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Enrichment of trace elements in red swamp crayfish: Influences of region and production method, and human health risk assessment

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ABSTRACT

Understanding the characteristics of trace element enrichment in crayfish from different regions is important for brand protection, quality control, and food safety. Here, we collected farmed and wild crayfish ($n = 660$) from four regions of China to explore differences in trace element enrichment and find an effective method of identifying their origin. The concentrations of most trace elements in tissue were ranked viscera > exoskeleton > muscle. The concentrations of Co, Ag, Rb, Ba, Se, Cs, and Tl were higher in wild crayfish than in farmed ones. A multi-element analysis shows that there are regional differences in elemental composition. Significant regional differences in $\delta^{13}C$ and $\delta^{15}N$ values were also found. The target hazard quotients are all <1, indicating that the normal consumption of crayfish has no significant health risks. This study demonstrates that a combination of multi-element and stable isotope ratio analyses is a practical way to identify the origin of crayfish products.

1. Introduction

The red swamp crayfish, *Procambarus clarkii*, is a freshwater species that originates from north-eastern Mexico and the south-central United States and is currently distributed worldwide (Barbaresi and Gherardi 2000; Gherardi, 2006; Yi et al., 2018). The red swamp crayfish has rapid reproduction and strong tolerance to various environmental conditions, including drought and salinity (Goretti et al., 2016; Piersanti et al., 2018; Dörr et al., 2020; Anandkumar et al. 2020a). The red swamp crayfish is popular with consumers due to its unique taste, low fat content, high nutritional value, and high economic value (Hobbs et al., 1989; Loureiro et al., 2015; Souty-Grosset et al., 2016; Adebiyi et al. 2020). The global production and consumption of freshwater crayfish have increased sharply over the last two decades (FAO, 2018; Rodriguez-Estival et al., 2019). China is now the largest producer of crayfish in the world (FAO, 2018; Xiong et al., 2020). In 2018, the annual production of crayfish in China reached 1.64 million tons and its total exports reached 10,801 tons (MOAC, 2019; China Crayfish Industry Development Report, 2019). The supply of red swamp crayfish in China is very extensive, and is mainly distributed in Hubei, Hunan, Jiangsu, Zhejiang, Anhui, Shanghai, and Shandong Provinces, of which Hubei Province accounts for about half of the total exports (MOAC, 2019).

Red swamp crayfish have the characteristics of rapid bioaccumulation and long residence times of trace elements in their tissues. Hence, they can be used as a biological indicator of heavy metal pollution in freshwater ecosystems (Suárez-Serrano et al., 2010; Kouba et al., 2010; Adebiyi et al. 2020; Anandkumar et al. 2020a, 2020b; Mistri et al., 2020). Crayfish, as a carrier of pollutants, can transfer trace elements to higher trophic levels (Geiger et al., 2005) and harm human health. For example, toxic elements such as mercury (Hg), cadmium (Cd), chromium (Cr), and lead (Pb) can induce a range of cancers, neurotoxicity, renal toxicity, and organ failure, even at very low doses (Jarup, 2003; Satarug et al., 2010; Jomova and Valko, 2011; Fu et al., 2019). Copper (Cu), zinc (Zn), nickel (Ni), manganese (Mn), and other essential trace elements, if excessive, can also be harmful to the human body (Chale, 2002; Bjorklund et al. 2017; Wang et al., 2019). In mining areas, contaminated industrial sites, and some regions where crayfish consumption is popular, the metals in the crayfish may pose a health risk to

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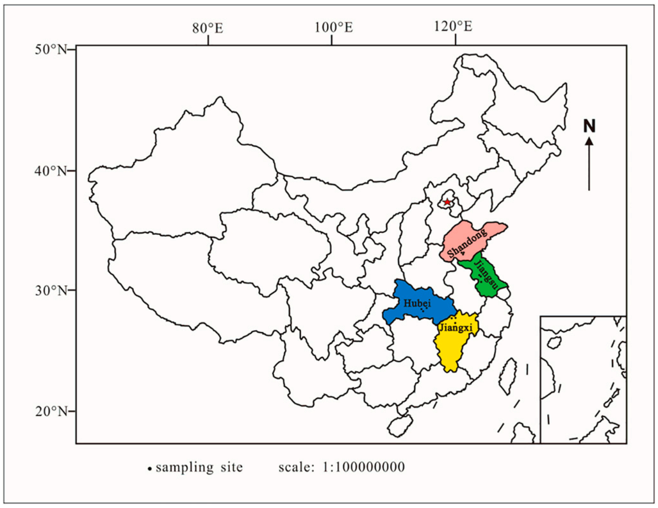

Fig. 1. Sampling sites within four crayfish-producing provinces of China with provinces highlighted. The red star represents Beijing, the capital of China. Different colors in the figure represent different sampling points.

high-rate consumers (Suárez-Serrano et al., 2010; Goretti et al., 2016; Peng et al., 2016). Therefore, the detection of trace elements in crayfish and the determination of the health risks of consuming it is of great significance to human health.

