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Analysis of trace elements and heavy metals in commercially important spiny lobster species from north east coast of Andhra Pradesh, India

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Abstract
The present study was aimed to evaluate the concentrations of different elements Li, Al, V, Cr, Mn, Fe, Co, Cu, Zn, As, Rb, Sr, Cd, Ba, Pb in muscle tissue of lobster species (*Panulirus homarus, Panulirus polyphagus, and Panulirus versicolor*) from the North East Coast of Andhra Pradesh. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine trace elements and heavy metals in lobster muscle tissue samples. The concentrations were expressed as µg/l wet weight. The order of trace metal concentrations were found as Zn>As>Cu>Fe>Al>Sr>Rb>Mn>Li>Co>Cd in *P. homarus* and Zn>Sr>As>Fe>Cu>Rb>Cr>Ba>Cd>Mn>Li>V>Al>Co>Pb in *P. polyphagus*. Whereas, the metal concentrations of *P. versicolor* were Zn>As>Cu>Sr>Al>Fe>Rb>Ba>Mn>Li>Cd>V>Co>Pb respectively. While the chromium element was not detected in *P. versicolor*. The present study in *Panulirus homarus, Panulirus polyphagus* and *Panulirus versicolor* species of lobsters the Zn element was showed highest concentration mean value than other 14 elements. The lowest mean values were obtained in Pb and Co elements in *P. polyphagus, P. versicolor,* and Pb was not detected in *P. homarus* species of lobsters. Throughout the North East Coast of Andhra Pradesh this is the first attempt of these elements in lobster species (*P. homarus, P. polyphagus, and Panulirus versicolor*) from the Bay of Bengal.

Keywords: Trace elements, lobsters, heavy metals, inductively coupled plasma mass spectrometry

Introduction

In recent decades, much attention has been paid to the study of essential and toxic trace element content in food stuffs (Arporn Busamongkol *et al.,* 2014) [5]. Consumption of seafood is an important dietary source of proteins, minerals, trace elements and essential fatty acids, including omega-3 acids and also seafood is an important source of food and essential nutrients to population of some regions, being part of the cultural traditions of many people. However, seafood may pose risks to human health because they may contain toxic substances, such as trace elements and persistent organic pollutants. Many studies have been conducted on the accumulation of trace elements in marine organisms in aquatic environment Fish incorporates trace elements by ingestion of suspended particulate matter in the water column and of food, by ion exchange of dissolved elements across lipophilic membranes (Edevaldo Silva *et al.*, 2016) [11].

Metalloids are toxic and bio accumulative thus, the contamination of aquatic environments by metals is a serious problem (Ramesh *et al.*, 2012; sun *et al*., 2010) [28, 31]. In fact, heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining and agricultural activities (Weher, 2008; Jaric *et al.*, 2011) [3, 17]. Industrial waste and mining can create a potential sources of heavy metal pollution in the aquatic environment (Gumgum *et al.,* 1994) [6]. Heavy metals discharged into the marine environment can harm both marine species and ecosystems, due to their toxicity and accumulative behaviour (Tuzen, 2009) [22]. Heavy metals are important for ecology of marine environment because they don't decompose, only change its chemical bonds. Metals thus gradually accumulate in the sea, sediment and in marine living organisms (DHI, 2001). Pollutants such as heavy metals in muddy sediments in the vicinity of the discharge point can be harmful to exposed benthic invertebrates and can also be carried higher in the food chain, including to humans through their consumption of seafood (Lee, 1992).

The commercial and edible species have been widely investigated in order to check for those hazardous to human health (Begüm *et al.,* 2005). Shrimps and crabs may provide useful means of monitoring of such elemental concentration levels and their impact on the aquatic environment. Benthic species take up heavy metals from the sediment in which they live and from food they consume (Soto-Jiménez *et al.*, 2001) [30]. Shrimp and lobster have numerous pathways for bioaccumulation of metals and other pollutants that include absorption at the gill surface, ingestion of water and sediment and consumption of organisms which have accumulated pollutants (Canli and Furness 1993). In particular, lobsters are known to absorb high concentrations of metals from their environment (Chou *et al.,* 1991) [9]. About 33 to 35% of absorbed metals accumulate in muscles and can be transmitted to humans through the food chain (Morales-Harnandez *et al.,* 2004) [21].

