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Concentration of Trace Metals in the Squids (*Loligo duvauceli, Sepioteuthis lessoniana*) and Cuttlefish (*Sepia latimanus*) from the North-Western Coast of Sri Lanka

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Abstract Concentrations of ten essentials and non essentialstrace metals Hg, Mg, Fe, Zn, Cu, Ni, Cr, Cd, Pb and Co were determined in the muscles of two squids (*Loligo duvauceli;* n=24, *Sepioteuthis lessoniana;* n=27) and one cuttlefish (*Sepia latimanus;* n=12) species collected from Kalpitiya and Mannar area of Sri Lanka in 2010. Trace metals were analysed using Varian Atomic Absorption Spectroscopy (VGA, GTA and Flame AAS). The mean values of all trace metals in muscles of studied species were within the international safety limits and pooled mean concentration of trace elements were following order; Mg > Zn > Cu > Fe > Cr > Ni > Hg > Cd > Co >Pb.

Keywords: *squids, cuttlefish, Sri Lanka, trace metals*

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1. Introduction

Cephalopods landings and consumption have been increasing worldwide during the past decades. The main reason for this increasing is that cephalopods are a good protein and lipid source [1,2]. Cephalopods consumed throughout the world, both as food and as feed supplements since they are rich in taste and have few inedible parts, hence have great commercial value [3,4]. Cephalopods are consumed not only fresh, but also also manufactured into processed food in huge quantities such as dry frozen and chilled products not only that but also as bait in many parts of the world [2,3]. The demand for cephalopods gradually increases with the depletion of oceanic fish stocks. The world fishery production of cephalopods from major fishing areas has been recorded at3,652,632 MT in 2010. Squid catches represented around 67% of total cephalopods while octopus and cuttlefish accounted for 9.5% and 16% respectively [5]. However, researchers suggested that world cephalopods landing is only few present of potential landings [6]. Historically, the consumption of cephalopod products has been highest in country like Japan and they still predominate in fisheries for cephalopods as well as in developing new technologies for catching them [7]. The consumption of cephalopods was increased recently, not in traditional consumers, mainly as chilled, frozen and ready to eat products [8]. There are about 80 species of cephalopods of commercial and scientific interest distributed in the Indian seas [2]. In the past there was a little interest incephalopod fishery in Sri Lanka and very little organized effort has been reported on harvesting cephalopods in commercial scale. But cephalopods are caught as by-catch species in trawl nets, beach seines, gill nets and scoop nets. In Sri Lanka there were good trawling grounds as Wedge bank, Pedro bank, Palk bay and Gulf of Mannar regions. But the accessibility to some of these grounds was lost due to establishment of EEZ in 1976 and civil disturbances in the last few decades [9,10]. As a results of FAO, Sri Lankan cephalopods catches contributes only two species; cuttlefish, bobtail squids nei and total catch was 780 MT in 2010 [5].

Cephalopods are exclusively marine predators and voracious carnivores with a very high metabolism and conversion rates. They constitute a class of marine mollusks which are found in a great variety of habitat from coastal waters to very deep-ocean environments. They are benthic (e.g. octopus), nectobenthic (cuttlefish, bottlesquid), neritic and oceanic (mainly squids), and constitute a primary food source for many marine predators such as fish, marine mammals or seabirds [4,11,12]. There are about 650 species of squids in the world's oceans, of these 80 species are commercial and scientific interest in the Indian Ocean [13]. Cuttlefish belongs to order Sepioidea and squid belongs to order Teuthoidea. In Sri Lanka, there are about six cuttlefish species (*Nautilus pompilius*, *Sepia aculeata*, *Sepia latimanus*, *Sepia pharaonis*, *Sepia prashadi* and *Sepiellainermis*) and four squid species (*Euprumnaberryi*, *Loligo duvauceli*, *Loligosinghalensis* and *Sepioteuthis lessoniana*) [14]. Among those species *Sepia aculeata* (Pothdella), *Sepia pharaonis* (Gembidella, Pothudella), *Loligo duvauceli*

(Bothaldella) and *Loligosinghalensis* (Ahindella) are economically important.