Moreover, the quality of red swamp crayfish differs according to geographical origin, especially in the enrichment of trace elements. Some aquatic products such as crab, shrimp, and fish may be intentionally or unintentionally mislabeled and counterfeited in regard to their country of origin, production method, or certification (Jacquet & Pauly, 2008; Li et al., 2017; Luo et al., 2019). This may seriously damage the rights of consumers and have very negative impacts on marketing and human health. Due to the high commercial value of crayfish, the crayfish industry, which has developed rapidly in recent years, may suffer the same problems. To improve the social and economic benefits of agricultural products, the Chinese government is promoting the "Protection of the Geographical Indication of Products (PGI)" policy to ensure food safety (Luo et al., 2019). More crayfish, such as Nanxian crayfish from Hunan Province, have been registered by the General Administration of Quality Supervision, Inspection, and Quarantine of China (AQSIQ). To avoid confusing certified crayfish products with other similar products, it is important to identify their geographical origin. Multi-element analysis and stable isotope ratio analysis are the two most widely used methods in product traceability. They have been used to determine the geographic origin of food products such as coffee, beef, mutton, crabs, prawns, clams, fish, and sea cucumbers (Franke et al., 2005; Kelly et al., 2005; Turchini et al., 2009; Liu et al., 2014; Ortea and Gallardo, 2015; Li et al., 2016; Zhang et al., 2017; Luo et al., 2019). However, no studies have been published on the traceability of the geographical origin of crayfish based on stable isotope ratio analysis and multi-element analysis.

Thus, the main objectives of the study are to (1) determine the enrichment characteristics of trace elements in Chinese crayfish, (2) clarify the multi-element patterns and stable isotope fingerprints of crayfish for improved discrimination of their geographical origin, and (3) evaluate the potential health risks of trace elements to humans via Chinese crayfish consumption.

2. Materials and methods

2.1. Sample collection and sample pretreatment

Crayfish samples ($n = 660$) were collected from four major crayfish production areas in China: Hubei ($n = 165$), Jiangxi ($n = 180$), Jiangsu ($n = 135$), and Shandong ($n = 180$) from June to August 2016 (Fig. 1). Crayfish with similar sizes and ages were selected to reduce the influence of factors specific to each sampling site (Luo et al., 2019). Farmed crayfish samples ($n = 345$) were caught at crayfish farms, while wild crayfish samples ($n = 315$) were captured from streams, lakes or rivers. Samples of the benthic mud that crayfish live in were collected from four regions.

To avoid contamination during sampling and transportation, all samples were sealed in separate plastic bags, stored in ice boxes, and immediately transported to the laboratory. Crayfish samples were dissected into muscle, exoskeleton, and viscera (including hepatopancreas, stomach, and intestine). Considering that the hepatopancreas, stomach, and intestine are usually discarded by consumer, in this study, they were mixed to form viscera samples. These tissues were dissected and then rinsed with ultrapure water and then freeze-dried. Subsequently, the freeze-dried samples were ground into fine powder, sealed, and stored under dry conditions for further analysis.

2.2. Multi-element analysis

For element analysis, approximately 0.25 g (dry weight) of each of crayfish sample was weighed in a 50 mL colorimetric tube and digested with 3 mL HNO_3 (65% v/v) at 80 °C for 24 h until clarification using a graphite digestion apparatus (JKI, China). The digests were cooled and diluted to 25 mL with ultrapure water. Then, the contents of trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Tl, Pb, and U) in the digests were determined by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7900, USA) at the Geochemical Laboratory of the China University of Geosciences (Wuhan). The content of total As was measured by cold atomic fluorescence spectrometry. The content of Hg was measured using a MERX cold atomic fluorescence mercury analysis system (Brooks Rand Instruments, Seattle, WA, USA). Blank and parallel samples and standard reference materials (TORT-3, National Research Council, Canada) were used for quality control. The results for the reference materials agreed with the certified values. The rates of recovery ranged from 80% to 110%. The concentrations of these elements are expressed in mg/kg (dry weight).

2.3. Stable isotope ratio analysis

To obtain sufficient analytical accuracy, the 660 crayfish samples were divided into 33 parts according to their sampling locations. For stable isotope ratio analysis (SIR), 2.00 mg of each pretreated sample was weighed. The $\delta^{13}C$ and $\delta^{15}N$ values of the samples were measured using an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific, Bremen, Germany) in the isotope analysis laboratory of the Shenzhen Entry-Exit Inspection and Quarantine Bureau. According to the International Union of Pure and Applied Chemistry (IUPAC) guidelines (Brand et al., 2014), the isotope ratios were expressed in δE (‰) versus Vienna Pee Dee Belemnite (V-PDB) for $\delta^{13}C$ and versus air for $\delta^{15}N$ following the formula:

$$\delta E(‰) = \left[\left(R_{sample} - R_{standard}\right)/R_{standard}\right] \times 1000 \quad (1)$$

where R_{sample} represents the $^{13}C/^{12}C$ ratio or $^{15}N/^{14}N$ ratio in the sample and $R_{standard}$ is the isotope ratio of the international standard. The international reference material was IAEA-600 caffeine with a $\delta^{13}C$ value of −27.771‰ and an $\delta^{15}N$ value of 1‰.

2.4. Human health risk assessment

The estimated daily intake (*EDI*) of heavy metals via crayfish consumption was calculated using the following equation (Adebiyi et al. 2020; Anandkumar et al., 2020a):

$$EDI = (C \times IR \times EF \times ED)/(BW \times AT) \quad (2)$$

Table 1
Average concentrations of trace elements in different crayfish tissues according to the region (mg/kg dry weight) with safety standard limits shown for comparison.

