Health risk assessment and bioaccumulation of heavy metals in Procambarus clarkii from six provinces of China

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Abstract

Contamination with heavy metals in wild red swamp crayfish (*Procambarus clarkii*) from 7 different geographical areas in six provinces of China (Hubei, Hunan, Jiangxi, Anhui, Jiangsu, and Shandong) was evaluated. Concentrations of chromium (Cr), arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg) in the abdominal muscle, gonad, and hepatopancreas were determined by inductively coupled plasma mass spectrometry (ICP-MS) and Atomic Fluorescence Spectrometer (AFS). Except for the Cd content in hepatopancreas, the contents of selected heavy metals in three different tissues were significantly lower than the proposed limits provided by United States Environmental Protection Agency (USEPA). The maximum accumulations of Cd and Pb were in hepatopancreas, while the maximum accumulation of As was in gonad, and the maximum accumulations of Hg and Cr were in abdominal muscle. The highest contents of Cr, Hg, and Pb were all detected in Dongting Lake, Hunan, which was consistent with the trend of the metal pollution index (MPI). Risk value of the target hazard quotient (THQ) was below 1.0, suggesting that the intake of selected heavy metals through crayfish consumption would not pose a significant health risk to consumers.

Introduction

With the rapid development of industrialization and urbanization, heavy metal pollution has become increasingly serious and received widespread attention in China (Huang et al. 2018). The majority of the known heavy metals can be readily absorbed and bio-accumulated in organisms via anthropogenic and natural emissions, and even those considered as essential, can be toxic if present in excess (Jaishankar et al. 2014).

Potential health risks of heavy metal exposure have been documented in the past few decades. In particular, recent studies have demonstrated that heavy metals have carcinogenic or noncarcinogenic risks to human beings (Peng et al. 2016; Jia et al. 2017). An increased risk of stomach cancer and lung cancer after ingesting chromium (Cr VI) in drinking water in Liaoning Province of China was reported (Beaumont et al. 2008). Arsenic (As), which is more toxic in its inorganic form than in its organic form, was established as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). Inhalation of inorganic As caused lung cancer in smelter workers (Enterline et al. 1987). Inorganic As might be associated with fetal loss, low birth weight, cognitive deficit, skin impairment, and cardiovascular disease (Smith et al. 2009). Cadmium (Cd) can cause acute kidney damage, spontaneous abortion, bone damage, and cancer through absorption of stomach, intestine, skin, and lung (Godt et al. 2006). Lead (Pb) is reported to be an accumulative metabolic poison, which affects the nervous, cardiovascular, renal, hematopoietic, and reproductive systems in human. It can also cause mental retardation and hyperactivity in children (Demayo et al. 1982). Elemental mercury (Hg) was harmful for the central nervous system, while inorganic Hg compounds primarily affected the kidney. Particularly, methylmercury (MeHg) was a potent neurotoxin (Beckers et al. 2017). Even low dose Hg was reported to decrease performance in motor function and memory not only in children but also in adults (Zahir et al. 2005). Therefore, it is very important to evaluate the potential risks to human health caused by food intake contaminated by heavy metals.

Red swamp crayfish (*Procambarus clarkii*) has been farmed extensively and become one of the most economically important farmed aquatic species in China since the 90's. Nowadays, China is the world's leading crayfish producer (Wang et al. 2005; Yi et al. 2018; Mo et al. 2019). The geographic conditions in Hubei, Hunan, Jiangxi, Anhui, Jiangsu, and Shandong make them be the main production areas of edible crayfish in China. Wild crayfish was most commonly found in warm fresh water, such as lakes, ponds, rivers, and wetlands. Chaohu Lake, Poyang Lake, Taihu Lake, Dongting Lake, and Weishan Lake are main sources of freshwater and aquatic products for local residents in these provinces. Evidences from previous studies suggested that the seven lakes mentioned above are endangered with various pollutants, especially heavy metals (Chi et al. 2007; Yang et al. 2009; Li et al. 2013; Wei et al. 2014). Crayfish camps on the special benthic lifestyle, and serves as a high-level consumer of benthic animals in natural water bodies. It can not only accumulate heavy metals in its own body through the food chain, but also can survive and multiply under the stress of heavy metals (Alcorto et al. 2006; Kuklina et al. 2014). Risk to human health via crayfish consumption has been reported based on a small-scale survey in Shanghai (Wu et al. 2010). Moreover, the consumption of crayfish in the peak summer season reached more than 100 metric tons per day in Nanjing, Wuhan, Hefei and other central cities in these six provinces (Mu et al. 2007). Given the above mentioned heavy metal pollution and wide population exposure, the health risk of crayfish consumption in these regions deserves attention. However, limited studies focus on contamination with heavy metals and health risk in the main production and consumption region of crayfish.

