

Concentrations and health risk assessment of 24 residual heavy metals in Chinese mitten crab (*Eriocheir sinensis*)

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Received: 24 December 2021; Accepted: 25 February 2022; Published: 24 March 2022

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ORIGINAL ARTICLE

Abstract

In this study, a total of 135 Chinese mitten crab (*Eriocheir sinensis*) samples were collected from four major crab-producing provinces (Jiangsu, Hubei, Anhui, and Liaoning) in China. The content of 24 trace elements in the edible parts of mitten crabs was determined by inductively coupled plasma mass spectrometry (ICP-MS). In addition, the content of trace elements in crab samples collected from different provinces was compared, and the heavy metal levels exceeding the standard were evaluated. Twenty four trace metallic elements were determined in Chinese mitten crab samples. The single factor pollution index (Pi), percentage of provisionally tolerable weekly intake (PTWI), and target hazard quotients (THQ) were used to evaluate the pollution level and food safety of Chinese mitten crabs. The final assessment results of dietary risks led to the health risk assessment accompanying food safety recommendations on Chinese mitten crabs. The overall finding of this study established that Chinese mitten crab aquatic products can be eaten for nutrition; however, attention should be paid on the potential food safety risks of some heavy metals. Moreover, efforts on developing the industrial economy by the local government and peoples' safety from food risks may also increase the awareness of environmental protection.

Keywords: China; Chinese mitten crab (*Eriocheir sinensis*); hazard index; trace metals; risk assessment

Highlights

- A total of 135 Chinese mitten crab (*Eriocheir sinensis*) samples were collected from four provinces in China.
- The sample collection area covered 86.7% of total Chinese river crab production.
- Pi, PTWI, THQ, and other indicators were used to evaluate the safety of crabs.
- A comprehensive evaluation resulted in the future food safety recommendation on Chinese mitten crabs.

- The findings of this study have given food safety and environmental protection opinions to the Chinese government.

Introduction

Chinese mitten crabs (*Eriocheir sinensis*) are very popular among consumers due to their delicious taste and high nutritional value (Ji *et al.*, 2015). In 2020, the

output of freshwater cultured river crabs in China was 775,887,000 kg. As a relatively high-end aquatic product in China, the food safety of river crabs is closely watched by consumers. In general, crustacean aquatic products have a strong ability to accumulate heavy metals and other pollutants (Feng *et al.*, 2021). Some of the trace elements in food are necessary for the human body, including copper, zinc, arsenic, etc., whereas some are harmful to the human body, including lead, cadmium, etc. Some essential trace elements when exceeding a certain limit or taken in excessive amount can cause harmful effects to the human body (Liu *et al.*, 2018).

Lead (Pb) is a nonessential highly toxic metal that is present in aquatic ecosystems (Zhang *et al.*, 2016). Long-term Pb exposure can lead to carapace malformations, abnormalities in the dorsal region of neonates, reddish extremities, ephippia (or dormant haploid egg), aborted eggs, and changes in the color of eggs (green and white) (Araujo *et al.*, 2019; Huang *et al.*, 2014). A contaminated aquatic environment may have a negative impact on the food chain and pose risks to human health. Lead and nickel can permeate the skin and accumulate at high concentrations in the dermis. The skin barrier was disrupted after metal exposure and this was accompanied by apoptosis, DNA damage, and lipid oxidation (Chavatte *et al.*, 2020). Zinc (Zn), cadmium (Cd), selenium (Se), nickel (Ni), and arsenic (As) are teratogenic metals (Lemly, 2014; Salvaggio *et al.*, 2016; Shabani *et al.*, 2015). Under environmental conditions with high concentrations of heavy metals, the ingestion of excessive amounts of heavy metals can lead to poisoning effects with possible serious consequences (Hong *et al.*, 2020; Rose *et al.*, 2015). Cd is a highly harmful environmental contaminant, which can cause reproductive toxicity (Liu *et al.*, 2020).

Wang *et al.* reported the presence of eight heavy metals (Cr, Mn, Ni, As, Se, Cd, Hg, and Pb) in crab samples collected from Jiangsu, Shandong, and Liaoning provinces (Wang *et al.*, 2020). Ke *et al.* analyzed breeding in crab samples collected from Jiangsu, Shandong, and Liaoning provinces (with 50% content of river crab), including the analysis of trace elements (Cd, Zn, Cu, Pb, Hg, As, Cr, and Ni) (Ke and Wang, 2012).