Most aquatic ecosystems contain trace metals to some extent released from domestic, industrial and other anthropogenic activities as well as natural phenomena like volcanic activity [15,16,17]. Some metal like copper (Cu), Iron (Fe) and zinc (Zn) are essential for normal metabolic and physiological functions of fish while other metals like mercury (Hg), lead (Pb) and cadmium (Cd) have no known biological role. The main functions of essential elements in the body include the formation of skeletal structure, maintenance of colloidal systems, as well as regulation of acid base equilibrium. They are important components of hormones, enzymes and structural proteins. Sodium, potassium and chloride maintain homeostasis and the acid-base balance, while phosphorus and calcium are required for the formation of skeletal structure of the body. Copper and zinc are firmly associated with metal-dependent enzyme. Zinc is involved in numerous protein functions such as the carbonic anhydrase [4]. Aquatic animals including the cephalopods take up essential and non-essential metals during the normal metabolic mechanism, taken up in the body through foods, gills and skin and accumulate in their body tissues [4,12,18,19]. The Sri Lankan continental shelf is of high economic interest for present and future hydrocarbon exploration. Oil production activities could lead to increased releases of heavy metals resulting in increased bioaccumulation in marine biota, particularly in cephalopods, since they are very efficient accumulators of various trace elements [20]. As in most marine animals, trace elemats concentration may vary with biological factors specific to an individual, a population or a species, or with environmenatal factor [7]. Trace metals, such as Hg, Cd and Pb etc. are persistent pollutant bio-accumulated by marine animals, risking public health through seafood. There has been increasing concern over the safety of food items that may contain harmful chemicals. Independendently of the species, the habitat or the life span they display, cephalopods share the ability to accumulate inorganic and organic pollutants such as metals, PCBs or organochlorine pesticides [11]. Therefore many fish and fish products importing and exporting countries instituted regulations and guideline limits for quality requirements for many chemical and biological hazards including trace metals. The EU directive of 1881/2006 and 629/2008 set the maximum level of trace metalcontaminants in cephalopods (without viscera) species as; Cd, 1 mg/kg, Pb, 1mg/kg, Hg, 0.5 mg/kg in wet weight basis [21,22].

Overall, the bilogy, behavior and life cycle of cephalopods are still poorly known in many aspects even if Sri Lankan cephalopods have received considerable attention over the last decade. Trace elemements in cephalopods have received increasing attention in recent decades, particularly in Europe and in Japan as these mollusks play a major role both as predator and prey in marine ecosystems [7]. In deep water condition, Hg bioavailability seems to be enhanced in these deep environments because the absence of solar radiation and the low oxygen concentrations in the deep environment favors a high methylation rate by bacteria [23] and Cd is enriched in mesopelagic waters while depleted in the surface ocean because of its regeneration from sinking biological debris from epipelagic zone and its uptake by organisms at the surface [11]. Cephalopods disply relatively short life sapns, typically from one to two years. Hence, they are major interest for monitoring variations of pollutant concentrations in the cephalopod tissues reflect the bioavailability and metal variations in their immediate environment over relative short time scale [7,24]. Trace metals in cephalopods species have been extensively studied in various parts of the world, only small numbers of study done within Sri Lankan water. In this framework, the objective of this study was to provide baseline data on a wide range of trace metals in the 3 cephalopods species in the northwestern coast of Sri Lanka. Such baseline information is needed to quantify the impact of anthropogenic contaminant sources (e.g. oil production activities) on these species. To this end, the concentrations and tissue distribution of 10 metals Mg, Fe, Zn, Cu, Ni, Cr, Cd, Pb, Co, Hg were determined in the tissues. The recorded values were then compared to the data from the current literature for other cephalopod species.

2. Materials and Methods

Samples were collected from commercial fishery landings in Kalpitiya and Mannar which situated in Nort-western coast of Sri Lankain April to Sept. 2010. Two squids (*Loligo duvauceli;* n=24, *Sepioteuthis lessoniana;* n=27) and one cuttlefish (*Sepia latimanus;* n=12) species were selected for analysis (Figure 1). Samples were preserved in ice and transported to the analytical chemical laboratory, Institute of Post Harvest Technology (IPHT), National Aquatic Research and Development Agency (NARA). At the laboratory individuals were numbered and their mantle length, fin length, head length, arm length and total weight were recorded. Then the head was separated and head weight and corn weight were recorded. The skin, viscera and wings were removed and washed with de-ionized water and fresh fillet weight was recorded. All samples were oven dried at 102°C to a constant weight, ground and stored in airtight bags. The dried samples were used directly for metal analysis.

