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Human health risk assessment and study of bioaccumulation of heavy metals in commercially important finfishes and shell fishes collected from Chennai coast, India

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ABSTRACT

Fishes and Crustaceans form the chief source of food for a peninsular country like India. Fish food production and Human health can be affected by heavy metal contamination. Heavy metals can be carcinogenic or non-carcinogenic based on their toxic effects and bioaccumulation. The present study aims to analyse the concentrations of four heavy metals (As, Cr, Cd, and Pb) in seawater and their bioaccumulation in commercially important fishes (*Nemipterus japonicus, Rastrelliger kanagurta*), Crab (*Portunus sanguinolentus*) and Shrimp (*Penaeus monodon, Penaeus indicus*). The samples were collected from Pulicat, Ennore, Pattinapakkam, Kovalam and Thiruvanmiyur regions of the Chennai coast and analysed using atomic absorption spectrophotometer, and the results were interpreted with the USEPA human health risk assessment. The hierarchy of the concentration of heavy metals in the seawater is in the order Pd> Ar> Cr> Cd. The distribution of the heavy metals concentrations in the tissues of *N. japonicus, P. indicus, and P. monodon* is in the order Pb > Cr> Cd > As, *R. kanagurta* and *P. sanguinolentus* is in the order Pb > Cr > As> Cd. The species studied showed the highest accumulation of heavy metal in the liver and lowest in muscles. Of all the species studied, the highest concentrations of all the metals were observed in *P. sanguinolentus*. The concentration of Cd and Pb in fish tissues were greater than the guidelines values suggested by USEPA, 2000 except for the muscles. THQ values of Pb and Cd in *P. sanguinolentus,* and Ar and Cd in *R.kanagurta* were above 1.0. Prolonged consumption of these two species from the Ennore and Pattinapakkam coasts may be potentially hazardous to human health. Application of effective preventive measures and appropriate strategies to minimize heavy metal pollution can be identified, developed and adopted, or integrated into coastal management practices by studies on the earlier and current incidents.

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1. Introduction

The global total marine capture fisheries production source was 79.3 million tonnes during the year 2016 and 84.4 million tonnes in the year 2018 (FAO, 2018 & 2020). For about 3.2 billion people, almost 20% of their average per capita intake of animal protein is contributed by fish food (FAO, 2018). Annual per capita fish consumption in India is 5-6 kg and 8-9 kg for the total population and for the fish-eating population, respectively (Salim, 2016). India is a major player among the fish exporting countries, ranking 6th among fish food exporters and importers (FAO, 2018), and contamination with metals or any hazardous substance in the fish produced can affect the country's economy and the health of the consuming population.

Metal concentration in the marine environment is affected by waste from urban mining, industrial and agricultural waste, and sewage waste. Though bioaccumulation of heavy metals depends on some environmental parameters, including pH, temperature, alkalinity, pollutant type, feeding behaviour, geological position, habitat, age and sex of the species (Aytekin *et al.,* 2019; Jonathan *et al.,* 2015), long-time exposure of living organisms to metal-contaminated environments leads to accumulation of metals and transfer of metals into the food chain. Fish can accumulate metals directly from water and through the food chain (Hadson, 1988). Humans are exposed to metals via the food chain (Rejomon *et al*., 2010). Elements such as Cu, Fe, Co and Zn are essential for fish growth and metabolism, metals such as Cd, As, and Pb are non-essential and toxic even when consumed at low levels in humans and can cause acute and chronic health effects (Rejomon *et al*., 2010).

Any aquatic organism living in a contaminated environment may accumulate trace metals and transfer the metals to the organisms in the higher hierarchy in the food chain. Finfish and shellfish are considered important bioindicator organisms as they are in the higher hierarchy of the food chain and, are consumed by humans and have a high tendency to accumulate metals. The growing interest of people in fish and fish products in recent years makes this study significant. Heavy metals can be carcinogenic or non-carcinogenic based on their toxic effects and bioaccumulation**.** USEPA suggested human health risk assessment associated with fish consumption, which includes (Oral reference dose (RfD) value and target hazard quotient (THQ).

The present study aims to analyse the concentrations of four heavy metals (As, Cr, Cd and Pb) in seawater and their bioaccumulation in commercially important fishes (*Nemipterus japonicus, Rastrelliger kanagurta*), Crab (*Portunus sanguinolentus*) and Shrimps (*Penaeus indicus, Penaeus monodon*).

