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Elemental analysis of wild *Eriocheir sinensis*: Determining the geographic origin and human health risk assessment

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RESEARCH ARTICLE

Abstract

Identification of composition characteristics of major and trace elements in wild mitten crabs (*Eriocheir sinensis*) from different water systems is important for protected geographical indication as well as food quality control and safety. In this study, inductively coupled plasma-mass spectrometry was employed to measure the contents of 23 elements in the muscle samples of wild *Eriocheir sinensis* from three water systems (Suifenhe, Nanliujiang, and Liaohe). The results of Kruskal–Wallis one-way ANOVA test comparisons revealed that most elements were significantly different in the samples collected from three water systems ($P < 0.05$). Geographic origin discrimination was achieved using principal component analysis in combination with hierarchical cluster analysis (HCA) and stepwise linear discriminant analysis (S-LDA). The HCA results showed the potential of eight characteristic elements for exploratory hierarchical clustering of the samples from three water systems; however, the clustering effect was unsatisfactory. The discrimination accuracy of S-LDA model for the samples from three water systems and that in cross-validation reached 100%. The health risk assessment further revealed that the normal level consumption of wild *Eriocheir sinensis* did not pose an appreciable health risk to consumers.

Keywords: wild *Eriocheir sinensis*; multi-element; geographic origin discrimination; health risk

Introduction

Eriocheir sinensis, one of the important aquaculture species in China (Che *et al.*, 2022), is an aquatic animal with a life history of catadromous migration that mates and spawns in brackish estuaries, and the hatched megalopa migrate to freshwater for growth (Cheng *et al.*, 2008). *Eriocheir sinensis* is widely distributed in the coastal water systems of China, from the Suifenhe (SFH) river system in Heilongjiang Province in the north to the Nanliujiang (NLJ) river system in Guangxi Zhuang autonomous region in the south. Among them, relatively large natural populations in the Yangtze, Liaohe (LH), and Oujiang rivers were once the main source of origin for *Eriocheir sinensis* pond culture in China (Wang *et al.*, 2022a; Zhang *et al.*, 2000). Owing to its benthonic and food habits, *Eriocheir sinensis* has a significantly higher enrichment capacity for heavy metals than fish (Yao *et al.*, 2014; Zhao *et al.*, 2012). Crustaceans have the characteristics of rapid bioaccumulation and long residence time of trace elements in tissues and high sensitivity to changes in different physicochemical properties in aqueous environments. These properties make them suitable for serving as biomarkers for the health status assessment of aquatic environments (Anandkumar *et al.*, 2020a, 2020b; Zhou *et al.*, 2021). As a carrier of pollutants, *Eriocheir sinensis* can transfer trace elements to higher nutrition levels to harm human health. Therefore, it is of great significance for human health to detect the content of trace elements in *Eriocheir sinensis* as well as determine the health risk of consuming *Eriocheir sinensis* (Yang *et al.*, 2022). With the increasing production of *Eriocheir sinensis*, its food safety has gradually attracted attention, and the contents of trace elements in cultured *Eriocheir sinensis* samples have been reported in various studies (Wang *et al.*, 2021; Xue *et al.*, 2022a; Zhao *et al.*, 2012). It has been illustrated that the contents of trace elements, such as Cu, Zn, Fe, and Mn, in the wild *Eriocheir sinensis* muscle samples are higher than those in cultured *Eriocheir sinensis* (Wu *et al.*, 2020). However, the edible security evaluation of heavy metals in wild *Eriocheir sinensis* has not been reported.

The quality of wild *Eriocheir sinensis* living in different water systems varied greatly, especially the enrichment of trace elements. Considering its high commercial value, the rapidly developing industry of *Eriocheir sinensis* is confronted with problems, such as intended or unintended error-tagging and falsified labels with respect to the origin, production method, or authentication. These problems seriously damage the rights of consumers and pose a great threat for marketing and human health (Bai *et al.*, 2022; Xue *et al.*, 2022b). In an attempt to improve the social and economic benefits of agricultural products, a series of origin traceability and protection measures, such as "protected geographical indications (PGI)" policy, have been taken to ensure food safety (Luo *et al.*, 2019).

