EVALUATION OF HEAVY METAL POLLUTION AND RISK ASSESSMENT OF DIETARY INTAKE OF POLLUTANTS IN CHINESE MITTEN CRAB (*ERIOCHEIR SINENSIS*) IN MAIN AQUACULTURE AREAS OF NORTHEAST CHINA

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Abstract. A systematic survey of heavy metal (Pb, Cd, Cr, As and Hg) concentrations in pond water, sediment and products of Chinese mitten crab (*Eriocheir sinensis*) aquaculture areas was carried out to assess their potential health risks to local people. The samples were all collected in the Liaoning Province, in the northeast of China. According to the results, the studied aquatic environment is not severely affected by heavy metal pollution at this time. As a result, local dietary exposure to these pollutants is still below the JECFA recommended values.
Keywords: *Crustacea, heavy metal, single factor pollution index method(SFPI), Nemero pollution index method (NPI), dietary exposure assessment*

Introduction

With the rapid development of industrialization and urbanization, the discharge of waste gas, waste water and waste residue containing heavy metals has greatly increased (Bhuiyan et al., 2011; Khillare et al., 2012; Rahman et al., 2014). Increasing attention has been paid to the human health risks caused by the release of these contaminants. Thus, it is important to assess the heavy metal pollution situation. The presence of heavy metal pollution in aquatic products has also raised more and more public health concerns in the last decades. In China, studies of the aquatic product heavy metal pollution situation have mainly focused on fish and shrimp (Yang, C.C., et al, 2013; Gu, 2012; Liu, et al., 2013) and shellfish (Tan et al., 2012; Sun et al., 2010; Wang et al., 2012; Conti et al., 2011; He, 1996). At this time, there is no research on toxic heavy metal pollution of Eriocheir sinensis crabs in this region. This study on the heavy metal pollution of crabs such as Eriocheir sinensis is the first such domestic study at present.

The Chinese mitten river crab (*Eriocheir sinensis* H. Milne-Edwards, 1853) is an arthropod crustacean, Order Decapoda of the Superfamily Grapsidae. It is an important aquaculture species in China. The natural breeding habitat of the river crabs is in Bohai bay, Liaoning Province, which is an appropriate habitat for breeding for large scale production. In 2015, crab farming in Bohai bay occupied 80000 hm^2 (2008), making it a major region for crab breeding in northeast China.

Based on its commercial importance, the evaluation of the river crab breeding environment, heavy metal pollution levels and product safety are vital. A better understanding of the crab breeding habitat will help the aquaculture industry to provide early warnings for supervision of food and health aspects. This research will provide

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knowledge for the establishment of quality control standards for the river crab commercial-scale-production, providing scientific guidance for the safety of river crab consumption as well as advice to the river crab breeding industry.

Materials and methods

Sample collection

The present study pilot is located in the main river crab aquaculture areas of Liaoning Province with a longitude range of 121°31'～ 122°28' and a latitude of 40°40'～ 41°27' (see *Figure 1*). Three categories of samples were collected: river water, sediment and adult crabs. These samples were collected from 9 breeding sites from September to October 2015. Each sample point was characterized by two parallel samples.

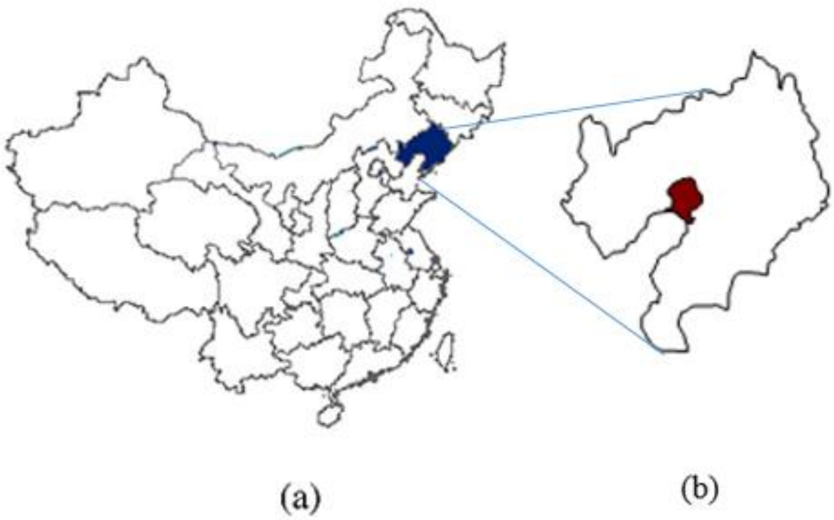

Figure 1. *(a) The mainland of China, and the location of Liaoning Province (blue area); (b)Sampling location of the study area in Liaoning Province (red area)*