2. Materials and Methods

2.1 Study Area

Five locations that orient between 13°N and 12°N namely Pulicat (13°25'41.2''N 80°18'33.4''E), Ennore (13°10' 54.0''N 80°18'58.6''E), Pattinapakkam (13°02'19.1''N 80°16'53.3''E), Thiruvanmiyur (12°58'32.3''N 80°16' 03.7''E) and Kovalam (12°47'28.5''N 80°15'07.4''E) are chosen for the present study. Study area is represented in Fig. 1.The study area is a centre of various human activities and has ports, harbours, industries of various kinds, places

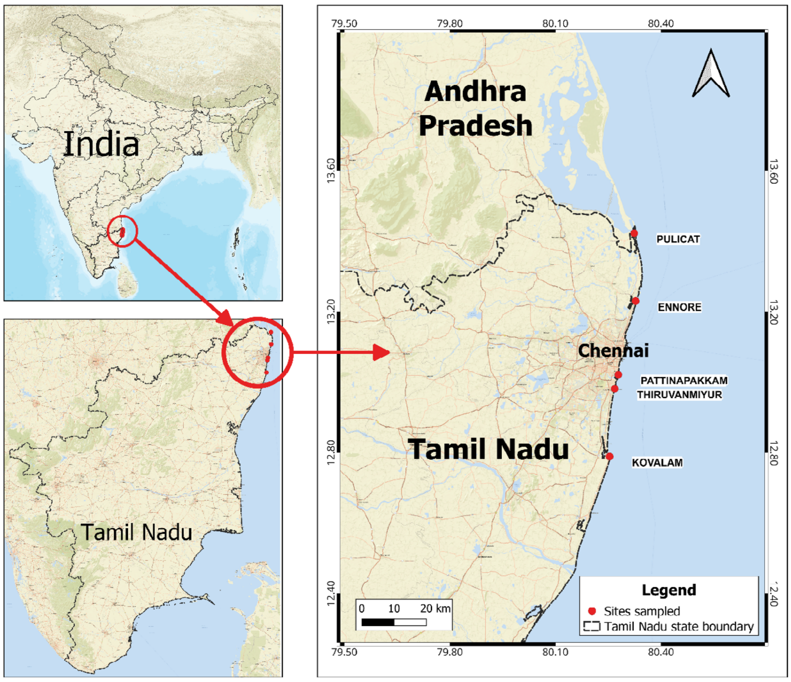

Fig. 1. Map of Chennai coast showing the 5 different sample collection locations of the present study

of historic importance, tourist beaches, etc. and coastal habitats like estuaries, lagoons, etc.

2.2 Sample collection and analysis

For the present study, seasonal samples of seawater were collected from five locations for four seasons (Winter, Summer, Pre-monsoon and Monsoon), in a 1-litre wide-mouth plastic bottle. All the bottles were rinsed with seawater from corresponding locations just before the collection of water to avoid contamination besides several washings with distilled water. Water samples collected were brought to the laboratory in an icebox and filtered. The samples were then tested for quantifying the concentrations of four heavy metals (As, Cr, Cd and Pb) to analyse the level of contamination.

Five specimens of similar size of each candidate species (fishes (*N. japonicus, R. kanagurta*), Crab (*P. sanguinolentus*) and Shrimps (*P. indicus, P. monodon*) were collected from the fishers in clean zip-lock pouches when the fishing boat arrived at the shore, kept in the icebox and transported to the laboratory. Morphometric measures and ecological characteristics of the samples are represented in Table 1.

Specimens were washed with distilled water, and different organs (liver, intestine, gills, skin, gonads, muscle) were dissected on a clean table with a sterile knife and blade. The samples were pooled to obtain a sufficient amount of tissues, then labelled and preserved at -20˚C. The preserved samples were lyophilised then powered in a clean mortar and pestle, and further stored at -20 ˚C until the analysis of heavy metal. The lyophilised samples were transferred into digestion flasks and digested with perchloric acid, nitric acid and sulphuric acid in the ratio of 1:5:1on a hot plate until all the tissues were dissolved and heavy metals in the tissues were analysed using a calibrated atomic absorption spectrophotometer with recovery rates above 90%. The accuracy of the tested metals is tested with reference material.

2.3 Bioconcentration Factor (BCFs)

BCF is the ratio of heavy metal concentration in an organism to its concentration of metal in the water. (Maurya *et al.,* 2019; Zhang *et al.,* 2018). BCF= Concentration of heavy metal in biological samples/ Concentration of heavy metals in water. Where BCF is represented as L/Kg dry weight; Con in biota mg/kg dw; C water mg/L.

