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Toxicity of trace metals in mud crab, *Scylla serrata* (Forsskal 1775) collected from Cochin backwaters, Kerala, India

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ABSTRACT

Trace metals are important environmental pollutants that may pose a potential risk to organisms and human health due to their toxicity, accumulation and difficult degradation. The present study was conducted to estimate the physicochemical parameters (water temperature, pH, dissolved oxygen, salinity, nitrite, nitrate, phosphate and silicate) and the trace metal (Fe, Ni, Zn, Cu, Pb, Cd and Cr) concentration in sediment and mud crab, *Scylla serrata* from the five stations of the Cochin backwaters. The seasonal overall average ranges of metals like Fe, Ni, Zn, Cu, Pb, Cd and Cr in crab (ppm dry weight) were recorded as 251.85 ± 105.29, 30.41 ± 11.73, 245.05 ± 112.67, 44.85 ± 21.99, 14.65 ± 6.62, 7.40 ± 3.24 and 36.38 ± 9.76 respectively. Trace metal contamination from industrial, domestic and agricultural sources affects the quality of water, sediment, and the biota of Cochin backwaters. The sediment can be regarded as either a sink or a source for trace metals in the aquatic environment, which may lead to bioaccumulation of toxic elements in the organisms through the food chain and cause adverse effects on human health.

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1. Introduction

Metal toxicity is of great environmental concern because of its bioaccumulative and non-biodegradable nature. Toxicity from trace metals is increasing due to the extensive release from industrial, agricultural, chemical, domestic and technological sources, which contaminate the water, sediment, soil and air (Aprile and Bellis, 2020). Water chemistry of the estuarine environment undergoes constant changes due to land-based discharges and is further influenced by environmental stressors, especially temperature, sunlight, humidity, rainfall and oxygen content (Vijayavel, 2010). It is also significant to determine the critical toxic concentrations of heavy metals in sediments to protect the aquatic biodiversity and maintain the stability of water ecology (Zhang et al., 2020). Sediments have a higher retention capacity than any other section of a water body; thus, they are the most important sink of trace metals in aquatic environments (Morin et al., 2007). They are one of the serious pollutants in aquatic habitats since these trace metals are flexible enough to enter the food chain of a marine ecosystem (Pandiyan et al., 2020). Notably, some trace metals are altered by living organisms and become organic complexes. Posing higher toxicity and greater health risks to animals and human beings (Su et al., 2011).

Aquatic species harbouring the estuarine ecosystem are under constant stress due to anthropogenic and environmental stressors, which harm their physiology and biochemistry (Walker et al., 2000). The health of any aquatic ecosystem or the survival of biota in the system is a function of its physicochemical variables. These indicators provide information on the impact on the aquatic ecosystem. Aquatic biota, which have the highest role in absorbing trace metals in the environment, are crustaceans such as crabs, shellfish and several types of shrimp. They are usually considered a bioindicator for the estimation of toxicological impacts. The mud crab *S. serrata* is a very important coastal resource due to its high demand and price in the international market (Jahan and Islam, 2016). The mud crabs constitute an important source of protein for local communities in the tropics and subtropics (Mirera, 2011). Crabs are often used as aquatic bioindicators because they can accumulate trace metals, which are quite high compared to other biotas (Yusni and Melati, 2019). They have a regulatory mechanism to detoxify and excrete accumulated metals (Deb and Fukushima, 1999). The present study aimed to (1) assess the physicochemical characteristics of water, (2) estimate the concentration of trace metals in the sediment samples (3) evaluate and quantify the bioaccumulation of trace metals in the flesh tissue of mud crab, *Scylla serrata* collected from the Cochin Backwaters.

