



## BIOACCUMULATION OF HEAVY METAL TOXICITY IN CRUSTACEANS & CEPHALOPODS FROM FISH MARKET IN COCHIN, KERALA, INDIA

### Biotechnology

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### ABSTRACT

The target of the present study was to determine and compare four heavy metal elements (Pb, Cd, Hg and As) in three Crustaceans species namely *Litopenaeus Vannamei*, *Penaeus indicus* & *Penaeus monodon* and three Cephalopods species namely *Loligo duvaucelii*, *Sepia pharaonis* & *Octopus vulgaris*, collected from fish market, Cochin coast, Kerala, India. Heavy metal concentration was determined by ICP-MS (Inductively Coupled Plasma Mass Spectrometer). Effluent from Industrial Activities reaches the wetland water and finally transferred to human being through food chain and causes toxicity. Hence these sea foods are commonly consumed and imported to other countries. It is matter of great concern to reduce such contamination. In this study, The mean concentrations (mg/kg) of toxic elements in Crustaceans and Cephalopods species were Pb: 0.1-0.58 and BDL-1.67, Cd: BDL-0.71 and 0.11-2.09, Hg: BDL-0.15 and BDL-0.16, As: BDL-0.51 and BDL-2.91. In few samples, level of Pb, Cd and As except Hg was found to be exceeded the recommended limits in EU regulation (EC1881/2006).

### KEYWORDS:

Heavy metals, Sea Food, ICP-MS, Contamination, BDL (below detection limit)

### INTRODUCTION

Seafood is widely consumed as part of the regular in take all over world due to its high nutritional value. Omega fatty acid, low saturated fat which results in good human health is present in sufficient quantity in sea food [1]. Seafood toxicity is increasing day by day due to bioaccumulation of heavy metals. Heavy metals are inorganic chemicals that are non-biodegradable and they cannot be metabolized by the body and tend to accumulate through time. These heavy metals cannot be degraded further and their toxic effects can be long lasting, unlike the organic contaminants that decompose into other chemicals with time [2]. Heavy metals hazardous to human health are mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As) [3].

Several anthropogenic pollutants such as increased industrialization, population growth and economic factor lead to bioaccumulation of heavy metals in food chain [4]. Industrial effluent comprising mainly of toxic and hazardous substances, including heavy metals [5] subsidize enormously to the aquatic ecosystem pollution instigating cytotoxic, mutagenic and carcinogenic effects in animals [6]. Major source of heavy metal contamination in coastal waters is due to industrial effluent and the Bay of Bengal and Arabian Sea is no exception [7]. Fish occupies the topmost position of aquatic food chain and may comprehend large amounts of metals from the water system [8].

The primary objective of this study was to survey heavy metal concentrations in Crustaceans (*Litopenaeus Vannamei*, *Penaeus indicus*, *Penaeus monodon*) and Cephalopods (*Loligo duvaucelii*, *Sepia pharaonis*, *Octopus vulgaris*) from Cochin fish market of Kerala district and to validate these results with other studies related.

### MATERIALS AND METHOD

#### Collection of Samples

Five samples of each kind of species i.e. Crustaceans species namely *Litopenaeus Vannamei*, *Penaeus indicus* & *Penaeus monodon* and Cephalopods species namely *Loligo duvaucelii*, *Sepia pharaonis* & *Octopus vulgaris*, were collected from Cochin, Kerala, India during Nov-Dec 2016 (Fig. 1) and were immediately preserved in an insulated ice box and transported to the laboratory where they were weighed and kept frozen at  $-20^{\circ}\text{C}$  until further analysis.

**Table 1:** Details of Species collected from Cochin fish market.

S. No.	Common Name	Scientific Name
Crustaceans Spp.		
1.	White leg Shrimp	<i>Litopenaeus Vannamei</i>
2.	Indian prawn	<i>Penaeus indicus</i>
3.	Asian tiger shrimp	<i>Penaeus monodon</i>
Cephalopods Spp.		
1.	Indian Ocean Squid	<i>Uroteuthis Sibogae</i>
2.	Pharaoh cuttlefish	<i>Sepia pharaonis</i>
3.	Common octopus	<i>Octopus vulgaris</i>



