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Spatio-temporal variability of trace metal concentrations in planktonic amphipods from an estuary of south-west coast of India

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Abstract
Spatio-temporal variations of 8 trace metals in planktonic amphipods were studied from a highly productive tropical estuary, the Cochin backwaters.The mean trace metal accumulation and bioaccumulation factors in amphipods were in the order Fe>Zn > Mn > Cu > Ni > Cr > Pb > Cd and Fe > Zn > Mn > Cu > Cr > Ni > Cd > Pb respectively. ANOVA revealed that the spatial and seasonal variations of trace metal content in amphipods were statistically significant.Hierarchical cluster analysis (HCA) has grouped the sampling stations into two clusters based on the trace metal concentrations in amphipods of Cochin backwaters. Thehigh bioaccumulating factors (BAF) reported in this studyindicating great trace metal accumulating capacityof amphipods. Two groups of elements were produced from statistical analysis using Principal Component Analysis (PCA). First group included Fe, Ni, Zn, Cr, Pb, Cd and Cu while second contained Mn. All metals exhibited higher concentrations in amphipods than their background values, signifying anthropogenic pollution in estuary.
Keywords: Trace metal, amphipods, Cochin estuary, India.

Introduction

The aquatic ecosystem encompasses the biggest portion of the biosphere and includes both freshwater and marine ecosystems. Estuaries are one of the most productive ecosystems on Earth and are vital to the life history of many aquatic species (Chapman and Wang, 2001).

Estuaries and coastal ecosystems are transition zones between rivers and the marine environment and act as a natural sink for several environmental pollutants. Many estuaries have been contaminated by pollutants like trace metals,impacting biotic communities and sensitive habitat areas. Itai Itai disease caused by cadmium pollution in Japan was one of the well-known environmental tragedies associated with trace metal pollution. Earlier studies testified that the ecological health of the Cochin estuary has depleted due to industrialization, sewage discharge, pollution from pesticides, fertilizers, metal discharge, and municipal wastes, dredging activities, conversion of land for agriculture, and land reclamation for tourism activities (Balachandran et al., 2005; Martin et al., 2008). The pollution load of Cochin backwaters increases due to waste disposal, dredging, transportation, industrialization and metal pollution (Martin et al., 2011; Anu et al., 2014). Over the past 50 years, the estuary has experienced detrimental alterations due to the release of untreated effluents from domestic and industrial sectors, culminating in 0.26 × 103 m^3 d-1 and 0.104 × 106 m3 d-1 in the estuarine ecosystem (CPCB, 1996; Menon et al., 2000). According to the Environmental (protection) Actof1985, Cochin backwater is classified under the “ecological sensitive zone”.

The bioavailable fraction of trace metals is of great significance when evaluating the environmental quality concerning trace metal pollution of an estuary. Bioavailability refers to a portion of the total quantity of metal that an organism takes up when encountering the environmental media, summed across all possible sources of metal, including water and food as appropriate (Rainbow and Luoma, 2011). Since concentrations of trace metals accumulated by certain organisms provide integrated measures of the local bioavailability of trace metals over a preceding period, the use of biomonitors to determine geographic and temporal variations in the bioavailability of potentially toxic metals in coastal waters is now established (Rainbow,1995). Amphipods have been proposed as model organisms because they are widespread, occur in high densities, are often the main food for predatory fish and birds, and have significant potential for metal accumulation in marine food chains (Marsden and Rainbow, 2004). Amphipods are widely used in trace metal pollution studies (Marsden et al., 2003; Seabrook, 2020). They are free-living Malacostracan crustaceans that function as keystone species in diverse habitats and are internationally used for sediment testing (Marsden and Rainbow, 2004). The majority of amphipods are detritivores, but some are also grazers of algae, omnivores, or predators of tiny insects and crustaceans. Bioindicators are organisms that can be used to provide information on the variation of pollutants over time and space. Without sound information on spatio-temporal variations, it will not be possible to differentiate the natural concentration of trace metals from anthropogenic metal inputs with a routine biomonitoring program. Moreover, this study is the first attempt to assess trace metal concentration in Amphipods of Cochin backwaters and its suitability as a bioindicator for pollution monitoring studies.

