifically analyzed using a selective hydride generation-atomic fluorescence spectrometer.

All the analyses were repeated three times by external calibration method and all the samples were blank-subtracted. The accuracy of the digestion method was validated by the determination of certified reference materials—mussel (GBW 08571) and *Pseudosciaena crocea* (GBW 08573), and the results are listed in Table S1. The recorded values of all elements were in good agreement with the certified values, with the recoveries ranging from 82.7 percent to 112.3 percent, suggesting that the applied method was feasible in the determination of trace metals in biota samples. The method detection limit was defined as three times the standard deviation of the blank samples or as the instrumental detection limit (*IDL*) if the blanks had no detectable contamination. *IDL* values were all lower than 0.1 ng/g. The relative standard deviations were within 5 percent for all trace elements. The concentrations of the studied trace elements were expressed as μg/g wet weight of the edible tissues of the collected bivalves.

All glass vessels were pre-cleaned by soaking in 50 percent (v/v) nitric acid solution for at least 12 h and then rinsed with deionized water for several times. The PTFE containers were boiled with 50 percent (v/v) nitric acid solution and the PET bottles were immersed in 5 percent (v/v) nitric acid solution for 24 h followed by rinsing with deionized water.

The nitric acid used was guarantee reagent. The stock calibration standards were adopted from the Chinese CRM/RM Information Center. The standards for calibration curves were prepared by diluting the stock solution with deionized water. The mixture of 5.0 ng/g ^{115}In in 1 N nitric acid solution was used as calibration internal standard.

2.3. Statistical analysis

Statistical analyses were performed by SPSS version 16.0 for Windows. One-way analysis of variance (ANOVA) and post-hoc least significant difference tests followed by correlation analysis (bivariate correlations) were used to extract information from the chemical analysis in order to find out the relationships among the trace elements (Capelli et al., 2000; Szefer et al., 2002). In this work, the results of chemical analysis were presented as mean ± SD. The value of $P<0.05$ was considered to indicate a significant difference in all statistical analysis. Cluster analysis was performed based on the analytical data by unweighted pair-group average method, using Kendall correlation coefficient as a measure of similarity, to obtain a visual representation of spatial and interspecific variations among the trace element concentrations.

2.4. Human exposure assessment

The health risk assessment for people from the northern China was conducted using the provisional tolerance weekly intake (*PTWI*), acceptable daily intake (*ADI*), and reference dose (*RfD*) established by the United States Environmental Protection Agency (USEPA) and the Joint FAO/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (USEPA (United States Environmental Protection Agency), 2011; JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003). The estimated daily intake (*EDI*) (μg/kg/day) was calculated using the following equation: $EDI = C_{bivalve} \times (dc_{bivalve}/bw)$, where $C_{bivalve}$ = average trace element concentration in bivalve (μg/g wet weight), $dc_{bivalve}$ = daily bivalve consumption (g/day) per capita of Chinese mainland residents in 2011 as reported by the FAO (Food and Agricultural Organization of the United Nations) (2011) and the BFMAC (Bureau of Fisheries of the Ministry of Agriculture of China) (2012), and bw = the average body weight (kg) of the target population. The hazard quotient (*HQ*) was calculated using the following equation: $HQ = EDI/RfD$. If the *HQ* was less than one, there would be no obvious risk of excessive intake of trace element from bivalve consumption.

3. Results and discussion

3.1. Levels of trace element concentrations

The concentrations of trace elements in the edible parts of the nine major marketed bivalves from six northern coastal cities of China are shown in Fig. 2 and summarized in Table 1. The results indicated that As, Cr, Hg, and Pb in all the collected bivalve species had comparable levels while Cd, Cu, and Zn exhibited apparent differences in different bivalve species. The concentrations of each trace element in marketed bivalves were described below and compared with earlier studies on bivalves (Table 1).

The concentrations of As had a narrow range of variation (0.87–2.94 μg/g) in different species, consistent with previous observations on bivalves (0.18–2.97 μg/g) (Lafabrie et al., 2007; Wang et al., 2007; Whyte et al., 2009). By estimating inorganic As concentration to be 10 percent of the total arsenic concentration according to the USFDA (United States Food and Drug Administration) (1993), the As levels in all the collected bivalve species were all within the restrictive range (inorganic As 1.0 μg/g wet weight) set up by the AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China) (2001).

The Cd levels had a wide range of variation (0.04–6.44 μg/g) and showed big differences among different species, which were consistent with previous observations on bivalves (0.04–6.5 μg/g) (Lafabrie et al., 2007; Liu et al., 2007; Ruan, 2008; Whyte et al., 2009). The Cd levels in *A. irradians* and *C. farreri* from all the sampled cities, *Crassostrea gigas* from Weihai and Yantai, and *S. subcrenata* from Qingdao and Rizhao were all higher than the safety level of 2.0 μg/g wet weight set up by the WHO (1982). *L. nipponica*, *M. meretrix*, *R. philippinarum* and *S. constricta* had comparably low Cd concentrations with insignificant differences among the sampled cities. *C. farreri* from Qingdao had the highest Cd level (6.44 μg/g), which was 160 times higher than the lowest Cd level (0.04 μg/g) of *S. constricta* from Weihai.

The concentrations of Cr in different bivalve species from different cities ranged from 0.09 to 4.91 μg/g, which were comparable with previous observations on bivalves (0.18–3.65 μg/g) (Lafabrie et al., 2007; Sivaperumal et al., 2007). *M. edulis* from Yantai had the highest Cr concentration, while the lowest Cr concentration was found in *S. subcrenata* from Weihai. The Cr levels in all the collected edible bivalve species were all within the restrictive range (2.0 μg/g wet weight) set up by the AQSIQ except *M. edulis* and *C. farreri* from Yantai (AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China), 2001). No significant difference was found among different species with a few exceptions.