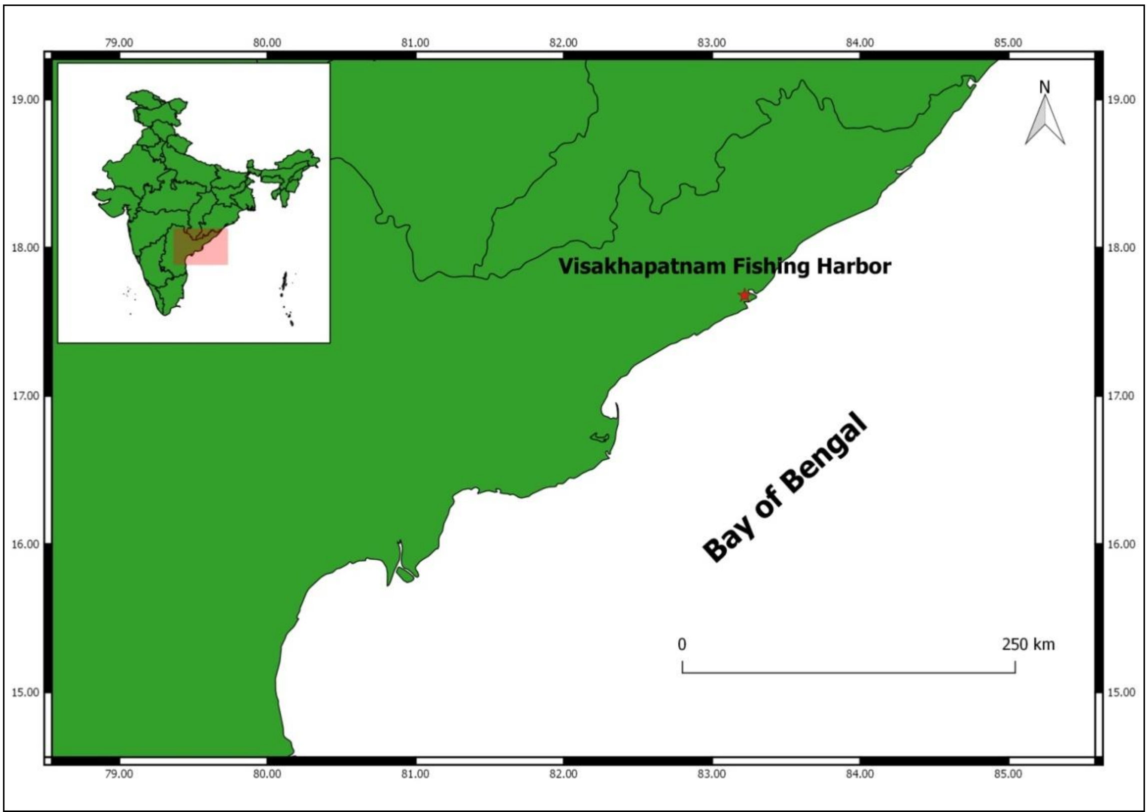
Lobsters are one of the most valuable and highly priced crustaceans in India, as well as an important export commodity. Lobsters are distributed widely in tropical and subtropical waters along with the Indian coast, major fisheries are located on the north-west, south-west, and south-east coasts (Radhakrishnan & Manisseri 2003). All lobsters are bottom dwelling species. This species inhabits rocky substrates, coral reefs (Holthuis 1991). Maximum ranging in size of total body length about 40 cm, average adult length about 30 cm. Males are usually much larger than females and they are occurring in depths ranging from the shoreline to over 1400 m. In this study *Panulirus homarus* (Linnaeus, 1758) commonly known as 'scalloped spiny lobster', *Panulirus polyphagus* (Herbst, 1793) commonly known as 'Mud spiny lobster' and *Panulirus versicolor* (Latreille, 1804) commonly known as 'Painted spiny lobster' were evaluated.

The present study analyzed the elements of Al, V, Cr, Mn, Fe, Co, Cu, Zn, As, Rb, Sr, Cd, Ba, Pb in *P. homarus, P. polyphagus,* and *P. versicolor* species. Heavy metals such as copper, Iron, chromium and nickel are essential metals, since their play an important role in biological systems, whereas cadmium and lead are non-essential metals, as they are toxic, even in trace amounts (Fernandes *et al.,* 2008). The purpose of this survey was to detect the levels of trace elements and heavy metals including aluminium, arsenic, lead, and cadmium in lobster samples and also to evaluate the levels of Zn, Cu, Fe and Mn from the north east coast of Andhra Pradesh, because these are essential for most living organisms. Zn and Cu play an important role in growth and cell metabolism, in the enzymatic and respiratory processes of aquatic animals and the relatively high level of these metals it is necessary to carry out these biological functions (Baboli and Velayatzadeh, 2013; Mitra *et al.,* 2012; Salam and Hamdi, 2014) [18, 14].