2. Materials and Methods

2.1 Study area

The Cochin backwaters form a complex network of shallow brackish water bodies, extending between 9.9835405 ºN to 76.26668 ºE and 9.6725436 ºN to 76.40586 ºE. Cochin backwaters were reported to be heavily polluted by trace metals through industrial and municipal sources (Balachandran et al., 2005; Martin et al., 2012; Bindu et al., 2015; Jayasooryan, 2015; Lallu, 2017). Three seasonal conditions prevail in the estuary viz., pre-monsoon (PrM), monsoon (M) and post-monsoon (PoM). The Cochin backwater is subjected to various organic as well as inorganic contaminants. The main source of metal pollution in the central part is the processing of metal-containing minerals (ores) at the FACT plant, paints and pigments used at the shipyard and ports. The case of the southern part of the estuary is subjected to the excessive use of fertilisers and pesticides in the agricultural fields in Kuttanad. Based on the special geographic features, anthropogenic activities and the inflow of pollutants from the different sources, five stations (Fig. 1.) were selected between Cochin bar mouth and Thanneermukkom bund of Cochin backwaters for the present study.

2.2 Sample collection

Water, sediment, and crab samples were collected bi-monthly from February 2017 to January 2018. Two litres of surface water samples were collected using a clean plastic bucket and stored in a pre-sterilised polythene bottle, which was rinsed with the sample water. Sediment samples were collected using Van Veen Grab. Crabs were collected from the five sites with the help of local fishermen. They were preserved in iceboxes packed with ice to maintain their freshness and later transported to the laboratory.

2.3 Analysis of samples

2.3.1 Physicochemical parameters

The water quality analysis of the backwaters gives the exact nature, cause and levels of pollutants if any. The physicochemical parameters like water temperature, pH, dissolved oxygen (DO), salinity, total hardness and the nutrients (nitrite, nitrate, phosphate and silicate) were analysed by adopting relevant methods from APHA (1998) and Grasshoff (1999).

2.3.2 Sediment samples

Sediment samples were air-dried; stones and plant fragments were removed by passing the dried samples through a sieve. The sieved sample was powdered in a mortar and pestle and finally passed through a 500µm sieve and stored in glass bottles. Then 5g powdered sediment sample was taken in the Teflon beaker and digested with H_2O_2, HNO_3 and $HClO_4$ in the ratio of 1:3:1 by using a microwave digestor. The digested samples were made up to 50ml with distilled water and aspirated in the AAS (APHA, AWWA, WEF. 1998).

2.4 Crab samples

Approximately equal size and weight of crab samples were taken, and its flesh was dissected and washed thoroughly with distilled water. Then, the sampled organ was dried in an oven at 80 °C for 48 hours and stored in a vacuum desiccator. Dried samples were powdered, and aliquots of about 300 mg were digested for 3h at 80 °C with 3ml HNO_3 (65% Merck, Suprapure). Additional nitric acid was added if the samples were charred, and 1ml $HClO_4$ (Merck Suprapure) was added to make the solution clear and evaporate to near dryness. Thick white fumes evolved at the endpoint. The digests were cooled and diluted to make the solution to 25ml with double deionised water and kept in plastic vials. Trace metals were analysed using an Atomic Absorption Spectrophotometer. The selected metals in the tissues is calculated as per the formula;

$$\text{Concentration of metal in the sample} = \frac{\text{AAS reading} \times \text{Vol.ofsample} \times \text{dilutionfactor (if any)}}{\text{dry weight of thes ample taken}}$$

2.5 Data analysis

Data obtained from physicochemical parameters were analysed using descriptive statistics. Pearson correlation coefficient was used to determine the level of variance among physicochemicals using the statistical package XLSTAT and was tested at $p<0.05$ for significance. Two-way ANOVA was used to determine the spatial and temporal variations of concentration of trace metals in sediment using the statistical package in MS-Excel.

