Heavy metal bioaccumulation in five bivalves from coastal areas of yellow sea and Bohai Sea, China: Evaluation of contamination and human health risk

Haiyan Ding
Chinese Academy of Fishery Science
Yanfang Zhao (zhaoyf@ysfri.ac.cn)
Chinese Academy of Fishery Science
Xiaofeng Sheng
Chinese Academy of Fishery Science
Xuming Kang
Chinese Academy of Fishery Science
Jinsong Ning
Chinese Academy of Fishery Science
Xihuang Zhong
Chinese Academy of Fishery Science
Derong Shang
Chinese Academy of Fishery Science

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Abstract

Heavy metal concentrations (Fe, Mn, Cu, Zn, As, iAs, Cr, Cd, Pb and Hg) in five commercially important bivalves from the coastal areas of Yellow sea and Bohai sea, China were determined, and their potential human health risks were evaluated. Results showed that there was a significant difference among these metal contents ($p<0.05$) in different bivalve species, except for Hg. The contents of all metals were below the maximum allowable limit regulated by China Food Standard Agency. The health risks from most bivalves' consumption were safe with the THQ being much lower than 1.0, except for Cd in some scallop samples. The average EDI/PTDI of Cd from *Chlamys farreri* consumption was 92%, and the average EDI of Cu and Cd in *Crassostrea gigas* occupied 61% and 62% of PTDI. Thus a moderate consumption of *C. farreri* and *C. gigas* was suggested to reduce the potential health risk of heavy metals.

1. Introduction

With the acceleration of urbanization and industrialization, a large number of contaminants including heavy metals have entered into the rivers, which eventually entered into the sea and worsened the marine pollution of heavy metals. Heavy metals are known to be nonbiodegradable and bioaccumulative. They can be accumulated by aquatic organisms and threat the human health through the food chain (Suami et al., 2019). Some of the heavy metals are essential elements for life, such as iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn). And some are nonessential elements, such as arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg), which are poisonous and harmful for human health. Although the essential elements are the vital components of some enzymes, excess intakes will be harmful. For example, Fe can cause gastrointestinal problems and Cu may cause kidney damage (Isidori et al., 2018). The nonessential elements could induce toxicity even at trace levels (Suami et al., 2019). In addition, different chemical forms of elements show different bio-toxicity. For example, the inorganic arsenic (iAs) which has been classified as human carcinogens, is more toxic than organic arsenic (USEPA, IRIS, 2020). Organic As in marine fish and bivalves is primarily presented as arsenobetaine (AsB), which is considered to be non-toxic. The methylmercury could cause nerve system disease and kidney damage, which is more toxic than inorganic Hg (Tang et al., 2020).

In China, marine bivalves occupy about 45 percent of marine products, and they can provide rich high quality of protein, vitamins and essential nutrients for human. At the same time, the bivalves are commonly used as biomonitors for heavy metal pollution in marine environment because they can accumulate heavy metal ions quickly for their filter-feeding habit (Jonathan et al., 2017). The coastal areas along Shandong and Dalian are belong to the Bohai Sea Economic Rim, which is one of the highly industrialized areas in China. Previous studies have indicated that the edible shellfish from the Bohai Sea and the Yellow sea had been contaminated with heavy metals at different levels, especially for As and Cd (Zhang et al., 2016). However, the balance between benefits and risks due to ingestion of heavy metals from marine aquatic products collected from this coastal area has been poorly characterized.

In present study, five most important commercially bivalves species including *Chlamys farreri*, *Ruditapes philippinarum*, *Mactra veneriformis*, *Cyclina sinensis* and *Crassostrea gigas* are selected to investigate the concentration of heavy metals (Fe, Mn, Cu, Zn, As, iAs, Cr, Cd, Pb, Hg), and the difference of these elements among different species of bivalves had been analyzed. In order to estimate the potential risk for human health derived from these bivalves, we have evaluated the provisional tolerable daily intake (PTDI) recommended by Joint FAO/WHO Expert Committee on Food Additive online database (JECFA, 2009), and the target hazard quotient (THQ) (USEPA, 1989), in order to evaluate possible alert regarding adverse effects.

2. Methods And Materials

2.1 Sample collection

A total of 366 bivalves samples including *C. farreri* (n=103), *R. philippinarum* (n=99), *M. veneriformis* (n=87), *C. gigas* (n=44), *C. sinensis* (n=33) were collected from the coastal areas of Shandong Province and Dalian along the Bohai sea and the Yellow sea in China in 2019. The sampling locations were Binzhou, Dongying, Weifang, Yantai, Weihai, Rizhao and Dalian (Fig. 1). Samples were immediately transported to the laboratory in an icebox. The soft tissues were removed, homogenized and stored at -20 °C until later analysis.