The Cu levels ranged from 0.68 to 95.27 μg/g and showed a big difference among different species. *C. gigas* from Yantai showed the highest Cu level (95.27 μg/g), about 140 times higher than the lowest Cu level (0.68 μg/g) of *L. nipponica* from Weifang. The Cu levels in *C. gigas* (26.82 μg/g from Dalian, 70.41 μg/g from Qingdao, 75.11 μg/g from Rizhao, 86.66 μg/g from Weifang, 61.10 μg/g from Weihai, and 95.27 μg/g from Yantai) were all the highest among the studied bivalve species from different sampled cities. Other bivalve species had comparable levels. The Cu concentrations in the collected edible bivalve species except *C. gigas* from Qingdao, Rizhao, Weifang, Weihai and Yantai, were all lower than the safety level of 50 μg/g wet weight set up by the AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China) (2001). The concentrations of Cu in bivalves have been previously reported at 1.38–10.25 μg/g (Wang et al., 2007) and 1.17–24.1 μg/g (Sivaperumal et al., 2007). Our results were thus 4–9 times higher than those previous measurements. Ruan (2008) reported comparable Cu levels of 0.88–60.3 μg/g in four bivalve species from Xiamen, China.

Meanwhile, the concentrations of Hg in different bivalve species were generally low (0.01–0.15 μg/g) among different sampled cities, which had comparable levels with previous observations on bivalves (0.006–0.12 μg/g) (Lafabrie et al., 2007; Liu et al., 2007; Wang et al., 2007; Whyte et al., 2009). The Hg levels in the collected edible bivalve species were all within the restrictive level (0.3 μg/g wet weight) set up by the AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China) (2001). No clear spatial difference was found among all the sampled cities. The highest

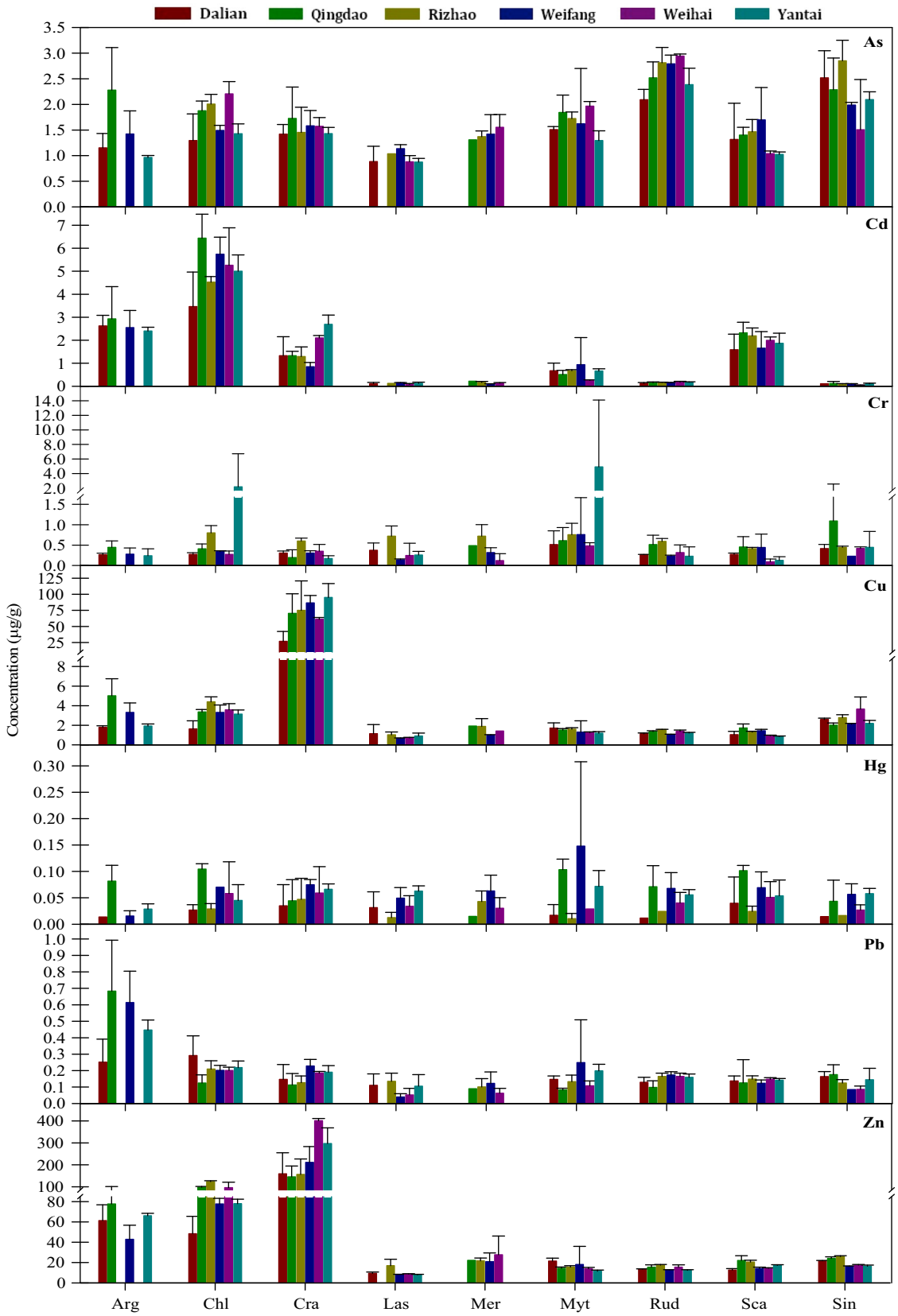

Fig. 2. Trace element concentrations (wet weight basis) in nine edible bivalve species from six northern coastal cities of China (Arg: *Argopecten irradians*; Chl: *Chlamys farreri*; Cra: *Crassostrea virginica*; Las: *Lasaea nipponica*; Mer: *Meretrix meretrix*; Myt: *Mytilus edulis*; Rud: *Ruditapes philippinarum*; Sca: *Scapharca subcrenata*; Sin: *Sinonovacula constricta*).

concentration was recorded in *M. edulis* from Weifang (0.15 μg/g), followed by *C. farreri*, *M. edulis* and *S. subcrenata* from Qingdao (0.10 μg/g).