Materials and Methods

Study area

Visakhapatnam (Lat: 17°.729" N and Long: 83°.219" E), located in the north east coast of Andhra Pradesh. It has a long coastline that stretches about 132 km and has significant economic activity yielding out of the fisherman population. The city is one of the most important place that surrounded by wide range of anthropogenic activities and polluting the sea extensively. Visakhapatnam has always been known for its healthy fishing, diverse resources and operating variety of crafts and gears. There are about 685 fishing crafts like longlines, Gillnets, trawl nets and more than 905 mechanized and motorized boats harboured, polluting the sea extensively. The gears operated for commercial fishing along Visakhapatnam coast. Finfishes and crustaceans were the two major groups contributing to the fishery of the region. It is also hosts a natural fishing harbor and port. Fishing harbor is the reference site used for this study.



Map of northeast coast of Andhra Pradesh showing sampling station and the Study area

Sample collection and preparation of samples

The lobsters *P. homarus*, *P. polyphagus*, and *P. versicolor* were collected from the landing center in the study area. After collection of samples the lobsters were immediately transferred in to the clean plastic containers for laboratory analysis. The samples were washed under running fresh tap water. The excess water was removed using blotting paper before taking body weight in mg on an electrical balance. The measurement of total length and standard length in cm. After recording the length and weights, the individual samples were used to identify the species was done according to Holthuis (1991). Muscle tissue samples were stored in deep freezer until further processing.

Determination of Heavy Metals

The muscle tissue was removed from the lobsters and was dried at constant temperature of 80 °C for 48 hr. Dried samples of individual tissues were ground into fine powder using mortar and pestle. For each lobster species dried powder samples were weighed accurately 5g. The muscle tissues were placed in a digestion vessel with 4 ml of concentrated nitric acid (HNO_3) and 1 ml of hydrogen peroxide (H_2O_2) were allowed to stand overnight at 70 ºC. Then the samples were digested to near dryness by evaporate up to 1 ml volume and make up to 25 ml volume. The concentration of heavy metals was analysed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS-Agilent 7700 series), which was available in the Centre for studies on Bay of Bengal, Andhra University. Visakhapatnam.

Operating Conditions of ICP-MS

RF power	1300-1350 W
Argon gas flow Nebulizer Auxiliary Plasma	 0.86 L/min 1.2 L/min 15 L/min
Lens voltage	5
Sample uptake rate	0.80 mL/min
Measuring mode	Peak hopping
Point per peak	1
Number of sweeps	50
Dwell time (microseconds)	50
Integration time (ms)	2500
Replicates	3
Internal standard	$^{103}Rh^{a}$

Results and Discussion

The mean and comparison of trace metals and heavy metal levels (µg/L) in muscle tissues for the selected lobster species (*P. homarus*, *P.polyphagus*, and *P. versicolor*) are presented in Table 1. This investigation showed variable amounts of different elements (Cr, Mn, Rb, Cd, Ba) and concentrations between the species.

In the present study, the highest concentrations of metals were Zn>As>Cu>Fe>Al in *P. homarus*, Zn>Sr>As>Fe>Cu found in *P. polyphagus* and *P. versicolor*. Whereas, the lowest concentrations of metals were found in three lobster species were Rb>Mn>Li>Co>Cd, Rb>Cr>Ba>Cd>Mn>Li and V>Al>Co>Pb respectively. The order of trace metal concentrations were found as Zn>As>Cu>Fe>Al>Sr>Rb>Mn>Li>Co>Cd in *P. homarus* and Zn>Sr>As>Fe>Cu>Rb>Cr>Ba>Cd>Mn>Li>V>Al>Co>Pb in *P. polyphagus*. Whereas, the metal concentrations of *P. versicolor* Zn>As>Cu>Sr>Al>Fe>Rb>Ba>Mn>Li>Cd>V>Co>Pb respectively. While, the chromium element was not detected in *P. versicolor*.

