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Trace elemental characterization of some food crustacean tissue samples by EDXRF technique

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Abstract

The trace elemental analysis of two species of mud crab and three species of prawn samples from Chilika lagoon, Orissa, India has been carried out by using Energy Dispersive X-ray Fluorescence (EDXRF) technique available at Institute of Physics, Bhubaneswar. Elements namely K, Ca, Mn, Fe, Cu, Zn, Se, Br, Sr and Pb have been measured in the present investigation. The study indicates the effectiveness of the technique in analyzing biological materials like tissue samples and opens a door for easy analysis of seafood items with a easy, fast, sensitive, simultaneous multi-elemental technique with a simple sample preparation procedure without any chemical treatment. Though all the specimens were collected from the same environment of the lagoon the elemental variation might be due to the differential migration pattern and metabolism or other biological factors.
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Keywords: Trace elements; Mud crabs; Prawn; Chilika lagoon; EDXRF

1. Introduction

Trace elements are continuously released into the aquatic environment via natural and anthropogenic influx. The determination of the trace elements in seafood is of interest because of the importance of many of these elements to human health (Yebra-Biurrun and Garcia-Garrido, 2001). This interest arises from three areas of concern i.e. nutritional, toxicological and environmental; nutritional because elements such as Mg, Al, Ca, Fe, Co, Cu and Zn are necessary for the maintenance of optimum health, toxicological since certain elements such as Pb, Cd, As and Hg are detrimental for optimum health, and environmental as tissue samples are used as bio-indicators to assess bioavailability and contaminant concentrations in coastal water. Many investigations have been carried out towards monitoring the concentration of various elements in aquatic and biological samples collected from various natural environments (Bryan, 1976; Bu-Olayan and Subramanyam, 1996; Hota et al., 2001; Alasalvar et al., 2002; Ashok et al., 2003). On the other side, the consumption of seafood is a significant pathway to metal exposure in the human population living in coastal areas (Hasmi et al., 2001) for which the accurate analysis of concentration of trace elements from different seafood items is essential.

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Various spectroscopic techniques like Particle Induced X-ray Emission (PIXE), Atomic Absorption Spectroscopy (AAS), Neutron Activation Analysis (NAA) etc. are used for the trace elemental characterization of biological materials. However this study has demonstrated the analysis the muscle tissue samples of food crustaceans by EDXRF technique for the first time. A very few investigations have justified the application of EDXRF technique in analyzing human blood samples (Hota et al., 2001; Custodio et al., 2005) and fish tissue samples (Carvalho et al., 2005). The present study is also a first time report on the elemental concentration of seafood crustaceans from Chilika lagoon, the largest coastal wetland along the east coast of India and a much focused *Ramsar* Site of international importance. Brachyuran mud crabs (*Scylla serrata* and *Scylla tranquebarica*), Penaeid prawns (*Penaeus monodon* and *Penaeus indicus*) and fresh water prawn *Macrobrachium rosenbergii* forms the major crustacean fishery from the Chilika lagoon. The interest in these species was motivated by the fact that these species are among the highly demanded crustacean food items both locally and as for export commodity.

2. Materials and methods

2.1. Study area

Chilika lagoon (Fig. 1) on the east coast of India 19° 28′ N–19° 54′ N and 85° 05′ E and 85° 38′ E is the largest coastal wetland with estuarine character in the Indian sub-continent, fluctuating in size during the year from a monsoon maximum of 1165 km^2 to a dry season minimum of 906 km^2; the linear axis is 64.3 km and the average mean width is 20.1 km (Mohapatra et al., 2007). The lagoon is separated from the Bay of Bengal by a sandbar between 100 m and 1500 m wide; an outer 30 km channel connects the main lagoon with the Bay of Bengal (World Bank, 2005). The lagoon is broadly divided into four ecological sectors; these sectors are called as northern sector, central, southern and outer channel sectors (Fig. 1). The lagoon is shallow with the minimum depth of 0.3 m and maximum of 6.8 m in northern and outer channel sectors respectively. Magarmukh is the gateway between the main lagoon and the outer channel. Hydrologically, Chilika lagoon is influenced by three sub-systems, the Mahanadi distributaries