The Pb levels in different bivalve species had a narrow range of variation (0.04–0.68 μg/g) and showed an insignificant difference among different locations. The highest level was recorded in

Table 1
Comparison of trace element concentrations (μg/g, wet weight) in bivalves from different areas (Arg: *Argopecten irradians*; Chl: *Chlamys farreri*; Cra: *Crassostrea virginica*; Las: *Lasaea nipponica*; Mer: *Meretrix meretrix*; Myt: *Mytilus edulis*; Rud: *Ruditapes philippinarum*; Sca: *Scapharca subcrenata*; Sin: *Sinonovacula constricta*).

Location	Sampling time	Species		As	Cd	Cr	Cu	Hg	Pb	Zn	References
Cities in the northern coast of China	Nov. 2011	Arg	Range	0.96–2.28	2.40–2.93	0.24–0.44	1.79–5.01	0.01–0.08	0.25–0.68	42.91–77.61	This study
			Mean ± SD	1.45 ± 0.58	2.63 ± 0.22	0.31 ± 0.09	3.01 ± 1.50	0.03 ± 0.03	0.50 ± 0.19	61.98 ± 14.44	
		Chl	Range	1.29–2.20	3.47–6.44	0.27–2.18	1.63–4.39	0.03–0.10	0.12–0.29	48.37–123.47	
			Mean ± SD	1.72 ± 0.36	5.07 ± 1.02	0.71 ± 0.74	3.23 ± 0.90	0.06 ± 0.03	0.21 ± 0.05	86.91 ± 25.24	
		Cra	Range	1.42–1.73	0.84–2.69	0.17–0.60	26.82–95.27	0.03–0.07	0.11–0.23	145.43–401.72	
			Mean ± SD	1.53 ± 0.12	1.60 ± 0.67	0.32 ± 0.15	69.23 ± 24.01	0.05 ± 0.01	0.16 ± 0.04	228.59 ± 101.88	
		Las	Range	0.87–1.13	0.08–0.15	0.14–0.72	0.68–1.14	0.01–0.06	0.04–0.11	7.94–16.93	
			Mean ± SD	0.96 ± 0.12	0.12 ± 0.03	0.35 ± 0.22	0.89 ± 0.20	0.04 ± 0.02	0.09 ± 0.04	10.28 ± 3.75	
		Mer	Range	1.31–1.55	0.10–0.22	0.12–0.72	0.98–1.92	0.01–0.06	0.06–0.12	20.90–27.66	
			Mean ± SD	1.41 ± 0.10	0.15 ± 0.05	0.41 ± 0.26	1.55 ± 0.44	0.04 ± 0.02	0.09 ± 0.02	23.09 ± 3.08	
		Myt	Range	1.29–1.97	0.25–0.94	0.48–4.91	1.18–1.70	0.01–0.15	0.08–0.25	11.45–21.52	
			Mean ± SD	1.66 ± 0.24	0.62 ± 0.23	1.34 ± 1.76	1.42 ± 0.21	0.06 ± 0.05	0.15 ± 0.06	15.85 ± 3.5 4	
		Rud	Range	2.09–2.94	0.12–0.18	0.23–0.59	1.06–1.55	0.01–0.07	0.10–0.17	12.34–17.60	
			Mean ± SD	2.59 ± 0.32	0.16 ± 0.02	0.36 ± 0.16	1.28 ± 0.18	0.05 ± 0.02	0.15 ± 0.03	14.52 ± 2.00	
		Sca	Range	1.02–1.70	1.59–2.32	0.09–0.46	0.85–1.44	0.02–0.10	0.12–0.15	12.61–21.98	
			Mean ± SD	1.32 ± 0.26	1.93 ± 0.29	0.30 ± 0.16	1.21 ± 0.33	0.06 ± 0.03	0.14 ± 0.01	16.81 ± 3.78	
		Sin	Range	1.51–2.85	0.04–0.12	0.23–1.09	2.01–3.64	0.01–0.06	0.08–0.18	16.27–25.83	
			Mean ± SD	2.21 ± 0.46	0.10 ± 0.03	0.51 ± 0.30	2.55 ± 0.61	0.04 ± 0.02	0.13 ± 0.04	20.31 ± 4.13	
		Summary	Range	0.87–2.94	0.04–6.44	0.09–4.91	0.68–95.27	0.01–0.15	0.04–0.68	7.94–401.72	
			Mean ± SD	1.68 ± 0.55	1.40 ± 1.68	0.53 ± 0.72	10.13 ± 23.63	0.05 ± 0.03	0.17 ± 0.12	54.89 ± 78.02	
Zhejiang coastline, East China Sea	Aug. 2002	Sin	Range	0.44–0.81	0.05–0.33	ND	2.27–9.11	0.006–0.021	0.06–0.22	6.92–17.90	Huang et al. (2007)
Montenegro, SE Adriatic	Sep. 2005–Feb. 2009	Myt	Range	0.3–4.1	0.38–0.60	ND	0.6–2.9	0.01–0.17	0.21–2.4	9.9–71.5	Stanković et al. (2011)
Bohai Sea	Jul.–Aug. 2002	Cra	Range	ND	0.51–3.29	ND	25.24–109.60	ND	0.21–0.62	115.95–379.55	Liang et al. (2004)
		Myt	Range	ND	0.34–1.96	ND	1.16–2.52	ND	0.16–0.60	11.87–22.42	
		Rud	Range	ND	0.14–0.63	ND	1.28–2.60	ND	0.13–0.34	9.95–20.06	
Bohai Sea	Jul.–Aug. 2003	Cra	Range	1.04–2.16	0.94–32.18	0.21–0.66	34.69–146.85	0.01–0.04	0.05–0.52	141.86–550.12	Wang et al. (2005)
		Mer	Range	0.90–1.78	0.17–0.70	0.17–0.30	1.69–33.83	0.02–0.02	0.06–1.33	22.21–53.97	
		Myt	Range	0.92–4.97	0.17–1.29	0.18–0.67	0.98–5.40	0.01–0.03	0.09–0.59	9.76–42.17	
		Rud	Range	1.98–2.85	0.14–0.29	0.16–0.35	1.44–3.56	0.02–0.04	0.08–0.28	10.84–32.38	
		Sca	Range	0.84–2.55	1.40–12.84	0.13–0.44	1.08–4.45	0.01–0.03	0.08–0.45	11.56–30.59	
The north-western Mediterranean[a]	Summer 2004 and 2005	Myt	Range	ND	0.23–0.36	0.09–0.60	ND	0.018–0.024	0.21–0.29	ND	Lafabrie et al. (2007)
The northern China seas	Oct.–Nov. 2005	Arg	Range	0.18–0.42	ND	ND	1.38–4.41	0.006–0.021	0.06–0.17	42.51–92.34	Wang et al. (2007)
		Chl	Range	0.27–0.50	ND	ND	2.29–10.25	0.014–0.023	0.11–0.25	81.29–209.72	
The Bay of Islands, northern New Zealand	Dec. 2005	Myt	Range	1.56–2.97	0.07–0.75	ND	ND	0.04–0.06	0.03–0.10	ND	Whyte et al. (2009)
Bohai Sea	Summer	Mussel	Range	0.29–12.15	0.27–6.50	ND	ND	0.006–0.108	0.033–0.895	ND	Liu et al. (2007)
Fish markets in and around Cochin, India	Jul. 2003–Jan. 2005	Myt	Range	BDL-0.69	BDL-0.98	0.18–3.65	1.17–24.1	BDL-0.33	BDL-0.98	3.8–37.7	Sivaperumal et al. (2007)
Shellfish culture areas of Xiamen	Apr. 2005	Cra	Range	ND	0.38–0.72	ND	20.9–60.3	ND	0.20–0.37	90.0–163.5	Ruan (2008)
		Rud	Range	ND	0.11–0.35	ND	1.02–2.26	ND	0.09–0.28	12.4–17.9	
		Sin	Range	ND	0.04–0.05	ND	2.69–4.39	ND	0.26–0.48	14.6–20.0	
		Tegillarca granosa	Range	ND	1.14–1.16	ND	0.88–0.97	ND	0.20–0.22	15.5–17.3	