All the representative samples of mitten crab used in this study were collected from the major breeding areas in China (the production of farmed river crabs in the four provinces accounted for 86.7% of the country's total production). A total of 24 trace elements (Li, Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ag, Cd, Cs, Ba, Tl, Pb, and U) were analyzed in each sample of mitten crab collected from Jiangsu, Hubei, Anhui, and Liaoning provinces in China. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the content of 24 trace elements in the edible parts of Chinese mitten

crabs. The differences in the content of trace elements in the crab samples collected from different provinces were analyzed. The single factor pollution index (Pi), provisionally tolerable weekly intake (PTWI), and target risk quotient (THQ) indicators were used to evaluate food safety.

Materials and Methods

Sample collection

All the Chinese mitten crab samples used in this experiment were collected from the four provinces of Jiangsu, Hubei, Anhui, and Liaoning in China. The crab samples of the Jiangsu province were mainly collected from Huai'an, Suqian, Changzhou, Wuxi, and Suzhou areas. The samples of the Anhui province were mainly collected from Anqing, Tongling, Wuhu, Xuancheng, and Ma'anshan areas. Hubei province crab samples were mainly collected from Xiaogan and Jingzhou cities, whereas the crab samples of the Liaoning province were collected from Panjin city. The sampling location of all the collected samples is shown in Figure 1.

Sample processing

The samples of cultured river crabs were collected in the year 2020 from September to October. A total of 135 crab samples were collected, including 40 samples from the Hubei province, 35 samples from the Anhui province, 35 samples from the Liaoning province, and 25 samples from the Jiangsu province. The collected river crab samples were kept in an incubator with ice cubes and brought to the laboratory for further analysis. Before the biopsy, the surface water of Chinese mitten crab samples was wiped out using absorbent paper and the samples were weighed using an electronic weighing machine. After the removal of the shell and crab gills, all the edible parts were mixed into a homogenizer with a meat grinder. The ground crab samples were put into polytetrafluoroethylene plastic bags and stored at -20°C for further analysis.

Chemical analysis

Five grams of the Chinese mitten crab sample (accurate to 0.001 g) were put into the microwave digestion inner tank followed by the addition of 5 mL nitric acid and 2 mL H_2O_2 to it. The sample mixture was covered and left for 1 h. The lid of the tank was then tightened, and the sample mixture was left for digestion following the standard operating procedures of the microwave digestion instrument. The microwave digestion program was set as microwave power of 1600 W (50%), climbing temperature to 185°C , heating time of 10.5 min, and holding time of

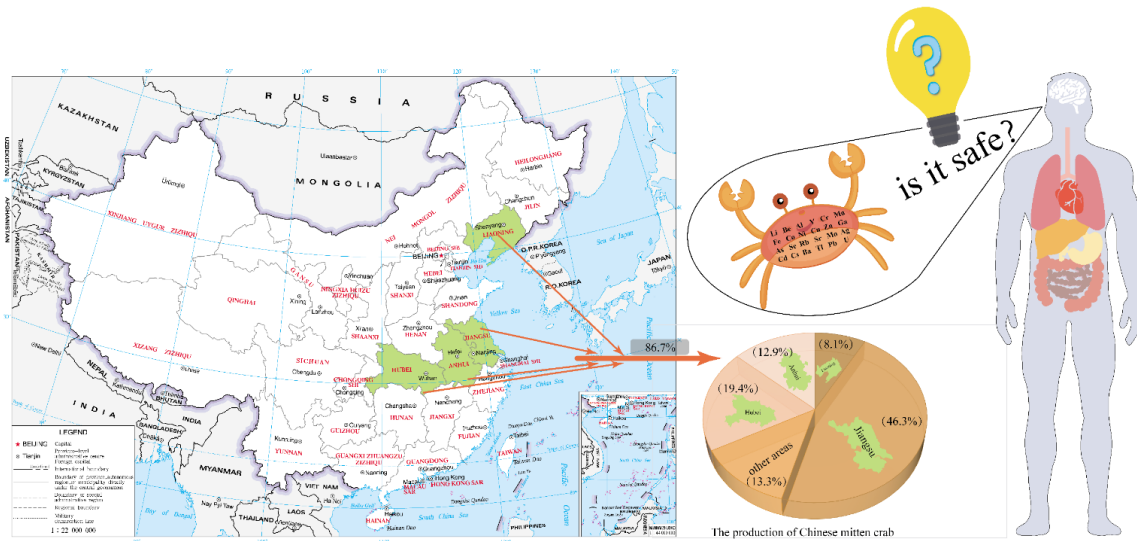


Figure 1. The sampling location of Chinese mitten crabs used in this study.

14.5 min. After cooling, the sample was slowly taken out from the tank, the inner lid rinsed with a small amount of water, the digestion tank placed on a temperature-controlled electric hot plate or an ultrasonic water bath, heated at 100°C for 30 min, diluted with 50 mL of water, and mixed well. A blank test was also run simultaneously.