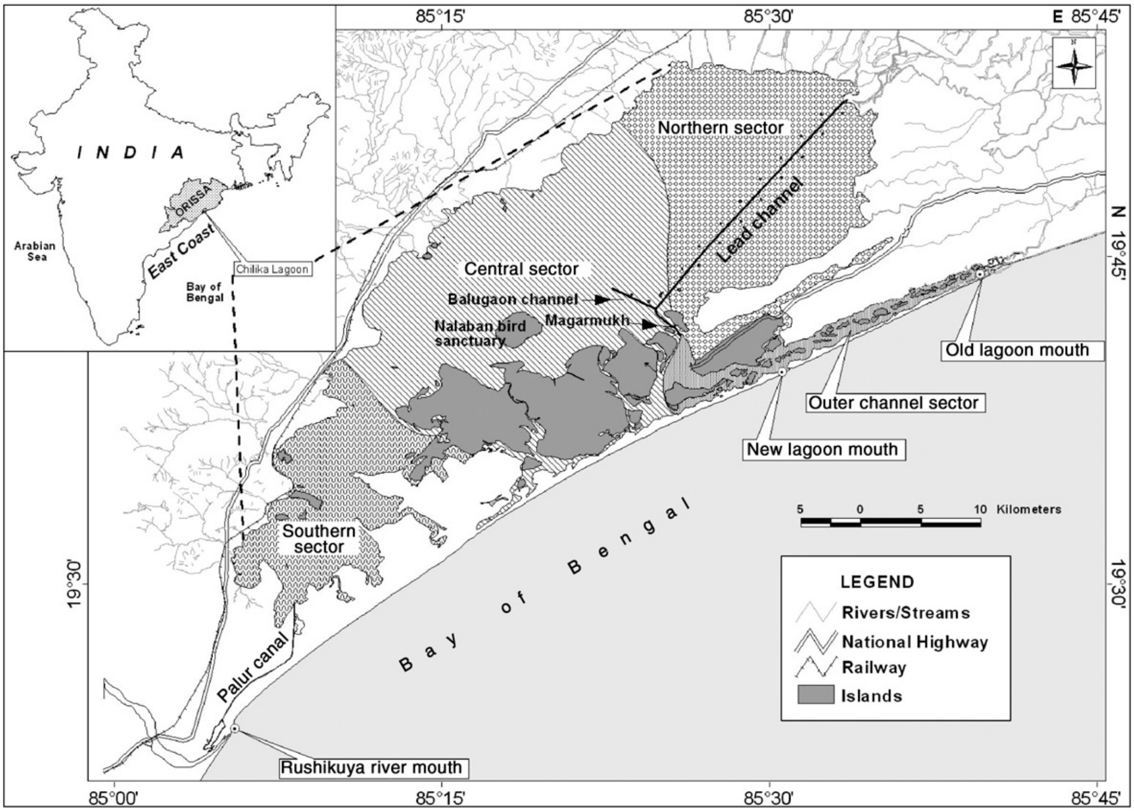

Fig. 1. Location map of Chilika lagoon showing the sampling station.

(Delta Rivers), 52 rivulets and streams from western catchments and the Bay of Bengal that maintain the estuarine character of the ecosystem (Mohapatra et al., 2007). The tidal range is about 80 cm in the sea inlet area and a few centimeters (<10 cm) in the inner portions, water circulation is mainly wind driven (Panda et al., 1995). The catchment area of the lagoon is 4406 km^2 and total inflow of fresh water has been estimated at 14,331 M m^3 (Panigrahi et al., 2007). The anthropogenic activities related to the processes influencing the quality of Chilika lake water are agricultural drainage, river runoff (Panigrahi et al., 2007) as well as untreated domestic solid and liquid wastages from 141 villages around the lagoon (Ghosh et al., 2006). Approximately 550 million litres day^{-1} of untreated sewage of capital city Bhubaneswar is discharged into the river Daya and ultimately to the Chilika lagoon (Ghosh et al., 2006). However the lagoon is free from industrial effluents.

The sampling station, in the outer channel of Chilika lagoon, is greatly influenced by the two antagonistic hydrological processes i.e. seaward flow of fresh water from Northern sector and tidal influx from the Bay of Bengal. This dynamic habitat with growth of sea grass meadows provides better habitat conditions for both crabs and shrimps (Mohanty et al., 2006). The mean depth at this site is 1.6±0.6 m.