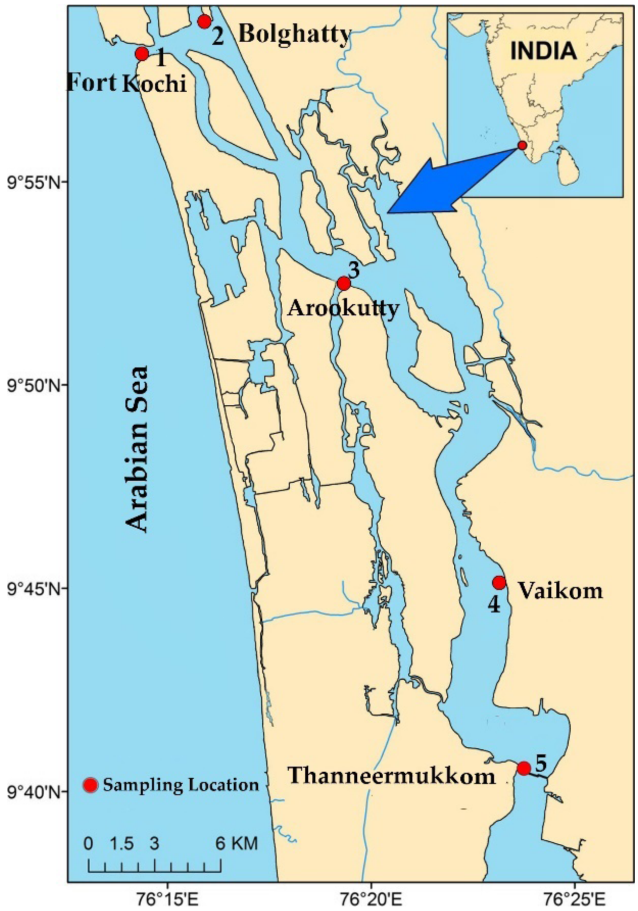

Fig. 1. Map of sampling locations at Cochin backwaters

3. Results and Discussion

3.1 Physicochemical parameters

Water is the most vital resource for all forms of life. The coastal areas are the first to face anthropogenic pressures and numerous types of waste, effluents and debris. The water quality in the coastal area is a critical feature that determines the health of the coastal ecosystem. The descriptive analysis of water quality parameters from the Cochin backwaters is given in Table 1. Karl Pearson's correlation analysis was used to determine the interrelationship between the physicochemical parameters. The correlation matrix of water quality parameters is given in Table 2.

The mean surface water temperature ranged between 28.30 and 33.85 °C in the Cochin backwaters. The surface water temperature is greatly influenced by atmospheric temperature (Meera and Nandan, 2010). The mean pH value recorded during the sampling period ranged from 6.51 to 8.30, with the highest pH value at S2 and the lowest at S5. pH < 7 was recorded in the southern region, but there is a transformation to alkaline towards S1 and S2. This is due to mixing fresh water in the southern region of the backwter and seawater in the bar mouth region (Selvam et

Table 1. Seasonal variation of physicochemical parameters of surface water from the Cochin backwaters

Stations	Seasons	Temp (°C)	pH	DO (ppm)	Salinity (ppt)	Total hardness (ppm)	Nitrite (µM)	Nitrate (µM)	Phosphate (µM)	Silicate (µM)
S1	PrM	31.50	7.98	4.24	33.69	7325.00	1.66	5.93	9.37	26.67
	M	28.30	7.88	6.84	15.89	582.50	2.19	19.79	1.92	42.95
	PoM	30.15	7.61	5.64	32.01	7650.00	0.58	9.79	4.36	36.00
S2	PrM	33.20	8.30	3.51	28.35	7590.00	1.38	13.03	10.41	17.95
	M	28.80	7.80	5.00	14.03	480.00	1.24	20.34	4.73	65.40
	PoM	30.75	7.61	3.42	24.20	5950.00	0.62	11.34	12.94	45.43
S3	PrM	32.95	7.40	4.30	26.29	3555.00	1.81	8.30	8.10	20.70
	M	29.60	7.80	5.18	10.25	450.00	2.27	14.01	2.24	57.00
	PoM	30.80	7.51	5.96	20.91	2675.00	0.56	10.60	2.82	53.35
S4	PrM	32.85	6.78	3.38	15.70	2904.50	1.42	15.80	7.86	39.91
	M	28.30	7.76	6.74	3.53	97.50	1.82	25.30	4.90	83.50
	PoM	30.80	7.49	4.88	15.05	694.00	1.96	17.60	13.17	71.00
S5	PrM	33.85	6.51	4.48	15.39	2325.00	1.00	9.16	4.58	50.57
	M	29.30	7.39	5.89	1.38	47.50	2.62	20.83	1.99	114.50
	PoM	31.05	6.89	4.62	12.24	1570.00	1.27	10.33	4.30	97.68