ND: no data.
BDL: below detection limit.
[a] The values have been converted to wet weight contents (calculated as 80% water content).

A. irradians from Qingdao, followed by *A. irradians* from Weifang (0.61 μg/g). Our results were consistent with previous measurements of bivalves (0.033–1.43 μg/g) (Lafabrie et al., 2007; Liu et al., 2007; Wang et al., 2007; Whyte et al., 2009). The Pb levels in all the collected edible bivalve species were all below the restrictive concentration (1.5 μg/g wet weight) set up by the EC (European Commission) (2006).

The concentrations of Zn in different bivalve species had a wide range of variation (7.94–401.72 μg/g) and showed a significant difference among different sampled cities. The results were rather comparable to the earlier observations on bivalves (2.51–379.55 μg/g) (Sivaperumal et al., 2007; Wang et al., 2007; Ruan, 2008). The Zn levels in all studied species were all within the restrictive range (inorganic As 1.0 μg/g wet weight) set up by the WHO (WHO, 1982). The concentrations of Zn in *L. nipponica*, *M. meretrix*, *M. edulis*, *R. philippinarum*, *S. subcrenata* and *S. constricta* from the sampled cities varied within relatively narrower ranges and the mean values of Zn in these species were apparently lower than in *A. irradians*, *C. farreri* and *C. gigas*. The highest Zn concentration (401.72 μg/g) was recorded in *C. gigas* from Weihai, which was about 50 times higher than the lowest Zn level (7.94 μg/g) of *L. nipponica* from Yantai. *C. gigas* from Yantai exhibited the second highest Zn concentration (297.13 μg/g) which was comparable with other areas. The concentrations of Zn in some species (*C. gigas* from all the sampled cities and *C. farreri* from Rizhao) exceeded the maximum permissible levels of 100 μg/g wet weight set up by the WHO (1982).

In general, among different bivalve species from different locations, Zn had the highest concentrations, followed by Cu, As, Cd, Cr, and Pb, while the Hg levels were the lowest. There were no clear spatial differences among locations for any studied trace element. Two possibilities may explain such a lack of spatial patterns. First, there was probably no obvious source of trace metal input. Second, some of these trace elements (e.g. Cu and Zn) are essential to marine animals, and it is likely that these elements may be regulated by the bivalves during the processes of their bioaccumulation. For example, the regulation of Cd, Cu, and Zn by bivalves has been demonstrated by previous studies (Blackmore and Wang, 2003; Pan and Wang, 2009). Information concerning whether bivalves can regulate Hg uptake is still not available.

A further comparison of the data obtained in this study with those previously reported indicates that the levels of trace elements found in bivalves are comparable to those reported for other areas home and abroad (Table 1).

It was reported that the concentrations of As, Cd, Cr, Cu, Hg, Pb, and Zn in marine bivalves from the Bohai Sea in northern China were generally within the range of 0.84–4.97 μg/g, 0.14–32.18 μg/g, 0.13–0.67 μg/g, 0.98–146.85 μg/g, 0.01–0.04 μg/g, 0.05–1.33 μg/g, and 9.76–550.12 μg/g, respectively (Liang et al., 2004; Wang et al., 2005). Our measurements of the bivalves consumed in the six northern coastal cities of China showed that the values obtained herein fell within the range of values commonly found in the previous measurements. The data of *M. edulis* in this study were rather similar to the previous measurements (e.g. 0.92–4.97 μg/g As, 0.17–1.29 μg/g Cd, 0.98–5.40 μg/g Cu, 0.01–0.03 μg/g Hg, 0.09–0.59 μg/g Pb, 9.76–42.17 μg/g Zn from the Bohai Sea; 0.38–0.60 μg/g Cd, 0.01–0.17 μg/g Hg, and 9.95–379.55 μg/g Zn from Montenegro, SE Adriatic) (Wang et al., 2005; Stanković et al., 2011). It could be found from Table 1 that the trace element contents in *C. gigas* at different sampling time and cities varied slightly except Cd contents. It was once up to 32.18 μg/g, indicating a severe Cd contamination status in the Bohai Sea at that time (Liang et al., 2004; Wang et al., 2005). Meanwhile, we found the trace element concentrations in *R. philippinarum, S. subcrenata,* and *S. constricta* in this study did not differ greatly from the previous reports of Liang et al. (2004), Wang et al. (2005) and Huang et al. (2007). In general, the levels of trace elements found in bivalves collected from the local seafood markets in the northern coastal cities of China were not very high and the contamination was moderate.