2.2. Sampling and experimental analysis

Mud crab and prawn samples were collected from a single sampling site near Magarmukh of Chilika lagoon, Orissa (Fig. 1). The specimens were caught by monofilament twine box net traps, a common gear used for the catch of crabs and prawns in Chilika lagoon. The specimens of *S. serrata*, *S. tranquebarica* and *M. rosenbergii* were in adult and intermoult stage where as the specimens of *P. monodon* and *P. indicus* were in sub-adult and intermoult stage. The prawn species, *P. monodon* and *P. indicus* used to migrate to the sea at their adult stage. The numbers of specimens selected for the each species were nearly of identical size and the detail body size of the specimens was presented in Table 1. The crab and the prawn samples were thoroughly washed in de-ionised water after sacrificing. All the samples were collected in the same day during post monsoon (October 2006). Their muscles were separated by using sterilized stainless steel scalpels and forceps in order to avoid contamination. The samples were lyophilized at the Regional Medical Research Center, Bhubaneswar and subsequently powdered by the help of agate mortar for EDXRF analysis. From each individual sample, 1 g of pure powdered sample was taken to make thick pellets of 25 mm diameter size. A low power (50 W) tungsten anode, air-cooled X-ray tube and a PC-based multi-channel analyzer (MCA) constitute the EDXRF system. A Mo secondary exciter was irradiated with X-ray tube (operating voltage: 30 kV, current: 0.6 mA) and then the characteristic K X-rays from the secondary exciter were used to irradiate the pellets. Characteristic X-rays from the samples were detected by using a Si (Li) detector, which has a resolution of 170 eV at 5.9 keV. The signals from the detector were amplified, fed to a PC-based MCA and recorded. The MCA was calibrated using a Fe-55 X-ray source.

Table 1
Biometric data of the crustacean samples from Chilika lagoon

Species	Sex	Average carapace width (crab)/length (prawn) in mm	Average body weight
S. serrata	M	106±8	243±17
	F	104±6	202±13
S. tranquebarica	M	141±6	481±22
	F	138±3	386±10
P. monodon	M	186±3	54.2±6.1
	F	179±4	49.6±4.7
P. indicus	M	128±4	19.6±2.8
	F	123±2	18.3±0.8
M. rosenbergii	M	184±5	70.3±8.6
	F	166±3	45.5±4.6

M: male F: female.

The spectral data were analyzed using the computer based program AXIL (Nayak et al., 2004). The elemental concentration was determined using the equation,

$$m_j = N_{ij}/I_0 G \varepsilon \sigma_{ij} \beta_i, \tag{1}$$

where m_j is the concentration (g/cm^2) of jth element present in the sample, N_{ij} is the net counts per unit time for the ith group of X-rays of jth element, I_0G is the intensity of the exciting radiation incident on the sample visible to the detector, ε is the detector efficiency for the jth element, σ_{ij} is the theoretical X-ray fluorescence cross-section at 17.8 keV excitation energy and β_i is the self-absorption correction factor that accounts for absorption of incident and emitted X-rays in the sample.

3. Results and discussion

In recent years, various spectroscopic techniques like Particle Induced X-ray Emission (PIXE), Neutron Activation Analysis (NAA) and EDXRF have attracted attention due to their prominent characteristics like multi-elemental and non-destructive nature. An inevitable demand for accurate charge measurement, an

Table 2
Comparison of EDXRF with other conventional trace elemental analysis techniques

Analytical methods	Minimum quantity of the sample	Minimum detectable quantity	Multi-elemental analysis	Non-destructive analysis	Data acquisition time	Cost effectiveness	Accuracy of high Zn elemental concentration
AAS	0.1–1 g	10^{-6}–10^{-7} g	No	No	Few hours	Cheaper	Better
ICP-MS	10–50 mg	10^{-9}–10^{-12} g	Yes	No	Few minutes	Expensive	Better
NAA	Few mg–few g	10^{-9}–10^{-12} g	Yes	Yes	Few minutes to few hours	Expensive	Good
PIXE	0.001–0.1 g	10^{-9}–10^{-12} g	Yes	Yes	Less than 10 min	Very expensive	Good
EDXRF	0.1–2 g	10^{-6}–10^{-9} g	Yes	Yes	Few minutes	Cheaper	Better