2.4 Target Hazard Quotient (THQ)

THQ is the estimation of the non-carcinogen risk level of consumption of heavy metals and it is calculated according to the Screening values for the target analyses table provided by USEPA region III risk- based concentration table suggested by USEPA, 2011(Baki *et al.,* 2018).

$$THQ = \frac{EF \times ED \times IR \times Cf \times C}{BW \times ATn \times RfD} \times 10^{-3}$$

Where EF is the exposure frequency (365 days/year), ED is the exposure Duration (here, we consider 60 years for adults to estimate non -carcinogen risk level), IR is the average food ingestion rate (55.8 g/day for adults) (Tong *et al.,* 2016; Zhang *et al.,* 2018), the Conversion factor (Cf) to convert from dry to wet weight (Maurya *et al.,* 2019) considering 79% moisture content is 4.8, C is the mean concentration of heavy metals in crustacean and fish (mg/kg dw), BW is the average body weight (60 kg) for an adult (Zhang *et al.,* 2018)**,** ATn is average exposure duration to non-carcinogen pollutant (ED × 365days/ year) (ATn for adults = 21900 days), RfD is the oral reference dose for the non-carcinogen risk level of heavy metal exposure (mg/kg /day, 3×10^{-4} for As, 1×10^{-3} for Cd, 3×10^{-3} for Cr, 4×10^{-3} for Pb) according to USEPA (2000, 2016).

Nemipterus japonicus *Rastrelliger kanagurta*

Portunus sanguinolentus *Penaeus monodon* *Penaeus indicus*

Fig. 2. Species collected for present analysis

3. Results and Discussion

3.1 Heavy metals in water samples

Mean concentrations of selected heavy metals present in seawater samples for four seasons from all the four locations of study are presented in Table-2, in the decreasing order of contamination, Pb> As> Cr> Cd.

The concentration of lead is the highest of all the four metals studied in all the locations throughout all the seasons. The highest concentration of Lead (23.88 mg/l) is found in Kovalam and the lowest (11.75 mg/l) in Pattinapakkam. The highest value of Arsenic (5.5 mg/l) was detected in Marina and the lowest value (0.35 mg/l) in Thiruvanmiyur. The highest concentration of cadmium (3.94 mg/l) is found in Kovalam and the lowest (0.89 mg/l) in Thiruvanmiyur. The highest concentration of Chromium (5.04 mg/l) is found in Kovalam and the lowest (0.78 mg/l) in Ennore.

3.2 Heavy metals in fish and crustaceans

The metal concentration in various organs (liver, intestine, gills, skin, gonads, and muscle) of the species (*N. japonicus, R.kanagurta*, *P.sanguinolentus*, *P.indicus, P.monodon)* is listed in the table3, 4 and 5.

The distribution of the heavy metals concentrations in the tissues of *N. japonicus, P.indicus, and P.monodon* is in the order Pb > Cr> Cd > As, *R.kanagurta* and *P.sanguinolentus* is in the order Pb > Cr > As> Cd.

The highest concentration of Pb is observed in the gonads of *R.kanagurta* collected from Pattinapakkam, whereas lowest concentration in muscle tissues of *N. japonicas.* The concentration of Pb (23.62- 0.59 mg/kg) is higher than the value recommended (0.3 ppm) by FSSAI (2015). Lead is a non-essential element; high levels of lead are known to cause blood pressure and cardiovascular disease in adults and cognitive development in children (Baki *et al*., 2018).

Chromium concentration ranged from 0.13 -6.7mg/kg in the studied species, which falls well within the value recommended (12 ppm) by FSSAI (2015). The highest load of Cr is observed in *P.sanguinolentus* and lowest *in R.kanagurta.* High loads of metals observed in Crabs are due to their benthic habitat, feeding on detritus and benthic prey, exposed in direct contact with sediments and tends to accumulate more heavy metal (Zhao *et al.,* 2012).

Cd occurs in low levels in the environment; highest concentration of Cd is recorded in the liver of *R.kanagurta* & *P.sanguinolentus* and lowest in shrimps with a range from (7.7- 0.16 mg/kg) in the present study. The maximum cadmium level in fish recommended by FSSAI, 2015 is 0.3ppm. Cd is capable of producing chronic health effects even at low concentrations (Friberg *et al*, 1971).