A CEM/MARS XP-1500+ microwave oven (CEM, Matthews, USA) were used for the microwave assisted digestion of cephalopods samples. The program has a working pressure 800 psi and can operate at temperatures up to 200°C. For microwave digestion 0.5 g of samples was accurately weighed directly into the PTFE microwave vessels. To each samples 10 mL of Conc. HNO_3 (Sigma-Aldrich, St. Louis, MO, USA) were added. The analytical reagent blanks were also prepared and these contained only the acids. All samples, reagent blanks and spiked samples were taken in duplicately. The vessels were sealed and placed into the rotor for microwave digestion. After the digestion process, the digested liquids were transferred to the 50 mL volumetric flask and volume up using de-ionized water. Metal concentrations were determined using Atomic Absorption Spectrophotometer (AAS) model Varian 240-FS Varian Australia, Pty Ltd, Mulgrave, Victoria, Australia). The Hg was determined using Varian VGA-77 after reducing with stannous chloride. The Mg, Fe, Zn and Cu were determined using Varian flame AAS an oxidizing air-acetylene flame. As well as Ni, Cr, Cd, Pb and Co were analysed using Varian GTA-120. Then the data were recorded in excel 2010 and statistically analysed. To avoid metal contamination, all glass and plastic utensils used were washed with ditergent, soaked in a bath of nitric (15%) acid for a minimum 24 h, rinsed three times in deionised (Milli-Q quality) water and dried in an oven at 50°C before use.

Data on the trace metals evaluation of different species was analyzed by one-way analysis of variance (ANOVA) using SPSS-16 version software followed by Tukey's test/Duncan's multiple range t est (DMRT). $P \leq 0.05$ were considered as significant.

L. duvauceli *S. lessoniana* *S. latimanus*

Figure 1. Studied squids and cuttle fish species

3. Results and Discussion

Quality assurance procedures included the use of analytical grade reagents, method blanks and matrix spikes. The suitability of the analytical method was evaluated in terms of their respective limits of detection, precision and recoveries using spiked samples. The recoveries were maintained between 80-120% and the relative standard deviation (RSD) values were less than 10%. The results obtained from spiked squids and cuttlefish samples indicated that the methods used were suitable for the determination of metal concentrations in the samples investigated.

The mantle length of *L. duvauceli*, *S. lessoniana* and *S. latimanus*were 10.8, 20.4 and 9.4 cm as well as totalweights were 23.4, 475.3 and 114.7 g respectively. The finding of the [7] mentioned that mantle length of the squids to be the most correlated to the concentration of trace elements.

Trace metals concentration in the mantle of 3 species is reported in Table 1.

Table 1. Trace element concentrations in 3 species, given as mean, standard deviations and ranges of the metal concentrations in dry weight (dw) basis. The symbol ND indicates concentrations below the detection limits

Metal	*L. duvauceli*		*S. lessoniana*		*S. latimanus*	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Cu (mg/kg)	20.33 ± 12.96	4.90-36.54	0.59 ± 0.45	0.11-1.60	0.92 ± 0.35	0.39-1.49
Fe (mg/kg)	3.32 ± 1.33	2.17-5.23	3.08 ± 3.19	0.04-13.16	2.80 ± 1.30	1.03-4.51
Mg (mg/kg)	71.96 ± 6.80	65.67-78.61	69.17 ± 14.22	39.54-112.14	83.16 ± 27.57	43.51-120.29
Zn (mg/kg)	26.53 ± 1.77	24.00-27.77	17.65 ± 3.27	11.50-25.41	17.70-4.61	9.11-24.01
Co (µg/kg)	18.28 ± 13.12	9.81-37.83	6.42 ± 13.71	ND-61.86	32.05 ± 29.77	7.26-106.31
Cd (µg/kg)	27.53 ± 10.42	17.05 ± 41.96	16.01 ± 8.67	ND-32.94	21.24 ± 28.05	ND-84.44
Cr (µg/kg)	182.29 ± 138.36	59.10-346.77	16.61 ± 22.03	ND-78.46	76.47 ± 34.01	33.77-124.55
Ni (µg/kg)	7.64 ± 1.70	5.91-9.47	109.97 ± 99.11	14.05-455.95	18.46 ± 11.87	5.54-40.78
Hg (µg/kg)	51.94 ± 12.95	32.75-61.05	33.08 ± 24.16	9.49-88.37	30.33 ± 17.86	10.16-68.20
Pb (µg/kg)	24.37 ± 12.18	7.63-36.72	5.88 ± 6.89	ND-26.92	6.43 ± 11.80	ND-34.83