2.2 Sample analysis

The homogenized samples of around 1.0 g were placed into digestion flasks and pre-digested with 8 mL HNO_3 (guaranteed grade) at room temperature for two hours. Then the samples were digested by a microwave digestion system (CEM MARS 6). The descriptive of the microwave digestive procedure was as follows: Initially, a temperature of 120 °C was reached and maintained for 10 min. Then, it was elevated to 160°C for another 20 min. Finally, the temperature was increased to 190 °C with a ramp time of 10 min and a dwell time of 25 min at 1500 W. After digestion, the HNO_3 was driven out from samples and the solution was diluted with deionized water to 50 mL. The concentration of heavy metals (Fe, Mn, Cu, Zn, As, Cr, Pb and Cd) were measured using inductively coupled plasma mass spectrometry (Elan DRC II, Perkin-Elmer, USA). The operating conditions for ICP-MS are according to our previous report (Kang et al., 2018). The concentration of total Hg was determined using hydride generation atomic fluorescence spectroscopy (AFS 9130, JiTian, China).

Inorganic arsenic speciation analysis was carried out by using high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) according to the National Standard Method of China (GB/T 23372-2009). About 1.0 g of each sample was weighted into a 50 mL centrifuge tube. To each tube, it was added 38 mL of deionized water and 2 mL of acetic acid which was used for sample protein precipitation. The tube was sonicated for 40 min and centrifuged for 10 min. After centrifugation, the supernatant was filtered (0.45 μm) prior to injection onto the column. In general, 30 μL samples were analyzed. The inorganic arsenic compounds in the samples were identified by matching the retention times of the peaks in the chromatograms with those of standards.

Water was purified in a Milli-Q system (18.2 MΩ cm, Millipore, USA) and used to prepare all standard solutions, mobile phase and reagents. All the reagents used in present study were of high purity and appropriate for heavy metal analysis. The standard solutions of arsenite [As(Ⅲ)] and arsenate [As(Ⅴ)] for iAs analysis and the stock solution for other heavy metals were purchased from the National Reference Material Center in China. A calibration curve for each heavy metal was prepared prior to every batch of analysis. In order to assess the precision and accuracy of the results, quality assurance was assessed using the standard reference material (SRM) of scallop (GBW10024) and shrimp (GBW10050). Five replicates were conducted for the determination of the heavy metals. The recovery of two standard reference materials ranged from 94.5% to 103.2% (n= 5).

2.3 Statistical analysis

The trace elements concentrations in soft tissues of the bivalves were expressed as means and standard deviations. The data were analyzed by SPSS statistical analysis software package (Version 24.0). The one-way ANOVA analysis was used to compare the difference between samples. The differences were considered significant at $p<0.05$.

2.4 Heavy metal pollution level evaluation

The Nemerow index (P_c) is an original multifactorial, integrated assessment for water pollution (Guan, 1979; Zhang et al., 2017). In recent years, it has also been used to evaluate the heavy metal pollution status in other fields, such as soils (Mohammadi et al., 2020), atmosphere (Sobhanardakani, 2019), rice (Li et al., 2020b), vegetables (Rukeya et al., 2018) and shellfish (Jiang and Yan, 2019). The single pollution index (P_i) are firstly calculated and then the maximum index value (P_{max}) and the average value (P_{avr}) are used to calculate the composite index. Both P_i and P_c are used to evaluate the pollution level of heavy metals in five edible bivalves. The single pollution index is as follows:

$$P_i = C_i / S_i \qquad (1)$$

Where P_i is the single pollution index that refers to the exceeding multiple of each index; C_i is the measured concentration of the heavy metals; S_i is the standard value of every heavy metal. When $P_i<0.2$, the pollutant is within the normal background value range; When $0.2 \le P_i \le 0.6$, the pollutant is slight contamination; When $0.6<P_i<1.0$, the pollutant is moderate contamination; When $P_i \ge 1.0$, it indicates that the pollutant is severe contamination. The standard limit values of each element are presented in Table 1.

It’s a convenient and effective way to evaluate the heavy metal pollution by the Nemerow index, which involves a simple mathematical process and employs basic physical concepts (Qu et al., 2018). The comprehensive P_c is as the formula (2).

$$P_c = \sqrt{\frac{P_{imax}^2 + P_{iavr}^2}{2}} \qquad (2)$$

Where P_{imax} is the maximum value of P_i, and P_{iavr} is the average vlalue of P_i. The classification standard of P_i and P_c are shown as Table 2.