al., 2012). The mean values of dissolved oxygen, salinity and total hardness ranged between 3.38 to 6.84, 1.38 to 33.69 ppt and 47.50 to 7650 ppm, respectively. The higher dissolved oxygen and salinity were recorded maximum at S1 (at Barmouth). The Barmouth region showed higher salinity during the pre-monsoon period. Salinity gradually decreased in the southern region. It was also reported by Selvam et al., 2012. The mean values of nutrients, nitrite, nitrate, phosphate and silicate ranged between 0.56 to 2.62 µM, 5.93 to 25.30 µM, 1.92 to 13.17 µM and 17.95 to 114.50 µM, respectively. Significant sources of nitrate include fertiliser, decayed vegetation, animal matter, and domestic and industrial effluents (Agwu et al., 2013). An increase in the concentration of phosphate level in water may be due to the use of detergents, and also the anthropogenic addition of phosphorous into the water has a considerable effect on the quality of water. That may be derived from domestic sewage and agricultural runoff (Usharani et al., 2010). The hydrobiological conditions of the estuary are greatly influenced by the intrusion of seawater associated with tides and the freshwater influx from the rivers, and the precipitation process based on the studies on the distribution of salinity and the temperature (Joseph, 2002). Due to the influence of monsoon rain, there is a variation in the salinity of backwater near freshwater conditions and gradual transformation into brackish conditions during the dry season (Selvam et al., 2012).

The correlation coefficients were used to determine the relationship among water samples' physicochemical parameters. From the results, it is clear that the temperature showed a significant negative correlation with pH, DO, nitrate, and silicate and a positive correlation with salinity, total hardness and phosphate at 0.05 level. The higher temperature in the water caused a decreased level of dissolved oxygen. Salinity showed a significantly high correlation with all the parameters except pH at 0.05 level. Total hardness showed a high positive correlation with salinity and a negative correlation with DO, nitrite and nitrate. Silicate showed a negative correlation with temperature, hardness and salinity.

3.2 Trace metal concentration in sediment samples

The average concentration of Fe, Ni, Zn, Cu, Pb, Cd and Cr in sediment (ppm) in the Cochin backwaters ranged from 16946.98 to 53652, 15.13 to 59.59, 101.15 to 743.68, 14.24 to 49.31, 13.89 to 44.39, 1.13 to 14.45 and 34.54 to 83.44 respectively (Table 3). The highest concentration of trace metals was recorded in S2, and the lowest was reported in S1 except in the case of Zn. The lowest concentration of Zn was reported in S2. The accumulation pattern of trace

Table 2. Pearson correlation matrix of physicochemical parameters in the Cochin backwaters

Variables	Temp (°C)	pH	DO (ppm)	Salinity (ppt)	Total hardness (ppm)	Nitrite (µM)	Nitrate (µM)	Phosphate (µM)	Silicate (µM)
Temp (°C)	1	-0.393	-0.761	0.465	0.482	-0.272	-0.553	0.365	-0.491
pH	-0.393	1	0.149	0.293	0.275	0.083	0.160	0.102	-0.264
DO (ppm)	-0.761	0.149	1	-0.451	-0.515	0.268	0.425	-0.560	0.397
Salinity (ppt)	0.465	0.293	-0.451	1	0.901	-0.459	-0.573	0.379	-0.805
Total hardness (ppm)	0.482	0.275	-0.515	0.901	1	-0.455	-0.512	0.391	-0.679
Nitrite (µM)	-0.272	0.083	0.268	-0.459	-0.455	1	0.319	-0.171	0.280
Nitrate (µM)	-0.553	0.160	0.425	-0.573	-0.512	0.319	1	-0.115	0.327
Phosphate (µM)	0.365	0.102	-0.560	0.379	0.391	-0.171	-0.115	1	-0.315
Silicate (µM)	-0.491	-0.264	0.397	-0.805	-0.679	0.280	0.327	-0.315	1

Values in bold are different from 0 with a significance level alpha=0.05

Table 3. Seasonal variation of trace metals (ppm) in sediment of the Cochin backwaters