3.1. Copper

Comparatively higher level of copper was found in *L. duvauceli* (20.33 mg/kg) and the lower level was recorded *S. lessoniana* (0.59 mg/kg) in wet weight basis. According to the previously studied of Prafulla*et al* (2001), showed that Cu concentration of muscle of *L. duvauceli* ranged from 0.15 – 13.8 ppm. An interesting elements of cephalopods is Cu, is an important component of respiratory pigments and hemocyanin represents 98% of the blood proteins in cephalopods [25,26]. As his study 85% of the total copper in *Octopus vulgaris* is bound to this respiratory pigment and the hemocyanin represents 98% of the blood protein in cephalopods. Therefore copper is required in large concentrations.

3.2. Iron

Iron is the most abundant transition element, and probably the most well known metal in biologic systems [19]. Iron is a component of a number of proteins such as ferreting [25]. The edible corn of the *L. duvauceli* is rich in iron content (3.32 mg/kg), particularly in *S. latimanus* is lower in iron concentration (2.80 mg/kg). Normally squid liver has high content of iron and it's ranged to 26.18-214.20 mg/kg [27].

3.3. Magnesium

Magnesium levels of all 3 studied species are relatively high and *S. latimanus* is recorded higher concentration (83.16 mg/kg) and *S. lessoniana* recorded lower concentration (69.17 mg/kg). It has been found that Mg participates in numerous reactions relating to immunocompetence such as growth and transformation of lymphocytes, synthesis of immunoproteins and chemotaxis [28]. For the fish, USDA (2009) provided a standard nutrient database reference for Mg level in fresh raw meat (muscle). According to this organisation, Mg level is calculated as 50 mg/100 g wet wt [28].

3.4. Zinc

Zinc acts as a cofactor in a variety of cellular processes, including DNA synthesis, structural components of proteins and as a cofactor in enzyme catalysis [3,19,25]. Zinc is always present in seafood, but concentrations found in mollusks are generally higher [3]. In generally *L. duvauceli* recorded higher concentration (26.53 mg/kg) while *S. lessoniana* recorded lower concentration (17.65 mg/kg). As per the literature much lower level of zinc was recorded in muscle comparatively liver and ink, though that value ranging from 3.52 to 42.41 ppm [27]. As his studied the concentrations of Zn in the different organs of *L. duvauceli* varied among stations and followed the order; liver>skin>gills>tentacles~muscles, in the sample. Interestingly Cu, Hg and Zn are metals that bind to metallothionein proteins, which play a role in the homeostasis of the essential metals and in the detoxicfication of the non-essential metals. The role of metallothionein proteins in metal sequestration in cephalopods is not completely clear and this issue clearly deserves further investigation [11].

3.5. Cobalt

Although the cobalt is an enzyme activator in human body and forms, the central metal atom in vitamin B_{12}, is a toxic element. The level of Co in varies 0.042 to 2.86 ppm in *L. duvauceli* in studied of Prafulla. As his studied the distribution pattern in general being ink > liver > gills > skin > muscle. In this study that values are much lower. The highest mean concentration was recorded *S. latimanus* (32.05 µg/kg) and lowest value was recorded in *S. lessoniana* (6.42 µg/kg).

3.6. Cadmium

The highest Cd content in the muscle of *L. duvauceli* (27.53 µg/kg) and lowest content was recorded in the muscle of *S. lessoniana* (16.01 µg/kg). Concentration of Cd in all studied species in this study is far below the EU safety limits. As the studied of Prafulla*et al*, Cd level of *L. duvaucelli* is much higher than this study; the average was < 2.0 ppm and the highest was 3.19 ppm and around 11% of the whole squids had Cd content > 3 ppm. The Cd is most attention trace metals in recent years, due to its cumulative effects and toxicity to the consumer.When ingested by animals and humans, it accumulates primarily in the renal cortex and may result in a variety of toxic effects, such as renal failure, bone degeneration and cancer [16]. Squids are a significant source of Cd for their predators [11]. This hypothesis was first proposed by [29]. Squid also plays a major role in transferring Cd through the food chain and Cd is efiiciently absorbed and strongly retained in the digestive gland of the cephalopods. Even if most of cephalopods display short life spans they can accumulate very high Cd concentration in their digestive gland reaching upto 1000 µg/g in wet weight basis [7,11,20,30].