The data were collected by the Agilent 7500 cx ICP-MS, and the operation parameters were set as follows: RF power, 1500 W; plasma gas flow rate, 15 L min⁻¹; nebulizer gas flow rate, 0.98 L min⁻¹; auxiliary gas flow rate, 0.24 L min⁻¹; collision gas H₂, 4.0 mL min⁻¹, collision gas He, 4.0 mL min⁻¹; nebulizer pump 0.10 rps; uptake speed, 0.40 rps; uptake time, 45 s; and stabilization time, 30 s.

Quality assurance and quality control

Every 10 samples were used as quality control samples, including occasional and quality control samples, to ensure data quality.

Data analysis

Excel 2010 statistical software was used for preliminary data analysis, followed by the application of SPSS statistics software for statistical analysis of data.

Results and Discussion

Sample area selection and the analytical performance

The Chinese river crab aquaculture industry is mainly located in East China, Central China, and Northeast

China. In this study, Chinese mitten crab samples were collected from the four provinces, ranked one to four, with the largest freshwater aquaculture production in China, namely, Jiangsu, Hubei, Anhui, and Liaoning. According to the 2021 China Fishery Statistical Yearbook (China Fishery Statistical Yearbook, 2021), the freshwater crab production in Jiangsu, Hubei, Anhui, and Liaoning provinces in 2020 was 359,121,000 kg (46.3%), 150,863,000 kg (19.4%), 99,770,000 kg (12.9%), and 62,620,000 kg (8.1%), respectively. These four provinces accounted for 86.7% of China’s total aquaculture production. Therefore, in this study, the mitten crab (*Eriocheir sinensis*) samples were collected from these four regions for follow-up analysis and research, and the data are representative.

The analysis results, including the limit of detection (LOD), limit of quantification (LOQ), and relative standard deviation (RSD) of ICP-MS are shown in Table 1. The value in the linear range represents the concentration of the solution on the IC-PMS; the LOD and LOQ represent the sample concentration.

The levels of trace elements in the edible parts of Chinese mitten crab

Table 2 represents the median value (25–75%) of crab samples. The chemical analysis results of four toxic and harmful heavy metal elements (Pb, Cd, As, and Cr) compared with the crab samples from other three provinces (Jiangsu, Hubei, and Anhui) showed the significantly higher difference in the Pb trace element content of Liaoning province crabs at the level of 95% (P < 0.05). Compared with the crab samples from Hubei or Anhui, samples collected from Jiangsu and Liaoning

Table 1. Summary of analyte masses, analytical conditions, LOD, LOQ, and RSD of the studied elements.

Element	Internal standard	Analysis mode	Linear range ($\mu\text{g L}^{-1}$)	Correlation coefficient	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	RSD (%)
^7Li	^6Li	Normal	0–5	0.9999	0.0010	0.0033	5.51
^9Be	^6Li	Normal	0–5	0.9991	0.0010	0.0033	4.84
^{27}Al	^{72}Ge	He	0–500	0.9998	0.0050	0.0165	6.99
^{51}V	^{72}Ge	He	0–5	0.9998	0.0003	0.0010	3.18
^{53}Cr	^{72}Ge	He	0–5	0.9997	0.0010	0.0033	3.27
^{55}Mn	^{72}Ge	He	0–100	0.9999	0.0004	0.0013	2.61
^{56}Fe	^{72}Ge	He	0–500	0.9999	0.0520	0.1972	3.30
^{59}Co	^{103}Rh	He	0–5	0.9987	0.0002	0.0052	2.84
^{60}Ni	^{103}Rh	He	0–5	0.9998	0.0054	0.0179	2.62
^{63}Cu	^{103}Rh	He	0–500	0.9997	0.0034	0.0114	1.41
^{64}Zn	^{103}Rh	He	0–500	1.0000	0.0458	0.1528	6.70
^{71}Ga	^{103}Rh	He	0–5	0.9989	0.0003	0.0008	2.80
^{75}As	^{103}Rh	He	0–50	1.0000	0.0022	0.0073	2.46
^{82}Se	^{103}Rh	He	0–50	0.9999	0.0206	0.0686	5.29
^{85}Rb	^{103}Rh	He	0–50	1.0000	0.0008	0.0027	1.07
^{88}Sr	^{103}Rh	He	0–500	0.9997	0.0003	0.0011	2.42
^{98}Mo	^{103}Rh	He	0–5	0.9998	0.0011	0.0036	0.84
^{107}Ag	^{103}Rh	He	0–5	1.0000	0.0022	0.0074	0.73
^{111}Cd	^{103}Rh	He	0–5	0.9998	0.0001	0.0003	4.08
^{133}Cs	^{103}Rh	He	0–5	0.9998	0.0003	0.0011	1.43
^{137}Ba	^{103}Rh	He	0–500	1.0000	0.0101	0.0337	0.46
^{205}Tl	^{209}Bi	He	0–5	0.9994	0.0003	0.0010	1.59
^{208}Pb	^{209}Bi	He	0–5	0.9996	0.0044	0.0147	4.97
^{238}U	^{209}Bi	He	0–5	1.0000	0.0009	0.0029	2.65

