Bio-accumulation of trace metals in Blue Swimmer Crab (*Portunus pelagicus*) in Sri Lanka

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Abstract

In this study, muscle of blue swimmer crabs (*Portunus pelagicus*) were analysed for the content of trace metal contaminants. The crabs were collected at the landing sites along the coast of Mannar, Negombo and down south. Totally 60 individuals were collected during the period of May to November, 2014 and the samples were kept in ice and sent to the laboratory. The weight, shell width, gender were determined and brown meat were taken for metal analysis. The concentration of Mercury (Hg), Cadmium (Cd), Arsenic (As), Lead (Pb), Copper (Cu), Ferrous (Fe) and Zinc (Zn) were determined after microwave digestion by Atomic Absorption Spectrophotometer (AAS). Cd, Pb and Hg concentrations in crab are important in food safety. Results showed that average Cd (ND-0.48 mg/kg), Hg (ND-0.26 mg/kg) and Pb (<0.52 mg/kg) concentration are lower than the maximum permitted level (Cd, Hg, and Pb, 0.5 mg/kg wet weight basis) by the European Union (EU) and Sri Lankan regulation. The EU and Sri Lanka have not set any maximum allowed levels for As in foodstuffs for human consumption. The study highlighted the suitability of *P. pelagicus* living in the studied area as a safe seafood when considering the contents of above trace metals present in their muscles.

Keywords: Blue swimmer crab, Trace metals, Spectroscopy, Food safety

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Introduction

Over 100 species of crabs are known worldwide, three species of edible crabs are popular among all communities in the country. They are in the order of preference *Scylla serrata*, *Portunus pelagicus* and *Portunus sanguinolentus* (Jayamanne, 1991). Blue Swimmer Crabs (BSC), *P. pelagicus* are generally found in sea, but also found in the lagoons. BSC also plays an important role in the Sri Lankan export fisheries. Creech (2014) reported that Sri Lanka had exported 2,842 tons including live, frozen and chill products of crabs during the period of January 2011-March 2012. The leading market for Sri Lankan BSC is Singapore (972 t) and United States (791 t) and the amounts were 37% and 28% respectively (Steve, 2014).

Trace metals are naturally found in relatively low amounts in the earth's crust. Investigation of the bioaccumulation of trace metals through aquatic food web is global importance and they can found in crab meat in high levels. If found at higher level and exceed the threshold level, they are not suitable for human consumption. The reason is bioaccumulation and biomagnification of heavy metals such as accumulation of Hg, Cd, Pb and As can be harmful to human health (Jinadasa *et al.*, 2010). Trace amounts of some metals, such as Fe, Zn and Cu are key metals for maintaining the metabolism of the human body. High concentrations of trace metals can be found in contaminated environments and when these trace metals enter into the human body through food, they can cause impaired mental and central nervous function and damages to vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative conditions including cancer (Jarup, 2003).

Trace metals such as As, Cd, Hg, Pb and inorganic tin (Sn) account for a majority of non-essential trace metals and causes food poisoning. Levels of As are naturally high in fish and seafood. Cd is mainly found in soil due to the contamination from insecticides, fungicides, sludge and commercial fertilizers. Thus, agricultural food products are mainly contaminated with Cd. Hg is an industrial pollutant as well as a by-product of volcanic emissions (Khan *et al.*, 2008).

The European Union (EU) has established maximum allowable concentrations of heavy metals in foodstuffs. The Food and Drug Administration (FDA) demands to enforce action to limit poisonous or deleterious substances in human food and animal feed, including Cd, Pb, Hg, etc. Toxic effects of non-essential trace metals such as Hg, Pb, Cd etc. had been reported in human, who consumed large amounts of seafood. Toxic trace metals can enter into marine organisms, which filter feed in contaminated seawater with such trace metal ions. Out of those metals, Cd is a commonly contaminated toxic metal in crab meat. Levels of Cd in the muscle meat of crab legs and claws (white crab meat) have been comparatively low. However, levels of Cd in the brown meat of crabs is often quite high and it is reported that this may be due to accumulation of Cd from crabs diet in their digestive organ (the hepatopancreas) which is a component of the brown meat (Davies *et al.*, 1981). Although there are maximum allowable limits set by EU for the level of Cd in some foods, including white crab meat, such limit is not recommended for the brown meat of crabs, due to the wide range of Cd concentrations reported by different countries (EU/EC, 2011).

Trace element concentrations in the tissues of BSC are well documented on a global basis, with a focus on non-essential elements such as Hg, Cd, Pb and As, and the essential elements like Cu, Zn and Fe for their role in human body (Ana *et al.*, 2012, Antonio *et al.*, 2010). The aim of this study was to evaluate concentration of the most common trace metals, Hg, Cd, As, Pb, Cu, Fe and Zn in BSC, *P. pelagicus* found in Sri Lankan waters and to conclude whether these levels are dangerous for human consumption, by comparing with the internationally recommended maximum allowable limits.