3.2. One-way ANOVA analysis and inter-elemental relationships in view of correlation

Significant differences in trace element concentrations were tested with one-way ANOVA for each species and for each trace element for the investigated samples. Table S2 shows the ANOVA results. Differences in trace element concentrations were found among the studied species. For example, *C. gigas* significantly accumulated Cu and Zn, while *M. edulis* accumulated Cr and Hg more efficiently than other bivalves. Similarly, a trace element can be accumulated by different bivalves. For example, As can be significantly accumulated by *R. philippinarum* and *S. constricta*. Results of correlation analyses (bivariate correlations with Pearson correlations coefficients) among these trace element concentrations are listed in Table S3 ($P<0.05$ or $P<0.01$). Cd was significantly correlated with Pb and Zn ($P<0.01$), while Cu was correlated with Zn ($P<0.01$). There were also some correlations between Cd and Hg ($P<0.05$).

To obtain a visual representation of spatial and interspecific variations among these trace element concentrations in the bivalve species, cluster analysis was performed based on the analytical data by the XLSTAT software. All the investigated samples could be grouped in terms of their similarity. The results obtained following hierarchical cluster analysis are shown as a dendrogram (Fig. 3) in which three well-defined clusters can be seen. A group of samples can be clearly discernible which is composed of adherent, benthic and sessile bivalves (YT-Chl, WH-Chl, WF-Chl, RZ-Chl, QD-Sca, DL-Arg, QD-Chl, DL-Chl, YT-Arg, WH-Sca, YT-Sca, WF-Sca, DL-Sca, RZ-Sca, YT-Cra, QD-Arg, WF-Arg, WF-Myt, WH-Mer, WH-Cra, WF-Cra, RZ-Cra, QD-Cra, DL-Cra and DL-Myt); There was no geographic variation in trace element concentrations in *C. farreri* and *S. subcrenata* with the similarity of 85 percent. At the same time, no geographic differences were found in *C. gigas* with the similarity of 90 percent. Zn and Cu made the greatest contribution (more than 98 percent) to the average similarity of 76.78 percent for *C. gigas* (Table S4). This is in agreement with the results of the trace element concentrations and *C. gigas* samples lay at some distance from the others. The second cluster consists of adherent bivalves (YT-Myt) alone because of the highest Cr levels in *M. edulis* from Yantai. The third cluster includes the rest ones which mainly belonged to adherent and sessile bivalves. *R. philippinarum* exhibited no geographic variation in trace element concentrations with the similarity of 90 percent. Comparable trace element levels in *R. philippinarum* samples may account for this. Zn and As made the contribution (88.83 percent, 92.88 percent, 85.94 percent) to the average similarity for *L. nipponica* (84.10 percent), *M. meretrix* (91.67 percent) and *M. edulis* (84.07 percent) (Table S4). Zn and Cd contributed more than 90 percent (93.43 percent, 94.08 percent) to the average similarity for scallops (84.42 percent for *A. irradians*, 82.04 percent for *C. farreri*) (Table S4). Moreover, the similarity (more than 90 percent for *C. gigas*, *R. philippinarum* and *S. constricta*; more than 85 percent for *C. farreri* and *S. subcrenata*) for the enrichment of trace elements indicated no significant difference among different locations. It is possible that the first cluster and the third cluster were well separated due to the variations in the bioaccumulation of trace elements between them. Assimilation efficiency, feeding activity (filtration rate), and efflux rate co-contributed to the observed interspecies differences which may explain such a phenomenon. The importance of efflux rate to intraspecies difference in Cu bioaccumulation has been demonstrated by the previous study (Pan and Wang, 2009).

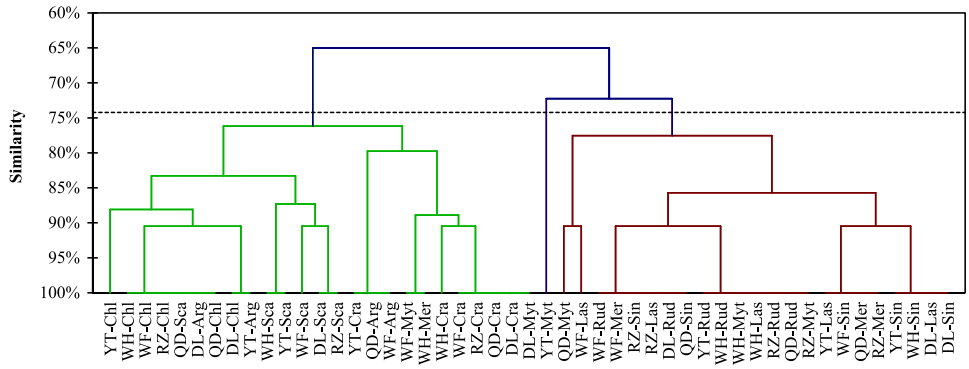

Fig. 3. The dendrogram for the similarity matrix of trace element concentrations in bivalves from six northern big coastal cities of China (DL: Dalian; QD: Qingdao; RZ: Rizhao; WF: Weifang; WH: Weihai; YT: Yantai; Arg: *Argopecten irradians*; Chl: *Chlamys farreri*; Cra: *Crassostrea virginica*; Las: *Lasaea nipponica*; Mer: *Meretrix meretrix*; Myt: *Mytilus edulis*; Rud: *Ruditapes philippinarum*; Sca: *Scapharca subcrenata*; Sin: *Sinonovacula constricta*).