Table 1 Limit value of heavy metal pollutants in marine organisms ($mg \cdot kg^{-1}$)

Element	Values	Reference
Fe	n,a	/
Mn	n,a	/
Cu	50	(NY 5073-2006, 2006)
Zn	150	(Liu et al., 2013)
iAs	0.5	(GB 2762-2017, 2017)
Cr	2.0	(GB 2762-2017, 2017)
Cd	2.0	(GB 2762-2017, 2017)
Pb	1.5	(GB 2762-2017, 2017)
Hg	0.5	(GB 2762-2017, 2017)

Note: n, a =value not available

Table 2 Single (P_i) and comprehensive Nemerow pollution index (P_c) classification standard

Pollution class	P_i	Pollution level	P_c	Pollution level
I	P_i<0.2	Normal background	P_c≤0.7	Safe
II	0.2≤P_i≤0.6	slight contamination	0.7< P_c ≤1.0	Precaution
III	0.6< P_i <1.0	moderate contamination	1.0< P_c≤2.0	Light pollution
[illegible]	P_i ≥ 1.0	severe contamination	2.0< P_c ≤3.0	Moderate pollution
[illegible]	/	/	P_c >3.0	Heavy pollution

2.5 Heavy metal health risk assessment

The concentrations of heavy metals in these bivalves were used to calculate the estimated daily intake of heavy metals (EDI) and target hazard quotient (THQ) to evaluate the health risk of heavy metal exposure. EDI was calculated by the following formula (Asantewah et al., 2021):

$$\mathrm{EDI} = \frac{EF \times ED \times FIR \times c}{WAB \times TA} \qquad (3)$$

Where EF represents the exposure frequency (365 $d \cdot a^{-1}$); ED is the age of exposure (70 a), equivalent to the average human life age; FIR is the food intake rate (40 $g \cdot d^{-1}$, (National Bureau of Statistics of China, 2019)), the data were taken from the average of the per capita aquatic product consumption of urban residents in China from 2014 to 2018; C is the metal content in seafood ($mg \cdot kg^{-1}$); WAB represents the average human body mass (60 kg, (WHO, 1989)), TA is the mean exposure time of non-carcinogenic sources (365 $d \cdot a^{-1} \times$ ED).

THQ shows the ratio between exposure and the reference dose, and the equation is expressed as follows (Huang et al., 2020):

$$\mathrm{THQ} = \frac{EDI \times 10^{-3}}{RFD} \qquad (4)$$

Where RFD is the reference dose. The daily reference dose of these elements is listed in table 3.

Table 3 Reference dose (RED) of heavy metals in bivalves ($mg \cdot kg^{-1} \cdot bw \cdot d^{-1}$)

Element	RFD ($mg \cdot kg^{-1} \cdot bw \cdot d^{-1}$)	Reference
Fe	0.8	(JECFA, 1983)
Mn	0.14	(USEPA, IRIS, 2020)
Cu	0.5	(JECFA, 2011)
Zn	0.3	(USEPA, IRIS, 2020)
As	n,a	/
iAs	3×10-4	(USEPA, IRIS, 2020)
Cr	3×10-3	(USEPA, IRIS, 2020)
Cd	1×10-3	(USEPA, IRIS, 2020)
Pb	3.5×10-3	(JECFA, 1999)
Hg	5.7×10-4	(JECFA, 1982)

This method is based on the assumption that the absorbed dose is equal to the intake, the ratio of the measured human intake to the reference dose is used as the evaluation criterion (Storelli, 2008). If the THQ values are below 1.0, there is no obvious health risk for the exposed population; otherwise, there will be a higher health risk.

3. Results And Discussion

3.1 Distribution of heavy metals

The concentrations of each trace metal in five species of bivalve were shown in Table 4. There was a significant difference in heavy metal accumulation in different species of bivalves.

For Cu, the highest level was in *C. gigas* which ranged from 1.01 to 70.2 $mg \cdot kg^{-1}$ and the average level was 37.3 $mg \cdot kg^{-1}$ which was much higher than other four kinds of bivalves (p<0.05). The second was for *C. farreri* with an average level of 3.64 $mg \cdot kg^{-1}$. and there was no significant difference in Cu concentrations among *C. farreri*, *R. philippinarum*, *M. veneriformis* and *C. sinensism* (Fig. 2).

For Zn, it was similar with Cu that *C. gigas* had the highest level with an average value of 110 $mg \cdot kg^{-1}$, which was two times more than that in *C. farreri* (51 $mg \cdot kg^{-1}$) and one-way ANOVA analysis showed that there was a significant difference between them (p<0.05). The third was for *R. philippinarum* and *C. sinensism*, and a lowest average content of 6.4 $mg \cdot kg^{-1}$ was detected in *M. veneriformis*. However, no significant difference was found for *R. philippinarum*, *C. sinensism* and *M. veneriformis* (seeing Fig.2).

For Fe, there was a significant difference among five kinds of bivalves (Fig.2). Among them, the highest value of 587.4 mg·kg^{-1} was detected in *M. veneriformis* which was about three times more than the second level in *R. philippinarum* (187.1 mg·kg^{-1}) (P<0.05). The lowest Fe content was found in the soft tissues of *C. sinensism* with 65.5 mg·kg^{-1}, and there was no significant difference among *C. farreri*, *C. gigas* and *C. sinensism* (P>0.05).