In order to avoid the confusing authentication of *Eriocheir sinensis* products with other similar products, it is important to determine their geographic origin. Multi-element-based origin discrimination technology can achieve relatively fast and accurate identification of the same species across different origins, thus showing a great potential for origin discrimination. Currently, the stated technology has been applied for identifying the origin of marine products, such as sea cucumber (Bai *et al.*, 2021; Kang *et al.*, 2018; Mamede *et al.*, 2022) and agricultural products, such as alcoholic drinks (Gao *et al.*, 2022; Gajek *et al.*, 2022), coconut oil (Amit *et al.*, 2022), honey (Maria *et al.*, 2020), tea (Zheng *et al.*, 2020), and cultured *Eriocheir sinensis* (Luo *et al.*, 2019). However, geographical origin tracing of wild *Eriocheir sinensis* based on multi-element analysis has not been investigated so far. Being a type of agricultural product, no research has been conducted on the geographical origin of wild *Eriocheir sinensis*. Regional identification and protection could be achieved by establishing the multi-element traceability fingerprinting of wild *Eriocheir sinensis*.

Therefore, this study selected wild *Eriocheir sinensis* from three water systems in China (NLJ, SFH, and LH rivers) as instances to analyze the content of four major elements and 19 trace elements in their muscle samples, and attempted to identify and analyze their geographical origins based on the fingerprint characteristics of these elements. The present study provides a new method for tracing wild *Eriocheir sinensis* population to fill gap in element content analysis and population identification of wild *Eriocheir sinensis*. The present study directed to achieve the following three functions: (1) to reveal the content levels and composition characteristics of trace elements in wild *Eriocheir sinensis* from different water systems, (2) to elucidate multi-element fingerprints of wild *Eriocheir sinensis* to establish a traceable model based on chemometric analysis for discrimination of their geographical origin, and (3) to evaluate the potential health risks of consuming wild *Eriocheir sinensis* consumption to human health with the contents of trace elements.

Materials and Methods

Reagents and apparatus

Superior-grade pure nitric acid and hydrochloric acid were sourced from Merck (Germany). Internal standard solution (lithium [Li], scandium [Sc], germanium [Ge], rhodium [Rh], indium [In], terbium [Tb], lutetium [Lu], and bismuth [Bi]) at a mass concentration of 100 mg/L and tuning solution (Li, yttrium [Y], cerium [Ce], thallium [Tl], and Co) at a mass concentration of 10 mg/L were procured from Agilent (USA). Standard solution (gallium [Ga], vanadium [V], chromium [Cr], cobalt [Co], nickel [N]i, selenium [Se], silver [Ag], arsenic [As], cadmium [Cd], lead [Pb], rubidium [Rb], mercury [Hg], cesium [CS], and uranium [U]) at a mass concentration of 0–20 μg/L and standard solution (sodium [Na], calcium [Ca], magnesium [Mg], potassium [K], iron [Fe], manganese [Mn], barium [Ba], copper [Cu], and zinc [Zn]) at a mass concentration of 0–1,000 μg/L were brought from the National Research Center for Certified Reference Materials of China. High-purity argon and helium (purity ≥ 99.999%) were also used.

Additionally, instruments used in this study comprised inductively coupled plasma-mass spectrometry (ICP-MS) 7500cx (Agilent) equipped with an octopole reaction system (ORS), microwave-assisted digestion system MARSX (CEM, USA), and water purifier (Millipore, USA).

Sampling and preparation of *Eriocheir sinensis*

Samples of wild *Eriocheir sinensis* (n = 36) were collected in August 2021 from the NLJ (n = 12), SFH (n = 12), and

LH (n = 12) water systems of China. Wild *Eriocheir sinensis* samples of similar size and age were chosen to decrease the influence of sampling site-specific factors. The body weight of samples ranged from 80 g to 100 g. The harvested *Eriocheir sinensis* samples were separately sealed in ziplock bags, numbered, and transferred immediately to the laboratory to prevent contamination during sampling and delivery. Prior to dissection, water on the body surface of *Eriocheir sinensis* samples was removed using absorbent paper, and the samples were weighed on an electronic scale. The limb and abdominal muscles were excised and homogenized. The harvested muscle samples were dried sufficiently in a vacuum freezer dryer to a constant weight, grounded, and strained through 100-mesh filter. Finally, the samples were preserved in dryer prior to testing.