Materials and Methods

Study area

The Cochin backwaters (Lat. 9º 30´-10º 10´ N and Long.76º15´-76º 25´E), are the largest of the backwaters on the Kerala coast and are regarded as India's second- largest estuarine system. The estuary has been polluted by trace metals laden from industrial, agricultural and municipal sources (Rejomon et al., 2016; Jayasooryan, 2015). Augmented anthropogenic activities of the Cochin city generate 0.104 M m^3 d-1 of industrial and 260 m^3 of domestic waste per day, which are being discharged directly into the estuary without any treatment (Rejomon et al., 2013). The geographic positions of each site were fixed using Global Positioning System and presented in the table 1. Theflow of pollutants from the different sources, geographical features and anthropogenic activities are the main criteria for the selection of different sampling stations (Fig. 1).

Collection of Samples

Field studies were conducted bimonthly from March 2017 to January 2019 in selected stations of Cochin backwaters. Three seasons prevail in the estuary namely pre-monsoon, monsoon and post-monsoon. Thus, sampling is following these seasons. Water sample collection and preservation were done as per standard methods Strickland and Parsons, 1972, APHA, 2012. Zooplankton was collected using a W.P. net (mesh size 200 µm) and kept in an icebox at 4-5 ºC and transported to the Laboratory for further process.

Estimation of Trace metals

In the laboratory, Zooplankton samples were sorted and amphipods were separated. Amphipods were rinsed with Milli-Q water to remove salts. Samples were completely dried overnight in an oven at 65˚C and stored in a vacuum desiccator. The dried samples were first powdered and a portion of about 300 mg was digested for 3 hours at 80°C with 3 ml of HNO3 and 1 ml of HClO4. The digests were made up to 25 mL with Milli-Q water. Trace metals in the water samples can be estimated following the standard protocol (Grasshoff et al., 1983).The dissolved metals were extracted using ammonium pyrrolidinedithiocarbamate (APDC, 2%) and Chloroform at acidic pH, brought back to the aqueous layer by back-extraction with concentrated nitric acid and made up to 20 ml with sterile di-ionized water.

The concentration of trace metals inamphipods and water samples were determined with an Atomic Absorption Spectrophotometer. The calculations of the selected metals in plankton were done as per the formula;

$$\text{Concentration of metal in the sample} = \frac{\text{AAS reading} \times \text{vol. of sample} \times \text{dilution factor(if any)}}{\text{dry weight of the sample taken}}$$

Bioaccumulation factor was calculated as the ratio of the concentration of the trace metals in the amphipods and in the surrounding water

$$BAF = CB/CW$$

Statistical analysis

The statistical analysis of range, mean, standard deviation, two-way ANOVA and Principal componentanalysis were performed using XLSTAT statistical software. The relationship between amphipod's trace metal accumulation in different sampling stations were assessed by hierarchical cluster analysis (HCA) using PAST (4.03) software.

Results

Trace elements in amphipods

In this study, the concentrations of 8 elements in the amphipods collected from the Cochin estuary wereassessed. The mean concentrations of different metals in amphipods were Fe (2325.04 ± 1749.8 ppm), Ni (27.43 ± 09.69 ppm), Zn (263.45 ± 89.3 ppm), Cu (33.4 ± 13.5 ppm), Cr (26.80 ± 09.69 ppm), Mn (54.27 ± 27.40 ppm), Cd (2.44 ± 00.70 ppm) and Pb (11.25 ± 6.10 ppm). The descending order of trace metal accumulation in amphipods was Fe >Zn > Mn > Cu > Ni > Cr > Pb > Cd.

Spatial and seasonal variability of trace metals in amphipods

Amphipods mean trace metal concentration across each sampling station was summarized in Table 2. Trace metal value of Fe in amphipods showed the maximum (3371.14 ppm) at station 3 and minimum (613.72 ppm) at station 5. Ni concentrations in amphipods ranged from 11.61 to 47.9 ppm. Lowest (20.94 ppm) and highest (33.4 ppm) mean concentration of

Ni was found in station 4 and 2 respectively. The maximum trace metal value (324.56 ppm) of Zn in amphipods was found at station 2 and minimum value (191ppm) at station 5. In the case of Cr and Pb, station 1 and 5 recorded the highest and lowest mean values of trace metals respectively. Highest mean trace metal value (46.57 ppm) of Cu was found at station 2 and lowest value (24.19 ppm)at station 5. In sampling station 5 data showed the highest mean trace metal concentration of Mn (72.33 ppm) and minimum (38.90 ppm) at station 1. The maximum station-wise mean value of Cd was found at station 2 (03.01 ppm) and minimum (01.73 ppm) at station 4. To find the similarity of amphipods trace metal accumulation in sampling stations, hierarchical cluster analysis (HCA) was executed. HCA demonstrates that stations 1, 2, 3 and 4 were all clustered together, while S5 was distant from others (Fig.2).