In order to provide information about heavy metal pollution in wild crayfish and further conduct risk assessment, this study investigated the contents of five heavy metal (Cr, As, Hg, Cd, and Pb) in three edible parts of field-collected crayfish from six provinces. Metal pollution index (MPI) and target hazard quotient (THQ) were used to evaluate the health risk of heavy metals after consumption of crayfish.

2 Materials And Methods

2.1 Sample collection and preparation

The wild red swamp crayfish (*Procambarus clarkii*) were obtained from the lakes in six provinces from August to October, 2018, including Hubei, Hunan, Jiangxi, Shandong, Anhui, and Jiangsu Provinces (Fig. 1), which have higher crayfish consumption levels. A total of 177 specimens (about 25 specimens every sampling sites) were collected. The crayfish were weighed (Table 1), killed and dissected. And then the gonad, hepatopancreas, and abdominal muscle were sampled. The hepatopancreas was a metabolically very active organ that could absorb and sequester heavy metals for detoxification (Chavez-Crooker et al. 2003). The dissected gonad, hepatopancreas, and abdominal muscle were washed with deionized water, dried at 60°C for 24 h to determine dry weight, grounded with a pestle and mortar, and then stored at − 20°C in sealed polyethylene bags until analysis.

Table 1
The date and body weight of wild crayfish obtained from 7 sampling sites

Study location	Province	Setting	Sample Size	Body weight (g)	Time
Chaohu Lake	Anhui	Wild caught	25	20.20	2018.10.03
Poyang Lake	Jiangxi	Wild caught	26	18.20	2018.09.15
Weishan Lake	Shandong	Wild caught	25	16.84	2018.09.07
Taihu Lake	Jiangsu	Wild caught	22	18.98	2018.09.26
Jianli	Hubei	Wild caught	26	23.33	2018.08.20
Anqing	Anhui	Wild caught	27	20.52	2018.09.07
Dongting Lake	Hunan	Wild caught	26	19.83	2018.09.20

2.2 Heavy metal analysis

Approximately 0.3 g of the tissue sample was weighed into a microwave digestion inner tank, and 10 ml of HNO_3 was added. The microwave digestion tank was covered, placed for one hour, and performed by a microwave digestion system (MARS-6, China Everbest Machinery Industry Co., Ltd., Shenzhen, China), according to the following program: 5 min to 120°C, 5min at 120°C, 5 min to 150°C, 10 min at 150°C, 5 min to 190°C, and 20 min at 190°C. After cooling, the digestion solution was diluted to 50 ml for later use. Simultaneously, 1 blank per 5 sample was test to eliminate interference. The contents of As, Cr, Pb, and Cd based on dry weight (d.w) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Tokyo, Japan). Sc, Ge, Rh, and Lu were applied for instrument calibration. The concentrations of Hg were measured by Atomic Fluorescence Spectrometer (AFS, Beijing Titan Instruments Co., Ltd., Beijing, China). All calibration straight lines had correlation coefficients > 0.999 and extraction recovery rate was 80%-120%. The contents of heavy metals in different tissues based on wet weight (w.w) were converted to the contents of heavy metals based on dry weight. In later analysis, the values of quantification limit were applied instead of the non-detected values of the corresponding heavy metals.

2.3 Health risk assessment

Total heavy metal accumulation in various tissues at different sampling sites was examined by the metal pollution index (MPI). The MPI was described by the following equation (Usero et al. 1997):

$$\mathrm{MPI} = (C_{Cr} \times C_{As} \times C_{Hg} \times C_{Cd} \times C_{Pb})^{\frac{1}{5}}$$

Where C_{Cr}, C_{As}, C_{Hg}, C_{Cd}, and C_{Pb} (mg/kg w.w) are the contents of Cr, As, Hg, Cd, and Pb based on wet weight in different tissue of crayfish sampled at each sampling sites.

Muscle and hepatopancreas are the main tissues of crayfish consumed by local residents. Therefore, this study evaluated the health risks of muscle and hepatopancreas consumption. Firstly, the daily heavy metal intake from crayfish consumption was estimated. Estimated daily intake (EDI) (μg/BWkg day) was calculated through the following formula:

$$\mathrm{EDI}_i = \frac{C_i \times DIR}{BW}$$

Where C_i (mg/kg w.w) is the content of heavy metal based on wet weight, DIR (g/day) is the average daily intake of crayfish by local residents, and BW is the average weight of consumers. The DIR for inhabitants are 2.0 g/day/person for abdominal muscle and 1.0 g/day/person for hepatopancreas. The BW for inhabitants is 60 kg (Peng et al. 2016a).