Tests on samples

Microwave-assisted digestion

Glassware for tests was soaked overnight in concentrated nitric acid, dried after 3 rinses in ultrapure water, and retained for later use. The samples were accurately weighed on an analytical balance and 0.15-g samples were placed in a digestion tank. After that, the digestion was performed by adding 2.5-mL nitric acid (having a mass concentration of 65%), 0.5-mL hydrochloric acid (mass concentration of 37%) as well as 7.0-mL ultrapure water (Low *et al.*, 2012). In order to fully digest the samples, the microwave digestion procedures were set by referring to the literature (Fan *et al.*, 2013) after validation by the following experiment specifies: microwave power of 1,600 W (50%); climbing temperature of 120°C, 150°C, and 190°C; heating time of 5 min; and holding time of 5, 10, and 20 min. The post-digestion solution was collected in a 50-mL volumetric flask, with addition of 0.5-mL internal standard solution (100 μg/L), and the final volume was settled to 50 mL. The same method was used for the digestion of blank control solution without a sample.

Multi-elemental ICP-MS measurements

After digestion, both samples and blank controls were measured by ICP-MS. The standard curves were plotted prior to each measurement. Tests were done when the fitness was greater than 99.9% under typical operating conditions (Table 1). In the process of sample analysis, we conducted parallel tests for each sample, and 10% of the samples were randomly selected for six repeated tests to examine test reproducibility. Prior to analysis, the standard substance was tested for qualification using the method reported by Qin *et al.* (2019).

Health risk assessment

The target hazard quotient (THQ) and estimated daily intake (EDI) values proposed by the US Environmental Protection Agency (USEPA) were used to evaluate the risk of each trace element in wild *Eriocheir sinensis* samples on human health (Qin *et al.*, 2015; Wang *et al.*, 2021; Zhao *et al.*, 2012). These values were calculated as follows:

Table 1. Operating parameters of ICP-MS (Agilent 7500 cx).

Operation parameters (optimized daily)	
RF power	1,500 W
Reflected power	<15 W
Carrier gas	1.03 $L{\cdot}min^{-1}$
Makeup gas	0.15 $L{\cdot}min^{-1}$
Collision gas He	4.0 $mL{\cdot}min^{-1}$
Nebulizer pump	0.1 revolutions/s (rps)
Uptake speed	0.4 rps
Uptake time	45 s
Stabilization time	30 s
Acquisition	Spectrum (multi-tune)
Peak pattern	Full quant (3)

$$EDI = [IR \times C/BWa] \times 10^{-3}, \quad (1)$$

$$THQ = EDI/RfD, \quad (2)$$

where EDI is the estimated daily intake (μg/kg/d) of trace elements in human body, IR is the per capita daily intake of aquatic products (g/d, wet weight); IR = 23.7 g/d was selected in this study according to the nutrition and chronic disease surveillance of Chinese residents conducted in 2015 (Disease Prevention and Control Bureau of National Health and Family Planning Commission of PRC, 2015); C represents trace elements in aquatic products (μg/kg, wet weight), and the mean value of C was calculated in this study; BWa is the mean adult body weight, calculated as 70 kg, RfD is the reference dose (μg/kg/d). THQ < 1 indicated no significant health risk, and THQ > 1 demonstrated a significant health risk. A larger THQ value represented a higher health risk.

Data processing

In this study, data were processed and tables and figures were generated using the Origin 2018 software (OriginLab, Northampton, MA, USA) and Excel 2016. Statistical analysis was carried out with the Statistical Package for Social Sciences software (version 22.0; IBM, USA). Differences in the multi-element profiles of wild *Eriocheir sinensis* samples from different water systems were analyzed utilizing Kruskal–Wallis one-way ANOVA test comparisons. The characteristic elements of samples collected from different water systems were screened using principal component analysis (PCA). The hierarchical cluster analysis (HCA) and stepwise linear discriminant analysis (S-LDA) were conducted on characteristic elements to explore the feasibility of elements for discrimination of the geographical origin of wild *Eriocheir sinensis* samples and to establish a discriminant model.

Results and Discussion

Multi-element contents in wild *Eriocheir sinensis* samples from three water systems

In this study, the contents of 23 elements were determined in wild *Eriocheir sinensis* muscle samples from three water systems (SFH, NLJ, and LH rivers) (Table 2). The content proportion of heavy metals, such as Cd (<0.5 mg/kg), Pb (<0.5 mg/kg), As (inorganic As <0.5 mg/kg), and Cr (<2.0 mg/kg) in all wild *Eriocheir sinensis* samples was much lower than the standard limit for crustacean aquatic products mentioned in GB 2762-2022 Limits of Contaminants in Foods (converted by 80% moisture content (State Health and Family Planning Commission of the People's Republic of China, State Food and Drug Administration, 2022; Wang *et al.*, 2022c). The data showed that the content of K in the wild *Eriocheir sinensis* muscle samples from three water systems was higher than that of Na, indicating that wild *Eriocheir sinensis* muscles could be a good source of K in human food for maintaining K and Na balance in the body and exert crucial role of K in maintaining the acid–base balance and osmotic pressure of the blood and body fluids. Among the trace elements in wild *Eriocheir sinensis* muscle samples from three water systems, Zn had the highest content, followed by Cu, which was significantly higher than that in the muscle samples of cultured river crabs (Luo *et al.*, 2019). Zn is an important coenzyme factor in animals involved in the synthesis of RNA, DNA, and protein and exerts a crucial role in regulating the absorption of other minerals and immune function (Chen and Luo, 2021).