provinces had a significant difference in the Cd trace element content at the level of 95% ($P < 0.05$). However, samples from Jiangsu and Liaoning showed Cd trace element content by 95%, and no significant differences ($P > 0.05$) were observed between both the groups. In addition, As trace element content from Anhui and Liaoning provinces showed significant differences at the level of 95% ($P < 0.05$) as compared to the samples collected from Jiangsu or Hubei provinces. However, no significant differences were observed in As trace element content between the river crab samples and the samples collected from Anhui and Liaoning provinces at 95% level ($P > 0.05$). Crab samples collected from Hubei and Anhui showed significant differences in the Cr trace element content as compared to the samples collected from Jiangsu or Liaoning provinces at 95% level ($P < 0.05$). However, compared with the crab samples from Hubei and Anhui provinces, there was no significant difference in the content of Cr trace elements in river crabs at the level of 95% ($P > 0.05$). The content distribution of 24 trace elements in the Chinese mitten crabs has been summarized in Table 2.

Factor pollution index method to evaluate the risk of Chinese mitten crab

Chinese national standard of food, GB 2762-2017 (2018), has established the threshold acceptable limit of Pb, Cd, As (inorganic), and Cr as 0.5 mg kg^{-1} , 0.5 mg kg^{-1} , 0.1 mg kg^{-1} , and 2.0 mg kg^{-1} , respectively. The standards from the ministry of agriculture of China, NY5073-2006 (NY 5073-2006, 2016) have decided the maximum permissible limit of As (inorganic arsenic), Pb, Cd, and Cu as 0.5 mg kg^{-1} , 0.5 mg kg^{-1} , 0.5 mg kg^{-1} , and 50 mg kg^{-1} , respectively. Final recommendations have been made by the Chinese legislation on setting the maximum permissible limit of Pb, Cd, As (inorganic), Cr, and Cu as 0.5 mg kg^{-1} , 0.5 mg kg^{-1} , 0.1 mg kg^{-1} , 2.0 mg kg^{-1} , and 50 mg kg^{-1} , respectively. The factor pollution index is shown in Table 3.

The average national P_{Cr} value has been set to less than 0.2 as a normal background level. In all the tested crab samples, the P_{Cr} was less than 0.2 with no pollution of Cr to crab samples. Although no pollution of Cu to crab samples was found, special attention should be paid to

Table 2. The content distribution of 24 trace elements in the Chinese mitten crabs collected from four major crab-producing regions.