Table 4 Trace metal concentrations in the soft tissues of different bivalves (mg·kg^{-1}, wet weight)

Bivalves		*C. farreri*	*R.philippinarum*	*M.veneriformis*	*C.gigas*	*C. sinensism*
Cu	Range	0.86-11.7	0.55-3.09	0.55-2.26	1.01-70.2	0.80-2.95
	Average	3.64	1.26	1.32	37.3	1.38
Zn	Range	18.0-137	7.06-20.4	3.34-11.6	12.9-201	7.78-16.5
	Average	51	12.2	6.4	110	12.1
Fe	Range	35.5-319.5	56.7-376.5	195.7-1083.6	17.7-174.1	37.8-122.2
	Average	99.4	187.1	587.4	74.9	65.5
Mn	Range	2.54-15.0	1.19-14.4	5.62-53.8	3.78-15.8	3.45-6.82
	Average	6.52	6.88	20.3	8.11	5.01
Total As	Range	1.49-8.41	1.29-5.22	1.21-4.77	1.33-4.28	1.40-3.35
	Average	3.91	3.23	2.71	2.41	2.35
iAs	Range	0.04-0.14	0.05-0.36	0.04-0.21	0.02-0.12	0.04-0.11
	Average	0.08	0.15	0.12	0.05	0.07
Cr	Range	0.11-0.94	0.07-4.78	0.45-3.19	0.06-0.89	0.12-1.26
	Average	0.36	0.55	1.47	0.4	0.33
Cd	Range	0.13-3.67	0.02-0.24	0.05-0.24	0.11-1.45	0.02-0.10
	Average	1.15	0.14	0.13	0.76	0.030
Pb	Range	0.06-0.51	0.08-1.10	0.10-1.19	0.05-0.34	0.05-0.17
	Average	0.19	0.19	0.49	0.16	0.09
Hg	Range	<0.01-0.05	<0.01-0.05	<0.01-0.06	<0.01-0.07	<0.01-0.05
	Average	0.020	0.022	0.020	0.024	0.020

For Mn, the highest content was also found in the soft tissues of *M. veneriformis* with an average of 20.3 mg·kg^{-1}, and it was significantly higher than other four kinds of bivalves (Fig.2). About 8.11 mg·kg^{-1} of Mn was found in *C. gigas*, and a similar concentration was wound in *C. farreri* and *R. philippinarum* (6.52 and 6.88 mg·kg^{-1}, respectively). The lowest Mn content was also found in *C. sinensism*, which was significantly lower than both *M. veneriformis* and *C. gigas* (P<0.05)

For total arsenic (As) and inorganic As, a different distribution character was found for different bivalves (Fig. 3). Total As in *C. farrier* ranged from 1.49 to 8.41 mg·kg^{-1} and the average level was 3.91 mg·kg^{-1} which was significantly higher than that in *R. philippinarum* (3.23 mg·kg^{-1}), and a similar value of total As was found in *M. veneriformis*, *C. gigas* and *C. sinensism*. While for inorganic As, the highest level was found in *R. philippinarum* and *M. veneriformis* with an average level of 0.15 and 0.12 mg·kg^{-1} respectively. And only 0.08 mg·kg^{-1} iAs was found in *C. farreri*. Present result further proved that the transformation of different arsenic species in different bivalves was different.

For Cd, an obvious difference was found for five different bivalves (seeing Fig. 3). For example, *C. farreri* had the highest Cd level which ranged from 0.13 to 3.67 mg·kg^{-1}, and the average content was 1.15 mg·kg^{-1}. The second was for *C. gigas*, which ranged from 0.11 to 1.45 mg·kg^{-1}, and the average value was 0.76 mg·kg^{-1}. The third level was found in *R. philippinarum* and *M. veneriformis*, both of them had a similar value (0.14 and 0.13 mg·kg^{-1}, respectively). The lowest value was for *C. sinensism*, which had only about 0.030 mg·kg^{-1} of Cd.

For Cr, *M. veneriformis* had the highest content with average level of 1.47 mg·kg^{-1}, and the lowest level was found in *C. farreri* and *C. sinensism* (0.36 and 0.33 mg·kg^{-1}, respectively). And for Pb, a similar phenomenon was found that the highest level was in *M. veneriformis* (0.49 mg·kg^{-1}), and the lowest level was also found in *C. sinensism*. For Hg, it was different from other trace metals that there was no obvious difference for five kinds of bivalves (P>0.05), and its' value ranged from 0.020 mg·kg^{-1} to 0.024 mg·kg^{-1}.

Thus, the clam *M. veneriformis* had the highest content of Fe, Mn, Cr and Pb among five kinds of bivalves. The oyster *C.gigas* had the highest content of Cu and Zn, and the scallop *C. farreri* had the highest content of Cd and total arsenic. The clam *M. veneriformis* and *R. philippinarum* had a slightly higher content of inorganic arsenic (iAs) than other three bivalves. In addition, the concentration of all heavy metals in present study in *C. sinensis* was the lowest among five kinds of bivalves.