Stations	Seasons	Fe	Ni	Zn	Cu	Pb	Cd	Cr
S1	PrM	20940.23	18.55	231.68	17.69	21.36	2.67	40.37
	M	16946.98	15.13	188.61	19.03	13.89	1.13	34.54
	PoM	19884.40	17.57	210.02	14.24	18.06	1.43	38.45
S2	PrM	53652.19	59.59	743.68	49.18	44.31	14.45	83.44
	M	41269.86	49.66	654.61	41.09	38.12	9.01	52.67
	PoM	50855.56	53.48	704.02	42.71	42.55	10.90	73.45
S3	PrM	37501.93	47.53	201.74	33.74	27.48	3.13	57.08
	M	26575.31	39.23	134.21	26.08	22.06	1.22	44.69
	PoM	32476.11	45.25	183.23	27.88	25.49	2.07	50.58
S4	PrM	47809.19	41.13	128.47	36.02	33.33	3.33	54.25
	M	37755.93	33.42	101.15	31.11	27.96	2.58	43.79
	PoM	45221.82	38.14	117.99	28.65	30.88	1.53	49.76
S5	PrM	43299.08	45.15	150.78	37.69	35.78	2.71	47.54
	M	31817.51	38.58	135.56	40.61	38.77	2.91	40.34
	PoM	39640.86	43.16	145.67	31.11	35.00	1.83	43.59

metals in the sediment samples from the Cochin backwaters was in the decreasing order Fe > Zn > Cr > Ni > Cu > Pb > Cd. The concentration of trace metals in the sediment of the Cochin estuary varies considerably with the textural characteristics of the sediments (Padmalal et al., 1997).

In the present study, Fe showed the highest mean concentration in the five sampling stations. In biological system, Fe is the most abundant and well-known transition element (Hoda et al., 2007). Followed by Fe, Zn showed a higher concentration in sediment. This may be due to industrial activities, associated vehicle activities, domestic constructions and jetties (Sörme and Lagerkvist, 2002; Zulkifli et al., 2015). The heavy load of Zn was to be expected in view of an annual loading of ~80 T of Zn in ~ 250 chemical industries situated in the northern region, of which the Zn manufacturing industry is the prominent unit. The concentration of Zn and Cd is high in the S2 may be due to the zinc industry located close to station 2 (Deepulal et al., 2012). Among the five sampling stations, S2 is in the northern part of the estuary, where concentrations of all the metals were high. Due to the flow restrictions and enormous input of industrial effluents are found to be primarily responsible for the contamination of the sediments with trace metals in these regions (Balachandran et al., 2005). Most of the metals in S2, i.e. in the bar mouth region, showed the lowest concentration. This indicates the progressive dispersal of metal contaminants toward the Cochin coastal waters (Nair et al., 1990; Balachandran et al., 2005). In this region, the seasonal variations of trace metals in sediments were found to be marginal because of the constant mixing of fluvial sediments with the marine sediments due to strong rectilinear currents. This process maintains effective flushing of metal contaminants through the Cochin inlet (Priju and Narayana, 2007; Balachandran et al., 2008; Purandara et al., 2011; Bindu et al., 2015; George et al., 2016). The main sources of Cr in the backwaters are from tanning, textile and steel industries on the northern part of the estuary.

Table 4. Results of two-way ANOVA for testing spatial and seasonal variations of trace metals in sediments

Metal	Source of variation	F	P-value
Fe	Spatial	118.92	0.000003
	Seasonal	38.45	0.00007
Ni	Spatial	295.71	0.00000001
	Seasonal	34.37	0.0001
Zn	Spatial	755.66	0.0000000002
	Seasonal	12.60	0.003
Cu	Spatial	37.33	0.00003
	Seasonal	5.06	0.04
Pb	Spatial	53.39	0.000008
	Seasonal	4.65	0.05
Cd	Spatial	41.71	0.00002
	Seasonal	4.39	0.05
Cr	Spatial	15.98	0.0006
	Seasonal	8.23	0.01