Then the potential health risks from exposure to heavy metal through crayfish consumption were assessed using the target hazard quotient (THQ) regulated by USEPA (1989), described as follows:

$$\mathrm{THQ}_i = \frac{EDI_i}{RfD_i} \times \frac{EF \times ED}{AT} \times 10^{-3}$$

Where ED is the exposure duration (year), EF is the exposure frequency (day/year), and AT is the average exposure time (day). In this study, the AT, ED, and EF were set as 25,550 days, 70 years, and 365 days/year, respectively. RfD_i (mg/kg/day) is the chronic oral reference dose for heavy metal regulated by USEPA (2019). The values of RfD_i for heavy metals is 3.0×10^{-3} mg/kg/day for Cr, 3×10^{-4} mg/kg/day for As, 1×10^{-4} mg/kg/day for Hg, 1.0×10^{-3} mg/kg/day for Cd, and 2×10^{-2} mg/kg/day for Pb, respectively (Wei et al. 2014; USEPA 2019).

3. Results And Discussion

3.1 Heavy metal contents in three different tissues of field-collected crayfish

The contents of the selected heavy metals (Cr, As, Hg, Cd, and Pb) in the hepatopancreas, abdominal muscle, and gonad of crayfish collected from 7 sampling sites were summarized in Table 2. Hexavalent chromium was a group 1 carcinogen with multiple complex mechanisms by which it triggers cancer development. Increased levels of DNA adduct formation, chromosome breaks, and oxidative stress were the main mechanisms of Cr VI causing cellular damage (DesMarais & Costa 2019). The highest content of Cr was 0.5959±0.0026 mg/kg d.w in abdominal muscle of crayfish from Dongting Lake. This result was consistent with these observations that the average of Cr content in sediment from Dongting Lake (88.29 mg/kg) was higher than Cr contents from Poyang Lake (70.77 mg/kg), Taihu Lake (41.5 mg/kg), and Chaohu Lake (63.36 mg/kg) (Yuan et al. 2011; Jiang et al. 2012; Tang et al. 2010; Li et al. 2013). The lowest content of Cr was 0.0029±0.0029 mg/kg in gonad from Anqing. The contents of Cr in abdominal muscle were significantly higher than those in other tissues collected from 5 sampling sites (Chaohu Lake, Waishan Lake, Dongting Lake, Jianli, and Anqing). Kuklina et al (2014) also found that Cr was more concentrated in abdominal muscle. However, the content of Cr in abdominal muscle was far lower than those in exoskeleton, hepatopancreas, and gills of crayfish after exposure to waterborne Cr (Bollinger et al. 1997). For fish, Cr was investigated to enrich in bladder and liver (Wei et al. 2014). Possible reason was that the enrichment of Cr might be specific and change according to the surrounding environment.

Organic As was non-toxic whereas inorganic As was toxic. Trivalent form (As_2O_3) and pentavalent form (As_2O_5) are two oxidation states of inorganic As. However, As (□) is 60 times more toxic than As (□) (Ratnaike et al. 2003). In this study, the highest content of As was 2.7379±0.0306 mg/kg in hepatopancreas of crayfish collected from Taihu Lake, while the lowest content of As (0.1411±0.0035 mg/kg) was observed in abdominal muscle collected from Chaohu Lake. It was reported that the content of As in crayfish muscle collected from Chaohu Lake (0.1402-0.2410 mg/kg d.w) was higher than those in crayfish muscle collected from Poyang Lake (0.010-0.080 mg/kg d.w.) and Xiang River (0.037-0.141mg/kg d.w) (Wei et al. 2014; Jia et al. 2017). These results may indicate that As is more easily enriched in crayfish than other aquatic invertebrates. Results showed that As contents in hepatopancreas and gonad were markedly higher than those in muscle. According to the United States Food and Drug Administration (1993), the content of inorganic As could be estimated as 10% of total As. Based on this parameter, the contents of inorganic arsenic in all the samples did not exceed the safety limit set by the China National Standards Management Department (CNSMD).

Since the Minamata incident in Japan in 1950s, methylmercury in the aquatic environment and aquatic organisms has raised global concerns (Harada et al. 1995). Peng et al (2016a) reported that MeHg constituted 92-99% of mercury in crayfish muscle. The highest content of Hg was in muscle from Dongting Lake (0.0613±0.0022 mg/kg d.w) and the lowest content of Hg was in gonad from Taihu Lake (0.0065±0.0003 mg/kg). These results were consistent with the content of Hg in the muscle of crayfish collected from 23 cities in China (58.1±19.2 μg/kg). Notably, unlike the other four heavy metals, the total contents of Hg in hepatopancreas and gonad were significantly lower than that in muscle. The same results were also observed in previous researches (Stinson & Eaton 1983; Goldstein et al. 1996; Wei et al. 2014).