Materials and Methods

Study area and sample collection

Adult BSC samples were collected to represent fish landing sites in Mannar, Negombo and Southern coast of Sri Lanka during the period from May to November, 2014. The total BSC sample size (n) was 60; where 30 females and 30 males. Selected samples were individually wrapped by clean polythene bags, stored in ice and were transported to the Analytical Chemistry Laboratory, at the National Aquatic Resources Research and Development Agency (NARA). Before dissecting each individual, the carapace width, (CW, cm) and the total weight, (TW, g) were measured. Then each individual was properly cleaned by rinsing with distilled water to remove debris, plankton and other external adherent. Then, males and females were separated based on the shape of the abdomen and colour of the claws. The carapace of the BSC was opened and the edible parts of muscle tissues were removed with sharp forceps. CW, TW and sex were recorded for each individual that was sampled. Then, each sampled portion was homogenized using a mixer grinder (Sonica domestic, India). From the homogenized sample nearly 25-50 g of muscle portion was obtained for the study. All these homogenized portions were packed separately in polythene bags with proper labelling and were stored at −20 °C until further analysis.

All glassware used were cleaned and dried properly. All the standards and reagents were prepared using ultrapure water (Barnsted, Easy pure LF system, Dubuque, USA). All the chemicals were analytical reagent grade or better (Sigma Aldrich, USA). The standard solutions of Hg, Cd, Pb, As, Fe, Cu and Zn at 1,000 mg/L (Fluka, Switzerland) were used to construct calibration curves.

Sample preparation

One gram of homogenised samples were accurately weighed and pre-digested by treating with 10 mL of 65% (v/v) HNO_3 acid for 15 min at room temperature. Predigested samples were digested again using a microwave accelerated system, MARS-6 (CEM, Matthews, USA). The digested samples were used to prepare 50.00 mL aqueous solutions. All the experiments were carried out in duplicate.

Trace metal analysis:

Atomic Absorption Spectrophotometer (AAS) (Varian240 FS, Varian Inc., Mulgrave, Victoria, Australia) was used to determine the Hg, Cd, Pb, As, Fe and Cu levels. A graphite tube atomizer (Varian GTA-120) was used for Pb, Cd and As determination. Vapour Generation Accessory (Varian VGA 77) with closed end cell was used for determination of Hg. Spectra AA Varian AAS with a flame (AAS-240 FS) was used for determination of Zn, Cu and Fe levels. The calibration curves for the absorption of all the metals were performed with a series of standard solutions of particular metal at optimum wavelength. The reagent blank samples and spiked samples were aspirated into the AAS, subsequent to the calibration and the readings were recorded. Each analytical batch was consisted of quality control sample, spiked samples and reagent blanks. The quality control samples were digested by following the same procedure used in the preparation of BSC samples for the trace metal analysis.

Quality control procedure:

The accuracy of the analytical procedure for total Hg, Cd and As were determined by analyzing certified quality control material in the same manner as samples were analysed (canned fish muscle, T/07194 and canned crab meat, T/07192QC from Food Analysis Performance Assessment Scheme, FAPAS, Sand Hutton, York, UK). Pb, Fe, Zn and Cu spiked samples were routinely analysed in the same manner as the samples. The Analytical Chemical Laboratory at NARA participated in the proficiency testing programme of the FAPAS for total Hg, Cd and As during the studied period, the results were satisfactory (the Z value for Hg: 0.0, Cd: −0.3 and As: −0.2), Proficiency Testing Report 07215, July–August 2014. The average field blank, derived from sample field blanks, and three times of its standard deviation were used to evaluate the limit of detection (LOD). The limit of quantification (LOQ) was 3 × LOD.

Statistical analysis

Statistical analysis was performed by Statistical Package for Social Sciences, version 20 for Windows. The statistical significance of the correlation was reported at the $p < 0.01$.

Results and Discussion

The suitability of the analytical method was evaluated in terms of their respective LOQ, recovery value using certified quality control materials (only for Hg, Cd and As) and recovery value of spiked samples (Cu, Fe, Zn and Pb). The recoveries were maintained between 80-120% and the relative standard deviation values (RSD) were less than 15%. Data on standard quality control materials and spiked samples are shown in Table 1. The results obtained from spiked BSC samples and quality control material results indicated that the methods used for the determination of metal concentrations in the samples are suitable.