24 residual heavy metals	The whole area Median mg kg ⁻¹ [IQR = 25–75%]	Jiangsu province median mg kg ⁻¹ [IQR = 25–75%]	Hubei province median mg kg ⁻¹ [IQR = 25–75%]	Anhui province median mg kg ⁻¹ [IQR = 25–75%]	Liaoning province median mg kg ⁻¹ [IQR = 25–75%]
Li	ND [ND-0.003]	0.003 [ND -0.018]	ND –	ND –	0.006 [ND-0.021]
Be	ND –	ND –	ND –	ND –	ND –
Al	2.246 [0.564–18.219]	11.780 [7.955–15.529]	0.234 [0.129–0.596]	1.195 [0.916–1.628]	38.795 [15.355–68.695]
V	0.003 [ND -0.040]	0.029 [0.015–0.035]	ND –	ND [ND-0.001]	0.081 [0.035–0.171]
Cr	0.011 [0.001–0.033]	0.022 [0.019–0.026]	ND [ND -0.003]	0.002 [ND -0.006]	0.057 [0.026–0.093]
Mn	1.975 [0.563–6.070]	3.209 [2.069–6.048]	0.301 [0.147–0.557]	1.475 [0.705–2.235]	10.541 [5.252–24.064]
Fe	25.841 [7.270–78.729]	80.947 [61.381–98.333]	3.076 [1.920–4.126]	18.869 [14.022–25.614]	97.115 [50.371–130.902]
Co	0.016 [0.006–0.044]	0.031 [0.018–0.041]	0.004 [0.003–0.006]	0.011 [0.008–0.016]	0.076 [0.050–0.099]
Ni	0.035 [ND -0.180]	0.131 [0.088–0.172]	ND –	0.007 [ND-0.027]	0.288 [0.239–0.393]
Cu	5.800 [3.650–11.586]	7.578 [6.228–10.079]	4.133 [3.276–6.206]	3.701 [3.051–4.639]	13.271 [11.022–15.386]
Zn	19.755 [14.371–28.137]	20.056 [17.010–25.313]	20.413 [17.951–24.811]	13.154 [11.232–14.380]	33.979 [26.560–39.021]
Ga	ND –	ND –	ND –	ND –	0.006 [ND-0.023]
As	0.530 [0.260–0.740]	0.696 [0.532–0.904]	0.226 [0.157–0.288]	0.635 [0.302–0.809]	0.577 [0.470–0.706]
Se	0.340 [0.275–0.420]	0.370 [0.296–0.420]	0.261 [0.233–0.290]	0.347 [0.293–0.403]	0.482 [0.379–0.522]
Rb	0.834 [0.686–1.078]	0.673 [0.587–0.831]	1.053 [0.719–1.279]	0.994 [0.768–1.241]	0.810 [0.700–0.881]
Sr	9.282 [5.483–15.381]	6.546 [4.503–14.413]	5.210 [3.519–6.722]	11.939 [7.901–19.547]	14.918 [12.293–17.782]
Mo	0.043 [0.018–0.081]	0.042 [0.032–0.051]	0.014 [0.012–0.016]	0.043 [0.037–0.056]	0.096 [0.081–0.110]
Ag	0.018 [0.011–0.026]	0.011 [0.006–0.014]	0.011 [0.008–0.015]	0.022 [0.014–0.028]	0.027 [0.024–0.031]
Cd	0.081 [0.016–0.169]	0.081 [0.052–0.162]	0.011 [0.007–0.014]	0.209 [0.167–0.262]	0.092 [0.075–0.117]
Cs	0.005 [0.002–0.009]	0 [ND -0.001]	0.004 [0.002–0.006]	0.009 [0.006–0.012]	0.005 [0.002–0.011]
Ba	2.756 [1.641–5.628]	2.322 [1.408–5.387]	1.863 [1.414–2.628]	9.872 [4.870–21.305]	2.476 [1.737–3.557]
Tl	ND –	ND –	ND –	ND –	ND –
Pb	ND [ND -0.003]	ND –	ND –	0.001 [ND -0.006]	0.009 [ND -0.037]
U	ND [ND -0.001]	ND –	ND –	0.023 [ND -0.0358]	ND –

Table 3. Factor pollution index of Chinese mitten crabs in major crab-producing areas of China.

	The whole area	Jiangsu province	Hubei province	Anhui province	Liaoning province
Cr	0.013	0.015	0.003	0.003	0.034
Cu	0.155	0.165	0.121	0.085	0.258
As	0.553	0.783	0.352	0.597	0.573
Cd	0.228	0.225	0.022	0.492	0.204
Pb	0.014	0.002	0.000	0.009	0.045

the crab samples collected from Liaoning province as they showed a mild P_{Cr} value of 0.258. The pollution level of P_{Cr} among 27 samples (out of 35 samples) collected from the Liaoning province was more than 0.2 with a pollution rate of 77.1%; however, the maximum P_{Cu} limit is set as 0.569, which is considered as mild pollution to the crab samples. As the total trace element contents were analyzed, we adopted the conversion coefficient method, where 10% value was selected as the conversion coefficient, and the national P_{As} of 0.553 was as an indication of mild pollution, although each province had a P_{As} value of more than 0.2 in the following manner, Jiangsu > Anhui > Liaoning > Hubei, but was found to be significant at the level of $P > 1.0$ for many places in Jiangsu and Anhui, indicating relatively serious concern of As pollution. Cd is considered at the light pollution level in China in the following manner: Anhui > Jiangsu > Liaoning; interestingly, no Cd pollution was found in the crab samples collected from the Hubei area. In addition, considering the national average Pb level, no Pb pollution was found in all the crab samples collected from all the provinces and cities, and each sample had more than 0.2. Pb level.

Taking a comprehensive look at these three pollution elements, the pollution ranking of Chinese mitten crab provinces was found in the following order: Liaoning > Jiangsu > Anhui > Hubei. Liaoning province showed higher heavy metal pollution which might be due to high background heavy metal content accumulation in Liaoning as compared to other provinces as an old industrial base of Northeastern China. The relatively low level of heavy metal contamination in Hubei province could be due to the Yangtze river flowing through Hubei to Anhui and Jiangsu, although there was no significant difference in the pollution level of the crab samples collected from Jiangsu and Anhui provinces.