Table 2 Heavy contents (Cr, As, Hg, Cd, and Pb) (mg/kg ww) of crayfish (hepatopancreas, muscle, and gonad) sampled from 7 locations in China (mean ± SD)

Location	Tissues	Heavy metal content (mg/kg w.w)				
		Cr	As	Hg	Cd	Pb
Chaohu Lake	Hepatopancreas	0.0965±0.0220	0.5660±0.0656	0.0116±0.0048	0.3086±0.0372	0.0319±0.0051
	Muscle	0.2123±0.0031	0.1411±0.0035	0.0484±0.0015	0.0011±0.0003	0.0156±0.0005
	Gonad	0.2058±0.0022	1.1402±0.0039	0.0201±0.0019	0.0017±0.0005	nd
Poyang Lake	Hepatopancreas	0.1508±0.0039	1.0742±0.0187	0.0188±0.0006	4.5671±0.0407	0.0518±0.0006
	Muscle	0.1122±0.0049	0.2106±0.0044	0.0279±0.0018	0.0016±0.0008	0.0141±0.0006
	Gonad	0.1607±0.0042	1.5155±0.0144	0.0101±0.0008	0.0015±0.0011	nd
Weishan Lake	Hepatopancreas	0.0181±0.0006	1.7004±0.0155	0.0096±0.0019	0.4277±0.0046	0.0561±0.0006
	Muscle	0.1936±0.0058	0.1976±0.0022	0.0197±0.0024	0.0006±0.0004	0.0240±0.0008
	Gonad	0.0602±0.0053	1.3303±0.0197	0.0054±0.0021	0.0002±0.0002	nd
Taihu Lake	Hepatopancreas	0.0723±0.0009	2.7379±0.0306	0.0107±0.0007	0.8850±0.0099	0.0400±0.0008
	Muscle	0.0811±0.0013	0.1718±0.0032	0.0158±0.0002	0.0004±0.0002	0.0168±0.0007
	Gonad	0.0998±0.0029	1.0660±0.0267	0.0065±0.0003	0.0014±0.0005	nd
Jianli	Hepatopancreas	0.0868±0.0016	1.9231±0.0245	0.0183±0.0018	2.1747±0.0236	0.1443±0.0010
	Muscle	0.1564±0.0167	0.2085±0.0136	0.0302±0.0014	0.0012±0.0006	0.0195±0.0033
	Gonad	0.0118±0.0434	1.2270±0.1511	0.0117±0.0011	0.0015±0.0010	nd
Anqing	Hepatopancreas	0.0877±0.0347	0.7771±0.1315	0.0125±0.0007	0.6907±0.1346	0.0464±0.0110
	Muscle	0.2984±0.0052	0.1863±0.0092	0.0475±0.0035	0.0010±0.0003	0.0062±0.0006
	Gonad	0.0029±0.0029	1.3641±0.0328	0.0150±0.0005	0.0013±0.0005	nd
Dongting Lake	Hepatopancreas	0.0447±0.0075	2.1492±0.1494	0.0190±0.0004	1.3947±0.1367	0.2141±0.0020
	Muscle	0.5959±0.0026	0.2945±0.0052	0.0613±0.0022	0.0014±0.0004	0.0373±0.0047
	Gonad	0.2656±0.0434	1.2510±0.1255	0.0168±0.0013	0.0089±0.1432	0.0589±0.0066

* ND = not detected

Cadmium was one of the global health problems that affected many organs and in some cases it could even cause deaths. Long-term exposure to cadmium through food, soil, water, and air might cause cancer and organ system toxicity such as cardiovascular, skeletal, reproductive, urinary, respiratory systems, and central and peripheral nervous (Rahimzadeh et al. 2017). The highest Cd content was detected in hepatopancreas of crayfish collected from Poyang Lake (4.5671±0.0407 mg/kg d.w). The contents of Cd in hepatopancreas of crayfish collected from Poyang Lake, Taihu Lake, Dongting Lake, Anqing, and Jianli exceeded the threshold values in the national food safety standards of China. The contents of Cd in sediment were 0.13-1.49 mg/kg in Poyang Lake, 1.71 mg/kg in Dongting Lake, and 0.20-2.88 mg/kg in Taihu Lake, which exceeded the content of class three (1 mg/kg) from the Chinese Environmental Quality Standard for sediment (Zhang et al. 2012; Qin et al. 2012; Hu et al. 2015). These evidences indicated that cadmium pollution was serious in these areas. Hepatopancreas is the main organ of cadmium accumulation and detoxification in crayfish (Kouba et al. 2010). The contents of Cd in abdominal muscle and gonad (<0.0089 mg/kg d.w) were far below the Cd contents in hepatopancreas in this study. The contents of Cd in hepatopancreas exhibited an apparent positive correlation between accumulation time

 (Zhang et al. 2014). This might be due to the existence of metallothionein proteins in hepatopancreas which could bind Cd for detoxification (Ploetz et al. 2007). The present study showed that the contents of Cd in muscle were lower than those reported in other studies, including 1.2-60.6 μg/kg d.w Cd from 12 provinces in China (Peng et al. 2016b), and 0.08 mg/kg d.w Cd from Lake Washington (Stinson & Eaton 1983).