3.3. Assessment of human exposure to trace elements

The element As exists in organisms in both organic and inorganic forms. According to most criteria for the amounts of trace elements in food, the concentration of inorganic As should be more restricted because it is more toxic than organic As. The results of As in this study were the sum of organic and inorganic As. The inorganic fraction was estimated by multiplying the As concentrations in this study by 10 percent according to the guidance document for arsenic in shellfish of the USFDA (United States Food and Drug Administration) (1993). As shown in Table 1, the concentrations of As, Hg, and Pb in all the collected edible bivalve species were all within the restrictive range (inorganic As 1.0, Hg 0.3, Pb 1.5 μg/g wet weight) set up by the AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China) (2001), EC (European Commission) (2006). However, Cd, Cu, and Zn exhibited apparent differences in different bivalve species (Fig. 2). Meanwhile, the Cu and Zn levels were extraordinarily high in all the collected *C. gigas* except those from Dalian and exceeded the maximum permissible levels (*MPLs*; Cu 50 and Zn 100 μg/g wet weight) set up by the AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China) (2001) and the WHO (1982), and the Cd content in those from Weihai and Yantai exceeded the restrictive value of Cd (2 μg/g wet weight) set up by the WHO (1982). Disturbingly, the Cd contents in *A. irradians* and *C. farreri* from all the studied cities exceeded the *MPL* set up by the WHO (1982). Obviously these three kinds of bivalves were not suitable for long-term consumption unless they are previously depurated to reduce the high levels of trace elements (Han and Hung, 1990; Chan et al., 1999).

In the present study, the estimated amount of daily bivalve consumption of 20 g wet weight/person/day in China (FAO, 2011; BFMAC (Bureau of Fisheries of the Ministry of Agriculture of China), 2012) and an assumed body weight of 58.1 kg for a Chinese person (Gu et al., 2006) were used to evaluate daily intake of the investigated elements by people in northern China through bivalve consumption. As shown in Table S5, the *EDI* of all trace elements except Cd by people were far below the *ADI* recommended by the FAO/WHO, indicating this intake would not pose a health risk in northern China at present. For Cd, this intake would pose a potential health risk for people in northern China. The *RfDs* were 0.3 μg/kg bw/day for inorganic As, 1 μg/kg bw/day for Cd, 3 μg/kg bw/day for Cr, 40 μg/kg bw/day for Cu, 0.1 μg/kg bw/day for Hg, 300 μg/kg bw/day for Zn, respectively (USEPA (United States Environmental Protection Agency), 2011). Our calculations suggested that the *HQs* of all trace elements examined in all studied bivalve species from all sampled cities except Cd in *A. irradians* from Qingdao and *C. farreri* from all sampled cities (excluding Pb, which does not have an agreed *RfD* value) were significantly less than one in this study, suggesting an overall situation that the consumption of most bivalve species at current levels does not present any risk of excessive trace element intake to people (Table 2). The calculated *EDI* of bivalve species suggested the consumption levels for some trace elements (As, Cr, Hg, Pb and Zn) were ~3–110 times lower than the corresponding *RfD* guidelines, and people would not experience significant health risks from the intake of trace elements through bivalve consumption. But the calculated *EDI* values of Cd and Cu were ~0.8–2.2 times higher than the corresponding *RfD* guidelines, and people could experience significant health risks from the intake of these trace elements through bivalve consumption.

3.4. Estimated daily intake of trace elements via bivalve consumption

In this study, the *PTWI* values were used for the calculation of the trace element concentrations associated with the consumption of investigated bivalves collected from the studied areas. As there are no data on the average national rate of bivalve consumption in China, the *PTWI* values were employed as the standards for the calculation of the trace element levels associated with possible amounts of bivalve consumed.

FAO/WHO Expert Committee on Food Additives have set a *PTWI* of inorganic As (As*) as 15 μg/kg bw/week, a *PTWI* of Cd as 7 μg/kg bw/week, a *PTWI* of Cu as 500 μg/kg bw/week, a *PTWI* of total Hg as 5 μg/kg bw/week, a *PTWI* of Pb as 25 μg/kg bw/week, a *PTWI* of Zn as 2100 μg/kg bw/week for human beings (JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1989; FAO/WHO (Food Additive Organization of the United Nations and World Health Organization), 2004). Based on these data, for normal adult Chinese people (58.1 kg body weight), a daily consumption of 423–1102 g, 9–485 g, 44–3640 g, 277–692 g, 71–184 g, 145–3432 g wet weight edible tissues of different bivalves is sufficient to reach the *PTWI* limit for As* (0.87 mg/person/week), Cd (0.41 mg/person/week), Cu (29.05 mg/person/week), Hg (0.29 mg/person/week), Pb (1.45 mg/person/week), Zn (406.7 mg/person/week), respectively (Table 3). On the whole, for all the sampled cities, As and Hg in all the studied species and Cu and Zn in all the studied species except *C. gigas* posed low risks to human health based on the calculated daily intake amounts presented in Table 3, which were large enough compared with the normal appetite of people; Cd and Pb in some species for all the sampled cities or some of the sampled cities posed high risks to human health.

For Cd, the calculated daily intake amounts were 20–24 g wet weight/day for *A. irradians*, 9–17 g wet weight/day for *C. farreri*,

Table 2
The calculated hazard quotients.

Species	As*[a]	Cd	Cr	Cu	Hg	Pb	Zn
Argopecten irradians	0.11–0.26	0.82–1.00	0.03–0.05	0.02–0.04	0.03–0.27	ND[b]	0.05–0.09
Chlamys farreri	0.15–0.25	1.19–2.20	0.03–0.25	0.01–0.04	0.10–0.34	ND	0.06–0.14
Crassostrea gigas	0.16–0.20	0.29–0.92	0.02–0.07	0.23–0.81	0.10–0.24	ND	0.17–0.46
Lasaea nipponica	0.10–0.13	0.03–0.05	0.02–0.08	< 0.01–0.10	0.03–0.21	ND	0.01–0.02
Meretrix meretrix	0.15–0.18	0.03–0.08	0.01–0.08	< 0.01–0.02	0.03–0.21	ND	0.02–0.03
Mytilus edulis	0.15–0.22	0.09–0.32	0.06–0.56	0.01–0.02	0.03–0.51	ND	0.01–0.02
Ruditapes philippinarum	0.24–0.34	0.04–0.06	0.03–0.07	< 0.01–0.01	0.03–0.24	ND	0.01–0.02
Scapharca subcrenata	0.12–0.19	0.54–0.79	0.01–0.05	< 0.01–0.01	0.07–0.34	ND	0.01–0.03
Sinonovacula constricta	0.17–0.32	0.01–0.04	0.03–0.12	0.02–0.03	0.03–0.21	ND	0.02–0.03

[a] As*: the concentration of inorganic As which was estimated as 10% of the total As concentration (USFDA (United States Food and Drug Administration), 1993).
[b] ND: no data.