Table 1. Morphometric measures and habitat of samples collected

Scientific Name	Common name	Inhabit stratum and distribution range	Feeding behaviour	Climate zone, depth	No. of samples	Length(cm) Mean(± SD)	Weight(g)
Nemipterus japonicus	Japanese Threadfin bream	Benthic	Carnivores	Tropical; depth range 5 - 80 m	25(5*5)	21.5±3.4	133.5±26.8
Rastrelliger kanagurta	Indian mackerel	Pelagic-neritic	Carnivores	Tropical; depth range 20 - 90 m	25(5*5)	27.6±4.5	187.7±43.7
Portunus sanguinolentus	Three spotted swimming crab	Benthic	Carnivores	depth of 30 m	25(5*5)	12.06±1.4	128.7±31.6
Penaeus monodon	Tiger prawn	Adult - Benthic	Omnivorous	Indo-West Pacific depths from 0 to 110 m.	25(5*5)	17.3±7.2 carapace (rostrum–telson)	41.3±7.2
Penaeus indicus	Indian prawn	Adult - Benthic	Omnivorous	Indo-West Pacific found at depths of 2 to 90 m.	25(5*5)	12.1±2.5 carapace (rostrum–telson)	14.3±5.5

Table 2. The concentration of heavy metals in the water samples of study area (Chennai coast)

Months	Stations	Heavy metals (concentration in mg/l)			
		As	Cd	Cr	Pb
WINTER	1	5.02±0.04	0.05±0.02	0.32±0.02	12.56±0.03
	2	2.55±0.02	1.76±0.02	1.56±0.02	16.81±0.01
	3	5.5±0.01	2.2±0.02	1.55±0.02	15.51±0.01
	4	0.35±0.02	0.89±0.01	1.25±0.02	20.9±0.01
	5	4.89±0.02	3.34±0.02	2.12±0.02	20.94±0.02
SUMMAR	1	3.31±0.01	2.75±0.03	1.26±0.02	13.23±0.02
	2	2.89±0.02	2.34±0.03	3.09±0.02	13.89±0.01
	3	3.56±0.02	1.56±0.02	0.8±0.01	17.41±0.01
	4	2.99±0.01	3.43±0.02	3.19±0.02	18.51±0.01
	5	5.15±0.02	3.56±0.03	4.11±0.01	20.51±0.01
PRE MONSOON	1	2.60±0.03	3.24±0.01	0.98±0.02	17.09±0.01
	2	2.78±0.01	1.89±0.01	0.78±0.01	13.21±0.01
	3	1.94±0.02	2.35±0.02	1.98±0.02	14.55±0.01
	4	2.13±0.02	3.11±0.01	2.45±0.02	19.23±0.02
	5	3.05±0.01	3.94±0.03	3.54±0.01	20.95±0.02
MONSOON	1	1.67±0.02	2.53±0.01	4.07±0.02	13.26±0.02
	2	2.55±0.02	2.78±0.02	2.65±0.01	12.7±0.01
	3	2.5±0.02	0.94±0.01	1.85±0.03	11.75±0.03
	4	0.94±0.01	2.03±0.01	1.56±0.02	15.5±0.01
	5	3.94±0.02	3.94±0.02	5.04±0.01	23.88±0.02

Legend: Stations – 1- Pulicut, 2- Ennore, 3- Pattinapakkam, 4- Thiruvanmiyur, 5- Kovalam.

Table 3. Average concentration (µg/g dry weight) of heavy metals (As, Cd, Cr & Pb) in tissues of Fish Species collected along the Chennai coast

Metal	Tissues	*N. japonicus*	*R. kanagurta*
As	Liver	3.63±0.8	6.79±2.2
	Gonad	3.04±1.4	5.41±1.7
	Gills	3.02±1.2	3.15±1.6
	Intestine	3.28±1.2	6.05±1.2
	Skin	2.31±0.7	4.13±0.8
	Muscles	0.03±0.02	0.07±0.03
Cd	Liver	3.89±0.7	5.26±1.7
	Gonad	3.13±0.4	4.48±2.5
	Gills	3.98±1.2	4.99±1.5
	Intestine	3.39±0.6	5.42±1.0
	Skin	2.81±0.7	4.43±0.8
	Muscles	0.12±0.1	0.87±1.1
Cr	Liver	5.72±1.3	5.92±2.4
	Gonad	5.29±1.2	5.49±2.4
	Gills	5.74±0.7	5.67±1.8
	Intestine	5.18±1.3	5.68±2.1
	Skin	4.42±1.2	4.90±2.0
	Muscles	0.39±0.2	0.35±0.2
Pb	Liver	15.55±3.1	16.95±4.8
	Gonad	14.84±2.4	18.00±3.6
	Gills	16.66±4.1	14.21±3.0
	Intestine	17.28±3.4	14.97±3.8
	Skin	9.44±2.4	15.11±3.6
	Muscles	0.59±0.1	1.72±1.8

Table 4. Average concentration (µg/g dry weight of heavy metals (As, Cd, Cr & Pb) in tissues of *P.sanguinolentus* collected along the Chennai coast.