unreasonably long time of irradiation and the necessary requirement of an accelerator constitute the main drawbacks of PIXE. AAS requires a complicated, time consuming, and destructive sample preparation procedure, although it is possible to analyze multi-elements without destroying the matrix using multi-elemental lamps, but lamps with four or more elements are not recommended for all applications (Nayak et al., 2001). In this context, EDXRF is multi-elemental and is highly sensitive to measurement of elements with accuracy, and hence well suited for the routine analyses of biological materials. The strength of this technique mainly relies on a simple method involving preparation of samples, a reasonably short time of measurement and a relatively simple data analysis system. However, it should be mentioned here that in comparison to PIXE and NAA, EDXRF is of special interest for biologists as this technique is one way fast, sensitive, simultaneous and multi-elemental, on the other way cheap, simple to handle and needs less space, and any size of sample material could be qualitatively and quantitatively analyzed without sample chemical pretreatment. The advantages and disadvantages of different traditional techniques with EDXRF are given in Table 2.

In EDXRF study, the validity of X-ray yield data for the tissue samples was evaluated by analyzing NIST Bovine liver (1577b) international standard and the comparison of measured values with the certified values is provided in Table 3. The NIST Bovine liver (1577b) was also used for standardizing EDXRF set up. It is evident that the analytical results of the standards are in good agreement with the NIST certified values. From this, it is evident that the experimental procedure adopted is reliable for analyzing these tissue samples.

The trace element data from tissues of mud crab species (*S. serrata*, *S. tranquebarica*) and prawn samples (*P. monodon*, *P. indicus* and *M. rosenbergii*) are presented in Table 4. The average K concentration in these tissue samples ranged from 8040 ppm in *S. tranquebarica* to 10,342 ppm in *S. serrata*. The average Ca concentration in tissue samples of crustaceans ranged between 616 ppm (*M. rosenbergii*) and 2846 ppm (*S. tranquebarica*). The Ca concentration in tissue samples of both mud crab species was observed higher than that of the three species of prawn samples. However, the K and Ca concentrations in the present study were observed to be lower as compared to the values reported for the fresh water crab from Vietnam (Brauer et al., 2001). The average Mn concentration in crustacean tissue samples ranged from 11.22 ppm (*S. tranquebarica*) to 15.17 ppm (*P. monodon*). The Mn concentration was observed comparatively higher in *P. monodon* tissues than the other four species. The average Mn concentration is much higher in comparison to other studies from crustacean samples as represented in Table 5 but the average concentration was lower than that reported for fresh water prawn from Hong Kong coastal water (Rainbow, 1986) and for the fresh water crab from Vietnam (Brauer et al., 2001). The average concentration of Fe ranged from 107 ppm in *P. indicus* to 297 ppm in *P. monodon*, which is observed to be much higher

Table 3
Observed concentrations (in ppm) of various elements in certified reference materials (CRMs) by EDXRF technique

Elements	Certified Reference Materials (CRMs) Bovine liver (NIST-1577b)	
	Certified value	Measured value
K	9940±20	9903±636
Ca	116±4	116±15
Mn	10.5±1.7	10.2±1.4
Fe	184±15	201±21
Cu	160±8	148±11
Zn	127±16	130±9
Se	0.73±0.06	0.69±0.05
Br	9.7	12.0±1.2
Sr	0.136±0.001	0.146±0.001
Pb	0.129±0.004	0.13±0.03

Table 4
Concentration of various trace elements (in ppm) in five different crustacean muscle tissue samples from Chilika lagoon, India