Kruskal–Wallis test is a nonparametric test to compare two or more continuous or discrete variables in groups without assuming a specific data distribution (Hong and Lee, 2014). The results of Kruskal–Wallis one-way ANOVA test on the elemental contents of wild *Eriocheir sinensis* in three water systems revealed significant differences in the contents of 10 elements (Na, Mg, K, Cu, As, Ni, CS, Hg, Cr, and Rb) ($P < 0.05$). The results also showed noticeable differences in nine elements, such as Ca, V, Mn, Fe, Co, Se, Ag, Cd, and Ba, between NJL and SFH water systems as well as between NJL and LH water systems ($P < 0.05$) but insignificant difference between SFH and LH water systems. In addition, the contents of major elements, such as Na, Mg, K, and Ca, were significantly higher in the *Eriocheir sinensis* samples collected from SFH water system, compared to those from NLJ and LH water systems ($P < 0.05$). This was probably attributed to the delayed sexual maturation of river crabs in SFH water system during the 2-year period of growth so that their life history extended to 3–4 years (Wang *et al.*, 2022b). The increased period of growth by 1–2 years directly affected the accumulation of major elements in the muscles of wild *Eriocheir sinensis* from SFH water system. The contents of six elements (Cu, As, Cs, Rb, Se, and Ag) were dramatically higher ($P < 0.05$), while the contents of four elements (V, Mn, Fe, and Ba) were lower ($P < 0.01$) in the samples collected from NLJ water system, compared to the samples from SFH and LH water systems. These results collectively demonstrated that the elemental composition in wild *Eriocheir sinensis* samples varied significantly in three water systems, which may be linked to regional variations in environmental conditions, such as geological background, climate, and pollution (Li *et al.*, 2016).

Principal component analysis of multi-element contents in wild *Eriocheir sinensis* samples from three water systems

By reducing the dimensionality of complex data, reproducible data were screened and original data were visualized in the form of various principal components (PCs) by PCA to concentrate and typically represent the data characteristics of original variables, thus fully reflecting the overall information (Ranamukhaarachchi *et al.*, 2017; Xu and Wang, 2020). In order to observe the clustering results of the samples and the presence of outliers, 15 elements with significant differences in wild *Eriocheir sinensis* samples in the three water systems were subjected to *Z*-score processing, followed by PCA. The results showed that the variance contribution proportions of top three principal components were 44.10%, 34.12%, and 6.76%, respectively, and the total contribution proportion was 84.98%. These three principal components generally comprised most of the information on the contents and composition of different elements that fully reflected the original data. Based on the loading patterns of principal components as shown in Figure 1, the main contributing elements that enriched the first principal component were Mn, Ca, Na, Fe, and Co in sequence; main contributing elements that enriched the second principal component were Zn and Cu in sequence, and the third principal component was mainly enriched by Pb. In all, eight elements (Mn, Ca, Na, Fe, Co, Zn, Cu, and Pb) were characteristic elements of wild *Eriocheir sinensis* samples collected from different water systems. After removing elements with highly overlapping information, the above-mentioned eight elements could collectively represent the data characteristics of original variables, providing basis for the construction of traceability models of wild *Eriocheir sinensis* samples from different water systems.

Table 2. Contents of mineral elements in wild *Eriocheir sinensis* samples from three water systems.