Metals	Tissue	Concentration
As	spawn	6.06±1.8
	gills	5.55±1.3
	muscle	0.87±1.4
Cd	spawn	4.96±2.2
	gills	4.42±2.4
	muscle	1.89±2.8
Cr	spawn	6.66±3.1
	gills	6.01±2.9
	muscle	1.64±2.7
Pb	spawn	16.44±6.2
	gills	16.75±5
	muscle	4.18±6.2

Arsenic concentration ranged from 8.7- 0.02 mg/kg. The highest loads of As is recorded in the liver of *R.kanagurta and lowest in N. japonicus, P.indicus & P.monodon.* The maximum Arsenic level in fish recommended by FSSAI, 2015 is 76 ppm. Chronic exposure to inorganic arsenic causes hematopoietic system, gastrointestinal tract, cardiovascular and nervous disorders. Skin disease, hypertension diabetes is also reported by authors (Centeno *et al*., 2005; Mandal and Suzuki, 2002).

Of all the 5 study locations, seawater from Pattinapakkam and Ennore coastal areas showed high concentrations of metal. This shows that both the areas are highly contaminated with metals.

Table 5. Average concentration (µg/g dry weight of heavy metals (As, Cd, Cr & Pb) in muscle tissues of Shrimp species collected along the Chennai coast

Metals	As	Cd	Cr	Pb
P.indicus	0.02±0.01	0.15±0.05	0.36±0.2	0.62±0.2
P.monodon	0.02±0.03	0.14±0.05	0.37±0.2	0.69±0.09