Origins	Tissue	Mn	Fe	Rb	Sr	Ba	Ni	Co	Se	V	U	Cs
Hubei	Viscera	393.50^{a}	1000.04^{a}	2.53^{a}	26.12^{a}	46.45^{a}	3.80^{a}	1.95^{a}	1.71^{a}	1.07^{a}	0.13^{a}	0.053^{a}
	Exoskeleton	619.83^{b}	678.60^{b}	2.25^{a}	263.59^{b}	401.56^{b}	0.72^{b}	0.46^{b}	0.60^{b}	1.42^{a}	0.031^{b}	0.094^{b}
	Muscle	17.30^{c}	31.39^{c}	3.59^{b}	7.40^{c}	7.59^{c}	0.068^{c}	0.028^{c}	1.03^{c}	0.081^{b}	0.003^{c}	0.018^{c}
Shandong	Viscera	271.51^{a}	447.38^{a}	3.63^{a}	142.63^{a}	49.83^{a}	4.06^{a}	1.33^{a}	2.06^{a}	0.77^{a}	0.23^{a}	0.057^{a}
	Exoskeleton	180.20^{b}	320.96^{a}	2.76^{a}	760.08^{b}	252.12^{b}	1.34^{b}	0.27^{b}	0.66^{b}	0.59^{a}	0.042^{b}	0.0637^{a}
	Muscle	6.29^{c}	16.25^{b}	5.29^{a}	53.33^{c}	6.33^{c}	0.32^{c}	0.021^{c}	1.03^{c}	0.03^{b}	0.004^{c}	0.0397^{b}
Jiangxi	Viscera	413.92^{a}	803.12^{a}	8.16^{a}	50.76^{a}	42.68^{a}	4.63^{a}	2.00^{a}	2.10^{a}	0.67^{a}	0.12^{a}	0.072^{a}
	Exoskeleton	303.24^{b}	344.16^{b}	7.15^{a}	364.79^{b}	294.15^{b}	0.60^{b}	0.35^{b}	0.62^{b}	0.53^{b}	0.023^{b}	0.079^{a}
	Muscle	9.95^{c}	15.79^{c}	12.80^{a}	11.49^{c}	4.82^{c}	0.40^{c}	0.029^{c}	1.21^{c}	0.017^{c}	0.002^{c}	0.085^{a}
Jiangsu	Viscera	484.76^{a}	853.49^{a}	8.58^{a}	58.46^{a}	47.74^{a}	7.26^{a}	3.67^{a}	1.52^{a}	0.83^{a}	0.12^{a}	0.060^{a}
	Exoskeleton	367.76^{a}	447.90^{b}	4.05^{a}	462.82^{b}	315.94^{b}	0.89^{b}	0.36^{b}	0.32^{b}	0.85^{a}	0.032^{b}	0.079^{a}
	Muscle	10.89^{b}	20.29^{c}	13.33^{a}	17.53^{c}	6.81^{c}	0.743^{b}	0.029^{c}	0.58^{c}	0.010^{b}	0.004^{c}	0.074^{a}
Origins	Tissue	Cu	Zn	Cr	Ga	Ag	Hg	Total As	Cd	Pb	Tl	
Hubei	Viscera	163.64^{a}	101.70^{a}	1.11^{a}	0.13^{a}	0.44^{a}	0.13^{a}	2.57^{a}	1.78^{a}	0.39^{a}	0.004^{a}	
	Exoskeleton	53.46^{b}	62.64^{b}	6.38^{b}	0.20^{b}	0.12^{b}	0.074^{b}	1.50^{b}	0.059^{b}	0.67^{b}	0.007^{b}	
	Muscle	35.74^{c}	94.67^{a}	0.50^{c}	0.003^{c}	0.089^{b}	0.35^{c}	0.64^{c}	0.026^{c}	0.07^{c}	0.001^{c}	
Shandong	Viscera	86.48^{a}	116.10^{a}	1.41^{a}	0.076^{a}	0.26^{a}	0.054^{a}	3.72^{a}	0.47^{a}	0.37^{a}	0.008^{a}	
	Exoskeleton	40.89^{b}	77.23^{b}	4.82^{b}	0.092^{a}	0.12^{b}	0.059^{a}	1.10^{b}	0.022^{b}	0.56^{b}	0.007^{a}	
	Muscle	22.77^{c}	85.11^{c}	0.11^{c}	0.002^{b}	0.072^{c}	0.095^{b}	0.94^{b}	0.005^{c}	0.007^{c}	0.003^{a}	
Jiangxi	Viscera	153.93^{a}	117.64^{a}	1.27^{a}	0.073^{a}	0.95^{a}	0.074^{a}	2.38^{a}	1.37^{a}	0.39^{a}	0.021^{a}	
	Exoskeleton	51.42^{b}	68.70^{b}	2.49^{b}	0.072^{a}	0.24^{b}	0.048^{b}	0.78^{b}	0.060^{b}	0.65^{a}	0.026^{a}	
	Muscle	24.36^{c}	88.23^{c}	0.25^{c}	ND	0.11^{c}	0.20^{c}	0.49^{c}	0.017^{c}	0.23^{a}	0.011^{b}	
Jiangsu	Viscera	148.68^{a}	114.80^{a}	1.53^{a}	0.11^{a}	0.39^{a}	0.050^{a}	3.47^{a}	2.19^{a}	0.45^{a}	0.018^{a}	
	Exoskeleton	48.59^{b}	69.52^{b}	4.56^{a}	0.12^{a}	0.16^{b}	0.034^{a}	1.13^{b}	0.047^{b}	0.63^{a}	0.013^{a}	
	Muscle	27.29^{c}	96.77^{c}	0.18^{b}	ND	0.078^{c}	0.14^{b}	0.96^{b}	0.045^{b}	0.55^{a}	0.008^{a}	
GB (2012)	–	NA	NA	2	NA	NA	0.5	0.5	0.5	0.5	NA	
WHO (1989)	–	30	100	50	NA	NA	NA	NA	1	2	NA	
USEPA (2000)	–	120	200	8	NA	NA	NA	NA	2	4	NA	