The highest seasonal mean values of trace metals in amphipods were observed during the monsoon season. The mean value of Fe concentrations in amphipods was higher than other metals during all seasons and Cd showed the lowest accumulation in amphipods in all seasons (Table 3).The results of ANOVA on metal concentrations in amphipods showed significant ($p<0.05$) seasonal and spatial variations (Table 4). The relationship between different trace metals in amphipods were visualized and assessed using principal component analysis (PCA). PCA results showed that the parameters with significant component matrices in component 1 were Ni (0.922) > Zn (0.900) > Fe (0.894) > Cr (0.861) > Cd (0.632) > Cu (0.546) but Mn (0.835) was the only trace metal with significant RCM in component 2 (Fig.3).

Bioaccumulation factor

The dissolved trace metal concentrations of Cochin backwaters were in the order Fe > Zn > Pb > Ni > Mn > Cr > Cu > Cd with concentrations 0.63, 0.18, 0.044, 0.041, 0.037, 0.03, 0.025, 0.007 ppm respectively.The result of dissolved metals from the present study was compared with WHO (2008) and EPA (1986) standards (Fig. 4). Among the studied trace metals all the metals were found to be below the maximum limit prescribed by WHO 2008 and EPA, 1986 except Fe, and Pb. The mean value of recorded Pb (0.044 ppm) and Cd (0.007 ppm) was compared with Class SW-I waters (shell fishing, Ecologically Sensitive Zone) water quality standard (EPA, 1986) (Fig. 4). The order of mean BAFs for trace metal studied in amphipods during the period of study is given as Fe > Zn > Mn > Cu > Cr > Ni > Cd > Pb (Fig. 5). The highest mean trace metal BAFs in amphipods during the period of study was 3684.68 reported for Fe and the lowest BAF was observed for Pb (257.99).

Discussion

Comparison of trace metal concentration in amphipods of cochin backwater with that of global data (Table 5) reveals its bio-accumulative capacity and its suitability as a fundamental tool for tracking trace metal pollution (Cai et al., 2024). Fe concentration ranges (210 to 5547 ppm) obtained for amphipods during the present study are higher and lower than the minimum and maximum Fe concentration (71.3 to 12900 ppm) reported for amphipods by an earlier study from the S.W Scotland (Rainbow and Moore, 1986). Amphipods collected from the from the Kerguelen waters of the Southern Indian Ocean (48 to 88 ppm) exhibited lower Zn concentration than observed (106.45 to 462.5 ppm) in the present study (Caio et al., 2017). Zn concentration ranges in Amphipods from the present study (106.45 to 462.5 ppm) are considerably higher than the Zn concentration ranges (59 to 128 ppm) reported for amphipods from the German Bight of the North Sea (Zauke et al., 1996). The minimum and maximum value of Cu in the concentration ranges (8 to 59.3 ppm) obtained for amphipods during the present study is higher than the minimum and maximum value of Cu in the concentration ranges (11.8 to 24.2 ppm) reported for Amphipods from the Kergulen waters (Caio et al., 2017). Moreover, Cd concentrations (2 to 8.8 ppm) in amphipods from the Northwestern Mediterranean Sea (Romeo et al., 1992) was typically higher than those found in amphipods (1.07 to 4.20 ppm) collected from the present study.

The mean Ni concentrations obtained in amphipods of the present study are ninety times higher than the mean Ni concentration (0.38 ppm) in amphipods reported from the German Bight of North Sea (Zauke et al.,1996). Amphipods of the present study also showed significantly higher levels ofZn than the mean Zn concentration (92.0 and 110 ppm) in amphipods reported from the Greenland Sea and Barents Sea (Ritterhoff and Zauke, 1997; Zauke and Schmalenbach, 2006). The mean Cu concentrations obtained in amphipods of the present study are higher than the mean Cu concentration (9 and 23.5 ppm) in amphipods reported from the Barents Sea and Greenland Sea (Zauke and Schmalenbach, 2006; Ritterhoff and Zauke, 1997). In Cochin backwaters mean Cd concentration in amphipods ismuch lower than the mean Cd concentration (10.5 and 28.2 ppm) in amphipods reported from the Barents Sea and Greenland Sea (Zauke and Schmalenbach, 2006;Ritterhoff and Zauke, 1997). The mean Pb concentrations obtained in amphipods of the present study are much higher than the mean Pb concentration (1.0 ppm) in amphipods reported from the Greenland Sea (Ritterhoff and Zauke, 1997).