Dietary risk assessment and human tolerance assessment

In this study, five heavy metal trace elements (Cu, Zn, Pb, Cd, and Cr) were selected out of 24 trace elements for human tolerance assessment and were evaluated for the actual weekly intake of adults (AWI) based on their

contents in river crabs and for weekly consumption report of aquatic products by Chinese residents. Assessment of these elements was compared using provisionally tolerable weekly intake (PTWI) parameter of adults to evaluate their edible safety level following the formula:

$$AWI (\%) = C_i \times WC \quad (\text{PTWI} = \text{weekly tolerable intake per unit weight} \times \text{adult weight}).$$

Where C_i represents the content of a certain trace element (mg kg^{-1}) in river crabs, and the average value was taken to calculate the average weekly intake of certain trace elements. WC represents per capita weekly consumption of aquatic products. According to the 2015 Chinese Resident Nutrition Reports on chronic disease conditions, it was calculated as $0.168 \text{ kg week}^{-1}$. Adult weight was calculated at 60 kg. Food safety was evaluated as the percentage of AWI to PTWI. The higher proportion of AWI and PTWI was considered as the lower food safety level. The World Health Organization (WHO) and the Food Additives Joint Expert Committee (JECFA) under the United Nations' Food and Agriculture Organization (FAO) have established the tolerable intake of pollutants per unit weight per week as the basis of food safety evaluation (WHO, 2004). Thus, the tolerable weekly intake per unit weight of Zn, Cu, Pb, Cd, and Cr was established as 7, 3.5, 0.025, 0.007, and $0.0233 \text{ mg kg}^{-1}$, respectively. According to the 2015 report of Chinese legislation on the status of nutrition and chronic diseases of Chinese residents, the limit of these five trace elements was calculated as $0.168 \text{ kg week}^{-1}$. In this way, the PTWI and AWI values, etc., of these five metals were calculated, and the relevant results are shown in Table 4.

The actual weekly intake (AWI) average values of five heavy metals (AWI average) of adults across the country accounted for 0.086 to 4.57% of PTWI, and the health risk of Cr ranked in the order of Jiangsu > Liaoning > Hubei > Anhui, followed by Liaoning > Jiangsu > Hubei > Anhui for Cu, Liaoning > Hubei > Jiangsu > Anhui for Zn, and Liaoning > Anhui > Jiangsu > Hubei for Pb. These findings revealed that the contents of heavy metals such as Cu, Zn, and Pb in the rice field cultured river crabs as a major production area of Northeast China

Table 4. Crab weekly intake of trace elements by adults (AWI) and their percentage of provisionally tolerable weekly intake (PTWI).

Parameter		Cr	Cu	Zn	Cd	Pb
China	PTWI mg	1.398	210.000	420.000	0.420	1.500
	Average mg kg ⁻¹	0.026	7.773	22.989	0.114	0.007
	Average of AWI mg	0.004	1.306	3.862	0.019	0.001
	Average of AWI/PTWI %	0.316	0.622	0.920	4.570	0.080
	Maximum content mg kg ⁻¹	0.221	28.456	58.467	1.018	0.104
	AWI Maximum content mg	0.037	4.781	9.822	0.171	0.017
	Maximum content/PTWI %	2.656	2.276	2.339	40.720	1.165
Jiangsu province	Average mg kg ⁻¹	0.029	8.262	21.282	0.112	0.001
	Average of AWI mg	0.005	1.388	3.575	0.019	–
	Average of AWI/PTWI %	0.351	0.661	0.851	4.493	0.013
	Maximum content mg kg ⁻¹	0.147	14.058	41.938	0.388	0.017
	AWI Maximum content mg	0.025	2.362	7.046	0.065	0.003
	Maximum content/PTWI %	1.767	1.125	1.678	15.520	0.190
	Hubei province	Average mg kg ⁻¹	0.007	6.062	24.718	0.011
Average of AWI mg		0.001	1.018	4.153	0.002	–
Average of AWI/PTWI %		0.078	0.485	0.989	0.436	–
Maximum content mg kg ⁻¹		0.048	20.689	58.467	0.022	–
AWI Maximum content mg		0.008	3.476	9.822	0.004	–
Maximum content/PTWI %		0.577	1.655	2.339	0.880	–
Anhui province		Average mg kg ⁻¹	0.006	4.244	12.818	0.246
	Average of AWI mg	0.001	0.713	2.153	0.041	0.001
	Average of AWI/PTWI %	0.071	0.340	0.513	9.839	0.051
	Maximum content mg kg ⁻¹	0.048	10.361	16.512	1.018	0.045
	AWI Maximum content mg	0.008	1.741	2.774	0.171	0.008
	Maximum content/PTWI %	0.577	0.829	0.660	40.720	0.504
	Liaoning province	Average mg kg ⁻¹	0.067	12.906	32.402	0.102
Average of AWI mg		0.011	2.168	5.444	0.017	0.004
Average of AWI/PTWI %		0.807	1.032	1.296	4.080	0.250
Maximum content mg kg ⁻¹		0.221	28.456	55.869	0.208	0.104
AWI Maximum content mg		0.037	4.781	9.386	0.035	0.017
Maximum content/PTWI %		2.656	2.276	2.235	8.320	1.165