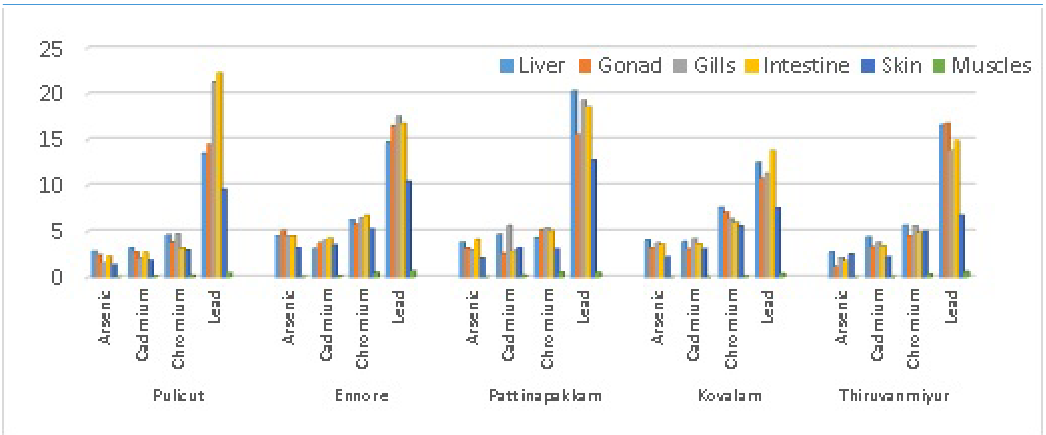

Fig. 3.1. Concentration of Heavy metals in tissues of *N. japonicas*

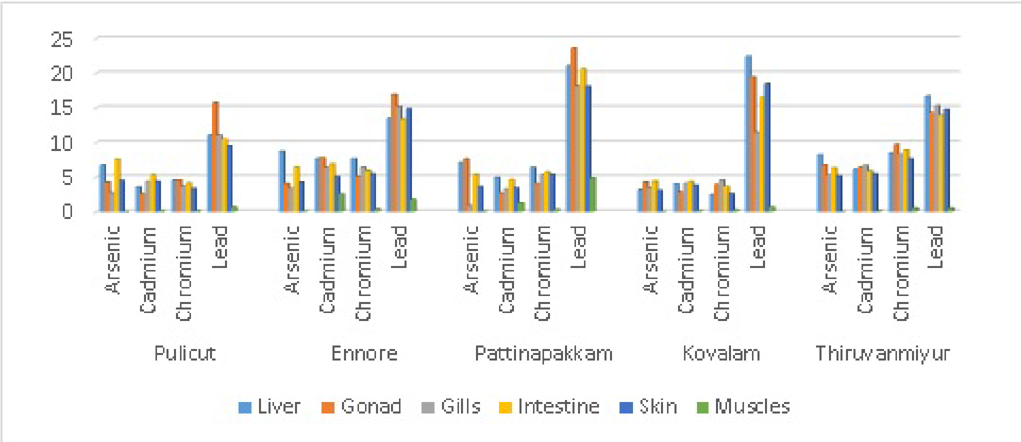

Fig. 3.2. Concentration of Heavy metals in tissues of *R.kanagurta*

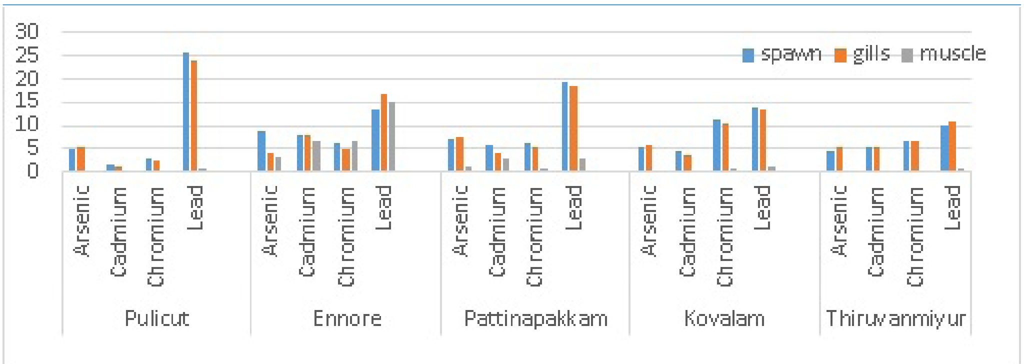

Fig. 3.3. Concentration of Heavy metals in tissues of *P.sanguinolentus*

Table 6. Recommended values of heavy metals (As, Cr, Cd and Pb) in fish and Crustaceans for Human consumption

Standard levels of metals	As	Cd	Cr	Pb
Tolerance level of heavy metals in fishes muscles(mg/kg) for consumption				
WHO- 1989		1		2
FAO-2003		0.05		0.2
USEPA 2000		2	8	4
EC-2014		0.5		0.3
FSSAI 2015	76	0.3-0.5	12	0.3
Tolerance level of heavy metals in crustaceans muscles(mg/kg) for consumption				
EU-2006	5	0.5	0.5	0.5