Sampling region	Element concentration (mg/kg, dry weight)							
	Na	Ca	Ni	Cu	Mn	V	Cr	Pb
SFH	9196.96±1334.559^{a}	3161.174±453.867^{a}	2.204±0.194^{a}	45.091±4.042^{a}	14.61±2.415^{a}	0.075±0.007^{a}	3.313±0.198^{a}	0.007±0.003^{a}
NLJ	4669.305±413.104^{b}	1946.753±192.260^{b}	1.794±0.09^{b}	53.677±7.255^{b}	1.381±0.262^{b}	0.059±0.002^{b}	2.702±0.123^{b}	0.011±0.004^{a}
LH	5375.116±228.274^{c}	2277.965±336.044^{b}	1.628±0.043^{c}	37.707±4.783^{c}	11.727±4.209^{a}	0.081±0.01^{a}	2.465±0.075^{c}	0.021±0.008^{b}
LOD	0.009	0.009	0.0015	0.0027	0.0018	0.0003	0.0006	0.0012
Sampling region	**Element concentration (mg/kg, dry weight)**							
	Mg	**Zn**	**Rb**	**As**	**Ag**	**Cd**	**Se**	**Co**
SFH	1784.666±194.428^{a}	171.558±7.386^{a}	6.301±0.168^{a}	1.121±0.033^{a}	0.127±0.012^{a}	0.003±0.004^{a}	1.035±0.089^{a}	0.136±0.007^{a}
NLJ	1290.907±56.73^{b}	176.465±8.469^{a}	14.184±0.928^{b}	2.681±0.615^{b}	0.235±0.027^{b}	0.007±0.006^{b}	1.622±0.185^{b}	0.102±0.006^{b}
LH	1090.172±37.462^{c}	139.141±6.497^{b}	3.728±0.368^{c}	1.333±0.128^{c}	0.132±0.033^{a}	0.004±0.006^{a}	1.023±0.071^{a}	0.102±0.006^{b}
LOD	0.009	0.0135	0.0003	0.003	0.0006	0.0003	0.006	0.0003
Sampling region	**Element concentration (mg/kg, dry weight)**							
	K	**Fe**	**Hg**	**Cs**	**Ba**	**U**	**Ga**	
SFH	14274.915±1170.631^{a}	39.021±4.285^{a}	0.85±0.088^{a}	0.012±0.001^{a}	4.142±0.682^{a}	0.003±0.001^{a}	0.032±0.002^{a}	
NLJ	10502.227±499.918^{b}	29.253±1.93^{b}	0.579±0.127^{b}	0.376±0.074^{b}	1.626±0.44^{b}	0.002±0.001^{a}	0.031±0.003^{ab}	
LH	9109.28±272.113^{c}	35.415±4.852^{a}	0.343±0.044^{c}	0.008±0.002^{c}	5.352±1.565^{a}	0.001±0^{b}	0.03±0.001^{b}	
LOD	0.009	0.0156	0.0003	0.0003	0.003	0.0003	0.0003	

Note: Different superscript letters ($^{a, b, c}$) in each column indicate significant differences ($P < 0.05$).

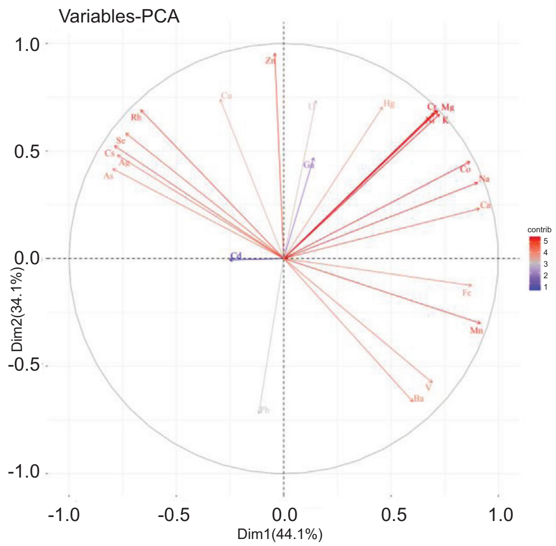

Figure 1. Principal component loading patterns of multi-element contents in wild *Eriocheir sinensis* samples from three water systems.

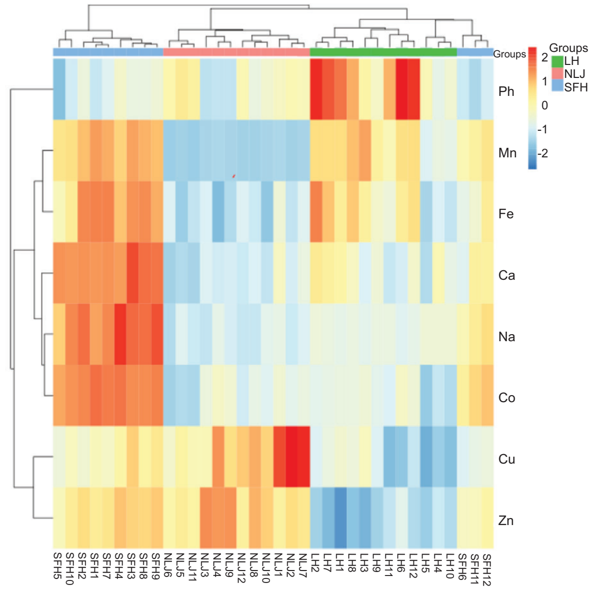

Figure 2. Hierarchical cluster analysis (HCA) heat map for geographic discrimination of wild *Eriocheir sinensis* samples from three water systems based on eight characteristic elements.