Elements	*S. serrata* (*n*=16)	*S. tranquebarica* (*n*=8)	*P. monodon* (*n*=6)	*P. indicus* (*n*=6)	*M. rosenbergii* (*n*=6)
K	10,342±375	8040±340	9095±342	8695±250	10,016±207
Ca	1961±11	2846±12	665±19	824±17	616±15
Mn	12.3±2.8	11.2±2.5	15.2±3.3	11.8±2.4	11.5±1.7
Fe	167±6	156±6	297±8	107±4	164±4
Cu	127±13	121±12	77±11	70±8	98±6
Zn	291±37	270±36	58±21	54±15	78±12
Se	0.36±0.10	0.40±0.10	0.33±0.14	0.37±0.10	0.24±0.02
Br	152±11	295±23	27±6	19±5	19±5
Sr	9.1±1.2	13.5±1.3	2.55±1.3	2.52±1.2	0.51±0.11
Pb	0.164±0.09	0.201±0.08	0.147±0.10	0.352±0.10	0.10±0.09

n=the number of specimens analyzed.

compared to fresh water crab (*Somaniathelphusa sinensis*) muscle (Brauer et al., 2001) but the concentration is within the limits of the observed value by Rainbow (1986). The Fe and Mn concentration in *P. monodon* samples were observed higher than that of the *P. monodon* samples of Bay of Bengal as observed by Hossain and Khan (2001). Paez-Osuna and Ruiz-Fernandez (1995a,b) observed the higher concentration of Fe and Mn in estuarine individuals than that of the marine individuals. Cu concentration ranged from 70 ppm in *P. indicus* to 127 ppm in *S. serrata*. The Cu concentration was observed higher in both mud crab tissue samples than the prawn samples. The Cu concentration in the present study was observed to be quite higher than other studies (Anderlini et al., 1982; Hossain and Khan, 2001). Zn concentration ranged from 54 ppm in *P. indicus* to 291 ppm in *S. serrata*. The Zn concentration in both mud crab species was observed to be very high in comparison to the prawn samples. Mean concentration of Zn is more than as reported by Anderlini et al. (1982) and Brauer et al. (2001) whereas it is less in comparison to the reported data of Al-Mohanna and Subrahmanyam (2001). The Zn concentrations in all the three species of prawn samples were observed closer to that of Paez-Osuna and Ruiz-Fernandez (1995a) from Pacific Coast of Mexico. Slightly higher availability of Zn in exchangeable phase may be contributed to the bioavailability of Chilika lagoon (Panda et al., 2006) and the higher concentration of Zn in the tissues samples of mud crabs might be due to the higher availability of Zn in exchangeable phase. There is evidence to suggest that the decapods are capable of regulation of the level of Zn in their body (Rainbow, 1985; Paez-Osuna et al., 1995). The Se and Pb concentrations in all the five crustacean

Table 5
Concentration of various trace elements in tissues samples of crustaceans from different geographical locations in ppm

Geographical Area	Species	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Sr	Pb	Reference
Bay of Bengal	*P. monodon*	–	–	3.1–6.5	9.1–15.7	12.1–21.2	24.1–35.7	–	–	–	0.8–1.2	Hossain and Khan (2001)
Vietnam	*Somaniathelphusa sinensis*	13,700	15,200	28	56	62	420	1.3	40	49	–	Brauer et al. (2001)
Hong Kong coastal water	*Metapenaeopsis palmensis*	–	–	26–90	32–781	31–84	39–146	–	–	–	–	Rainbow (1986)
Worldwide	From 24 references	–	–	1.0	–	70	80.0	–	–	–	1	Bryan (1976)
Kuwait	From crustacean samples	–	–	0.98	–	14	46	–	–	–	0.7	Anderlini et al. (1982)
Kuwait	*Portunus pelagicus*	–	–	0.95	–	123.8	206	–	–	–	1.9	Al-Mohanna and Subrahmanyam (2001)
Pacific Coast of Mexico	*Penaeus vannamei*			4.54 ±3.84	53.9 ±9.0	27.5 ±2.4	70.4 ±4.9					Paez-Osuna and Ruiz-Fernandez (1995a,b)
Crab samples	*Scylla* sp.	9191	2404	12	161	124	280	0.38	223	11	0.18	Present study
Prawn samples	Three prawn species	9269	701	12.8	189	82	64	0.31	21	1.86	0.20	Present study

Table 6
Concentrations of trace elements (ppm) in muscle tissues of female and males in five crustacean samples from Chilika lagoon, India