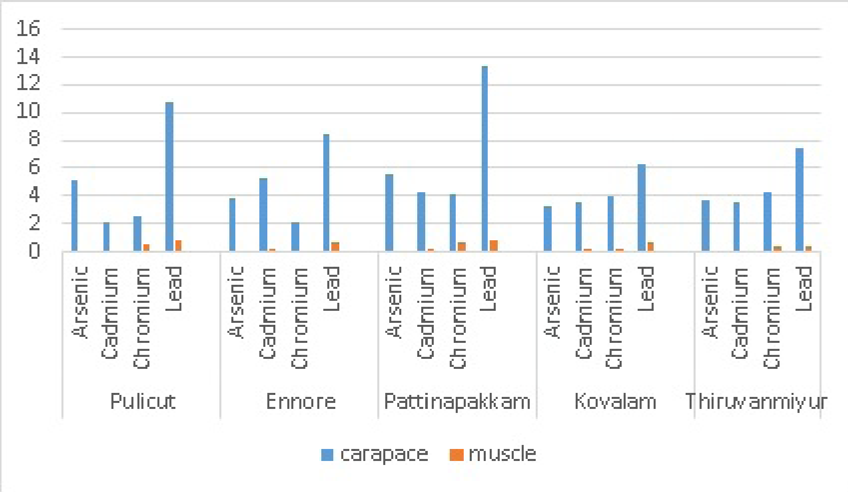

Fig. 3.4. Concentration of Heavy metals in tissues of *Penaeus indicus*

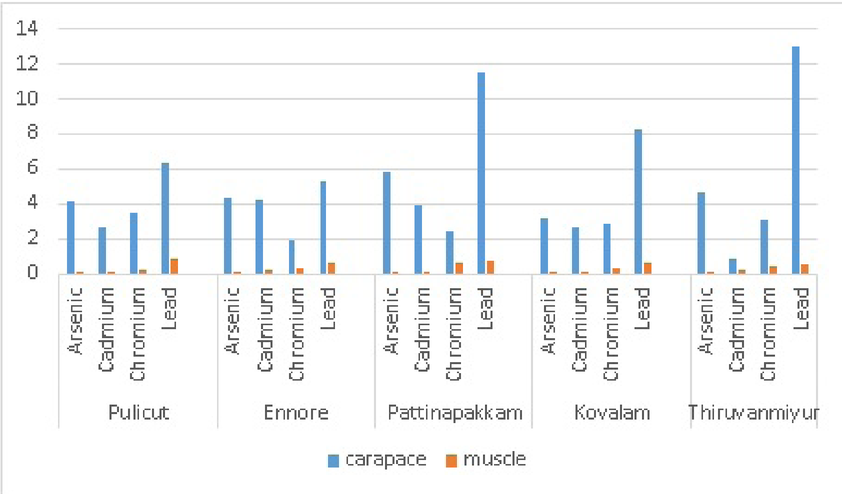

Fig. 3.5. Concentration of heavy metals in tissues of *Penaeus monodon*

3.3 Bioconcentration Factor (BCFs)

In the present study, heavy metal concentration in different tissues (liver, intestine, gills, skin, gonads, and muscle) showed bioaccumulation of heavy metals in various organs of fish and crustaceans. The liver of fish showed higher BCF, followed by gonads, intestine, gills and skin, BCF of muscle is much lower than the other tissues. The liver showed high BCF as they are metabolically active in breaking down toxic elements. In crabs, BCF was in the order of Spawn, gills and muscle. The BCF of shrimps was comparatively low than the other species studied.

3.4 Health risk assessment of the trace metals in fish and crustaceans

Diet is the most common way of exposure to toxic metals in humans. Muscle tissues of fish and Crustaceans are explored more than other organs because it is the part consumed by humans (Aytekin *et al*., 2019). Fish form the chief source of food to a peninsular county like India and dispenses exposure to toxic metals as fish and crustaceans have a strong potential to accumulate metals (Aytekin *et al*., 2019). Accumulation of heavy metals in fish could affect the health conditions of consumers on consume fish on a daily basis (Maurya *et al*., 2019).

Therefore it is important to calculate Health risk assessment for fish from polluted coastal areas. The Target Hazard Quotient (THQ) is estimated for all four metals studied through consumption of fish and crustaceans species and is represented in Table- 7. THQ values of Pb and Cd in *P.sanguinolentus* and Ar and Cd in *R.kanagurta* were above 1.0. The values stipulate that prolonged consumption of these two species from the Ennore and Pattinapakkam coast may be potentially hazardous to human health.

This study exhibits that the mean concentrations of metals As, Cd, and Pb detected in all tissues except muscle tissue in Pattinapakkam and Ennore are higher than the value recommended by USEPA. Arsenic, Cadmium, Chromium and lead have deleterious effects on human health even at a marginal concentration (Ahmed *et al*., 2015; Zhang *et al.* 2018). Differences in metal accumulation in different species could be due to differences in their ecological niche, physiology, habitat and feeding. Invertebrates accumulate heavy metals in their body organs in different concentrations; this is due to various factors like their feeding habitat, striatum inhabited, sexual maturity, age, metabolism, absorption, excretion and efficiency of the invertebrate in regulating and detoxification of the

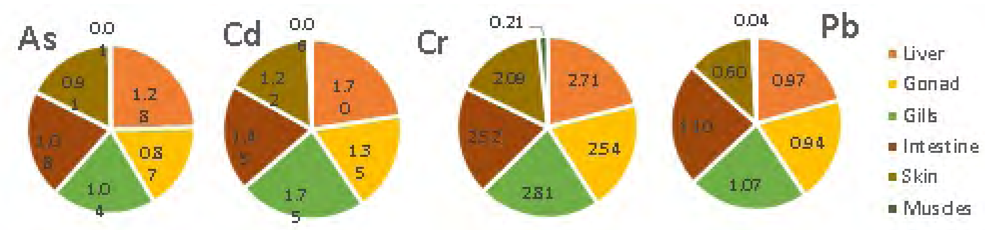

Fig. 4.1. Bio- concentration factor of heavy metals (As, Cd, Cr, Pb) L/kg in different tissues of *N. japonicus*