Different letters in the same column indicate statistical differences among different crayfish tissues (viscera, exoskeleton, and muscle) from the same region ($P < 0.05$). ND: not detected; NA: not available. The safety standards are GB2762-2012 (SAPRC, 2012); WHO: World Health Organization; USEPA: United States Environmental Protection Agency. The safety standard of inorganic As is 0.5, assuming that 10% of As is inorganic (USFDA, 1993).

where *EF* is exposure frequency (365 days/year); *ED* is exposure duration (70 years for adults, equivalent to the average lifetime); *IR* is ingestion rate (in this case, 0.02 kg person^{-1} day^{-1} for adults); *C* is the metal concentration in crayfish (mg/kg, wet weight); *BW* is average body weight (70 kg for adults); and *AT* is average exposure time for non-carcinogens (365 days year^{-1} × *ED*).

The target hazard quotient (THQ) represents the risk of non-carcinogenic effects. If it is less than 1, the exposure level is less than the reference dose (Adebiyi et al. 2020). This indicates that daily exposure at this level is unlikely to cause adverse effects during a person's lifetime. It can be calculated using the following equation:

$$\mathrm{THQ} = \mathrm{EDI}/\mathrm{RfD} \tag{3}$$

where *EDI* represents the estimated daily intake and *RfD* represents the reference dose.

2.5. Statistical analysis

Origin 2018 software (OriginLab, Northampton, Massachusetts, USA) and Excel 2016 were used for data processing and chart generation. Statistical analyses were carried out using SPSS 22.0 software (SPSS, Chicago, IL, USA). Student's *t*-tests were used to determine significant differences between crayfish tissues, wild and farmed samples, and origins ($p < 0.05$). Principal component analysis (PCA) was used to describe the sample clusters from different origins.

3. Results and discussion

3.1. Enrichment characteristics of trace elements in crayfish

Table 1 summarizes the average concentrations of the 21 analyzed trace elements in different organ tissues of crayfish sampled from the four geographical locations. Concentrations of Hg, As, and Cd were below the Chinese national safety standards (GB2762-2012; SAPRC, 2012) and the WHO (1989) and USEPA (2000) standards (Table 1). However, the concentrations of Cd in viscera exceeded the Chinese and WHO (1989) standards. The concentrations of Pb and Cr in the exoskeleton exceeded Chinese standards but not the USEPA (2000) and WHO (1989) standards. The concentrations of Cu in all crayfish tissues exceeded the WHO (1989) standards but not the USEPA (2000) standards. The concentrations of Zn in the viscera slightly exceeded the WHO (1989) standards; however, Zn concentrations in all crayfish tissues did not exceed the USEPA (2000) standards.

The elements Cu, Zn, Fe, Mn, Cr, Co, Ni, and Se are essential trace elements in this organism, while the other analyzed elements are non-essential and toxic (WHO, 1989; Rejomon et al., 2010). The results show that the accumulation and distribution of most trace elements in crayfish tissue occur from highest to lowest in viscera > exoskeleton > muscle, except for Cr, Ba, Sr, Cs, and Pb, which are exoskeleton > viscera > muscle. This is consistent with previous research (Suárez-Serrano et al., 2010; Goretti et al., 2016; Xiong et al., 2020). The hepatopancreas, a part of the viscera, is a very metabolically active organ that can sequester contaminants and contribute to the detoxification process. The lowest concentrations of pollutants were observed in abdominal muscle (Goretti et al., 2016; Xiong et al., 2020). Unlike those of other metals, the concentrations of Hg and Rb in tissues were ranked in the order of muscle > viscera > exoskeleton. For Hg, this could be attributed to the high affinity of Hg for the sulfur-containing amino acid cysteine, which is found in muscle protein (Henriques et al., 2014; Rodríguez-Estival et al., 2019). For Rb, although similar results have been reported in a previous study (Anandkumar et al. 2018), the cause is unclear.

Muscle tissue is of most concern from a health viewpoint, as it is the part most commonly eaten by humans (Anandkumar et al. 2020a). The mean concentrations of some essential elements in crayfish muscle tissue decreased in the order of Zn > Cu > Fe > Mn > Ni > Cr. The mean concentrations of toxic elements in crayfish muscle tissue decreased in the order of As > Pb (Hg) > Cd > Tl. The contents of essential trace elements in crayfish are generally higher than those of non-essential (except for Rb, Sr, and Ba) or toxic elements. For example, the