3.3 Trace metal levels in crabs

The average concentrations of trace metals Fe, Ni, Zn, Cu Pb Cd and Cr in crab, *Scylla serrata* (ppm dry weight) during pre-monsoon season in the Cochin backwaters was ranged from 281.48 to 418.24 (average 349.08 ± 62.24), 18.45 to 53.50 (average 38.48 ± 12.69), 153.29 to 477.63 (average 309.75 ± 112.20), 14.62 to 80.79 (average 53.86 ± 26.52), 1.80 to 24.22 (average 10.92 ± 7.85, 4.28 to 12.06 (average 9.18 ± 3.38) and 36.99 to 52.91 (average 43.51 ± 8.64) respectively (Table 5 and 6). Fe, Ni, Zn, Cu Pb Cd and Cr in crab (ppm dry weight) during monsoon season ranged from 156.45 to 387.70 (average 245.06 ± 89.66), 16.60 to 36.04 (average 27.65 ± 6.99), 109.46 to 291.66 (average 172.11 ± 80.21), 24.55 to 74.72 (average 46.28 ± 20.96), 8.82 to 17.11 (average 14.74 ± 3.47), 3.06 to 9.92 (average 6.54 ± 2.94) and 20.28 to 32.89 (average 29.45 ± 6.87) respectively (Table 5 and 6). Fe, Ni, Zn, Cu Pb Cd and Cr in crab (ppm dry weight) during post-monsoon season 70.36 to 244 (average 161.85 ± 64.50), 12.36 to 41.32 (average 25.11 ± 11.01), 165.54 to 396.18 (average 253.29 ± 94.36), 14.68 to 49.52 (average 34.42 ± 14.21), 11.28 to 26.23 (average 18.28 ± 6.13), 2.99 to 8.72 (average 6.49 ± 2.92) and 29.04 to 45.02 (average 36.18 ±

Table 5. Spatial and seasonal variation of trace metals (ppm dry weight) in mud crab of the Cochin backwaters

Stations	Seasons	Fe	Ni	Zn	Cu	Pb	Cd	Cr
	PrM	385.05	18.45	477.63	14.62	1.80	4.28	49.88
S1	M	156.45	16.60	291.66	24.90	8.82	3.06	32.89
	PoM	183.77	12.36	396.18	14.68	11.28	2.99	45.02
	PrM	337.53	53.50	366.38	78.42	24.22	12.06	52.91
S2	M	184.34	36.04	116.02	60.45	17.11	7.78	31.91
	PoM	188.62	41.32	280.95	47.02	26.23	8.66	43.13
	PrM	281.48	42.19	153.29	45.20	9.11	7.65	37.43
S3	M	204.45	28.48	138.58	24.54	16.30	5.38	20.28
	PoM	70.36	30.79	165.54	27.49	21.77	4.81	29.04
	PrM	323.11	41.42	285.62	50.30	10.71	10.61	40.34
S4	M	292.37	31.29	109.46	46.82	16.84	6.55	32.43
	PoM	119.88	24.82	201.76	33.39	18.33	7.30	32.57
	PrM	418.24	36.86	265.84	80.79	8.79	11.29	36.99
S5	M	387.70	25.86	204.86	74.72	14.61	9.92	29.74
	PoM	244.38	16.25	222.06	49.52	13.81	8.72	31.14

8.68) respectively (Table 5 and 6).

The seasonal overall average ranges of metals like Fe, Ni, Zn, Cu, Pb, cd and Cr in crab (ppm dry weight) ranged from 245.06 to 349.08 (average 251.85 ± 105.29), 25.11 to 38.48 (average 30.41 ± 11.73), 172.11 to 309.75 (average 245.05 ± 112.67), 34.42 to 53.86 (average 44.85 ± 21.99), 10.92 to 18.28 (average 14.65 ± 6.62), 6.49 to 9.18 (average 7.40 ± 3.24) and 29.45 to 43.51 (average 36.38 ± 9.76) respectively. The accumulation of Zn was higher than Fe in S1 in all the seasons. Zn plays a major role in different enzymatic activities; hence, all the body parts can accumulate it in higher concentrations (Kamaruzzaman et al., 2012). The seasonal bioaccumulation of trace metals in the crab was in the decreasing order of pre-monsoon > monsoon > post-monsoon. The substantial increase in the trace metal concentrations from the monsoon to post-monsoon to pre-monsoon in the backwater provides a time-integrated indication of environmental contamination. This results from the semi-enclosed topography of the backwater, which intensifies the weak flushing of contaminants and the influence of salinity on the bioaccumulation process (George et al., 2013).