**Fig.1.** Sampling site in Cochin, Kerala



**Fig.2.** Fish Shop in fish market in Cochin, Kerala

**Determination of Heavy Metals**

All the glassware and plastics were soaked overnight in 10% (v/v) nitric acid rinsed with distilled water and de ionized water and dried before being used. 5 gm of boneless muscle tissue was removed using stainless steel knife and was digested to a strong acid digestion (H2O2+HNO3 conc.) mixture at 1:3 ratios at 150°C for 20 minutes and allowed to cool at room temperature. Samples were processed in duplicate and then diluted to a total 50 ml with milli Q water and filtered through 0.45 m micro pore membrane filter paper for analyses and then prepared samples were analyzed by inductively coupled plasma mass spectrometer (ICP-MS). Test Method being used in analysis was validated as per Regulation (EC) 882/2004 to meet Commission Regulation (EC) 333/2007 & Commission Regulation (EC) 582/2016 (Table 2 & Table 3). The concentrations of heavy metals were presented in mg/kg, wet weight for samples.

**Reagents**

All reagents were of analytical grade and Milli Q water was used for all dilutions. The element standard solutions from Merck were used for the calibrations.

**Table 2:** Method validation data for analysis of heavy metal in Crustaceans Species as per EC regulation

S. No.	Parameters	LOD	LOQ	Recovery	MU (±)
1.	Pb	50	100	107.16	100 ± 6.24
2.	Cd	50	100	94.28	100 ± 4.17
3.	Hg	10	25	88.50	25 ± 0.84
1.	As	50	100	114.76	100 ± 8.36

#Result were in µg/kg unit, MU= Measurement Uncertainty, LOD (limit of Detection) and LOQ (Limit of Quantification)

**Table 3:** Method validation data for analysis of heavy metal in Cephalopods Species as per EC regulation

S. No.	Parameters	LOD	LOQ	Recovery	MU (±)
1.	Pb	30	60	92.16	200 ± 5.31
2.	Cd	50	100	93.91	100 ± 3.0
3.	Hg	100	200	105.49	200 ± 7.10
4.	As	100	200	91.07	200 ± 4.93

#Result were in µg/kg unit

**RESULT & DISCUSSION:**

**Heavy metals content**

It showed, the levels of Pb, Cd Hg and As that were expressed in mg/kg. The data clearly shows variation in the level of heavy metals in cephalopods and crustacean sub species of the same fish market (Table 1 and table 2).

**Table 4:** Level of Heavy Metal concentration in Crustaceans Species Samples

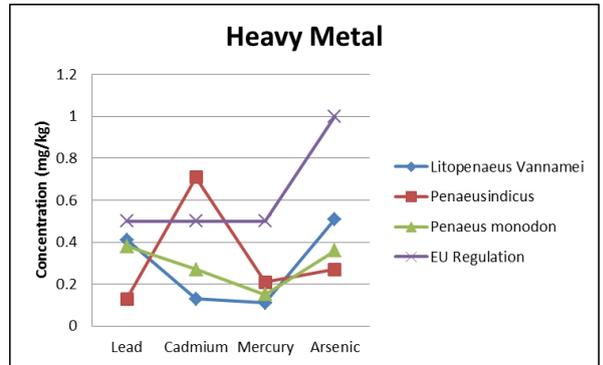
S. No.	Parameters	Results			EU Regulation
		Litopenaeus Vannamei	Penaesusindicus	Penaesus monodon	
1	Pb	0.12-0.41	BDL-0.13	0.11-0.38	0.5
2	Cd	BDL-0.13	BDL-0.71	0.10-0.27	0.5
3	Hg	BDL-0.11	BDL-0.21	BDL-0.15	0.5
4	As	0.14-0.51	BDL-0.27	0.20-0.36	1

#Result were in mg/kg unit with recovery between 80-110%, BDL (below detection limit)

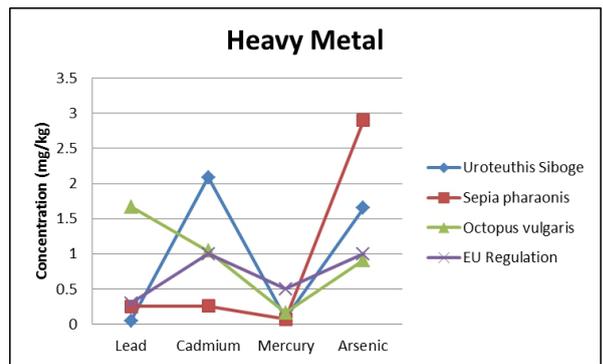
**Table 5:** Level of Heavy Metal concentration in Cephalopods Species Samples