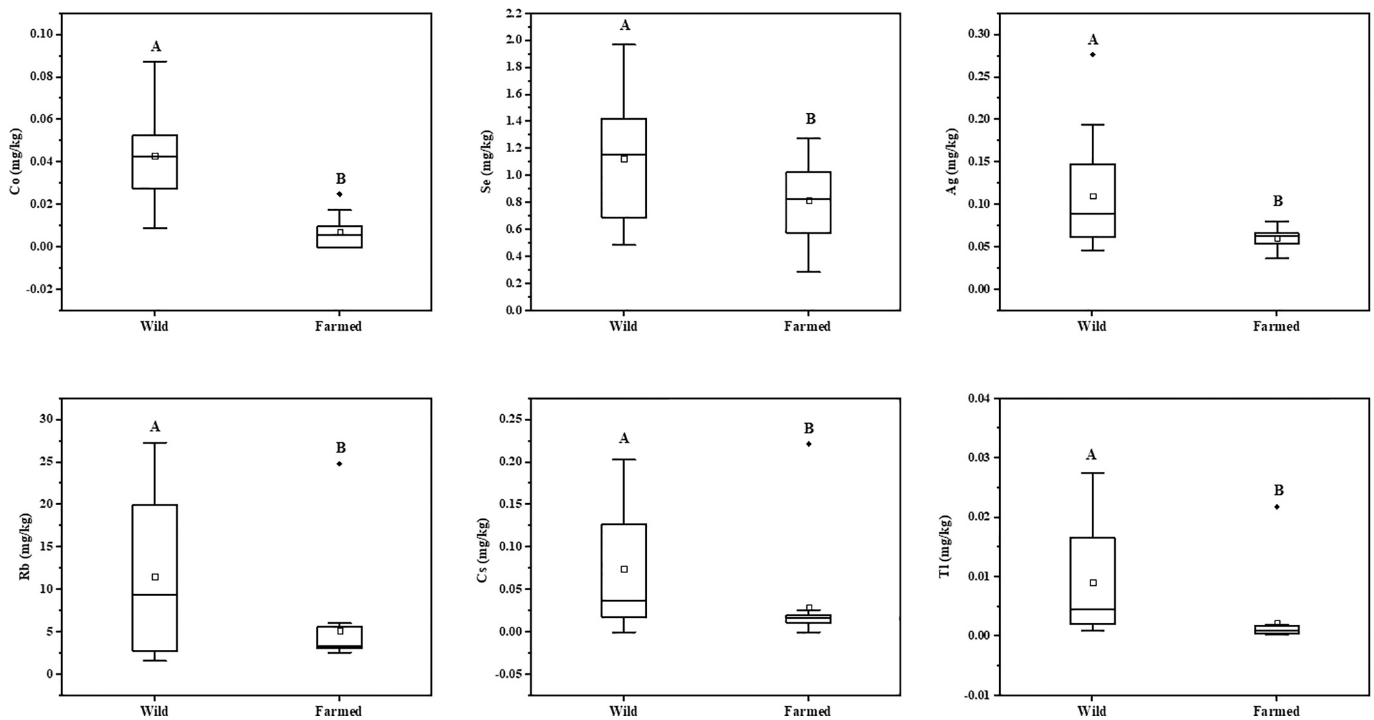

Fig. 2. Analyses of the concentrations of six trace elements in crayfish muscle samples. Comparison between wild and farmed production methods. Different letters indicate a statistical difference ($P < 0.05$).

contents of essential elements such as Cu, Zn, Fe, Mn, Ni, and Se were higher than those of toxic elements such as Hg, Pb, Cd, and As. Similar research shows that essential elements (such as Cu and Zn) are more highly bioaccumulated than toxic non-essential elements (such as Pb, Cd, and Hg) in some aquatic species (Wu & Yang, 2011; George et al., 2011; Anandkumar et al., 2020a,2020b). This may be related to the fact that Zn and Cu are essential for normal growth and enzymatic activity in aquatic organisms (Handy, 1996). In addition, the elemental bioaccumulation in aquatic organisms is not only related to environmental parameters (such as the temperature and alkalinity of the surrounding environment), but also to internal factors such as life cycle, habitat, and feeding properties (Zhou et al., 2008; Anandkumar et al. 2019).

The contents of trace elements in the muscle of wild and farmed crayfish are compared in Fig. 2 and Table S1 (see Supporting information). The contents of most trace elements were comparable ($P > 0.05$; Table S1); however, several (Co, Ag, Rb, Ba, Se, Cs, and Tl) were significantly higher in wild crayfish than farmed ones ($P < 0.05$; Fig. 2). This may be related to the longer trace-element exposure times and greater contact with surrounding sediment experienced by wild crayfish (Xiong et al., 2020). Considering that the aquaculture base was located in farmland far away from industrial activity, the sources of toxic elements in the farmed crayfish were mainly attributed to geogenic and farming activities (e.g. pesticides, manure, and fodder). However, wild crayfish may be distributed across many aquatic systems and may receive pollutants from industry, agriculture, and domestic waste.

3.2. Geographical distribution of trace elements in crayfish muscle

The distribution of trace elements in crayfish muscle samples from different regions is shown in Table 1. Among the four regions, the contents of Cu, Zn, Mn, Fe, and Co (essential elements) and Hg, Pb, Cd, and As (toxic elements) were lowest in crayfish muscle samples from Shandong, but the Sr content was the highest. Hubei samples had the highest concentrations of Cr, Mn, Fe, Cu, V, and Ba; Jiangxi samples had the highest concentrations of Ag, Cs, and Tl; and Jiangsu samples had the highest contents of Ni and Zn (essential) and Cd and Pb (toxic). These results indicate that the elemental composition of crayfish differs significantly by region. This could be related to regional variations in environmental conditions such as the geological background, climate, and pollution (Li et al., 2016; Zhao et al., 2013). The minerals and trace metal components in the crayfish are reflections of the trace mineral components in the soil and environment where they grow (Anderson and Smith 2005). Moreover, the feed is an important factor that must be carefully considered in aquaculture products. Regional differences in the

Table 2
PCA of multi-element composition of crayfish muscle samples displayed as factor loadings and cumulative variance contribution rates.