Spatial and seasonal assessment of trace metals in amphipods

The mean Fe, Zn, Cu, Cr and Pb content in amphipods during the period of the study showed a 4.0 to 4.8, 1.2 to 1.7, 1.1 to 1.9, 1.2 to 1.4 and 1.1 to 1.5 times enrichment at stations 1 to 4 when compared to station 5. Ni content in amphipods showed a 1.4 to 1.6 times enrichment at stations 1 to 3 when compared to station 4. Mn content in amphipods showed a 1.1 to 1.9 times enrichment at stations 2 to 5 when compared to station 1. Cd content in amphipods showed a 1.3 to 1.7 times enrichment at stations 1 to 3 and station 5 when compared to station 4 (Table 2). The spatial variations of Fe, Ni, Zn, Cu, Cr, Mn, Pb and Cd contents (ANOVA, Table 4) noted in amphipods were found to be statistically significant ($p \leq 0.05$). HCA showed that S1, S2, S3, and S4 formed a cluster which was distant from S5. The element concentration at station 1 is more similar to those in station 4. Similarly, station 2 and 3 showed more similar trace metal concentration profile Figure 2. The high enrichment of trace metals like Fe, Ni, Zn, Cu, Cr, Cd and Pb in amphipods at station 2 is due to the influence of effluent discharges from the nearby chemical industries and domestic sewage inputs in this area from the Cochin city through the Periyar river. The similar high enrichment of trace metals like Ni, Zn, Cr, Pb and Cd in amphipods at station 1 is due to the influence of effluent discharges associated with port and shipyard activities at this locality (Balachandran et al., 2005). High enrichment of Mn in amphipods at station 5 is due to the influence of agricultural effluent discharges at this locality from the Kuttanad agricultural fields (Selvam et al., 2012; Rejomon et al., 2013).

Trace metal accumulation in amphipods during different seasons showed the following decreasing order: monsoon > post-monsoon > pre-monsoon. Seasonally the mean metal contents in amphipods showed higher values during the monsoon period when compared to post-monsoon or pre-monsoon periods. For instance, the mean values of Fe, Ni, Zn, Cu, Cr, Mn, Cd and Pb in amphipods showed a 3.6, 1.5, 1.4, 1.1, 1.3, 2.0, 1.2- and 2.5-times enrichment during the monsoon when compared to the pre-monsoon period (Table 3). Thus, the major factor which causes an increase of trace metal concentrations in amphipods is probably related to the high influx of metals as a result of pollution due to increased inputs of domestic sewage and discharge of industrial and agricultural effluents by tides and freshwater discharges to the estuarine environment of Cochin (Ouseph, 1992; Rejomon et al., 2016). The seasonal variations of Fe, Ni, Zn, Cr, Mn, Cd and Pb contents (ANOVA, Table4) noted in amphipods were found to be statistically significant ($p \leq 0.05$).

Distributions of trace metals in organisms depend upon their bioaccumulation. By applying PCA, it seems that metals such as Fe, Ni, Zn, Cr, Pb, Cd and Cu highly contribute positively to the first axis. These strong associations were because of efficient uptake and low elimination rate of these metals in amphipods (Rainbow et al., 1990). Mn contributes positively to the second axis. This observation suggests that these two groups of trace metals do not have the same origin.The physical forcing that regulates the dispersion and seasonal dynamics of metals, along with local point sources of their addition, are the main causes of the bioavailability in source water in general (Achary et al., 2020).

Bioaccumulation factor

The aquatic organism's ability to take in trace metals from its surroundings is assessed using BAF. The BAF values for studied metals in amphipods are given in Figure 5. It was noted during the study that, each metal showed high BAF exceeds 100. Bioaccumulation of metals in zooplankton depends upon bioavailability, the amount of dissolved metal uptake, the physiological efficiency of the organism to excrete excess metals as well as on the feeding rate, and prey availability (Rainbow, 1997). The high concentrations of metals and high bioaccumulation values indicate that the amphipods have an enormous capacity for the accumulation of trace metals and are thus potentially excellent bioindicators for the evaluation of the contamination of estuarine ecosystems by metals (Rejomon et al., 2008; 2010; Achary et al., 2020).

Conclusion

A routine biomonitoring approach will not be able to distinguish between natural background concentrations and anthropogenic metal inputs without a solid understanding of spatio-temporal changes. This study presents baseline information on trace metal concentration in water and amphipods.The highest metal concentration in amphipods was detected during the monsoon season. Most of the metals in amphipods are high in the northern stations of the estuary.Metal concentration in amphipods provides a great deal of information of practical relevance in terms of the spatio-temporal variabilities of trace metals in the Cochin backwaters.