Mud crabs are bottom feeders, and their burrowing behaviour influences the accumulation of trace metals in their tissues from the surrounding sediment (Kamaruzzaman et al., 2012; Chainy and Paital, 2012). The crabs are closely related to the sediment because it is their main habitat and feeding site (Sultana et al., 2015). Thus, the trace metals in the sediment are easily absorbed and easily accumulated in the crab tissue through bioaccumulation and biomagnification (Amin et al., 2018). Mu'nisa and Nurham, 2010 reported that the accumulation of trace metals in the body of an organism depends on its concentration in water, temperature, species, physiological condition and feeding habits of organisms (Sarong et al., 2015). In crabs, the metal accumulation is directly proportional to the bioavailability of metals from the water and food chain (Viswanathan et al., 2013). Many crustaceans play a significant role in efficiently regulating trace metal burden, among which moulting is one such key mechanism through which substantial quantities of metals may be expelled by the moulted carapace (Anandkumar et al., 2019).

3.4 Assessment of trace metal in crab

Analysis of variance

The statistical significance of spatial and seasonal differences in trace metal concentration in crab was tested using two-way ANOVA. Results indicate that spatial and seasonal variability in concentrations of all the metals was statistically significant ($p<0.05$; Table 7) in crabs. The special variations of trace metal concentrations in crab were significantly different among the five stations during all the seasons.

4. Conclusion

The present study highlights the seasonal changes in the physicochemical characteristics of surface water and, the spatial and seasonal distribution of trace metals in the sediments, and the bioaccumulation of trace metals in the mud crab *Scylla serrata,* from the Cochin backwaters. This study reveals that the backwater is under the stress of anthropogenic activities. The accumulation of trace metals in the muscle tissue of mud crab was in the order of Fe >

Table 6. Season-wise averages of trace metals (ppm) in crab from the Cochin backwaters

Seasons	Fe	Ni	Zn	Cu	Pb	Cd	Cr
PrM	349.08 ± 62.24	38.48 ± 12.69	309.75 ± 112.20	53.86 ± 26.52	10.92 ± 7.85	9.18 ± 3.38	43.51 ± 8.64
M	245.06 ± 89.66	27.65 ± 6.99	172.11 ± 80.21	46.28 ± 20.96	14.74 ± 3.47	6.54 ± 2.94	29.45 ± 6.87
PoM	161.40 ± 64.50	25.11 ± 11.01	253.29 ± 94.36	34.42 ± 14.21	18.28 ± 6.13	6.49 ± 2.92	36.18 ± 8.68
Overall	251.85 ± 105.29	30.41 ± 11.73	245.05 ± 112.67	44.85 ± 21.99	14.65 ± 6.62	7.40 ± 3.24	36.38 ± 9.76

Table 7. Results of two-way ANOVA for testing spatial and seasonal variations of trace metals in crab, *Scylla serrata*

Metal	Source of variation	F ratio	P value
Fe	Spatial	5.05	0.03
	Seasonal	18.24	0.001
Ni	Spatial	12.29	0.002
	Seasonal	22.73	0.001
Zn	Spatial	9.58	0.004
	Seasonal	9.68	0.007
Cu	Spatial	6.75	0.01
	Seasonal	9.59	0.008
Pb	Spatial	12.54	0.002
	Seasonal	6.86	0.02
Cd	Spatial	36.61	0.00003
	Seasonal	19.62	0.0008
Cr	Spatial	9.23	0.004
	Seasonal	20.36	0.0007

Zn > Cu > Cr > Ni > Pb > Cd. The higher concentration of trace metals in the sediment reflects the toxicity of trace metals in the crab. The higher metal concentration in the Cochin estuary poses considerable ecological risk due to environmental quality and the concentration of trace metal in sediment and the edible crab. The long-term monitoring of metal distribution, bioaccumulation and health risk assessment will provide information regarding pollution management strategies, especially for backwaters and elsewhere in general.

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