Compared to previous studies of heavy metals in bivalves from coastal areas, the present results of most metals are similar or within the range of reported studies. The differences of trace elements in bivalves from different regions are mainly reflected in the content of Fe, As, Cr, Cd and Hg. For example, the present result of As concentration in *C. farreri* was below the result from the Southern New Caledonian (Metian et al., 2008), and As concentration in *C.gigas* reported by Liu et al (2020) was nearly ten times of the present study. The levels of Hg in *R. philippinarum* in this study were obviously lower than those from the Atlantic Coast, Southern Spain (Usero et al., 1997) and Venice lagoon, Italy (Sfriso et al., 2008). The concentration of Fe and Hg in *C.gigas* from Atlantic Coast was several times of the present study (Suami et al., 2019). As have been reported by previous researches that there is a complicated process about heavy metals accumulation in aquatic organisms, which can be affected by species, age, physiological stage, and environmental parameters (bioavailability, salinity, and temperature of living environment) (Kang et al., 2018; Maurya et al., 2019).

3.2 Pollution levels of heavy metals

Table 5 gives the single factor pollution index (P_i) of heavy metal in five bivalves calculated by formula (1). For *C. farreri*, Zn and Cd were slightly contaminated (P_i value was 0.340 and 0.576 respectively). For *R. philippinarum*, iAs and Cr were slightly contaminated, and the P_i values were very low between 0.2 and 0.3. For *M. veneriformis*, Cr was moderately contaminated (P_i, 0.736), iAs and Pb were slightly contaminated. The P_i of Cr was highest which meant that Cr may be more easily accumulated by *M. veneriformis*. For *C. gigas*, the P_i of Cr and Cd was 0.200 and 0.328 respectively, and they were slightly contaminated. In *C. gigas*, Cu and Zn were moderately contaminated (P_i values were 0.746 and 0.734 respectively), and in some samples, P_i was much higher than 1.0. The *C. sinensis* was the most safety bivalves with all elements in normal background levels.

Present study proved that Cu and Zn in *C. gigas* were moderately contaminated, which was similar with the previous result reported by (Qin et al., 2010). Although Cu is an essential element for various metabolic activities, long-time over tolerable level exposure could cause anaemia, kidney and liver damage (Schumann et al., 2002). As an essential element, Zn is the cofactor of various enzymes and plays a vital role in metabolic activity (Suami et al., 2019). However, high level exposure of Zn can also lead to nausea, abdominal pain and lethargy (Denil et al., 2017). In addition, the average P_i values of Cd in *C. farreri* and Cr in *M. veneriformis* were both below 1.0, while in some samples, the P_i values were over 1.0. And it showed that some of *C. farreri* and *M. veneriformis* were heavily contaminated by Cd. Table 6 showed the Nemerow index (P_c) of heavy metals in five species of bivalves. And it could be found that the P_c of Cu and Zn in *C. sinensis*, Cd in *C. farreri* and Cr in *M. veneriformis* were all slightly contaminated with P_c>1.0. Thus, both P_i and P_c results proved that there was some contamination in present *C. sinensis*, *C. farreri* and *M. veneriformis* samples. Although all these heavy metals levels are below the maximum allowable limit regulated by China Food Standard Agency (GB 2762-2017, 2017), they should attract much attention when considering the health risk of the bivalves consuming.

Table 5 Pollution index (P_i) of heavy metals in bivalves

Bivalves	*C. farreri*		*R. philippinarum*		*M. veneriformis*		*C. gigas*		*C. sinensis*	
	average	max	average	max	average	max	average	max	average	max
Fe	n,a	n,a	n,a	n,a	n,a	n,a	n,a	n,a	n,a	n,a
Mn	n,a	n,a	n,a	n,a	n,a	n,a	n,a	n,a	n,a	n,a
Cu	0.073	0.233	0.025	0.062	0.026	0.045	0.746	1.404	0.028	0.059
Zn	0.340	0.914	0.082	0.136	0.043	0.078	0.734	1.340	0.081	0.110
iAs	0.161	0.283	0.299	0.607	0.244	0.417	0.098	0.247	0.150	0.213
Cr	0.181	0.470	0.275	0.762	0.736	1.596	0.200	0.443	0.166	0.630
Cd	0.576	1.834	0.072	0.121	0.066	0.119	0.380	0.725	0.014	0.048
Pb	0.128	0.34	0.126	0.450	0.328	0.791	0.107	0.229	0.057	0.115
Hg	0.040	0.094	0.045	0.102	0.040	0.116	0.047	0.138	0.041	0.105

Table 6 Nemerow index (P_c) of heavy metals in bivalves

Bivalves	*C. farreri*	*R. philippinarum*	*M. veneriformis*	*C. gigas*	*C. sinensis*
Fe	n,a	n,a	n,a	n,a	n,a
Mn	n,a	n,a	n,a	n,a	n,a
Cu	0.17	0.05	0.04	1.12	0.05
Zn	0.69	0.11	0.06	1.08	0.10
iAs	0.23	0.48	0.34	0.19	0.18
Cr	0.36	0.57	1.24	0.34	0.46
Cd	1.36	0.10	0.10	0.58	0.04
Pb	0.26	0.33	0.61	0.18	0.09
Hg	0.07	0.08	0.09	0.10	0.08