Table 3
The calculated daily intake amounts (g wet weight/day) for the major marine bivalves from six northern coastal cities of China based on the provisional tolerance weekly intake of trace elements.

Species	As*[a]	Cd	Cr	Cu	Hg	Pb	Zn
Argopecten irradians	546–1297	20–24	ND[b]	828–2318	519–4150	91–216	749–1354
Chlamys farreri	566–965	9–17	ND	945–2546	415–1383	94–161	471–1201
Crassostrea gigas	720–877	22–69	ND	44–155	593–1383	120–146	145–400
Lasaea nipponica	1102–1431	388–727	ND	3640–6103	692–4150	184–239	3432–7317
Meretrix meretrix	803–950	264–581	ND	2161–4235	692–4150	134–158	2101–2780
Mytilus edulis	632–965	62–233	ND	2441–3517	277–4150	105–161	2700–5074
Ruditapes philippinarum	423–596	323–485	ND	2677–3915	593–4150	71–99	3301–4708
Scapharca subcrenata	732–1221	25–37	ND	2882–4882	415–2075	122–203	2643–4607
Sinonovacula constricta	437–825	485–1454	ND	1140–2065	692–4150	73–137	2249–3571

[a] As*: the concentration of inorganic As which was estimated as 10% of the total As concentration (USFDA (United States Food and Drug Administration), 1993).
[b] ND: no data.

22–69 g wet weight/day for *C. gigas*, and 25–37 g wet weight/day for *S. subcrenata*. These results were consistent with high Cd levels of these three bivalves. The Cd concentrations in bivalve species (*A. irradians* and *C. farreri* from all the studied cities, *S. subcrenata* from Qingdao and Rizhao) exceeding the *MPL* may account for this. *C. farreri* with significantly accumulated Cd contributed more to the less daily intake amounts than other bivalve species. Disturbingly, Cd in *C. farreri* from all the studied cities, especially from Qingdao could pose a potential leaching risk to human health.

For Cu and Zn, the calculated daily intake amounts were 44–155 g wet weight/day and 145–400 g wet weight/day for *C. gigas*. Significantly accumulated Cu and Zn would allow only very restricted daily intake amounts for *C. gigas*. Cu and Zn in all the collected *C. gigas* (except Cu in *C. gigas* from Dalian) exceeded the *MPLs* set up by the AQSIQ and WHO. In particular, over consumption of *C. gigas* from Yantai may put people in danger with excessive intake of Cu.

A daily consumption of as low as 9 and 94 g of *C. farreri* is sufficient for Cd and Pb to reach the *PTWI* limits, respectively, which is the amount that may result in a risky weekly intake of Cd and Pb if the exposure is long-term. In terms of the studied trace elements, especially Cd daily consumption would be the limiting factor for bivalves as a food for a large number of consumers and coastal residents from the six northern coastal cities of China. Besides, care must be taken considering that most coastal residents regularly consume large quantities of bivalves.

4. Conclusion

Accumulation of trace elements (such as As, Cd, Cr, Hg, Pb) in food is a major health safety concern worldwide. The present study was a large-scale investigation of trace elements in nine edible bivalve species from six northern coastal cities of China. The results indicated that trace element levels varied in different bivalve species, possibly due to the differences in trace element bioaccumulation. The concentrations of the essential trace elements were higher than those of the non-essential trace elements, with Zn concentrations being the highest. There were significant correlations between some elements (Cd–Pb, Cd–Zn, Cu–Zn). Risk assessments indicated that we should pay attention to human health risks associated with the exposure to Cd and Cu via the consumption of marketed bivalves. Human health risks associated with the exposure to the other studied trace elements through the consumption of marketed bivalves were negligible. However, care must be taken considering that most coastal residents regularly consume large quantities of bivalves.

Acknowledgments

This study was co-supported by the Department of Science and Technology of Shandong Province (2012GHY11535) and the CAS/SAFEA International Partnership Program for Creative Research Teams (Representative Environmental Processes and Their Effects on the Resources in Coastal Zone). The help of Drs. Boquan Li and Qingxi Han in the identification of sampled bivalve species is greatly appreciated.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2014.07.023.

References

AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China), 2001. Safety Qualification for Agricultural Product-Safety Requirements for Non-environmental Pollution Aquatic Products (GB 18406.4-2001).

Australian Agency for International Development, 1996. Country Environmental Profile of China. Commonwealth of Australia, Canberra, Australia p. 115.

BFMAC (Bureau of Fisheries of the Ministry of Agriculture of China), 2012. China Fishery Statistical Yearbook 2011. China Agriculture Press, Beijing p. 138.

Blackmore, G., Wang, W.X., 2003. Comparison of metal accumulation in mussels at different local and global scales. Environ. Toxicol. Chem. 22, 388–395.

Capelli, R., Drava, G., De Pellegrini, R., Minganti, V., Poggi, R., 2000. Study of trace elements in organs and tissues of striped dolphins (*Stenella coeruleoalba*) found dead along the Ligurian coasts (Italy). Adv. Environ. Res. 4, 31–43.

Chan, K.W., Cheung, R.Y.H., Leung, S.F., Wong, M.H., 1999. Depuration of metals from soft tissues of oysters (*Crassostrea gigas*) transplanted from a contaminated site to clean sites. Environ. Pollut. 105, 299–310.

Du, R.X., Fan, Z.X., Wen, A.L., Cai, L.J., Zhang, B., 2009. Content analysis of heavy metal in economic shellfish from Shandong coast. (In Chinese). Shandong Agric. Sci. 8, 58–63.

EC (European Commission), 2006. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L 364, pp. 5–24.

FAO (Food and Agricultural Organization of the United Nations), 2011. Food Supply—Livestock and Fish Primary Equivalent. FAOSTAT. 〈http://faostat3.fao.org/faostat-gateway/go/to/download/FB/CL/E〉.

FAO (Food and Agriculture Organization of the United Nations), 2012. The State of World Fisheries and Aquaculture 2012. Rome, Italy, 209p.