Table 1: Concentrations of trace elements and heavy metals in muscle tissue of lobsters

Muscle tissue sample Conc. (µg/l)				
Elements	**Atomic number**	***P. homarus***	***P. polyphagus***	***P. versicolor***
Li	3	0.09±0.04	0.08±0.02	0.12±0.03
Al	13	21.4±0.41	0.05±0.03	22.5±0.04
V	23	ND	0.08±0.01	0.06±0.04
Cr	24	ND	1.49±0.20	ND
Mn	25	4.13±0.02	0.12±0.02	0.87±0.04
Fe	26	26.7±0.01	43.4±0.11	16.57±0.05
Co	27	0.08±0.04	0.02±0.01	0.05±0.03
Cu	29	40.6±0.28	32.3±0.19	66.4±0.26
Zn	30	106.43±0.07	171.13±0.03	108.94±0.05
As	33	84.81±0.18	51.13±0.03	74.48±0.13
Rb	37	5.18±0.04	2.74±0.08	3.36±0.03
Sr	38	12.92±0.07	51.34±0.04	26.62±0.03
Cd	48	0.017±0.01	0.16±0.01	0.07±0.02
Ba	56	ND	0.52±0.04	1.65±0.07
Pb	82	ND	0.01±0.00	0.1±0.01

Note: ND: Not Detected, Mean ± SD (n= 3)