3.3 Assessment of the health risks of heavy metals

3.3.1 Estimated daily intake (EDI)

The EDI of heavy metals here represented the daily intake of heavy metals through the consumption of selected bivalves, which was listed in Table 7. The PTDI values proposed by JECFA (FAO/WHO, 1985; WHO, 1989; Mok et al., 2015) and the Chinese Nutrition Society (2013)were shown in Table 7 as the reference value to evaluate the hazard risk from these bivalves consumption. From Table 7, it can be calculated that the average EDI/PTDI of the selected metals for these bivalves ranged from 1% to 92%. The average EDI/PTIDI of Cd in *C. farreri* reached to 92%, which meant that Cd was the most hazard element for *C. farreri* consumption. Although the average EDI value were all below the PTDI, the coastal residents exposed higher dose, may have been exposed under excess dose of Cd from the regular consumption of *C. farreri*. For *R. philippinarum*, the average EDI of Cr occupied 13% of the PTDI. The average EDI of Cu and Cd in *C. gigas* occupied 61% and 62% of the PTDI, respectively. This result was similar to the previous research (Liu et al., 2019), which proved that there was potential health risk for people to be exposed to Cu and Cd from mass consumption of *C. gigas*, and Cd from *C. farreri* consumption. For *M. veneriformis*, the average EDI of Fe occupied 48.9% of the PTDI and this value was highest in present studied bivalves. Since Fe is the essential element for human, *M. veneriformis* can be considered as the Fe supplement food for iron deficiency group.

Table 7 The estimated daily intake (EDI) of heavy metals in bivalves ($\mu g \cdot kg^{-1} \cdot bw \cdot d^{-1}$, wet weight)

Bivalves	EDI										PTDI
	C. farreri		*R. philippinarum*		*M. veneriformis*		*C. gigas*		*C. sinensis*		
	AVG	EDI/PTDI	AVG	EDI/PTDI	AVG	EDI/PTDI	AVG	EDI/PTDI	AVG	EDI/PTDI	
Fe[a]	66.3	8.3	125	15.6	391	48.9	50	6.3	43.7	5.5	800
Mn[b]	4.35	2.4	4.59	2.5	13.5	7.4	5.41	3.0	3.35	1.8	183
Cu[d]	2.43	6.1	0.84	2.1	0.88	2.2	24.87	62.2	0.92	2.3	40
Zn[d]	34.0	11.3	8.13	2.7	4.267	1.4	73.33	24.4	8.067	2.7	300
iAs[c]	0.053	2.5	0.129	6.0	0.081	3.8	0.033	1.5	0.053	2.5	2.14
Cr[d]	0.241	8.0	0.396	13.2	0.980	32.7	0.265	8.8	0.221	7.4	3.00
Cd[d]	0.767	92.4	0.096	11.6	0.089	10.7	0.507	61.1	0.019	2.3	0.83
Pb[d]	0.128	3.6	0.127	3.6	0.328	9.2	0.107	3.0	0.057	1.6	3.57
Hg[d]	0.014	2.5	0.015	2.6	0.013	2.3	0.016	2.8	0.014	2.5	0.57

[a] PTDI values were summarized by FAO/WHO (1985).

[b] PTDI values were summarized by Chinese Nutrition Society (2013).

[c] PTDI values were summarized by WHO (1989).

[d] PTDI values of other elements were all summarized by Mok et al. (2015).

3.3.2 Target hazard quotient

THQ is a method to estimate non-carcinogenic health hazard. The THQ value of heavy metal in present studied bivalves was calculated according to Formula (2). The THQ was integrated risk index, and has been used widely for risk assessment of various contaminants (Mok et al., 2015). The THQ values for individual metals from the selected bivalves consumption are shown in Table 8. The average THQ values of all metals in all samples were all below 1.0, while the THQ values of Cd in some *C. farreri* samples were exceed 1.0, and the highest value was to 2.366, which meant that there was potential health risk with long-term consumption exposure to these *C. farreri*. Some researchers found that there are some other organic Cd forms in *C. farreri and R. philippinarum* which have less toxicity than inorganic one (Choi et al., 2007; Zhao et al., 2012). So the toxicity of Cd here may be overestimated, and the *harmful* effect still should be paid more attention. For *R. philippinarum*, all the average THQ values were below 0.5, the THQ values of Cr in some samples exceeded 1.0, which indicates that there is a factor for health concern. The THQ value of iAs in *R. philippinarum* was the highest among the five bivalves. And it reveals that iAs was more easily accumulated by *R. philippinarum*. For *M. veneriformis*, the top three metals were Fe, Cr and iAs. The max THQ value of Fe was 0.907. For *C. gigas*, the top three metals was Cd, iAs and Zn, which was similar with the previous research (Denil et al., 2017). The max THQ values of Fe in *M. veneriformis* and Cd in *C. gigas* were both more than 0.9 and close to 1.0, which might pose health risk. The THQ values of Zn and iAs in *C. gigas* were also in accord with the results in Taiwan area of Chien et al. (2002). For *C. sinensis*, the average THQ values of all metals were below 0.1 except iAs. This result showed that *C. sinensis* was most safe when compared to other four kinds of bivalve.