Hierarchical cluster analysis based on characteristic elements

Hierarchical cluster analysis is an unsupervised data analysis for visualizing intrinsic structure of datasets without presuppositions about the origin of samples. HCA based on the measured characteristics can identify relatively homogeneous clusters in different sample groups (Zhang *et al.*, 2020). For preliminary observation of discriminatory ability of eight characteristic elements (Mn, Ca, Na, Fe, Co, Zn, Cu, and Pb), these elements were used as variables to cluster the samples from three water systems using a systematic clustering method. Distance between samples was calculated through complete clustering using Euclidean distance. The clustering results (Figure 2) demonstrated that cut in dendrogram at a Euclidean distance of 4 could roughly classify samples into three categories, with the first category consisting of 9 samples, all from the SFH water system, the second category consisting of 12 samples, all from the NLJ water system, and the third category comprising 15 samples (3 from SFH and 12 from LH water systems). Overall, the results of clustering analysis set up samples from the NLJ water system individually as a category; however, there was erroneous discrimination between SFH and LH samples, possibly because of the close geographical distance and similar fingerprint characteristics of geological and environmental background elements between two river systems. The clustering analysis suggested that the aforementioned eight characteristic elements had certain ability in exploratory hierarchical clustering of samples from three water systems, although the clustering effect was not satisfactory.

Stepwise linear discriminant analysis based on characteristic elements

Linear discriminant analysis was a supervised data classification method with known samples used as training sets under the premise of assumed sample classification, in which a discrimination model could be established based on correlations between samples of different categories and then applied to discriminate and classify unknown samples. A comprehensive and quantifiable origin traceability model was achieved. The "leave-one-out method" was used for cross-validation of model, that is, samples in the training set, except a certain sample, were used as the training set, and the relevant discriminant function was established to discriminate samples (Luo *et al.*, 2019). In this way, each sample could be used as a test set to verify the discriminant function established using the remaining samples, thereby examining the stability of discrimination model. S-LDA was performed to determine the most useful variables on the basis of LDA, to eliminate the interfering information, and to discriminate geographical origin with fewer variables (Mamede *et al.*, 2022). In this study, characteristic elements (Mn, Ca, Na, Fe, Co, Zn, Cu, and Pb) derived from PCA were used to construct S-LDA model. The results exhibited that the predicted classification accuracy achieved for the samples from the three systems was 100%, and the discrimination accuracy in cross-validation reached 100% (Table 3).

Through this S-LDA model, two canonical discriminant functions (Function 1 and Function 2) were established

Table 3. The predicted classification and accuracy of discriminant function.

	Classification results[a,c]									
	Original					Cross-validated[b]				
	Sampling region	Predicted group			Total	Sampling region	Predicted group			Total
		SFH	NLJ	LH			SFH	NLJ	LH	
Count	SFH	12	0	0	12	SFH	12	0	0	12
	NLJ	0	12	0	12	NLJ	0	12	0	12
	LH	0	0	12	12	LH	0	0	12	12
%	SFH	100.0	0	0	100.0	SFH	100.0	0	0	100.0
	NLJ	0	100.0	0	100.0	NLJ	0	100.0	0	100.0
	LH	0	0	100.0	100.0	LH	0	0	100.0	100.0

[a]100.0% of original grouped cases were accurately classified.
[b]Cross-validation was performed for analyzed cases only. In cross-validation, each case was classified by the functions derived from all cases except that case.
[c]100.0% of cross-validated grouped cases were accurately classified.

with five elements (Na, Mn, Co, Cu, and Zn) finally screened from eight characteristic functions. Function 1 demonstrated a variance contribution rate of 81.6% and Function 2 had a variance contribution rate of 18.4%. With Function 1 as abscissa and Function 2 as ordinate, wild *Eriocheir sinensis* samples from three water systems were visualized by scatter plots (Figure 3), which could intuitively reflect that discriminant functions achieved good classification results for the samples from these three water systems.