Lead toxicity is a major public health problem in developed and developing countries. Both acute and chronic exposure to lead have the potential to cause many deleterious systematic effects, including immune imbalances, frank anemia, hypertension, cognitive deficits, vitamin D deficiency, infertility, gastrointestinal effects, and delayed skeletal and deciduous dental development (Mitra et al. 2017). The highest content of Pb was observed in hepatopancreas collected from Dongting Lake (0.2141±0.0020 mg/kg d.w). The contents of Pb in abdominal muscle ranged from 0.0062 mg/kg d.w to 0.0373 mg/kg d.w, which was consistent with the values (mean 0.023 mg/kg d.w) reported by Peng et al (2016b). The contents of Pb in gonad were not detected, except those in Dongting Lake. The contents of Pb in hepatopancreas were slightly higher when compared with the those in abdominal muscle and gonad. The same results were also reported by previous studies (Wei et al. 2014; Jia et al. 2017). Roldan and Shivers (1987) indicated that Pb could store in metal-containing vacuoles of hepatopancreatic cells.

Metals showed different affinity to organs, which might be due to the different functions of organs and metabolic roles of metals (Ashraf 2005). As presented in Table 2, it was concluded that the hepatopancreas was the primary organ for Cd, As, and Pb deposition, the abdominal muscle was the ideal organ for Cr and Hg deposition, and the gonad was the primary organs for As deposition. The maximum limit required of these heavy metals for crayfish in the national food safety standards of China were 2 mg/kg w.w (Cr), 0.5 mg/kg w.w (Inorganic As), 0.5 mg/kg w.w (MeHg), 0.5 mg/kg w.w (Cd), and 0.5 mg/kg w.w (Pb), respectively (GB 2762 2017). The values of Cr, As, Hg, and Pb contents in these three different tissues of crayfish all met national food safety standards of China. However, the content of Cd in hepatopancreas exceeded the lowest limit. However, the studies couldn't just focus on the heavy metal content of crayfish. Health risk assessment is essential.

3.2 Health risk assessment

Fig. 2 presented the MPI values for heavy metal in three tissues collected from seven sampling points. Considering the MPI in different tissues, the distribution of the heavy metals was in the ascending order of abdominal muscle < gonad < hepatopancreas for the seven sites. Generally, the muscle is weak to accumulate heavy metals. The liver and gill, as metabolically active organs, have a great tendency to store high levels of heavy metals (Monikh et al. 2013). Considering the MPI in same tissues, the order of MPI in hepatopancreas was as follows: Jianli > Poyang Lake > Dongting Lake > Taihu Lake > Anqing > Weishan Lake > Chaohu Lake; the order of MPI in abdominal muscle was as follows: Dongting Lake > Chaohu Lake > Jianli > Anqing > Poyang Lake > Weishan Lake > Taihu Lake; the order of MPI in gonad was as follows: Dongting Lake > Chaohu Lake > Poyang Lake > Taihu Lake > Jianli > Anqing.

The THQ provided an indication of the risk level associated with pollutant exposure. This method of risk estimation had recently been used by many researchers and had been shown to be valid and useful (Yi et al.

 troduced to estimate potential health risk of chronic exposure to heavy metals in crayfish. As shown in Fig. 3, the average THQ of individual heavy metal in abdominal muscle followed the order 1 > Hg > Cr > As > Cd > Pb for all sampling sites. It was worth noting that hepatopancreas was the favorite food of crayfish for Chinese, while the heavy metals tended to be enriched in the hepatopancreas, so it could not be ignored when evaluated. The average THQ of individual heavy metal in hepatopancreas followed the order 1 > Cd > As > Hg > Cr > Pb (Fig. 4), suggesting that risk of heavy metals exposure via crayfish consumption in these locations is extremely low.

Conclusions

Heavy metal pollution has become a crucial environmental problem in China. The aim of this paper was to investigate the contamination of five heavy metals (Cr, As, Hg, Cd, and Pb) of wild crayfish in China. The results showed that the contents of these five heavy metals in crayfish in the main production and consumption area were mostly below the national safety standards, suggesting heavy metal pollution in these regions has been controlled. Health risk assessment indicated that exposure to selected heavy metals from crayfish consumption had no non-carcinogenic health risk to local inhabitants. However, no evidences proved that excessive consumption could not pose health risks. Whether other persistent pollutants except heavy metal would be detected in crayfish, and related health risk needed further evaluation.

Declarations

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Data availability Data and material access are not available.

Declarations

Ethical approval and consent to participate Not applicable.

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Competing interests The authors declare no competing interests.