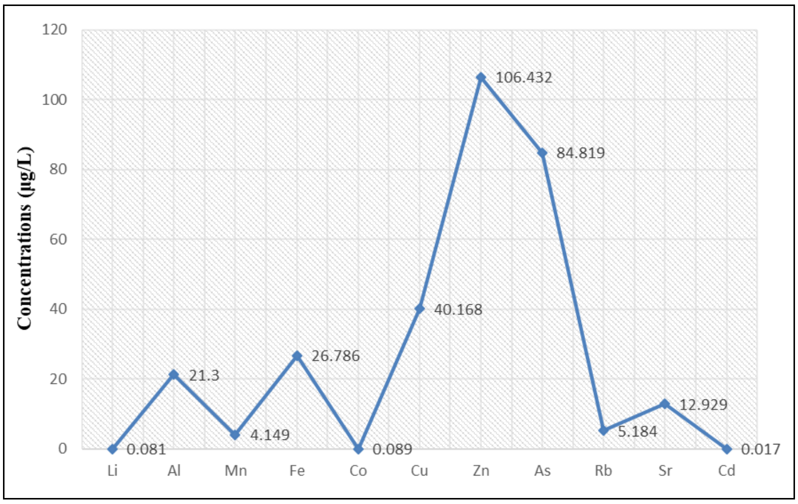


Fig 1: Mean values of elements in *P. homarus*

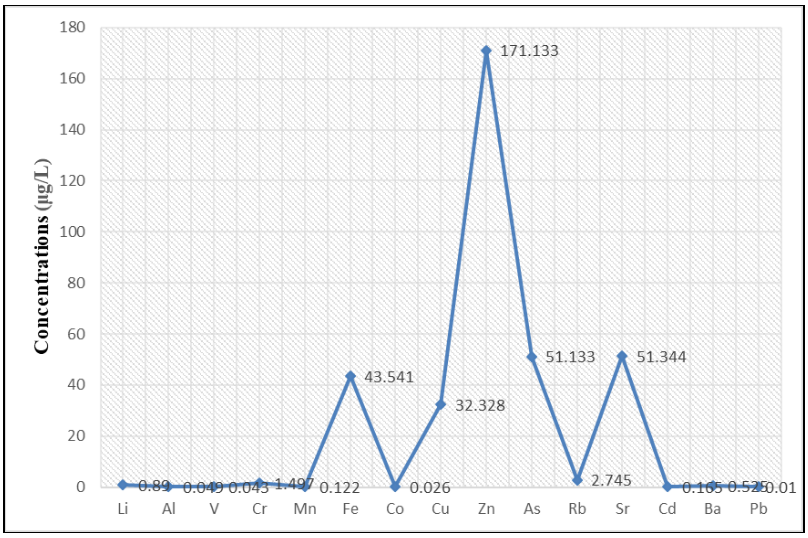


Fig 2: Mean values of elements in *P. polyphagus*

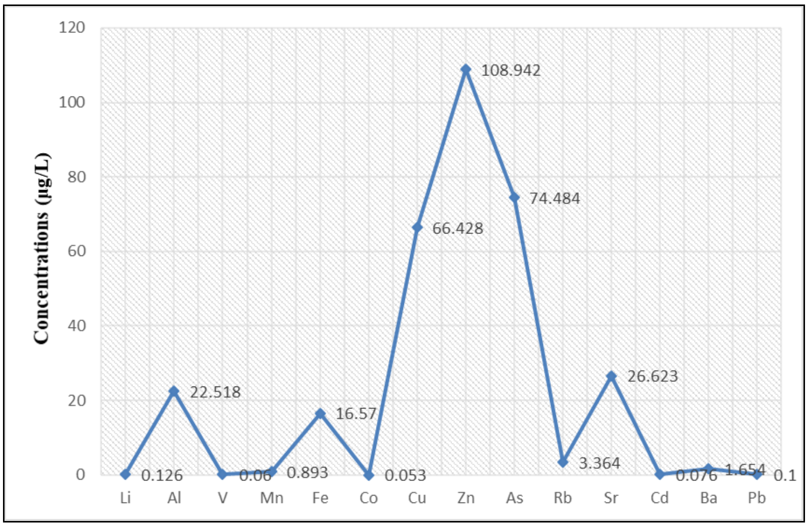


Fig 3: Mean values of elements in *P. versicolor*

The Zn concentration in muscle tissue of the lobster species was higher than other elements and the lowest concentrations were Co, Cd in *P. homarus*, Pb, Co in *P. polyphagus* and V, Co were *P. versicolor* lobster species. Zn, Cu, Fe, Mn, and Al are essential for most living organisms. In the present study, Zn exhibited the highest accumulation in these lobster species, followed by Sr, As, Fe and Cu. That could be explained because Zn and Cu play an important role in growth and cell metabolism, in the enzymatic and respiratory processes of aquatic animals and the relatively high level of these metals it is necessary to carry out these biological functions (Baboli and Velayatzadeh, 2013; Mitra *et al.*, 2012; Salam and Hamdi, 2014) [18, 14].

The present study obtained the mean values of Li and Al elements were 0.09±0.04, 21.4±0.41 in *P. homarus* and 0.08±0.02, 0.05±0.03 in *P. polyphagus*. Whereas, 0.12±0.03, 22.5±0.04 in P. versicolor respectively. Though, there was no differentiation in mean values in *P. homarus*, *P. polyphagus, and P. versicolor* species. The concentration of Aluminium clearly reflects the effects on living things, so it is an appropriate indicator for acidification of the environment (Ezoe *et al.*, 2001) [33]. The present study V mean values in the lobster species were 0.08±0.01 and 0.06±0.04 in *P. polyphagus* and *P. versicolor* species. Among these two species *P. versicolor* was higher mean value than the *P. polyphagus.* The V element was not detected in *P. homarus.*

Chromium does not occur freely in nature. The main chromium mineral is chromite. In the present study chromium concentration 1.49±0.20 in *P. polyphagus.* Whereas, chromium was not detected in *P. homarus and P. versicolor*.

Much of the Manganese in natural waters is present in suspended forms, thus resulting in the removal of this metal from the water column by sedimentation before toxic levels can be attained. Manganese is therefore less toxic than most other metals (Gokoglu *et al.*, 2008). For the this study *P. homarus*, *P. polyphagus* and *P. versicolor* showed the mean values of Mn in the muscle tissue were 4.13±0.02, 0.12 ± 0.02 and 0.87 ± 0.04 respectively.

The mean values of Fe and Co were 26.7±0.01, 0.08±0.04 in *P. homarus*, 43.4± 0.11, 0.02±0.01 in *P. polyphagus and* 16.57±0.05, 0.05±0.03 in *P. versicolor* species. Among these two elements Fe showed higher mean value in *P. polyphagus* than the other two species. Whereas, the cobalt element was showed slightly similar results in three species.

A high concentration of Cr, Fe and Mn has been reported in the exoskeleton of *Panulirus Gracilis* from the Gulf of California. Shahadat Hoossain and Yusuf Sharif Ahmed Khan studied trace metals in penaeid shrimp and spiny lobster from the Bay of Bengal (Cr, Fe, Mn) and reported levels of accumulation lower than our results. However, most of the trace elements and heavy metals studied in the edible muscle tissue were within the recommended limits for human consumption.