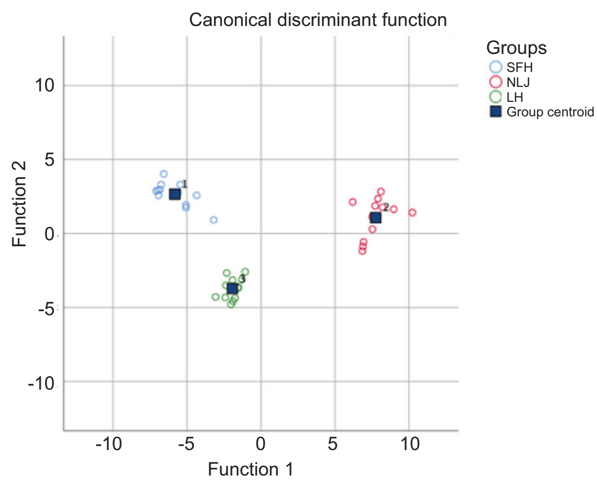

Figure 3. Classification diagram of canonical discriminant functions

Human health risk assessment

A quantitative health risk assessment was conducted for 19 detected trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Ag, Cd, Cs, Ba, Hg, Pb, and U). The reference dose (RfD) of each element was decided as reported in the literature (Bai *et al.*, 2022; Zhao *et al.*, 2012; Zhou *et al.*, 2021). The EDI of trace elements in human body was calculated based on the mean value of each trace element in wild *Eriocheir sinensis* samples (converted by 80% moisture content) (Wang *et al.*, 2022c) using Equation (1), and the THQ of each trace element was calculated using Equation (2), as outlined in Table 4. The EDI values of trace elements were lower than RfD values (Zhou *et al.*, 2021). Additionally, the THQ value of each element in the muscle of wild *Eriocheir sinensis* from three water systems was less than 1. Therefore, normal (not excessive) consumption of wild *Eriocheir sinensis* would not be a significant health risk to consumers.

However, in this study, the THQ values of Hg and As were relatively high. The THQ values of Hg in wild *Eriocheir sinensis* from three water systems were 0.58, 0.39, and 0.23, respectively, and the THQ values for As in three water systems were 0.25, 0.61, and 0.30, respectively. Hg and As are global pollutants extensively distributed in the environment. After Hg and As pollutants enter the water, they firmly bond with massive suspended particulate matters present in the water, which are then sunk and adsorbed by sediments into the bottom of the water. After pollution, their resolution rates are very slow (Jannetto and Cowl, 2023; Wang *et al.*, 2011). Aquatic organisms have a strong enrichment ability for As and Hg, and aquatic products are regarded as the main source of these two heavy metals for human consumption (Song *et al.*, 2016; Sun *et al.*, 2013). Hg entering aquatic products exists in the form of alkyl mercury, in which methyl mercury is hypertoxic and easily penetrates the blood–brain barrier, causing severe damage to the nervous system (Hedayati *et al.*, 2012). As includes organic As and inorganic As, with stronger toxicity of inorganic As. Long-term exposure to inorganic

Table 4. Estimated daily intake (EDI, mg/kg/d) and target hazard quotient (THQ) of different elements in wild *Eriocheir sinensis* samples from three water systems.