S. No.	Parameters	Results			EU Regulation
		Uroteuthis Siboge	Sepia pharaonis	Octopus vulgaris	
1	Pb	BDL-0.05	BDL-0.25	BDL-1.67	0.3
2	Cd	0.11-2.09	0.19-0.26	0.31-1.04	1
3	Hg	0.21-0.09	BDL-0.07	0.10-0.16	0.5
4	As	BDL-1.65	BDL-2.91	BDL-0.91	1

#Result were in mg/kg unit with recovery between 80-110%



**Fig.3.** Maximum Heavy Metal Concentration in different Crustaceans Species Samples



**Fig.4.** Maximum Heavy Metal Concentration in different Cephalopods Species Samples.

The International official regulatory agencies like EU, FAO & WHO have set prescribed limit for heavy metal concentrations beyond which the fishery product is considered inappropriate for human consumption. However in the Indian, there is no safety level of heavy metals in fish and shrimp tissues [17].

For Crustacean species, the metal concentrations decrease in the order: Cd>As> Pb>Hg, whereas heavy metal concentration in Cephalopods species showed result As>Cd>Pb>Hg. Many previous literatures stated that the occurrence of trace mineral and heavy metals is related to length, weight, age, species, and location of fishes [9].

Since Kochi is the major industrial region of Kerala, these waste water discharges from industries is major source of pollution and affect the ecosystem hence accumulation of heavy metals in cephalopods occurs at a higher rate [16]. Additionally, environmental factors such as season, place of development, nutrient availability, temperature and salinity of the water also may contribute to the variation of heavy metals concentration in different fish species [18]. The EU limits levels of heavy metals and other environmental contaminants that can be present in fish and fishery products. In general, levels are disallowed if dietary intake would likely exceed acceptable daily or weekly intake for humans (Directive

91/493/EC) [15].

For Pb in Table 1, the highest level was observed in *Litopenaeus Vannamei* at 0.41 mg/kg which was within range as per EU Regulation, while in Table 2 the highest level was in *Octopus vulgaris* at 2.91 mg/kg which exceeds EU range. Headache, petulance, abnormal pain and various nervous system disorders are related to Pb poisoning [10]. Cadmium was detected in each sample of cephalopods from Kochi market and some even showed result out of EU range. Cd poisoning may have adverse effect on kidney, lung, bones, brain as well as central nervous system in humans [11]. Furthermore, the environmental surroundings and seasonal changes of lipids may influence the heavy metals to accumulate [12]. Hg is one of the most toxic elements among the studied heavy metals and exposure to high level of this element could adversely affect the brain, kidneys and developing fetus [13]. But in this study, Hg was found to be below detection limit or within EU commission range. Arsenic was found to be the most abundant heavy metal in the edible tissue of the species studied. Among different forms of arsenic, inorganic As is most toxic and considered as a Group A human carcinogenic and can have adverse effect mainly to lung, kidney, and skin disorder if present in large amount [13]. In Crustacean Species, As (Arsenic) were found with range of prescribed limit by EU regulation and only in one *Sepia pharaonis* sample, it were higher i.e. 2.91 mg/kg. The above result clearly shows the impact of aquatic environment [14] as well as surrounding human activities at particular location may contribute to accumulation of heavy metals in fishery products. Accumulation of heavy metals in the aquatic environments have been associated with urban runoff, sewage treatment plants, industrial effluents and wastes, mining operations, boating activities, domestic garbage.

## CONCLUSIONS

The purpose of this study was to provide information on heavy metal concentrations in different Crustaceans & Cephalopods samples from local fish market. In the present study, the majority of toxic metal concentrations in the Crustaceans & Cephalopods samples analyzed were well within the limit prescribed for human consumption proposed by European Community (EC1881/2006) [19]. At another end, the contamination of Pb, As, Cd and Hg in these positive samples from Cochin showed that major industrial effluent was discharged in this area which in turn increased heavy metal levels.

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