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Figures

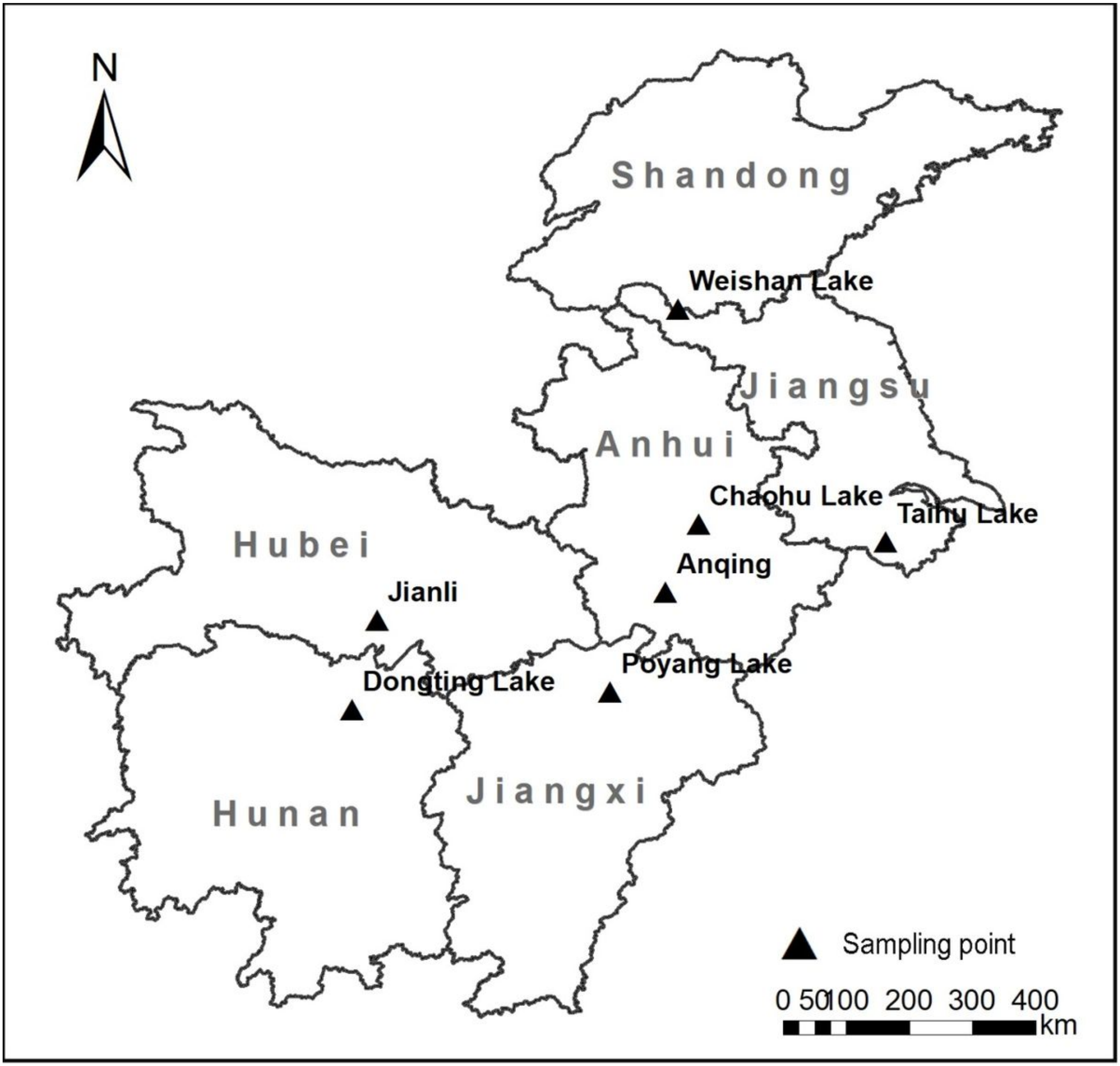

Figure 1

Diagram of 7 different sampling points in 6 provinces of China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