3.7. Chromium

Chromium is essential trace elements for human and ncessary for glucose tolerance [3]. As well as Cr is one of the least toxic trace elements although its toxicity at high doses [19]. The highest Cr level was recorded *L. duvauceli* (182.29 µg/kg) and the lowest was recorded in *S. lessoniana* (16.61 µg/kg). Levels in our commercial cephalopods were low, suggesting that they would not be at risk from Cr if they catch around in the Sri Lankan waters. As Prafulla values, Cr in muscle ranged from 0 to 1.98 ppm and it was highest in ink, followed by skin and liver. Very little data is available on the variation of Cr and Ni concentrations in cephalopods tissues. For example, in the squid Sthenoteuthisoualaniensis Cr concentrations were higher in juweniles than in adults, whereas juveniles displayed lower Ni concentrations than adults. Such difference was explained by the evolution of food habits between the juvenile and adult stages, juvenile feeding more on crustacean while adults primarily preyed on fish [11].

3.8. Nickel

The level of Ni in *S. lessoniana* (109.97 µg/kg) was highest and *L. duvauceli* (7.64 µg/kg) was the lowest. Prafulla said that the liver has high amount of Ni (4.70 ppm) in *L. duvauceli* and the distribution of Ni in other body component were more or less similar. Nickel is categorized as of highly toxic and non essential element to biological systems. But a few years ago physiological function of Ni was unclear. Nowadays, it is thought that Ni plays an important role in hormone, lipid and cell membrane metabolism. Several studies on Ni indicated to the role of nickel in various enzymatic system as well as activating enzymes associated with the glucose breakdown and use [3].

3.9. Mercury

Mercury is one of the most studied and toxic metals concern in seafood sector in recent. As well as, Hg is considered as a most problematic metal in marine ecosystems, because of its bioaccumulation and biomagnifications in marine food webs. The predominant and stable form of Hg in seafood is methyl mercury, this is the form that is most toxic to organisms, which it provokes deleterious effects on the nervous system and interferes with the process of cell division [12,25]. In this study Hg studied as total Hg (ΣHg), and highest value recorded in *L. duvauceli* (51.94 µg/kg) and lowest value was recorded in *S. latimanus* (30.33 µg/kg). The concentrations of mercury in these 3 species are far below the EU safety limits (0.5 mg/kg).Prafulla mentioned that Hg is the least abundant toxic metal found in squid and it was a range of 0.01 to 0.07 ppm. The levels of Hg in the different body components were also low, and distribution followed the order liver> skin > gills > tentacles - muscles [27]. But in other study showed that Hg concentrated muscles tissues of giant squid (*Architeuthis dux*) than other metals [11]. Trace elemant concentration of cephalopods could vary according the location where individuals were captured. This is clerly exemplified by the much Hg in the tissues of the specimen from Mediteranean [11]. This was explained by high temperature and absence of solar radiation in the deep environment. This condition is favour for methylisation process and that much of Hg accumulation was not examined the specimen collect from the Indian Ocean in this study. To the best of my knowledge, Hg uptake and retention has not been fully investigated in cephalopods.

3.10. Lead

Lead is a classic chronic or cumulative poison, which produces a continuum of deleterious effects and cause behavioral deficits on animal and humans [19]. Aquatic organisms take up and accumulate lead from water, sediment or and food. In animals, lead affects a great number of physiological systems The high blood Pb level can cause kidney dysfunction, brain damage, anaemia and can inhibit the normal functioning of many enzymes [3,25]. Prafulla was recorded Pb content of *L. duvauceli* 0 to 1.83 ppm. But in this study, the highest mean value recorded also in *L. duvauceli* (24.37 µg/kg) and lowest value was recorded in *S. lessoniana* (5.88 µg/kg).