Pond water

Clean polyethylene bottles where used to collect and store the water samples. Immediately after the samples were collected, 2 ml/liter nitric acid was added to guarantee the acidification of a XY molarity. The collection, storage, transportation and treatment of the water samples were performed according to the standards set forth in GB 12997-91 and GB 12998-91.

Sediment

Upper layer (0 ~ 10 cm) sediment samples were collected, stored and transported to the lab for analysis according to GB 17378.3-2007 standards. Gravel and plant debris were removed. The sediment samples were sealed and stored in a -18°C freezer. The samples were dried at 60°C, grinded, sieved through an 80 mesh sieve and stored in airtight bags after homogenization.

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Adult crab

The crab samples were washed and cleaned using a plastic knife and tweezers. The edible parts of the muscle tissues and ovaries were homogenized and stored in double layer polyethylene plastic bags in a -18℃ freezer.

Data determination

Concentrations of Pb, Cd, Cr, As and Hg were determined by graphite furnace atomic absorption spectrophotometer atomic fluorescence according to the National Standards NY 5361-2010 and GB 2762-2012. Pb, Cd and Cr concentrations were analyzed by graphite furnace atomic absorption spectrophotometry, while As and Hg concentrations were determined by atomic fluorescence spectrophotometry. The concentration of each element was expressed based on the quality control where the relative standard deviation was less than 10% of the concentration averages of 2 test samples. Statistical data analyses were performed using SPSS 16.0 software, and the standard of significant difference was set to P=0.05.

Criteria of evaluation and methods

Pollution degree

The single factor pollution index (SFPI) and Nemerow pollution index (NPI) were determined by the method set forth by Pang et al. (2015) (Zhu et al., 2014) to evaluate heavy metal pollution status and pollution risk. The SFPI was used to evaluate the pollution status of a single heavy metal element. It was calculated as follows:

$$p_i = \frac{C_i}{S_i} \quad \text{(Eq. 1)}$$

where P_i is the heavy metal element *i* pollution index, C_i is the heavy metal element *i* content in the specimen (pond water, sediment and crab tissues), and S_i is the heavy metal element *i* evaluation standard (*Table 1*). The Pi value indicates the degree of pollution; its size is directly proportional to the pollution degree. However, the NPI takes into account both the average pollution status of a single heavy metal element and the degree of heavy metal pollution. It was calculated as follows:

$$p = \sqrt{\frac{(P_{i\max})^2 + (P_{i\,ave})^2}{2}} \quad \text{(Eq. 2)}$$

where P is the comprehensive pollution index of Nemero, $P_{i\ max}$ is the maximum pollutant value of all investigated single factor pollution indices, and $P_{i\ ave}$ is the average pollutant value of all single factor pollution indices.

According to the pollution index calculations, the level of heavy metal pollution is divided into grades to evaluate the overall degree of pollution (*Table 2*).

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Table 1. *Evaluation standard of heavy metals (NY 5361-2010; GB 2762-2012)*

Sample species	Heavy metals				
	Pb	Cd	Cr	As	Hg
Pond water (mg L^{-1})	0.05	0.005	0.1	0.05	0.0001
Sediment (dry weight) (mg kg^{-1})	60	0.5	80	20	0.2
Crab Product (mg kg^{-1})	0.5	0.5	2.0	0.5[a]	0.5[b]

Note that (a) and (b) represent methyl mercury and inorganic arsenic, respectively; the same holds for Tables 3, 4, 5 and 6.