Element	Factor 1	Factor 2	Factor 3	Factor 4
V	**0.889**	−0.325	−0.066	−0.177
Cr	**0.808**	0.025	0.361	0.099
Mn	0.441	0.057	0.184	−0.594
Fe	**0.914**	−0.133	0.074	−0.055
Co	**0.520**	0.430	0.309	0.268
Ni	−0.225	0.101	**0.661**	0.474
Cu	**0.907**	−0.158	0.003	0.015
Zn	**0.792**	0.006	−0.195	0.272
Ga	**0.624**	−0.312	−0.120	0.195
As	0.041	−0.706	−0.287	**0.408**
Se	0.462	0.429	−0.247	0.069
Rb	0.007	**0.869**	−0.067	0.191
Sr	−0.095	−0.368	−0.378	**0.654**
Ag	**0.526**	0.533	−0.244	0.221
Cd	0.089	−0.061	**0.920**	0.190
Cs	0.060	**0.906**	−0.191	0.193
Ba	**0.872**	−0.113	0.003	0.239
Tl	0.076	**0.918**	−0.179	0.228
Pb	−0.023	−0.040	**0.916**	0.259
U	0.186	−0.477	−0.225	0.365
Hg	**0.621**	0.121	0.089	−0.665
Variance contribution rate (%)	30.15	20.11	13.91	11.03
Cumulative contribution rate (%)	30.15	50.26	64.17	75.20

Note: The four factors represent Hubei, Jiangxi, Jiangsu, and Shandong, respectively. The bold represents the elements that contribute more to each principal component.

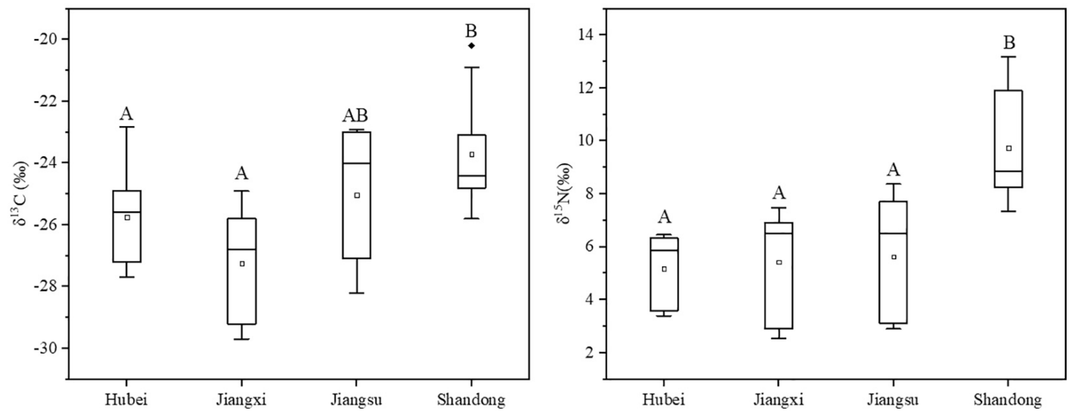

Fig. 3. Boxplots of $\delta^{13}C$ and $\delta^{15}N$ values in crayfish sampled from four different areas (centreline = median; □ = mean; different letters indicate a statistical difference between regions, $P < 0.05$).

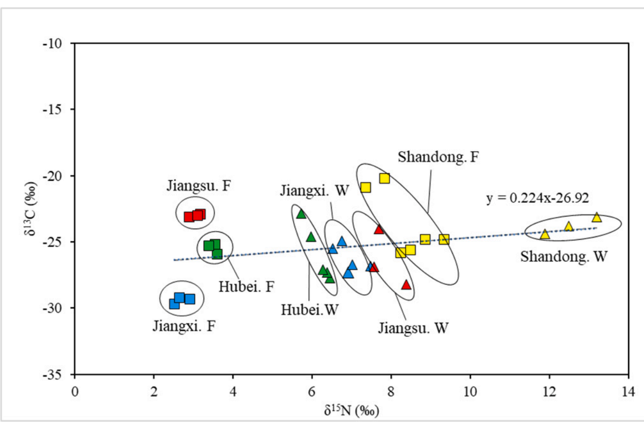

Fig. 4. Scatterplot of stable carbon and nitrogen isotope ratios in wild (W) and farmed (F) crayfish muscle tissue sampled from four areas.

macronutrients or trace minerals added to feed may affect the elemental contents (Luo et al., 2019).

The 21 trace elements in crayfish samples from four regions were analyzed using PCA. The first four factors explained 75.20% of the total variability (Table 2). The values of V, Cr, Fe, Co, Cu, Zn, Ag, Ga, Hg, Mn, Se, and Ba had the highest impact on the first principal component, and Hubei samples could be distinguished. The contents of Rb, Cs, and Tl dominated the second principal component, and Jiangxi samples could be separated from other samples. The Ni, Pb, and Cd contents had the greatest influence on the third principle component, and the Jiangsu samples were distinguishable. The contents of Sr, U, and As had the highest weight on the fourth principal component. The contents of Sr and U in Shandong were higher than in other regions. So, the fourth principal component represents the characteristics of trace element concentrations in Shandong samples. Therefore, it can be concluded that PCA analysis of trace elements in crayfish can be used to distinguish their geographical origin. (See Table 2.)