Table 1. Assigned values, determined values and recovery (%) of quality control samples (T-07192 and 07194 QC) and spiked recovery values (n = 7, *µg/kg)

Sample	Value	µg/kg	mg/kg					µg/kg
_	_	Hg	Cd	As	Cu	Fe	Zn	Pb
T-07192 QC	AV	95.68	5.62	13.36	_	_	_	_
	DV	94.47 ± 14.05	5.9 ± 1.02	11.0 ± 1.31	_	_	_	_
	R (%)	98.73	101.40	82.93	_	_	_	_
T-07194 QC	AV	141	4.99	1063	_	_	_	_
	DV	155.2 ± 17.2	*5.5 ± 0.7	*1,011 ± 114	_	_	_	_
	R (%)	110.07	*110.22	*95.1	_	_	_	_
Spiked sample	R(%)	_	_	_	89.30	99.12	83.80	86.57
LOQ (mg/kg)	_	0.07	*0.006	*0.34	0.01	0.15	0.43	0.52

AV-Assigned values   DV- Determined values R-Recovery

The body size distributions of the BSC show that there is a size difference between female and male crabs (Table 2). The largest size of female and male were 18.0 cm (502.58 g) and 17.5 cm (490.45 g) respectively. There was no significant difference ($p>0.05$) in the length and weight data between male and female crabs. According to the literature, CW of the crabs changes with maturation and habitat. In Egypt, India and Australia, the CW values are in the range of 11.2-17.5, 9.0-11.0 and 9.6-10.9 cm respectively (Deniz, 2013).

Table 2. The body size distribution of BSC samples

	Total weight (range), g	Width (range), cm
Average (all)	257.83 (58.02-502.58)	13.9 (9.2-18.0)
Male	267.62 (79.41-490.45)	13.8 (9.2-17.5)
Female	248.38 (58.02-502.58)	14.1 (10.0-18.0)

Table 3. Mean concentration of trace metals in BSC (mg/kg, wet weight basis)

	Hg	Cd	As	Pb	Cu	Fe	Zn
Avg (all)	0.08±0.06	0.15±0.11	4.45±3.50	0.22±0.21	7.97±5.30	13.41±13.24	22.04±8.96
Male	0.07±0.06	0.16±0.11	4.67±3.68	0.25±0.24	6.44±2.57	7.72±7.41	21.82±8.19
Female	0.09±0.08	0.14±0.09	4.22±3.36	0.20±0.18	9.49±6.77	19.10±15.32	22.25±9.80

According to Deniz, 2013, the level of Hg, Cd, As, Pb, Cu, Fe and Zn in the muscle tissues of BSC had been <LOD-0.26, <LOD-0.48, 0.10-18.47, <LOD-0.78, <1.52-23.52, <ND-49.03 and <2.51-44.21 mg/kg respectively. The quantitative relationship of the metal levels of the BSC generally have followed the sequence Hg<Cd<Pb<As<Cu<Fe<Zn. Deniz (2013) has reported that Cd, Pb, Cu, Zn and Fe levels in muscle tissue of BSC as 0.50-1.47, 0.10-0.52, 9.64-72.52, 37.80-178.61 and 7.31-25.51 mg/kg, respectively. In a similar study, Al-Mohanna and Subrahmanyam (2001) have reported that Pb, Cu, and Zn levels in BSC from the Kuwail Bay as 1.7-2.1, 110.2-142.8, and 188.7-221.7 mg/kg respectively. Deniz and Ozogul (2011) reported that at Mersin Bay in Turkey, the Cd, Pb, Cu, Fe and Zn concentrations of carapace muscle of BSC were 0.84-1.17, 0.22-0.43, 25.39-31.05, 13.15-23-90 and 101.40-108.64 mg/kg respectively. The above values are higher than the value observed in the present study. This may be due to the different geographical regions and their physio-chemical parameters, sexual maturation and size of individuals (Deniz and Ozogul, 2011; Viswanathan *et al.*, 2013). Chitrarasu *et al.,* (2013) have reported comparatively high level of trace metals in BSC in India and the levels (mg/kg) were 21.1±0.76 (Cu), 2.5±0.5 (Cd), 47.8±0.76 (Zn) and 2.8±0.35 (Pb). The authors have reported that it may be due to industrial anthropogenic activities such as fertilizer and cement factories.

In the present study, the levels of Cu and Fe were significantly higher in females than males. Soundarapandian *et al.,* (2010) have reported that Fe and Zn levels in the edible crab *Podophthalmus vigil* also were higher in males than females. Mehdi *et al.,* (2014) have recorded that there is no significant difference in Hg level between males and females of BSC living in Persian Gulf, Iran.

According to the maximum permissible level for Hg, Cd and Pb, recommended by the European Commission for crustaceans is 0.5 mg/kg (EU/EC-1881, 2006). The studied samples are safe to be consumed with respect to those trace metals.

Acknowledgement

The authors are grateful to the staff of the Analytical Chemistry Laboratory (ACL), National Aquatic Resources Research and Development Agency (NARA) for the necessary help provided for the laboratory analysis. Authors are also thankful to the staff of the Quality Control Unit, Ministry of Fisheries and Aquatic Resources Development for the support given during the collection of samples.

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