FAO/WHO (Food Additive Organization of the United Nations and World Health Organization), 2004. Summary Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956–2003). Internet Edition. ILSI Press, Washington, DC 〈http://jecfa.ilsi.org〉.

Gao, X.L., Zhou, F.X., Chen, C.T.A., 2014. Pollution status of the Bohai Sea: an overview of the environmental quality assessment related trace metals. Environ. Int. 62, 12–30.

Gu, D.F., He, J., Duan, X.F., Reynolds, K., Wu, X.G., Chen, J., Huang, G.Y., Chen, C.S., Whelton, P.K., 2006. Body weight and mortality among men and women in China. J. Am. Med. Dir. Assoc. 295, 776–783.

Han, B.C., Hung, T.C., 1990. Green oysters caused by copper pollution on the Taiwan coast. Environ. Pollut. 5, 347–362.

Huang, H., Wu, J.Y., Wu, J.H., 2007. Heavy metal monitoring using bivalved shellfish from Zhejiang coastal waters, East China Sea. Environ. Monit. Assess. 129, 315–320.

Jacinto, G.S., 1997. Preliminary assessment of marine pollution issues in the East Asian Seas region at the end of the millennium. Trop. Coasts 4, 3–7.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1989. WHO Technical Report Series 759, WHO, Geneva, Switzerland.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Summary and Conclusions of the 61st Meeting of the Joint FAO/WHO Expert Committee on Food Additives, JECFA/61/Sc, Rome, Italy, 10–19.06.03, pp. 1–22.

Lafabrie, C., Pergent, G., Kantin, R., Pergent-Martini, C., Gonzalez, J.L., 2007. Trace metals assessment in water, sediment, mussel and seagrass species—validation of the use of *Posidonia oceanica* as a metal biomonitor. Chemosphere 68, 2033–2039.

Liang, L.N., He, B., Jiang, G.B., Chen, D.Y., Yao, Z.W., 2004. Evaluation of mollusks as biomonitors to investigate heavy metal contaminations along the Chinese Bohai Sea. Sci. Total Environ. 324, 105–113.

Liu, W.X., Chen, J.L., Lin, X.M., Fan, Y.S., Tao, S., 2007. Residual concentrations of micropollutants in benthic mussels in the coastal areas of Bohai Sea, North China. Environ. Pollut. 146, 470–477.

Pan, K., Wang, W.X., 2009. Biodynamics to explain the difference of copper body concentrations in five marine bivalve species. Environ. Sci. Technol. 43, 2137–2143.

Rawson, P.D., Burton, R.S., 2002. Functional coadaptation between cytochrome *c* and cytochrome *c* oxidase within allopatric populations of a marine copepod. Proc. Nat. Acad. Sci. U.S.A. 99, 12955–12958.

Ruan, J.S., 2008. A preliminary study of heavy metal contents in sea water, sediments and cultured shellfish in shellfish culture areas of Xiamen (In Chinese). J. Trop. Oceanogr. 27 (5), 47–54.

Sivaperumal, P., Sankar, T.V., Viswanathan Nair, P.G., 2007. Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards. Food Chem. 102 (3), 612–620.

Stanković, S., Jovic, M., Milanov, R., Joksimovic, D., 2011. Trace elements concentrations (Zn, Cu, Pb, Cd, As and Hg) in the Mediterranean mussel (*Mytilus galloprovincialis*) and evaluation of mussel quality and possible human health risk from cultivated and wild sites of the southeastern Adriatic Sea, Montenegro. J. Serb. Chem. Soc. 76, 1725–1737.

Steffen, W., Sanderson, A., Tyson, P.D., Jäger, J., Matson, P.A., Moore III, B., Oldfield, F., Turner, I.I.B.L., Wasson, R.J., 2004. Global Change and the Earth System: A Planet under Pressure. Springer-Verlag, Berlin-Heidelberg New York p. 336.

Szefer, P., Frelek, K., Szefer, K., Lee, C.h.B., Kim, B.S., Warzocha, J., Zdrojewska, I., Ciesielski, T., 2002. Distribution and relationships of trace metals in soft tissue, byssus and shells of *Mytilus edulis* trossulus from the southern Baltic. Environ. Pollut. 120, 423–444.

USEPA (United States Environmental Protection Agency), 2011. Risk-Based Concentration Table. U.S. EPA, Region 3, Philadelphia, PA, USA. 〈http://www.epa.gov/reg3hwmd/risk/human/index.htm〉.

USFDA (United States Food and Drug Administration), 1993. Guidance Document for Arsenic in Shellfish. Department of Health and Human Services, United States Food and Drug Administration.

Wang, H., Xu, Q., Yang, H.S., 2007. Heavy metal contents and quality evaluation of scallop in northern China seas (In Chinese). Mar. Sci. 31, 11–18.

Wang, Y.W., Liang, L.N., Shi, J.B., Jiang, G.B., 2005. Study on the contamination of heavy metals and their correlations in mollusks collected from coastal sites along the Chinese Bohai Sea. Environ. Int. 31, 1103–1113.

Whyte, A.L.H., Raumati Hook, G., Greening, G.E., Gibbs-Smith, E., Gardner, J.P.A., 2009. Human dietary exposure to heavy metals via the consumption of greenshell mussels (*Perna canaliculus* Gmelin 1791) from the Bay of Islands, northern New Zealand. Sci. Total Environ. 407 (14), 4348–4355.

WHO, 1982. Toxicological Evaluation of Certain Food Additives and Contaminants (WHO Food Additives Series No. 17). World Health Organization, Geneva, Switzerland, pp. 28–35.

Xu, S.S., Song, J.M., Yuan, H.M., Li, X.G., Li, N., Duan, L.Q., 2010. Effects of the combined pollution of Cd, Hg, Pb and petroleum hydrocarbons on common fishery species in the Bohai Bay (In Chinese). Asian J. Ecotoxicol. 5, 793–802.

Zhang, Y., Song, J.M., Yuan, H.M., Xu, Y.Y., He, Z.P., 2010. Concentrations of cadmium and zinc in seawater of Bohai Bay and their effects on biomarker responses in the bivalve *Chlamys farreri*. Arch. Environ. Contam. Toxicol. 59, 120–128.