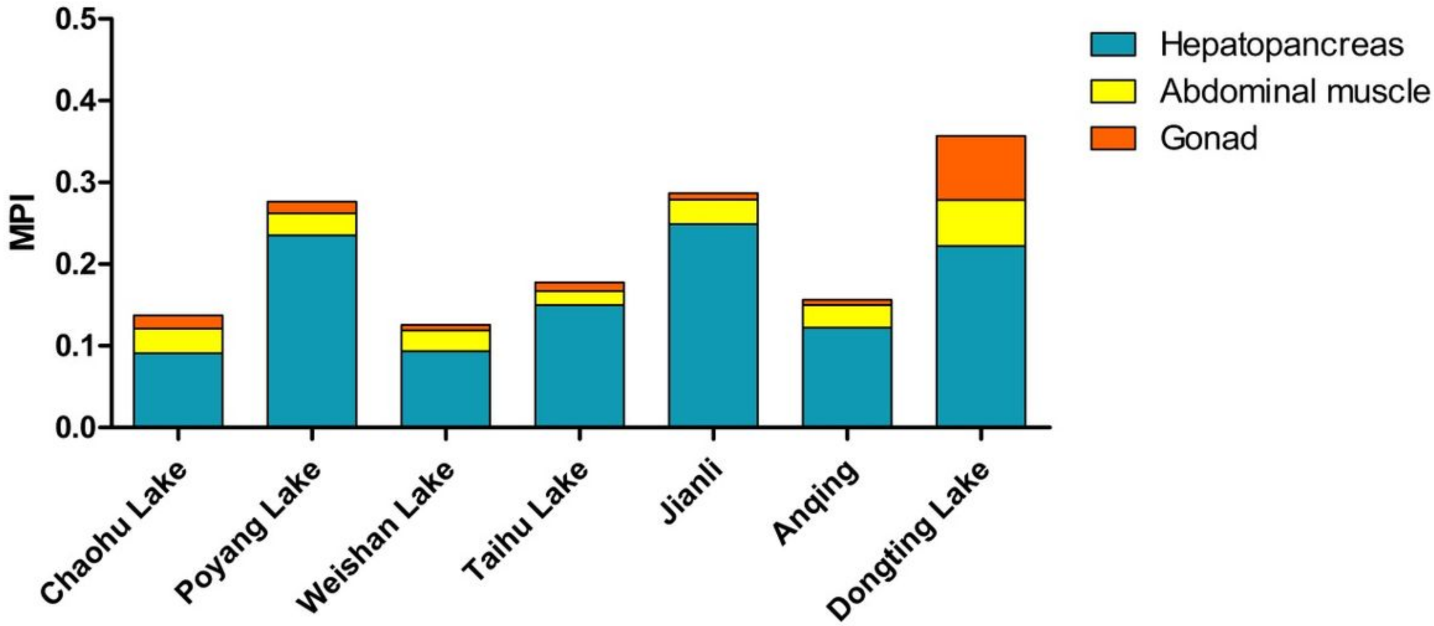

Figure 2

The MPI in different tissues and sampling sites.