Table 2. Investigated trace metal level in squids and cuttlefishsp in previous studies

Species	Country	Metals	Concentration	Reference
Todarodesfilippovae	Tasmania	Cd	0.06-0.70 µg/g, dw	[7]
	Amsterdam		0.13-3.56 µg/g, dw	
	Tasmania	Co	<DL	
	Amsterdam		0.03-0.35 µg/g, dw	
	Tasmania	Cr, Ni, Pb	<DL	
	Amsterdam		<DL	
	Tasmania	Cu	2.90-14.2 µg/g, dw	
	Amsterdam		4.60-9.70 µg/g, dw	
	Tasmania	Fe	4.30-25.5 µg/g, dw	
	Amsterdam		5.30-22.1 µg/g, dw	
	Tasmania	Hg	0.28-1.94 µg/g, dw	
	Amsterdam		0.11-0.57 µg/g, dw	
	Tasmania	Ni	<DL	
	Amsterdam		<DL	
	Tasmania	Zn	49.3-83.6 µg/g, dw	
	Amsterdam		50.4-65.6	
Sthenoteuthisoualaniensis	India	Cd	0.26-5.26 µg/g, ww	[31]
Loligopatagonica	Argentina	Cu	7.8 mg/kg, dw	[32]
		Fe	2.3 mg/kg, dw	
		Zn	12 mg/kg, dw	
		Cd	0.32	
Loligoforbesi	British Channel	Cu	4 mg/kg, dw	[33]
		Zn	59 mg/kg, dw	
		Pb	0.20 mg/kg, dw	
Loligoopalescens	California	Cu	4.3 mg/kg, dw	[34]
		Fe	2 mg/kg, dw	
		Zn	25 mg/kg, dw	
Sepia pharonis	Saudi Arabia	Pb	0.25-0.54 mg/kg, ww	[3]
		Zn	5.74-9.44 mg/kg, ww	
		Cd	0.04 mg/kg, ww	
		Cu	2.19 mg/kg, ww	
Sthenoteuthisoualaniensis	India	Hg	0.04 µg/g, ww	(Suwanna et al., 2007)
		Cd	3.759 µg/g, ww	
		Pb	0.035 µg/g, ww	
		Zn	16.54 µg/g, ww	
		Cu	10.99 µg/g, ww	
Sepia recurvirostra	Indonesia	Mg	64.87 mg/kg, ww	[4]
		Fe	4.03 mg/kg, ww	
		Zn	19.62 mg/kg, ww	
		Cu	5.70 mg/kg, ww	
		Cd	0.04 mg/kg, ww	
		Pb	ND	
		Hg	ND	
Sepia sp.	Egypt	Cu	9.7 µg/g, dw	[19]
		Fe	75.7 µg/g, dw	
		Zn	55.0 µg/g, dw	
		Cr	2.2 µg/g, dw	
Todarodessaggitatus	Greece	Cd	0.421 µg/g, ww	[16]
		Cr	0.115 µg/g, ww	
L. vulgaris	North Eatsern Atlantic	Hg	264 ng/g, dw	[12]

As for coastal and oceanic targeted cephalopods, the digestive gland and the branchialherats contained the highest concentration of Ag, Cd, Co, Cu, Fe, Ni, Zn [11], but in this study did not concern the body parts seperately. The trace elemant concentration of cephalopods may vary with biological and environmental factors such as age (size and weight), sex and geographical origin [11]. Our limited sampling did not allow making comparisions for all these factors. Although globally poorly documented, metal and metalloid concentration in cephalopods tissues have received increasing interest over the last decades. In Sri Lanka and most European countries, viscera are removed before consumption. In Italy and Spain, however, small *Loligo* spp. is eaten as whole. Moreover, in Japan, the digestive gland is consumed and is considered to be a delicacy. It was therefore important to evaluate the implication of metal levels in squid tissue for public health [20]. The trace metal content of squids and cuttlefish has variously been reported in previous studies were given in Table 2.

4. Conclusion

The present study shows that the average concentrations of all trace metals were significantly lower in the edible parts of studied species. They don't seem to cause any health hazard risk to consuming these three cephalopods. The data generated in this study may form baseline values for trace metals commonly eaten in squids and cuttlefish of Sri Lanka. As referred literature further studied required about the trace metals in various body parts and geographical area arround Sri Lanka.

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