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Disclosure of Interest

The authors have no conflict of interest.

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Figure captions

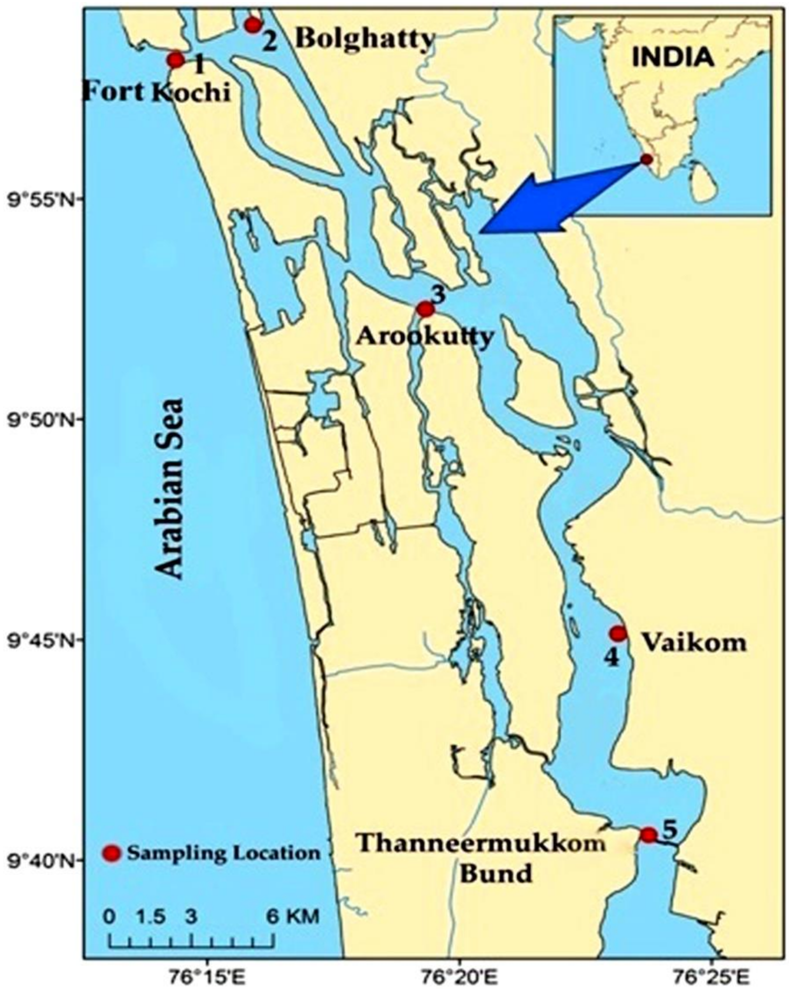
Figure 1. *Study area*

Figure 2. *Hierarchical cluster analysis (two way) demonstrating the similarity among sampling stations based on the trace metal concentrations in amphipods of Cochin estuary*

Figure 3. *Principal component analysis (PCA) demonstrating trace element profiles in amphipods of Cochin estuary*

Figure 4. *Mean concentration of metals in surface water compared with international standards*

Figure 5. *Mean BAFs of trace metals in Amphipods of Cochin estuary*



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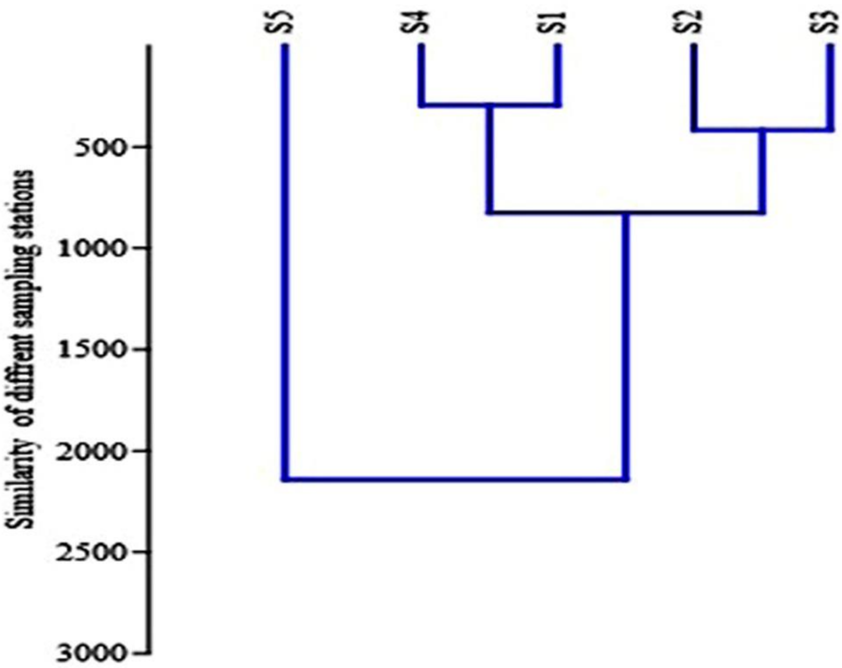