have the higher eating risk of mitten crabs as compared to the crab samples collected from the other three places. Severe concerns were observed on Cd pollution of crab samples collected from Jiangsu and Anhui provinces. Among the five heavy metals analyzed, Cd has the highest food safety risk; hence, more attention should be paid in the future to avoid the accumulation of such water contaminants to prolong the food safety of mitten crabs. The contents of these five heavy metals in the crab samples collected from the Hubei province were relatively safe compared to other production areas due to the water flow of the Yangtze river, avoiding the accumulation of heavy metal trace elements in the crab samples from the Hubei province. The AWI and PTWI values in the crab samples collected from different areas in China are shown in Table 4.

The target hazard quotients and hazard index analysis

The target hazard quotient (THQ) proposed by the United States Environmental Protection Agency (USEPA) (USEPA, 2011) was used to assess the risk of a single trace element in river crabs in aspects of human health, and calculated by the formula:

$$EDI = [IR \times C/BW_a] \times 10^{-3} \text{ and } THQ = EDI/RfD$$

Where EDI represents the daily intake of human trace elements ($\mu\text{g kg}^{-1} \text{d}^{-1}$), and IR is the average daily intake of aquatic products (g d^{-1} , wet mass). According to the Chinese Resident Nutrition and Chronic Disease status report (Year 2015), this study took 23.7 g d^{-1} EDI, where

C is the content of trace elements in aquatic products ($\mu\text{g kg}^{-1}$), BWA is the average adult weight, calculated as 60 kg, and RfD is the reference dose ($\mu\text{g kg}^{-1} \text{d}^{-1}$). If THQ is <1 , there is no significant health risk; however, there is a significant health risk to the food intake when THQ reaches >1 . A high THQ value represents a greater corresponding risk.

Five heavy metal trace elements (Cu, Zn, Pb, Cd, and Cr) and three harmful trace elements (Ba, U, and Ag), which have been widely reported in the literature, were selected from the detected 18 trace elements for quantitative health risk assessment. The reference doses (RfD) of Cr, Zn, Cd, Ba, U, and Ag were retrieved through the official website of USEPA; RfD values were not retrieved for Cu, and the reported values in the literature were used. Pb reference data were used from the database of the European Food Safety Authority (EFSA). The daily intake of trace elements (EDI) and target hazard quotient (THQ) were calculated based on the median value of each trace element obtained from the river crabs. As a result, the national standard level of EDI and the THQ value of each trace element in the crab samples collected from each province were less than 1. The results are depicted in Table 5.

Where THQ_i is the target hazard quotient of trace element i . The national HI value of compound pollutants is 0.1221 at the average intake level, which was found to be 0.1387, 0.0766, 0.1609, and 0.2215 for all the samples collected from the four provinces, namely, Jiangsu, Hubei, Anhui, and Liaoning, respectively. All the samples represented less than one HI value, indicating that there was no obvious health risk in the intake of crab samples containing five heavy metals and three harmful trace elements analyzed. However, the HI values of crab samples collected from Anhui and Liaoning provinces were higher than the national standard average value, requiring certain attention.

Further analysis showed that the contribution rate (%) of different elements to HI of the compound pollutant was quite different. The THQ values of single trace element analyzed in the Chinese mitten crabs collected from four respective provinces were as follows. $\text{THQ}_{\text{China}}$, Cu (46.9%) $>$ Cd (26.2%) $>$ Zn (21.3%) $>$ Ba (4.4%) $>$ Ag (1.1%) $>$ U = Pb = Cr; $\text{THQ}_{\text{Jiangsu}}$, Cu (53.9%) $>$ Cd (23.1%) $>$ Zn (19.0%) $>$ Ba (3.3%) $>$ Ag (0.6%) $>$ U = Pb = Cr; $\text{THQ}_{\text{Hubei}}$, Cu (53.3%) $>$ Zn (35.1%) $>$ Cd (5.6%) $>$ Ba (4.8%) $>$ Ag (1.2%) $>$ U = Pb = Cr; $\text{THQ}_{\text{Anhui}}$, Cd (51.4%) $>$ Cu (22.7%) $>$ Ba (12.1%) $>$ Zn (10.8%) $>$ U (1.9%) $>$ Ag (1.1%) $>$ Pb (0.1%) $>$ Cr; and $\text{THQ}_{\text{Liaoning}}$, Cu (59.2%) $>$ Zn (20.2%) $>$ Cd (16.4%) $>$ Ba (2.2%) $>$ Pb (1.1%) $>$ Ag (0.9%) $>$ U = Cr.