Species	Sex	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Sr	Pb
S. serrata	F (*n*=8)	10,630±380	1897±12	13.47±2.94	172±7	132±13	307±39	3.05±0.11	148±10	9.78±1.22	0.20±0.01
	M (*n*=8)	10,054±360	2026±11	11.22±2.62	162±6	122±12	274±35	2.59±0.10	157±12	8.51±1.18	0.13±0.01
S. tranquebarica	F (*n*=4)	8451±391	2639±10	12.27±2.81	163±7	129±14	293±21	1.76±0.11	279±21	14.10±1.41	0.23±0.09
	M (*n*=4)	7628±289	3054±15	10.17±2.3	149±6	113±10	247±51	1.62±0.09	311±25	12.82±1.20	0.17±0.07
P. monodon	F (*n*=3)	9198±351	639±11	16.27±3.60	319±9	72±9	61±21	0.71±0.16	24±5	2.8±1.30	0.17±0.10
	M (n=3)	8993±333	691±27	14.08±2.92	274±7	83±12	56±18	0.61±0.11	29±7	2.30±1.30	0.12±0.10
P. indicus	F (*n*=3)	8867±261	786±13	12.36±2.51	128±5	64±7	61±18	0.39±0.10	17±4	2.70±1.30	0.37±0.10
	M (*n*=3)	8523±239	862±21	11.32±2.29	86±3	76±8	48±12	0.34±0.11	20±6	2.36±1.10	0.33±0.10
M. rosenbergii	F (*n*=3)	10,816±232	598±14	12.61±1.78	176±4	91±5	84±13	0.29±0.02	18±5	0.53±0.10	0.10±0.09
	M (*n*=3)	9216±182	633±16	10.40±1.62	152±3	106±8	73±12	0.20±0.02	20±5	0.49±0.12	0.10±0.09

M — males, F — females, *n*=number of samples.

species from Chilika lagoon were well within the permissible limits of sea foods in a human consumption point of view. The Pb concentration in the present study was very low in comparison to the studies of crustaceans from other parts of the globe (Bryan, 1976; Anderlini et al., 1982; Al-Mohanna and Subrahmanyam, 2001; Hossain and Khan, 2001). Also the average Se concentration observed in the present study was comparatively lower in comparison to the study from Vietnam (Brauer et al., 2001). The Br concentration in the present study was observed to be quite higher in both mud crab species in comparison to the fresh water crab muscle from Vietnam (Brauer et al., 2001) but in all three prawn species the Br concentration is comparatively lower. Also Brauer et al. (2001) indicated the incorrect reporting of the Br by microwave digestion procedure due to evaporation of unknown fraction for volatile nature of the element.

Sex based difference in the trace elemental concentrations were marked in the present study (Table 6). Some elements like K, Mn, Fe, Zn, Se, Sr and Pb were observed to be higher in the female tissues of all the five species. However Br was observed to be higher in male tissues of all the five species. Ca concentration was observed to be higher in males of both mud crab species but in all the three prawn species Ca concentration was observed to be higher in females. The Cu concentration was observed to be higher in females of both mud crab species but in all the three prawn species a higher concentration of Cu was observed in the males. Higher concentrations of Cu, Mn, Fe and Zn in the females than that of the males of *Panulirus inflatus* were also observed from the Mexican Pacific Coast (Paez-Osuna et al., 1995). Also higher concentrations of Fe, Zn and Pb in the muscles of females than males of *L. stylirostris* and Cu, Zn and Pb in females of *L. vannamei* than males from Altata-Ensenada del Pabellon Lagoon (S.E. Gulf of California) were observed by Ruelas-Inzunza and Paez-Osuna (2004). Differences in the trace elemental concentration among individuals of different sex could occur because of the variation in growth rates between the sexes (Paez-Osuna et al., 1995). Also differences in feeding habit (Paez-Osuna and Tron-Mayen, 1996) and metabolism in both sexes might be the result of differential trace elemental concentration.

The study indicated the effectiveness of the technique in analyzing biological materials like tissue samples and opens a door for further extensive study. Though all the five species were collected from the same environmental conditions, the wide variation of the different trace elements in the tissue samples gives an indication of differential migration pattern, feeding habit and metabolism of the species. A similar feeding habit (Mohapatra et al., 2004–05) or similar migration pattern and similar habitat might be the cause of comparatively similar composition of the trace elements like Mn, Fe, Cu, Zn, Se and Pb in both mud crab species.

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