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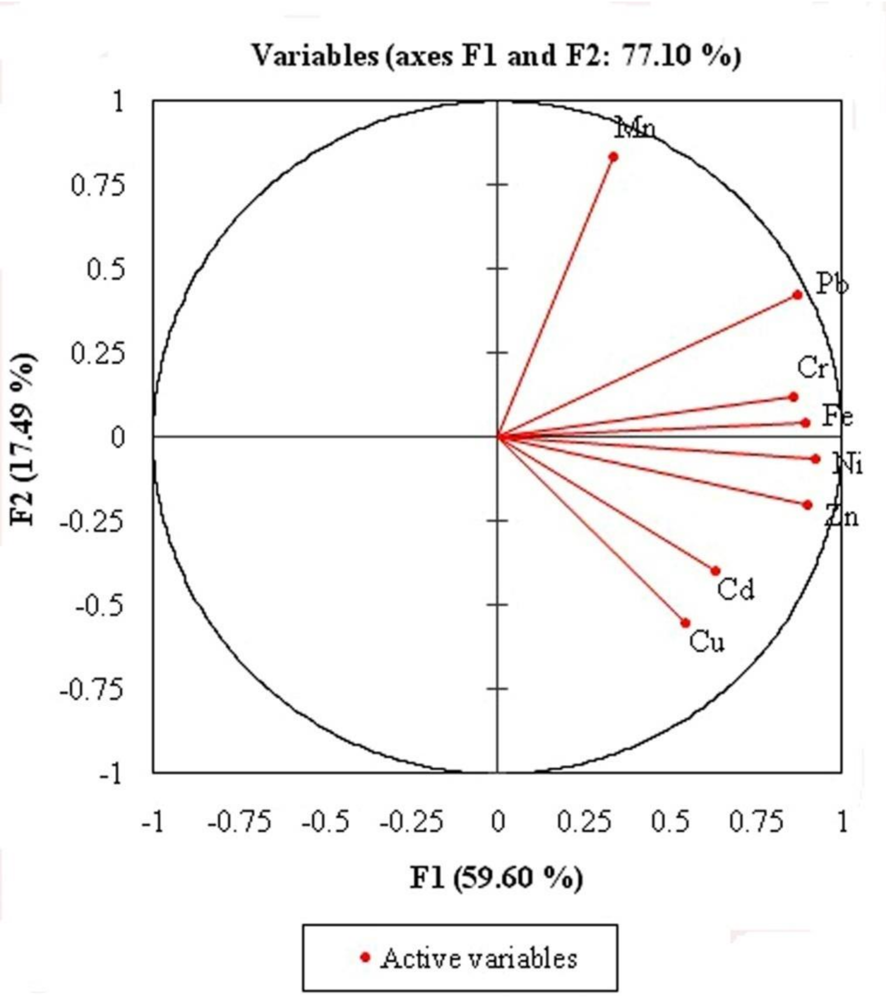

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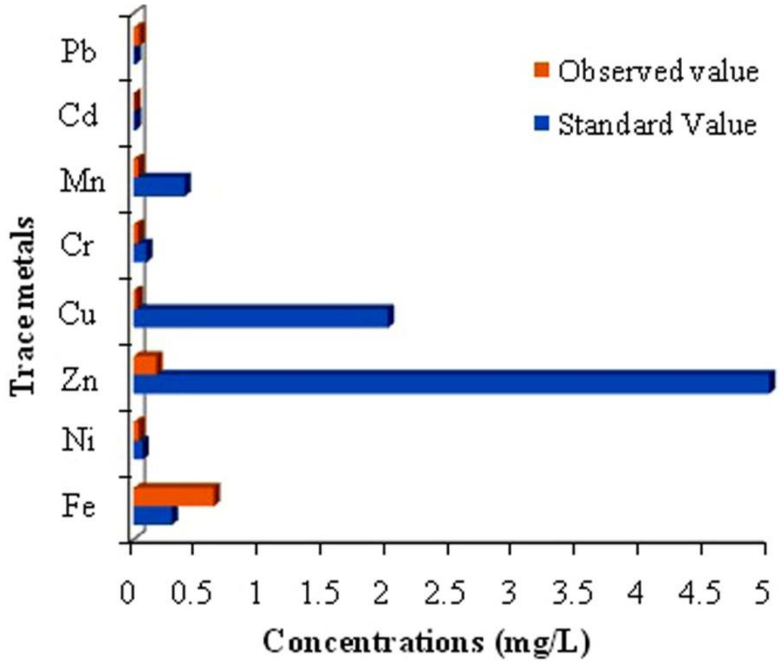