Table 2. *Classification standard of heavy metal pollution index (Zhu et al., 2014)*

Level	SFPI	Pollution level	NPI	Pollution level
1	$Pi \le 1$	clean	$P \le 0.7$	safety
2	$1 < Pi \le 2$	slight	$0.7 < P \le 1$	warning
3	$2 < Pi \le 3$	moderate	$1 < P \le 2$	slight
4	$Pi > 3$	heavy	$2 < P \le 3$	moderate
5			$P > 3$	heavy

Dietary exposure assessment method

In this study, the evaluations complied with the declarations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) were determined from the crab tissue (mg kg-1) to evaluate the safety of crab dietary intake for tenants of Liaoning province (Wang and Wang, 2014). Thus:

$$PTWI/PTMI = \frac{\bar{x} \cdot L \cdot D}{M} \quad \text{(Eq. 3)}$$

where *PTWI* is the provisional tolerable weekly intake (μg kg^{-1}), and D =7days; *PTMI* is the provisional tolerable monthly intake (μg kg^{-1}), and D=30days; $\bar{x}$ is the concentration (mg kg^{-1}); L is the daily intake of food (g/person/day); and M is the adult body weight (set M=60kg). According to the dietary survey data for the Liaoning province adults, the average per capita fish and shrimp consumption is 44.3 g/day (Zhu et al., 2014).

Result and discussion

Accuracy and precision

According to the standard methods (NY 5361-2010; GB 2762-2012), the spiked recoveries ranged from 108.9% ~ 91.3%, and the relative standard deviation (RSD, n=6) was between 7.4% and 4.2% (*Table 3*).

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***Table 3.** Average recoveries and RSD (n=6)*

Sample species	Quality Control	Heavy metals				
		Pb	Cd	Cr	As	Hg
Pond water	Addition levels (mg L^{-1})	0.05	0.005	0.1	0.05	0.0001
	Recovery (%)	91.3	96.4	108.9	95.1	94.1
	RSD (%)	4.3	4.2	5.1	6.4	6.3
Sediment	Addition levels (dry weight) (mg kg^{-1})	2.0	0.5	5.0	3.0	0.2
	Recovery (%)	92.3	103.1	104.3	93.5	92.1
	RSD (%)	4.2	4.7	4.9	6.6	7.4
Crab Product	Addition levels (mg/ kg^{-1})	0.5	0.5	2.0	0.5[a]	0.5[c]
	Recovery (%)	96.3	98.2	96.1	94.2	93.1
	RSD (%)	4.3	4.2	4.7	6.4	5.3

Note: (c) represents total mercury; the same is true for Tables 4, 5 and 6.

Assessment of heavy metal pollution

Tables 4 and *5* show the average concentrations and pollution evaluations, respectively, of heavy metals in river water, sediment and crab products. According to these results, all of the samples were 100% suitable for human consumption (*Table 4*). There was significant variation of the heavy metal concentrations in all samples, even within the same kind. The highest heavy metal concentrations were for Cr, followed by Cd, Pb and As. The lowest concentrations for Hg were in the river water and sediment samples. In crabs, the concentration of As was the highest, followed by Cr, Pb, Cd and Hg. These findings are consistent with the general law of heavy metal content in fish and shellfish (Gu and Zhao, 2012). It can be deduced that bioaccumulation of As and Hg in crabs was relatively high, although their concentrations were not overly high in pond water and sediment.

***Table 4.** Concentrations of heavy metal ($\bar{x} \pm s$)*

Sample species	Sample size	Heavy metals				
		Pb	Cd	Cr	As	Hg
Pond water (mg L^{-1})	18	0.020±0.013	0.0030±0.0019	0.043±0.004	0.0115±0.0072	0.000021±0.000004
Sediment (dry weight) (mg kg^{-1})	18	22.2±2.1	0.21±0.06	27.2±3.1	6.2±0.5	0.044±0.021
Crab Product (mg kg^{-1})	36	0.06±0.01	0.05±0.01	0.12±0.03	0.22±0.07[a]	0.05±0.01[c]

Note that (*s*) represents standard deviation.

The values of the single factor pollution index (*Pi*) ranged between 0.06 ~ 0.60 in pond water, sediment and crabs (*Table 5*). The *Pi* values for the river water and sediment samples were the highest for Cd (0.60 and 0.42 respectively), while the *Pi* values for As and Hg in the river crabs were both 0.44. The values of the Nemero pollution index *P* ranged from 0.50 to 0.35 in pond water, sediment and crabs. These values were significantly lower (0.7) than the classification standard of the heavy metal pollution index (*Table 2*), which suggests that the crab breeding environment of the

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river is not subject to toxic heavy metal pollution. Therefore, this river is an excellent breeding area for river crabs.