3.3. Stable isotope ratios in crayfish with different geographical origins

The results of stable C and N isotopic ratio analysis of crayfish muscle samples are shown in Figs. 3 and 4. The $\delta^{13}C$ values of the crayfish samples ranged from −20.2‰ to −29.7‰. Among them, the mean ± SD of $\delta^{13}C$ values were − 25.8‰ ± 1.62‰ (Hubei), −27.2‰ ± 1.77‰ (Jiangxi), −25.0‰ ± 2.28‰ (Jiangsu), and − 23.7‰ ± 1.98‰ (Shandong). As shown in Fig. 3, the $\delta^{15}N$ values of the crayfish samples ranged from 2.53‰ to 7.49‰ in Jiangxi, from 2.90‰ to 8.38‰ in Jiangsu, from 3.40‰ to 6.46‰ in Hubei, and from 7.36‰ to 13.2‰ in Shandong. The $\delta^{15}N$ values from different regions were ranked in the order of Hubei < Jiangxi < Jiangsu < Shandong. The changes observed in the stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ may mainly reflect significant differences in the daily diets of crayfish from different geographical regions.

Red swamp crayfish (*Procambarus clarkii*) are omnivorous aquatic animals. The $\delta^{13}C$ values showed distinct regional differences that might be associated with different food sources (Fig. 3). In general, the $\delta^{13}C$ values in animal tissues such as those of crayfish are primarily determined by their feed type, especially the proportions of C_3 and C_4 in plant material (Molkentin et al., 2007; Bong et al., 2010; Guo et al., 2010; Park et al., 2018). C_4 and C_3 plants are commonly used as carbohydrate sources for aquatic feed (Niu et al., 2012). C_4 plants (e.g., maize) can cause higher $\delta^{13}C$ values in animal tissues than C_3 plants (e.g., some grasses; Jiang et al. 2020). Shandong is a major agricultural province of China and corn is one of its main crops. The $\delta^{13}C$ values of Shandong crayfish in this study were higher than those of other regions ($P < 0.05$; Fig. 3), which reflects the effect of food source on isotope ratio. Furthermore, the carbon source of sediments is mainly organic matter from plant remains (Boutton et al., 1998). The $\delta^{13}C$ values in the benthic mud of Shandong were higher than in other regions ($P < 0.05$; Table S2), indicating that the surrounding environment may affect the carbon isotope ratio of crayfish.

The $\delta^{15}N$ values of crayfish samples from different origins also exhibited differences (Fig. 3). The $\delta^{15}N$ values in crayfish tissues are dependent on dietary and regional conditions. Metabolism (e.g. assimilation and dissimilation) leads to further enrichment of heavier isotopes in aquaculture species, causing predatory species to have higher $\delta^{13}C$ and $\delta^{15}N$ values than their food (Serrano et al., 2007; Li et al., 2016). The soil fertilization regimes of feedstuff crops may also influence $\delta^{15}N$ values (Bateman & Kelly, 2007; Zhao et al., 2020). The $\delta^{15}N$ values in crayfish samples from Shandong were much higher than those from other regions ($P < 0.05$; Fig. 3), which is related to the higher $\delta^{15}N$ values found in the benthic mud of Shandong ($P < 0.05$; Table S2). Soil/sediment nitrogen isotope composition can be affected by changes in the composition and structure of the local vegetation community (Quan et al., 2016). These results reflect the influences of food sources and regional conditions on $\delta^{15}N$ values in crayfish.

Previous studies have shown that stable isotope analysis is a promising way to identify the geographic origins and production methods of some aquatic products (Serrano et al., 2007; Ostermeyer et al., 2014; Gamboa-Delgado et al., 2014). In this study, the stable carbon and nitrogen scatterplot for crayfish shows a clear distinction between farmed and wild crayfish from all regions (Fig. 3). Fig. 4 shows that the $\delta^{15}N$ values of wild crayfish from all regions were higher than those of farmed crayfish, which is related to different food sources. The habitat and life history of wild crayfish are much more complex than those of farmed

Table 3
Estimated daily intake (EDI, mg/kg/day) and estimated target hazard quotient (THQ) of analyzed elements in crayfish from four regions.