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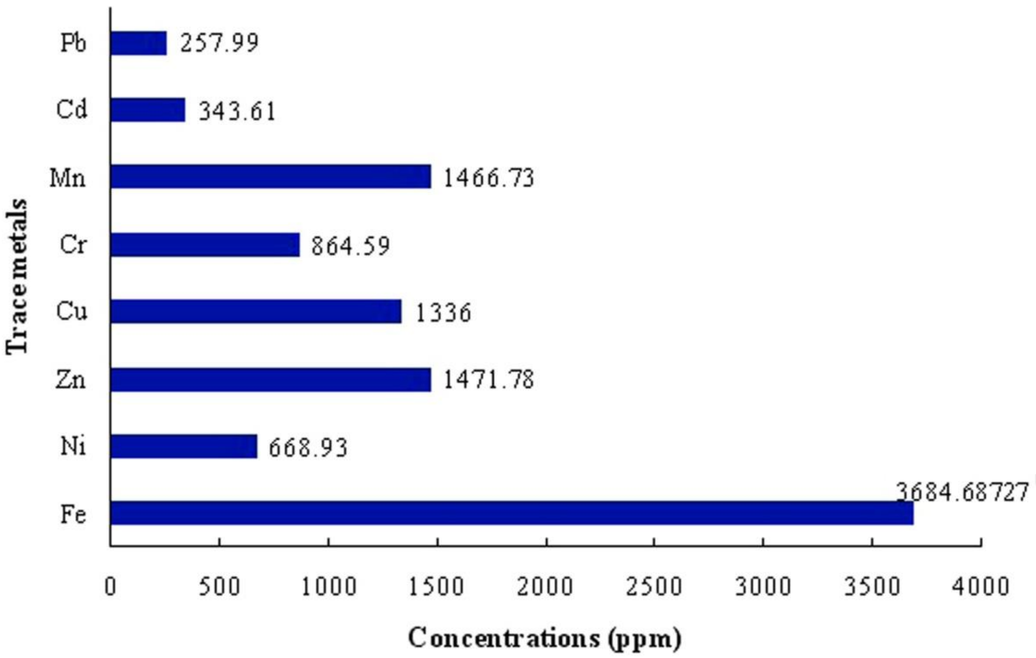

Figure 5. *Mean BAFs of trace metals in Amphipods of Cochin estuary*

Table 1. Location of sampling stations in Cochin backwaters

Sl No.	Location	Latitude	Longitude
1	Fort Kochi	9° 58'. 12" N	76° 14' 22" E
2	Bolghatty	9°59' 44" N	76°15' 40" E
3	Arookutty	9°52' 16" N	76°19' 12" E
4	Vaikom	9°45' 07" N	76°23' 15" E
5	Thanneermukkom	9°40' 28" N	76°24' 01" E

Table 2. Mean trace metals (ppm, dry weight) concentration in planktonic amphipods of Cochin backwaters.

Trace Metals	Stations						
	S1	**S2**	**S3**	**S4**	**S5**	**Min.Max.**	**Mean ± S. D**
Fe	2197.75 ±1906.34	2957.81 ± 1892.86	3371.14±1301.38	2484.77±1571.87	613.72 ±412.10	210.00 5547	2325.04 ±1749.80
Ni	29.60 ± 5.10	33.40 ± 8.90	32.12 ± 9.28.00	20.94±6.60	21.04±8.80	11.61 47.90	27.43 ± 9.69
Zn	296.50 ±86.87	324.56±83.86	274.04± 93.99	231.15±61.37	191.00 ±57.63	106.45 462.50	263.45 ±89.30
Cu	27.06 ±13.48	46.57± 10.07	34.49± 8.80	34.65 ± 13.65	24.19 ± 10.70	8.00 59.30	33.4 ± 13.50
Cr	29.89 ± 9.98	28.50± 7.85	28.67±12.39	26.17±8.75	20.83 ±7.39	7.00 48.00	26.80 ±9.69
Mn	38.90 ± 19.25	44.55 ±26.67	53.86±24.37	61.68 ±21.59	72.33 ±33.13	13.40 120.00	54.27 ± 27.40
Cd	02.52 ± 0.60	03.01 ± 0.47	02.69± 0.62	01.73 ± 0.55	02.23 ± 0.44	1.07 3.20	2.44 ±0.70
Pb	13.32 ± 5.74	10.92 ±6.10	12.72 ± 7.21	10.11± 5.43	09.16±5.55	2.50 24.90	11.25 ± 6.10