Table 5. *Evaluation of pollution index of heavy metals*

Sample species	*Pi*					*P*	Pollution level
	Pb	Cd	Cr	As	Hg		
Pond water	0.40	0.60	0.43	0.23	0.21	0.50(n=5)	safety
Sediment	0.37	0.42	0.34	0.31	0.22	0.38(n=5)	safety
Crab Product	0.12	0.10	0.06	0.44a	0.44c	0.35(n=5)	safety

Edible safety evaluation

Compared with the PTWI/PTMI recommended by JECFA, the dietary intake of toxic heavy metal contaminated crabs was evaluated (*Table 6*). The *PTWI/PTMI* intakes of heavy metals in river crab were significantly below 10% of the values recommended by JECFA. The *PTWI/PTMI* intakes values recommended by JECFA were the highest for Cr, followed by As and Hg, and the lowest were for Pb and Cd.

Table 6. *Estimated weekly intakes of heavy metal (Li et al., 2010)*

Heavy metals	Concentrations (mg kg^{-1})	*PTWI/PTMI* (μg kg^{-1})	JECFA recommended values(μg kg^{-1})	Proportion in JECFA recommended value(%)
Pb	0.06	0.31	25	1.2
Cd	0.05	1.11	25*	4.4 *
Cr	0.12	0.62	6.7	9.3
As^{a}	0.22	1.14	15	7.6
Hg^{c}	0.05	0.26	5	5.2

Note: The * represents a tentative monthly tolerable intake of cadmium (PTMI) (WHO,2012)

Not all heavy metals are toxic. Some are essential for physiological purposes, but can have adverse health effects at higher concentrations. Other heavy metals are toxic even at relatively low concentrations. Most heavy metal pollution is due to anthropogenic and industrial activities (Al-Musharafi, 2016). Toxic heavy metals interact with protein and DNA, causing mutations and affecting physiological activities and metabolic processes (Al-Musharafi, 2016; Yao et al., 2014). Also, some heavy metal valences create different effects on metabolic activity. For example, Cr is an essential trace element in the human body, but is also a highly toxic metal element. The toxicity of Cr^{6+} is much higher than that of Cr^{3+}. Cr can cause skin reactions and even skin disease. It can also stimulate and/or corrode the respiratory tract, lead to cancer, or induce gene mutation (Teng et al., 2010). Lung cancer caused by chromium compounds is recognized worldwide as a chromium cancer (WHO, 1997). The most toxic metals include As, Hg, Pb and Cd, which are non-essential elements for the human body (Al-Musharafi, 2016). As and Hg take a variety of different forms. Inorganic arsenic is divided into pentavalent arsenic and trivalent arsenic, which are both more toxic than organic arsenic (Zhao et al., 2009).

Some organic forms of heavy metals are less toxic than the related inorganic forms. For example, the toxicity of methyl mercury is much higher than that of inorganic mercury (Jia et al., 2015). In the present study, the content of total mercury was

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significantly lower than the limit value of methyl mercury; therefore, it may not have a significant effect on humans.

In this study, bioaccumulation of toxic metals in crab shells was similar to that in tissue. However, accumulation in the viscera was significantly higher than that in the muscle tissue. Normally, only muscle tissue is consumed, which therefore reduces the risk of heavy metal toxicity from crabs. At present, the proportion of people's consumption of crab is very small (Li et al., 2010). Strict attention should be paid to the reduction of toxic heavy metal pollution. Public awareness is crucial to local residents to reduce crab dietary intake to avoid potential health risks, such as cancer risk.

Conclusion

All of the heavy metal values in the aquaculture water, sediment and river crab products were within the Chinese national standards. The results of this study indicate that the target aquatic environment is currently safe with respect to pollution by the investigated heavy metals (Pb, Cd, Cr, As and Hg). Therefore, the present-day dietary exposure to these pollutants is still well below the JECFA recommended values. However, people should work to reduce the pollution of industrial and domestic waste, which will protect human health and survival and ensure food safety.

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