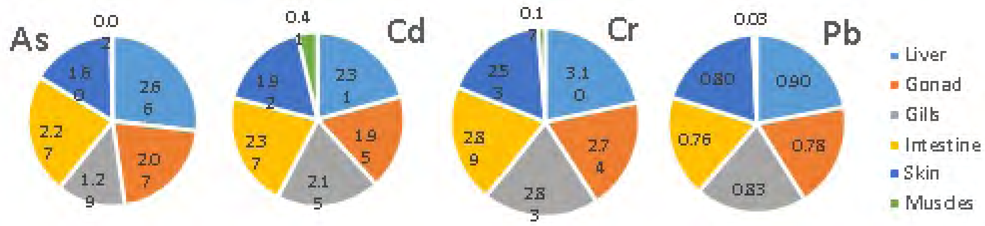

Fig. 4.2. Bio- concentration factor of heavy metals (As, Cd, Cr, Pb) L/kg in different tissues of *R. kanagurta*

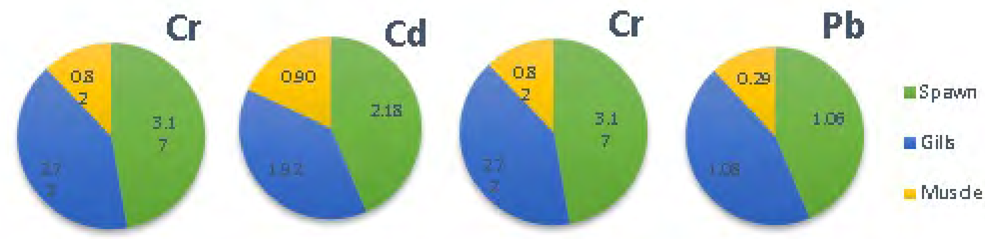

Fig. 4.3. Bio- concentration factor of heavy metals (As, Cd, Cr, Pb) L/kg in different tissues of *P. sanguinolentus*

Table 7. Target hazard quotient (THQ) of Fish and Crustaceans collected along the Chennai coasts

Species						
N. japonicus	**As**	0.4464	0.8928	0.5952	0.2976	0.2976
	Cd	0.6696	0.7142	0.9821	0.1786	0.3125
	Cr	0.3274	0.9077	0.8333	0.9374	0.6250
	Pb	0.5692	0.8147	0.6473	0.5245	0.7366
R. kanagurta	**As**	0.7440	1.6368	1.3392	0.7440	0.5952
	Cd	0.8035	11.3832	5.8478	0.7589	0.5357
	Cr	0.1934	0.6250	0.5803	0.4166	0.7886
	Pb	0.7589	1.9642	5.4349	0.8035	0.6138
P. sanguinolentus	**As**	0.5952	0.8928	1.0416	0.7440	0.8928
	Cd	0.4018	4.5533	11.6064	0.5803	0.8482
	Cr	0.3274	0.9374	0.7142	0.9077	0.4018
	Pb	0.9932	4.2073	3.1360	1.3504	0.9151
P. monodon	**As**	0.1488	0.2976	0.5952	0.4464	0.2976
	Cd	0.4910	0.8482	0.7142	0.9821	0.4018
	Cr	0.8333	0.2083	0.8779	0.2381	0.5506
	Pb	0.8258	0.7366	0.8705	0.6584	0.3683
P. indicus	**As**	0.2976	0.1488	0.7440	0.1488	0.1488
	Cd	0.5357	0.9374	0.4910	0.4018	0.7589
	Cr	0.3125	0.5357	0.9077	0.5208	0.5506
	Pb	0.9263	0.7142	0.8035	0.7366	0.6473

inculcated heavy metals (Bryan, 1971; Baki *et al*., 2018; Eisler, 1981). Out of all the above, food source, dermal, and breathing contribute more sources of heavy metal through bioaccumulation and bio transmission in shrimps, and crab (Baki *et al*., 2018).

4. Conclusion

The study becomes highly significant as the study area is a centre of various human activities, has ports, harbours, industries of various kinds, places of historic importance, tourist beaches, etc. and has coastal habitats like estuaries, lagoons, etc. Heavy metals in the fish and crustaceans tissues widely vary among species and within the tissues of species. Bioaccumulation is more in the liver and gonads, followed by gills, skin, intestine and muscle. The study revealed that the *R. kanagurta* and *P. sanguinolentus* pose carcinogenic risk from cd and Pb consumption on Ennore and Pattinapakkam, south-east coast of India. These results can be used to provide baseline information on human health risks associated with the consumption of fish and crabs. Application of effective preventive measures and appropriate strategies to minimize heavy metal pollution can be identified, developed and adopted or integrated into coastal management practices.

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5. References

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