Table 3. Seasonal variation of trace metals (Mean ± S.D) in amphipods of Cochin estuary.

Trace metals (ppm)	**Pre-monsoon**	**Monsoon**	**Post-monsoon**
	Mean ± S. D	**Mean ± S. D**	**Mean ± S. D**
Fe	1086.50±641.62	3925.16 ±1791.03	1963.45±1200.27
Ni	22.29 ± 7.22	34.10 ± 9.5	25.88 ± 07.22
Zn	232.08± 89.60	317.29 ± 82.40	240.97 ±72.84
Cu	33.20±11.50	35.73±15.36	31.30 ±13.67
Cr	22.75±9.51	31.28±8.88	26.4 0± 09.10
Mn	39.16±13.17	80.45±27.43	43.19±17.56
Cd	2.13± 0.76	2.6±00.52	2.56± 00.681
Pb	7.02±2.80	17.47± 4.21	9.21± 04.90

Table 4. Two-way ANOVA of metal concentrations in Amphipods

Trace metals	**Source of variations**	**F value**	***p*-value**
Iron	Stations	5.20	0.02*
	Seasons	16.43	0.001*
Nickel	Stations	6.5	0.01*
	Seasons	11.08	0.004*
Zinc	Stations	6.46	0.012*
	Seasons	8.41	0.010*
Copper	Stations	10.51	0.002*
	Seasons	1.16	0.36ns
Chromium	Stations	4.02	0.044*
	Seasons	9.49	0.007*
Manganese	Stations	4.76	0.029*
	Seasons	23.13	0.004*
Cadmium	Stations	12.69	0.001*
	Seasons	6.08	0.024*
Lead	Stations	3.84	0.049*
	Seasons	63.06	0.000*

* Significant at the 0.05 probability level

Table 5. Comparison of trace metal concentration in amphipods from the Cochin estuary with those from worldwide published literature(range/mean ± SD in ppm).

Sampling area	Fe	Ni	Cd	Cu	Zn	Cr	Mn	Pb	References
Greenland Sea		3.0 ± 0.1	28.2±2.6	23.5±1.3	92±6			1.0±0.1	Ritterhoff&Zauke, 1997
Framstrait		3.6 ± 05	27.7±3.22	21.8±2.5	86±5			1.2±0.2	Ritterhoff&Zauke, 1997
N.E Atlantic			69.5	38.6	76.3				Rainbow,1989
N.WMediterraneanSea			2-8.8	17.6-34.9	108-197				Romeo et al., 1992
N.E Atlantic			35.5-108	18.6-66.7	58.5-109				Henning et al., 1985
Polar Front			118 ± 81	34±26	529±238				Henning et al., 1985
Southern Indian Ocean			21.2-81.7	11.8-24.2	48-88				Caio et al., 2017
German Bight			0.38 ±0.07	96.3±7.4	59±4				Zauke et al.,1996
Central North Sea		0.3±0.7	7.42±2.12	14.0±3.6	128±34			2.7±1.8	Zauke et al.,1996
Milliport, Scotland			1.6	77.5	188				Marsden&Rainbow,2004
Dunedin,NewZealand			8.9	15.6	133				Marsden&Rainbow,2004
Scotland	218-18900			58.5-117	104-3920				Rainbow&Moore 1986
Barents Sea			10.5 ± 4	9±3	110±16				Zauke&Schmalenbach,2006
Poland	131-554		2.41-3.02	39.2-57.4	94.2-148				Fialkowski et al., 2000
Italy	108.7-540		0.82-3.88	32.6-87.7	112-236			0.53-3.52	Ugolini et al., 2004
Cochin estuary	325.04±1749.8	27.43±9.69	2.44± 0.7	33.4±13.5	263.45±89.3	26.8±9.6	54.27±27.4	11.25±6.1	Present study