C. farreri, *R. philippinarum* and *C. gigas* are the three most popular shellfish for people living in the coastal area. And the results show that there is higher potential risk of consuming these three kinds of bivalves than others due to their high max THQ values. The daily intake FIR used to calculate THQ values is the average consumption of aquatic products rather than bivalves all over the country. In particular, the intake of fisherman and people living by the coastal area might consume much more than the average FIR and the health risk might be undervalued for them.

Table 8 The target hazard quotient (THQ) of heavy metals in bivalves

Bivalves	THQ									
	C. farreri		*R. philippinarum*		*M. veneriformis*		*C. gigas*		*C. sinensis*	
	average	max	average	max	average	max	average	max	average	max
Fe	0.083	0.266	0.156	0.314	0.489	0.903	0.063	0.145	0.055	0.102
Mn	0.031	0.071	0.033	0.069	0.097	0.256	0.039	0.075	0.024	0.032
Cu	0.005	0.016	0.002	0.004	0.002	0.003	0.05	0.094	0.002	0.004
Zn	0.113	0.305	0.027	0.045	0.014	0.026	0.244	0.447	0.027	0.037
iAs	0.177	0.310	0.430	0.810	0.270	0.470	0.110	0.273	0.177	0.253
Cr	0.080	0.209	0.132	1.062	0.327	0.709	0.088	0.199	0.074	0.28
Cd	0.767	2.447	0.096	0.161	0.089	0.16	0.507	0.967	0.019	0.063
Pb	0.037	0.097	0.036	0.209	0.094	0.227	0.031	0.065	0.016	0.033
Hg	0.025	0.063	0.026	0.061	0.023	0.07	0.024	0.082	0.025	0.056

4. Conclusion

Bivalves is one of the most popular and favorite seafood in China. Present research revealed that the trace element concentrations in five kinds of bivalves from the Yellow sea and Bohai sea were generally low and safe as food. Different species of bivalves had significantly different accumulation capacity for different heavy metals. The concentration of Fe, Mn, Cr and Pb in M. veneriformis, Cu and Zn in C. gigas, and As and Cd in *C. farreri* were significantly higher than others ($p<0.05$). Although the contents of all the studied heavy metals was below the maximum allowable limit regulated by China Food Standard Agency, Pi and Pc value showed that Cu and Zn in *C. sinensi*, Cr in *M. veneriformis*, and Cd in *C. farreri* were lightly polluted. Compared to the PTDI, the average EDI of Cd in *C. farreri* was the highest and occupied 92% of PTDI, the EDI of Cu and Cd were 62% and 61%, respectively. The max THQ values of Cd in *C. farreri* and Cr in *R. philippinarum* were both more than 1.0, which indicated that the over intake of the two bivalves for long-term represented an appreciable hazard to human.

Declarations

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Conflicts of interest/Competing interests (include appropriate disclosures)

All the authors declare that they have no conflict of interest.

Availability of data and material (data transparency)

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Code availability (software application or custom code)

Not applicable.

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

Haiyan Ding performed the data analyses and wrote the manuscript.

Yanfang Zhao designed experiments and modified the manuscript.

Xiaofeng Sheng performed the experiment.

Xihuang Zhong performed the experiment and analysed the data.

Xuming Kang analysed the data.

Jinsong Ning performed the data analysis.

Derong Shang performed the analysis instruments.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Ethics approval (include appropriate approvals or waivers)

Not applicable.

Consent to participate (include appropriate statements)

Not applicable.

Consent for publication (include appropriate statements)

Not applicable.