It can be seen from the parentheses that the total contribution rate of Cu, Cd, and Zn was reached more than 80%. Although Cu and Zn are essential trace element heavy metals for living organisms, they may also cause harmful effects to the human body if ingested in excess amounts. In view of the fact that the calculated EDI of these two elements was much lower than their reference doses, and $\text{THQ} < 1$, both Cu and Zn trace elements were considered as the main risk elements. Cd is not an essential trace element for the human body and is considered a harmful element with a relatively high contribution rate to HI. Therefore, it is considered the main risk element among the 24 elements tested. Although its THQ is <1 , it can also be included in the focus of monitoring the health complications in the future.

Conclusion

In this study, Chinese mitten crab samples were collected from four major crab-producing areas accounting for 86.7% of China's total aquaculture production. Through the analysis of the heavy metal content in the Chinese mitten crab samples, it is believed that under normal

Table 5. Estimated daily intake (EDI) and target hazard quotient (THQ) of trace elements during crab consumption in China.

Element	Reference dose RfD $\mu\text{g kg}^{-1} \text{d}^{-1}$	Median		Jiangsu province		Hubei province		Anhui province		Liaoning province	
		EDI	THQ	EDI	THQ	EDI	THQ	EDI	THQ	EDI	THQ
Cr	1500	0.0043	–	0.0087	–	–	–	0.0008	–	0.0225	–
Cu	40	2.2910	0.0573	2.9933	0.0748	1.6325	0.0408	1.4619	0.0365	5.2420	0.1311
Zn	300	7.8032	0.0260	7.9221	0.0264	8.0633	0.0269	5.1958	0.0173	13.4217	0.0447
Cd	1	0.0320	0.0320	0.0320	0.0320	0.0043	0.0043	0.0826	0.0826	0.0363	0.0363
Ba	200	1.0886	0.0054	0.9172	0.0046	0.7359	0.0037	3.8996	0.0195	0.9780	0.0049
Pb	1.5	–	–	–	–	–	–	0.0002	0.0001	0.0036	0.0024
U	3	–	–	–	–	–	–	0.0091	0.0030	–	–
Ag	5	0.0071	0.0014	0.0043	0.0009	0.0045	0.0009	0.0089	0.0018	0.0107	0.0021

The hazard index (HI) was used to evaluate the health risks of compound pollutants and calculated by the formula: $\text{HI} = \sum_{i=1}^n \text{THQ}_i$.

eating conditions, these crab samples have no excessive eating health risks, and can be consumed regularly for nutritional benefits. However, some of the data obtained are worthy of in-depth analysis. For example, among Cr, Cu, Zn, Cd, and Pb metals, Cd has the highest food safety risk, which is worth noting. Moreover, a certain percentage of arsenic content is regarded as the inorganic arsenic content, which is highly toxic than the organic form of arsenic. In the future, there is a need to analyze the organic and inorganic forms of heavy metals such as Cd and As, and accumulate more reliable data to assess the dietary risks of mitten crabs in China. Overall findings conclude and reinforce the suggestion of safe eating practices of Chinese mitten crab in China.

Author's Contributions

Lei Gao wrote the article; Xiaoli Huang analyzed the data; Peng Wang and Zhongxiang Chen guided the experiments; Qirui Hao and Shuyan Bai collected the samples; Shizhan Tang and Chenhui Li did the ICPMS analysis; and Dongli Qin performed ICPMS analysis, data analysis, and guided the experiments. Besides, the authors would like to thank Shiyanjia Lab (www.shiyanjia.com) for the language editing service.

Funding

This study was funded by the National Key Research and Development Program of China (2020YFD0900301), the National Natural Science Foundation of China (32002445, 32102855), Natural Science Foundation of Heilongjiang Province (YQ2021C039), and the China Postdoctoral Science Foundation funded project (2021MD703899).

Data Availability Statement

Data available on request due to restrictions.

Conflicts of Interest

The authors declare no conflict of interest.

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