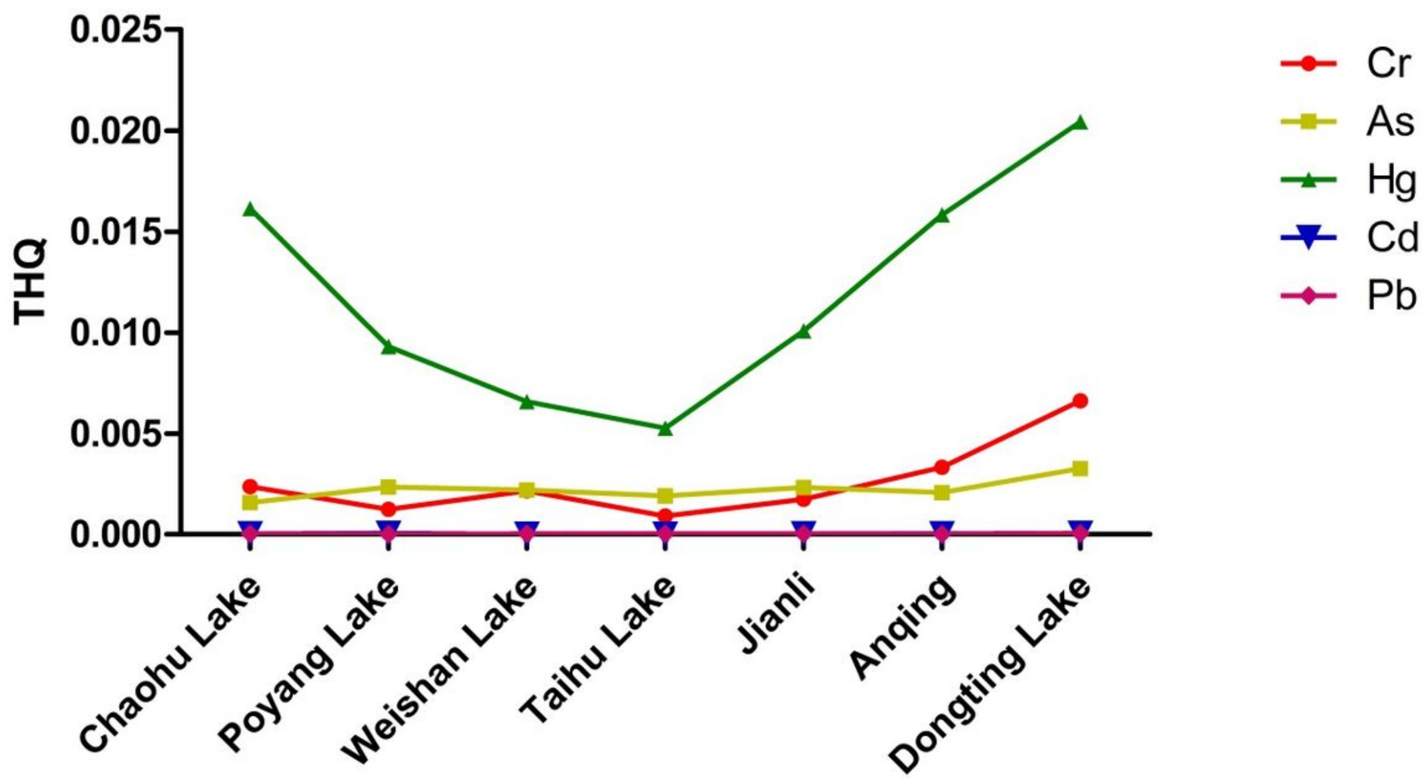

Figure 3

The THQ of heavy metals through abdominal muscle consumption of crayfish collected from 7 sampling

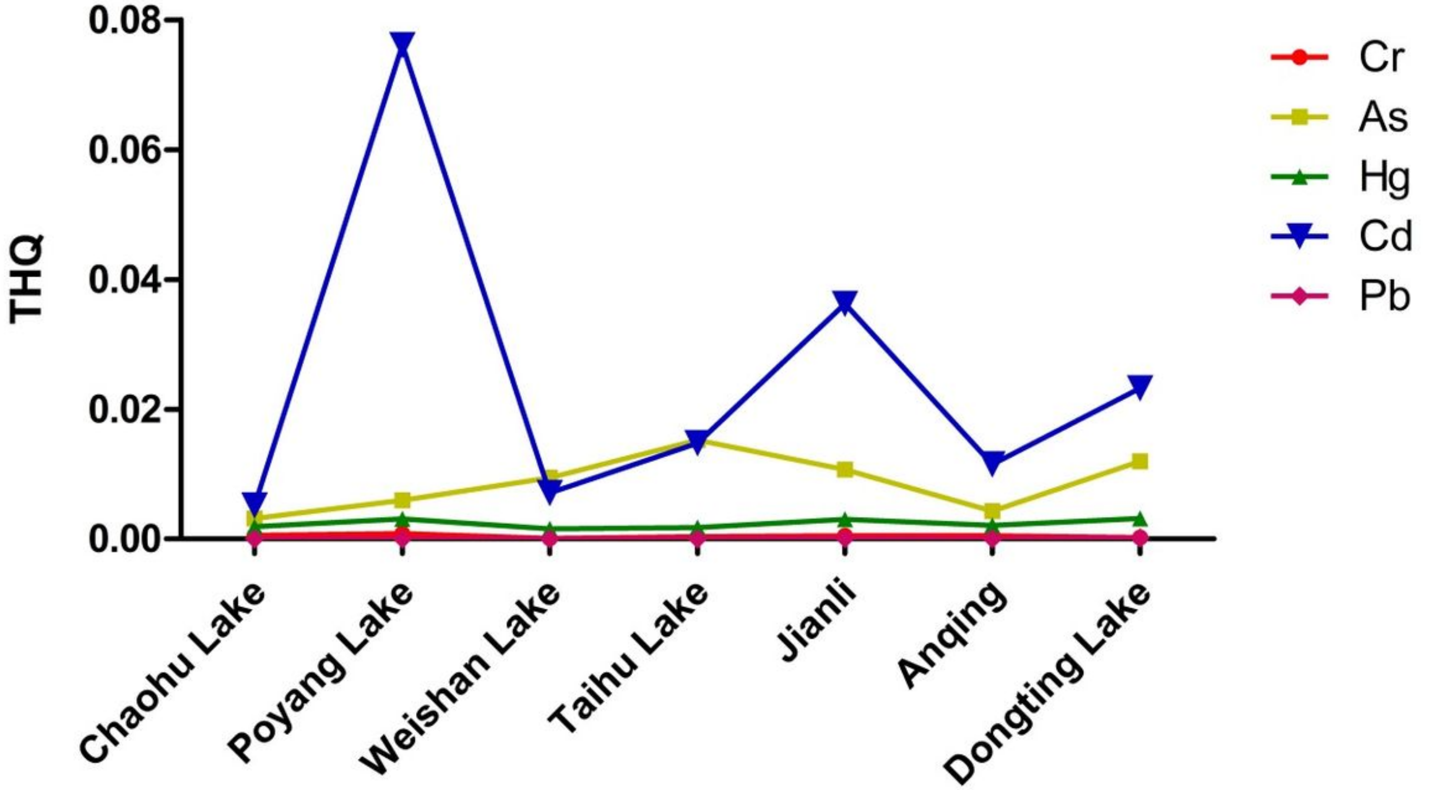

Figure 4

The THQ of heavy metals through hepatopancreas consumption of crayfishfish collected from 7 sampling sites.