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Figures

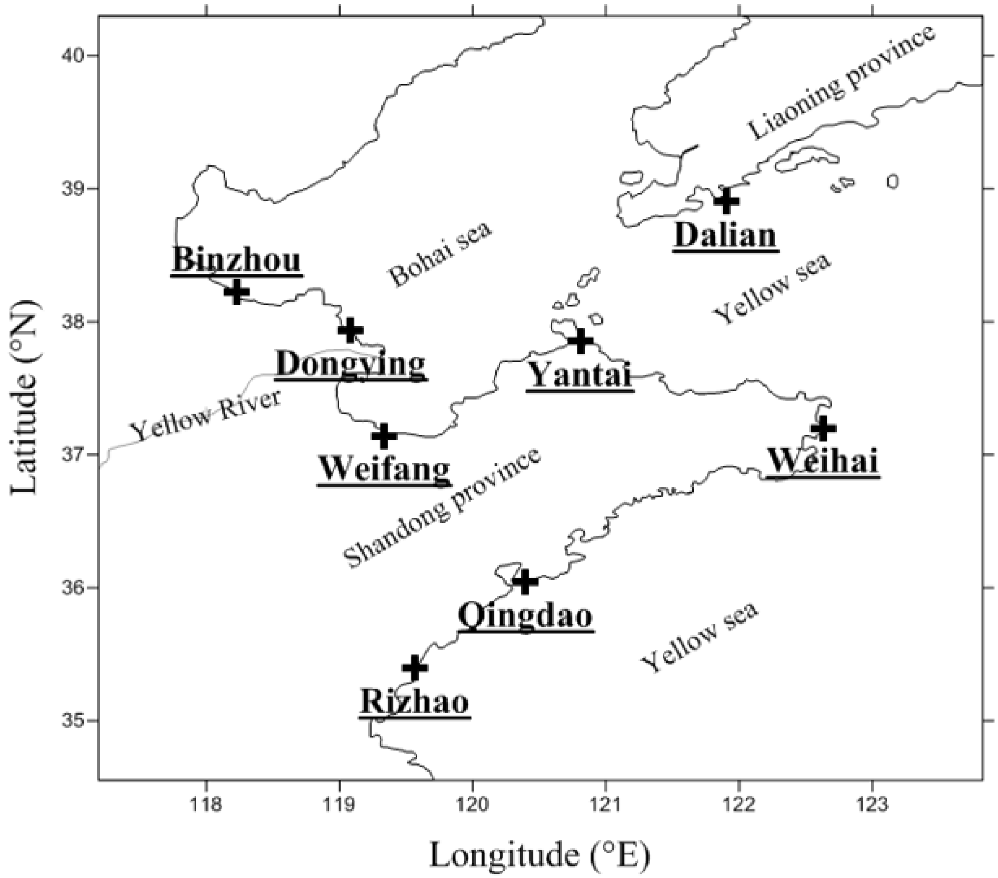

Figure 1

The sampling sites (symbol of cross) of bivalves

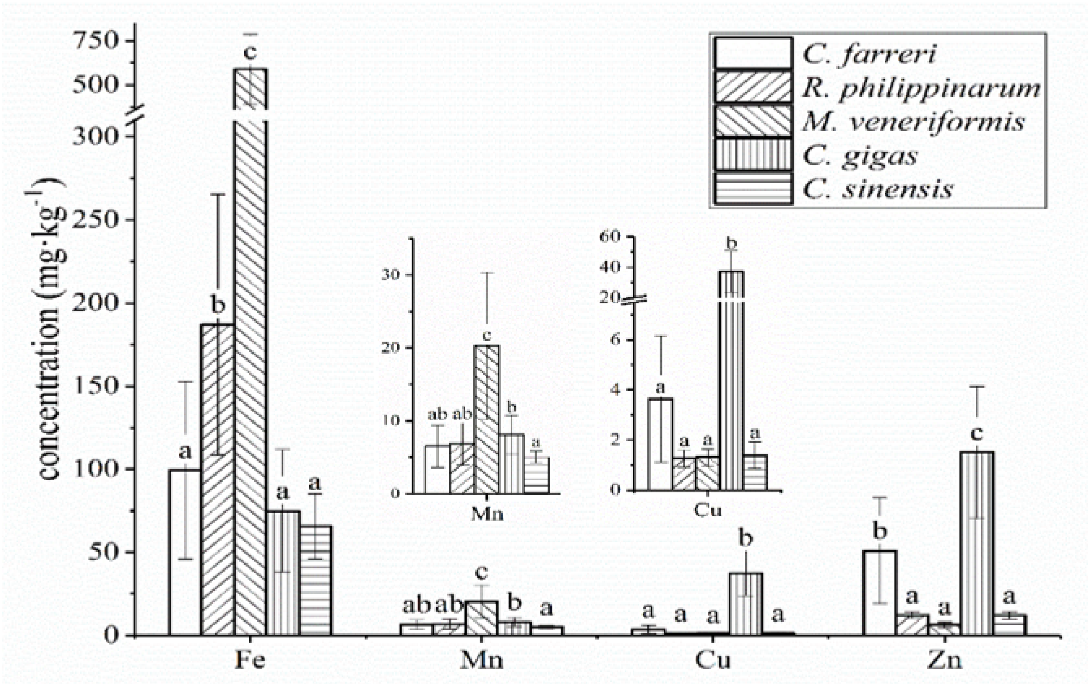

Figure 2

The concentrations of some essential elements in different bivalve samples (Different letters for the same metal indicate the significant differences, $p<0.05$).

Figure 3

The concentrations of some non-essential elements in different bivalve samples (Different letters for the same metal indicate the significant differences, $p<0.05$).