Element	EDI (mg/kg/d)				RfD	THQ			
	Shandong	Hubei	Jiangxi	Jiangsu	(mg/kg/d)	Shandong	Hubei	Jiangxi	Jiangsu
V	2.32×10^{-6}	6.21×10^{-6}	1.28×10^{-6}	7.99×10^{-7}	0.009	2.60×10^{-4}	6.90×10^{-4}	1.42×10^{-3}	8.89×10^{-5}
Cr	8.60×10^{-6}	3.83×10^{-5}	1.93×10^{-5}	1.38×10^{-5}	1.5	5.73×10^{-6}	2.55×10^{-5}	1.29×10^{-5}	9.22×10^{-6}
Mn	4.81×10^{-4}	1.32×10^{-3}	7.62×10^{-4}	8.33×10^{-4}	0.14	3.44×10^{-3}	9.46×10^{-3}	5.44×10^{-3}	5.95×10^{-3}
Fe	1.24×10^{-3}	2.40×10^{-3}	1.21×10^{-3}	1.55×10^{-3}	0.7	1.78×10^{-3}	3.43×10^{-3}	1.73×10^{-3}	2.22×10^{-3}
Co	1.63×10^{-6}	2.15×10^{-6}	2.24×10^{-6}	2.21×10^{-6}	0.03	5.43×10^{-5}	7.17×10^{-5}	7.47×10^{-5}	7.36×10^{-5}
Ni	2.46×10^{-5}	5.24×10^{-6}	3.05×10^{-5}	5.64×10^{-5}	0.02	1.23×10^{-3}	2.62×10^{-4}	1.53×10^{-3}	2.82×10^{-3}
Cu	1.74×10^{-3}	2.74×10^{-3}	1.87×10^{-3}	2.09×10^{-3}	0.04	4.36×10^{-2}	6.84×10^{-2}	4.66×10^{-2}	5.22×10^{-2}
Zn	6.51×10^{-3}	7.25×10^{-3}	6.76×10^{-3}	7.41×10^{-3}	0.3	2.17×10^{-2}	2.42×10^{-2}	2.25×10^{-2}	2.47×10^{-2}
Ga	2.07×10^{-7}	2.45×10^{-7}	ND	ND	0.3	6.90×10^{-7}	8.17×10^{-7}	–	–
As	7.23×10^{-5}	4.87×10^{-5}	3.71×10^{-5}	7.35×10^{-5}	0.0003	2.41×10^{-1}	1.62×10^{-1}	1.24×10^{-1}	2.45×10^{-2}
Se	7.92×10^{-5}	7.90×10^{-5}	9.29×10^{-5}	4.47×10^{-5}	0.005	1.59×10^{-2}	1.58×10^{-2}	1.86×10^{-2}	8.94×10^{-3}
Rb	4.05×10^{-4}	2.75×10^{-4}	9.81×10^{-4}	1.02×10^{-3}	0.005	8.10×10^{-2}	5.50×10^{-2}	1.96×10^{-1}	2.04×10^{-1}
Sr	4.08×10^{-3}	5.66×10^{-4}	8.79×10^{-4}	1.34×10^{-3}	–	–	–	–	–
Ag	5.56×10^{-6}	6.82×10^{-6}	8.34×10^{-6}	6.02×10^{-6}	0.005	1.11×10^{-3}	1.36×10^{-3}	1.67×10^{3}	1.20×10^{-3}
Cd	4.16×10^{-7}	2.02×10^{-6}	1.28×10^{-6}	3.45×10^{-6}	0.001	4.16×10^{-3}	2.02×10^{-3}	1.28×10^{-3}	3.45×10^{-3}
Cs	2.99×10^{-6}	1.37×10^{-6}	6.50×10^{-6}	5.67×10^{-6}	0.001	2.99×10^{-3}	1.37×10^{-3}	6.50×10^{-3}	5.67×10^{-3}
Ba	4.85×10^{-4}	5.81×10^{-4}	3.69×10^{-4}	5.21×10^{-4}	0.2	2.42×10^{-3}	2.90×10^{-3}	1.84×10^{-3}	2.61×10^{-3}
Tl	2.61×10^{-7}	1.11×10^{-7}	8.46×10^{-7}	6.23×10^{-7}	0.00008	3.27×10^{-3}	1.39×10^{-3}	1.06×10^{-2}	7.79×10^{-3}
Pb	5.36×10^{-7}	5.34×10^{-6}	1.78×10^{-5}	4.22×10^{-5}	0.0015	3.57×10^{-4}	3.56×10^{-3}	1.19×10^{-2}	2.81×10^{-2}
U	3.16×10^{-7}	2.24×10^{-7}	$1.0.42 \times 10^{-7}$	3.11×10^{-7}	0.003	1.1×10^{-4}	7.46×10^{-5}	4.73×10^{-5}	1.04×10^{-4}
Hg	7.26×10^{-6}	2.70×10^{-5}	1.52×10^{-5}	1.07×10^{-5}	0.0001	7.26×10^{-2}	2.70×10^{-1}	1.52×10^{-1}	1.07×10^{-1}

Notes: RfD: oral reference dose; ND: Not detected.

crayfish. Wild crayfish have wider and more complex food sources, while the activity and feeding of farmed crayfish are more controlled (Luo et al., 2019). Therefore, stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$) is a powerful tool for determining the geographical origins and production methods of crayfish from different regions.

3.4. *Human health risk assessment*

In the study, we evaluated the human health risks of consuming trace elements in crayfish. The estimated adult daily intakes (EDIs) and target hazard quotients (THQs) of consuming trace elements in crayfish from the four regions are listed in Table 3. It shows that the EDIs of the analyzed trace elements are lower than the oral reference doses (RfDs; Varol et al., 2017; Adebiyi et al. 2020; Xiong et al., 2020). Therefore, normal crayfish consumption should not pose a severe health risk to consumers, although care should be taken not to consume too much. Moreover, the trace element THQs of crayfish are generally less than 1, further confirming that the consumption of trace elements in crayfish will not cause significant health risks.

4. Conclusions

Of the analyzed organs, visceral tissue stored the most trace elements in crayfish, followed by the exoskeleton and muscle tissue. The contents of some essential trace elements (Cu, Zn, Fe, Mn, Ni, Cr, and Se) in muscle samples from all regions were higher than those of toxic elements (Hg, Cd, As, and Pb). The contents of some essential trace elements in wild crayfish in Hubei, Jiangxi, and Jiangsu were higher than those in farmed crayfish, which may be related to the greater elemental exposure time and surrounding sediment contact of wild crayfish. The multi-element analysis showed that different areas have characteristic elemental compositions, which indicates that there is an influence of regional environmental conditions such as geological background and climate. The SIR analysis showed that the isotopic ratios differed between the four crayfish production areas, which is related to diet and region. These results demonstrate that the combination of multi-element and stable isotope ratio analyses is a practical way to distinguish crayfish products by geographical region. The health risk assessment suggests that intake of trace elements from crayfish consumption is not a serious health risk, although excessive consumption should be avoided.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

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