Element	EDI (mg/kg/d)			RfD (mg/kg/d)	THQ		
	SFH	NLJ	LH		SFH	NLJ	LH
V	5.10×10^{-6}	4.00×10^{-6}	5.50×10^{-6}	0.009	0.57×10^{-3}	0.44×10^{-3}	0.61×10^{-3}
Cr	2.24×10^{-3}	1.83×10^{-3}	1.67×10^{-3}	1.5	0.15×10^{-3}	0.12×10^{-3}	0.11×10^{-3}
Mn	0.99×10^{-3}	0.09×10^{-3}	0.79×10^{-3}	0.14	7.07×10^{-3}	0.67×10^{-3}	5.67×10^{-3}
Fe	2.64×10^{-3}	1.98×10^{-3}	2.40×10^{-3}	0.7	3.77×10^{-3}	2.83×10^{-3}	3.43×10^{-3}
Co	9.23×10^{-6}	6.88×10^{-6}	6.90×10^{-6}	0.03	0.31×10^{-3}	0.23×10^{-3}	0.23×10^{-3}
Ni	0.15×10^{-3}	0.12×10^{-3}	0.11×10^{-3}	0.02	7.46×10^{-3}	6.07×10^{-3}	5.51×10^{-3}
Cu	3.05×10^{-3}	3.63×10^{-3}	2.55×10^{-3}	0.04	76.3×10^{-3}	90.9×10^{-3}	63.8×10^{-3}
Zn	11.6×10^{-3}	11.9×10^{-3}	9.42×10^{-3}	0.3	38.7×10^{-3}	39.8×10^{-3}	31.4×10^{-3}
Ga	2.14×10^{-6}	2.13×10^{-6}	2.01×10^{-6}	0.3	7.12×10^{-6}	7.09×10^{-6}	6.71×10^{-6}
As	0.08×10^{-3}	0.18×10^{-3}	0.09×10^{-3}	0.0003	0.25	0.61	0.30
Se	0.07×10^{-3}	0.11×10^{-3}	0.07×10^{-3}	0.005	14.0×10^{-3}	22.0×10^{-3}	13.9×10^{-3}
Rb	0.43×10^{-3}	0.96×10^{-3}	0.25×10^{-3}	0.005	0.09	0.19	0.05
Ag	8.60×10^{-6}	15.9×10^{-6}	8.97×10^{-6}	0.005	1.72×10^{-3}	3.19×10^{-3}	1.79×10^{-3}
Cd	0.17×10^{-6}	0.46×10^{-6}	0.24×10^{-6}	0.001	0.17×10^{-3}	0.46×10^{-3}	0.24×10^{-3}
Cs	0.78×10^{-6}	25.5×10^{-6}	0.57×10^{-6}	0.001	0.78×10^{-3}	25.5×10^{-3}	0.57×10^{-3}
Ba	0.28×10^{-3}	0.11×10^{-3}	0.36×10^{-3}	0.2	1.40×10^{-3}	0.55×10^{-3}	1.81×10^{-3}
Hg	0.06×10^{-3}	0.04×10^{-3}	0.02×10^{-3}	0.0001	0.58	0.39	0.23
Pb	0.49×10^{-6}	0.76×10^{-6}	1.41×10^{-6}	0.0015	0.32×10^{-3}	0.51×10^{-3}	0.94×10^{-3}
U	0.17×10^{-6}	0.16×10^{-6}	0.05×10^{-6}	0.003	0.06×10^{-3}	0.05×10^{-3}	0.02×10^{-3}

As can lead to liver, kidney, and lung diseases as well as damage to the skin and reproductive and nervous systems. The International Agency for Research on Cancer (IARC) has classified As as a human carcinogen element (Tchounwou *et al.*, 2012). Agricultural activities, industrial discharges, and urban sewage were the three main sources of accumulation of heavy metals in wild *Eriocheir sinensis*. The use of additives and pesticides containing As must be strictly restricted, and exposure risk assessment and sources of As and Hg must be further studied in wild *Eriocheir sinensis*.

Conclusions

In this study, inductively coupled plasma-mass spectrometry identified 23 elements in muscle samples of wild *Eriocheir sinensis* from three water systems, and the content proportion of heavy metals, such as Cd (<0.5 mg/kg), Pb (<0.5 mg/kg), As (inorganic arsenic <0.5 mg/kg), and Cr (<2.0 mg/kg), in all *Eriocheir sinensis* samples was far below the standard limit stipulated in GB 2762-2022 Limits of Contaminants in Foods. The elemental composition of different water systems has certain characteristics. Three water systems exhibited significant differences in 10 elements, such as Na, Mg, K, Cu, As, Ni, Cs, Hg, Cr, and Rb ($P < 0.01$). Cd had no noticeable difference in three water systems, while other elements exhibited differences of different degrees in three water systems, indicating the impact of regional environmental conditions, such as geological background and climate. These results suggest that the multi-element analysis combined with chemometrics is able to realize the discrimination of geographic origins in wild *Eriocheir sinensis*. The food health risk assessment demonstrated that the THQ values of the analyzed trace elements in wild *Eriocheir sinensis* muscle samples from three water systems were all less than 1; therefore, normal consumption of wild *Eriocheir sinensis* would not present an obvious health risk to consumers, but excessive consumption must be avoided. However, the THQ values of two elements, Hg and As, were relatively high and should be enrolled as key monitoring elements in the future studies.

Conceptualization

The manuscript was written and revised by Shuyan Bai and Dongli Qin. Experiment design, execution, sample collection and were performed by Shihui Wang, Dongli Qin, Lei Gao, Qirui Hao, Ningning Du. Data collection and analysis were performed by Shuyan Bai, Shihui Wang and Dongli Qin. Financial support by Shuyan Bai, Peng Wang, and Dongli Qin. All authors read and approved the published version of the manuscript.

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