Our study reveals that the mean values of Cu and Zn were 40.6±0.28, 106.43±0.07 in *P. homarus*, 32.3±0.19, 171.13±0.03 in *P. polyphagus and* 66.4±0.26, 108.94±0.05 in *P. versicolor*. Whereas, the Zn exhibits the higher mean value in *P. polyphagus* then the other two species. Among these two elements Cu showed higher mean value in *P. versicolor* than the *P. homarus* and *P. polyphagus*.

According to Pourang *et al.,* 2005 [25], Zn is the most abundant element in muscle, followed by other metals. Krishna *et al.,* 2014 reported that the Zn shows the highest concentration levels in the metals of the muscle tissues in marine organisms. Our results were co-related with the reported results. Shahadat Hoossain and Yusuf Sharif Ahmed Khan reported that the Zn, and Cu were lower than our results. Raju Subha *et al.*, 2016, was studied accumulation of Zn, Fe, Cu and Cd in Panulirus homarus homarus species of lobsters. The mean values of Zn, Fe, Cu and Cd were (7.93 ±1.54, 4.23 ± 0.96, 2.36 ± 0.27 and 0.21±0.03) respectively. These results showed lower mean values than our study.

The present study was observed As and Rb mean values of *P. homarus*, *P. polyphagus* were 84.81±0.18, 5.18±0.04 and 51.13±2.74±0.08 as well as 74.48±0.13, 3.36 ± 0.03 in *P. versicolor.* Whereas, the Sr mean values of three species were 12.92±0.07, 51.34±0.04 and 26.62 ±0.03 respectively. Among those three elements as was showed higher mean value in *P. homarus* than the other two species. While, Rb exhibits the slightly similar mean values in three lobsters of species. The higher mean values of Sr were showed in *P. polyphagus* than the *P. homarus and P. versicolor*.

The present study showed the mean values of Ba were found in two species of lobsters were 0.52±0.04 and 1.65±0.07 respectively. Whereas, the Ba element was not detected in *P. homarus.*

Arsenic, Cadmium and lead are classified as potentially toxic heavy metals that are very harmful even at low concentration when ingested over a long period. Cadmium concentrations of the present study were 0.017±0.01 in *P. homarus*, 0.16±0.01 in *P. polyphagus* and 0.07±0.02 in *P. versicolor* species. The high average concentration of cadmium was accumulated in *P. homarus* than the *P. polyphagus* and *P. versicolor*.

Lead compounds are generally toxic pollutants. In this study the mean values of lead were obtained at 0.01±0.00 in *P. polyphagus* and 0.1±0.01 *P. versicolor* respectively, but there was no differentiation in mean values of two species. The lead was not detected in *P. homarus*. This metal showed very lowest mean values than the other 14 elements. Discussed the metal accumulation in lobsters (*Panulirus homarus*); the mean values of Arsenic, Cadmium and Lead were 103.9±50.6, 163.1±77.4 and 640.6±84.2 respectively. Shahadat Hoossain and Yusuf Sharif Ahmed Khan discussed that the accumulation levels of Cd and Pb. These results show higher mean values than our study.

Conclusions

In the present years, the health benefits related to seafood consumption has been extensively publicized. Seafood is rich in protein low cholesterol and high percentage of (n-3) polyunsaturated fatty acids, liposoluble vitamins and essential minerals. Epidemiological studies have indicated that populations with a rich seafood diet have low risk of coronary heart disease, hypertension and cancer. In recent years, attention has been focused on determination of elements in seafood due to nutritional benefits of essential elements and toxicological concerns related to anthropogenic influx of contaminants.

To the best of our knowledge, this is the first detailed report of trace elements and heavy metals in muscle tissues of spiny lobster species from the Bay of Bengal, Andhra Pradesh, India; therefore, only limited comparisons of our results with other data can be made. Our study was analyzed the 15 elemental concentrations in *P.homarus*, *P. polyphagus* and *P. versicolor* species. According to the present study Zinc element was showed highest mean concentration value than other 15 elements. Zinc is an essential element that is necessary for all cells. It helps the immune system fight off invading viruses and bacteria. Zinc is a key element that cells use to metabolize nutrients. Immune function, DNA and protein production, and cell division are all related to zinc levels in the body.

The findings of the study are the spiny lobsters of the off shore fishing grounds of the Bay of Bengal were found to have high protein levels, good nutritional values and safe levels of metal concentrations for human health. Conversely, there are number of elements that are toxic into the human body, interpose with its functioning and undermine health such as Cadmium, Lead, Arsenic, and Aluminium. These toxic metals have no known physiological functions.

Acknowledgments

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