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TRACE METALS ACCUMULATION IN CRUSTACEAN COPEPODS FROM THE COCHIN ESTUARY, KERALA, INDIA

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ABSTRACT

Concentrations of trace metals like iron (Fe), nickel (Ni), zinc (Zn), copper (Cu), chromium (Cr), manganese (Mn), cadmium (Cd) and lead (Pb) were estimated in Copepods that collected during the period of February 2017 to January 2018 from the Cochin Estuary. The concentration of trace metals in Copepods showed significant spatial and seasonal variations. The average concentration of trace metals in Copepods were found to be in the decreasing order: Fe > Zn > Mn > Cu > Ni > Cr > Pb > Cd. Correlation analysis revealed a strong positive affinity between the most of metals analysed in Copepods.

Keywords: Bioaccumulation, Trace metals, Copepods, Cochin backwaters

INTRODUCTION

Metal pollution in estuaries is a global problem as it affects the sustainability of marine living resources **[1]**. Metals are ubiquitous in aquatic environments, persistent, potentially bioavailable, and toxic to biota at higher concentrations **[2]**. Zooplankton is the most abundant group of animals in estuarine ecosystems. They are the major secondary consumers in aquatic ecosystems. They have a significant capacity to bioaccumulate metals from water and food and have the ability to transfer metals to higher trophic levels **[3]**. As zooplankton will slowly metabolize or excrete metals, these pollutants may bioaccumulate within the organism (e.g. mysids) at high levels **[4]**. So zooplankton has been recommended as one of the groups for baseline studies of metals in the aquatic environment **[3]**. The Cochin estuary forms a transitional ecotone between terrestrial and marine ecosystems and is characterized by both freshwater and seawater mixing in which the high productivity supports a large amount of biodiversity **[5]**. Copepods are small aquatic crustaceans in mesozooplankton communities which are playing a primary role in the energy transfer pathways from primary producers to tertiary level consumers **[6]**. Copepods are very sensitive to trace metal toxicity and have a high capacity to accumulate trace metals **[6, 7]**. Copepods are the best biomarkers for monitoring trace metals due to their huge biomass, easy availability, sensitivity, and vital role in the trophic food chain **[8]**. Studies on metal concentrations in the Cochin backwaters revealed that a higher concentration of soluble metals exerts a negative influence on the abundance of copepods **[9]**. Therefore it is important to assess the suitability of zooplankton (e.g. copepods) as biomonitors for trace metals in the estuarine environment. The present study attempts to evaluate the concentration of trace metals like Fe, Ni, Zn, Cu, Cr, Mn, Cd and Pb in Copepods from February 2017 to January 2018 from the Cochin backwaters.

MATERIALS AND METHODS

Study area

Cochin estuary (Lat. 9º 30´-10º 10´ N and Long.76º15´-76º 25´E) is a monsoonal tropical estuary which is connected to the Arabian Sea through two inlets one at Cochin and the other at Azhikode and receives large volumes of freshwater from seven major rivers such as Periyar, Chalakudi, Muvattupuzha, Meenachil, Manimala, Pamba and Achancovil. Metal pollution in the estuary appears mainly from the effluents that discharge through the polluting chemical industries located in the banks of the Periyar and Muvattupuzha rivers. The main polluting chemical industries are Fertilizers and Chemical Travancore Ltd (FACT), Indian Rare Earth Limited (IRE), Hindustan Insecticides, Travancore Cochin Chemicals (TOC), Cochin Refineries Ltd and Merchem, and Cominco Binani Zinc, etc. In addition to the above metal effluents, the release of waste oil, antifouling paints from Cochin port and Shipyard also pollutes the estuary. The domestic sewage from Cochin City and wastes from aquaculture fields also drain into the study area **[10]**. Based on anthropogenic activities and the inflow of pollutants from different sources, five sampling stations were selected **(Figure 1)**. The features of these stations selected for study are as follows. Station 1 (Fort Kochi) which is located nearby Bar mouth has close connectivity to the Arabian Sea. Cochin port trust, International container tranship terminal (ICTT), and several boat jetties are also located close to the site of Station 1, Station 2 (Bolghatty) is located at the confluence point of the river Periyar. This site receives effluents from industries located on the banks of the river Periyar, Station 3 (Arookutty) receives effluents from the Cochin City, the Naval Base, Cochin Port and shipyard, Station 4 (Vaikom) receives industrial effluents from the Hindustan Newsprint factory, agricultural effluents from the hinterlands and effluents from the industries located on the banks of Muvattupuzha River and Station 5 is located near Thanneermukkom bund which receives large amounts of agricultural effluents from Meenachil river.

Figure 1: Map showing sampling locations in Cochin backwaters

Sampling and analysis

Bimonthly sampling of zooplankton was carried out during pre monsoon, monsoon and post monsoon of February 2017 to January 2018 from the Cochin backwaters. Zooplankton samples were collected by horizontal towing using a Working Party (WP) net (mesh size 0.2 mm, mouth area 0.6m²) from the surface water. Samples collected were kept in an icebox and transported to the laboratory. Samples were sorted under a binocular microscope, rinsed with Milli-Q water to remove salt and visually observed to ensure the absence of any foreign particles. Samples were dried in an oven (65ºC) and stored in a vacuum desiccator. The dried samples were digested for 3 hours at 80°C with 3 ml of nitric acid and 1 ml of perchloric acid (65%, Merck, Suprapure) in tightly closed Eppendorf reaction tubes. The digests were diluted to 25 mL with MilliQ water and metals were analysed using a Flame Atomic Absorption Spectrophotometer after calibration with elemental standards. All metal concentrations in copepods were reported in µg/g, dry weight.

Statistical analysis

Pearson's correlation coefficient (r) used to find out the relationship among different trace metals in Copepods at 5% level of significance. Statistical Analysis was carried out by using XL-STAT pro software package. ANOVA (two-factor without replication) was used to assess whether the trace metal concentrations in copepod varied significantly among study region spatially and seasonally.

RESULTS

Average concentration of trace metals in copepods during different seasons are given in **Table 1**. The average concentration ranges of trace metals in copepods were as follows: Fe, 272 - 5673 µg/g; Ni, 3.55 - 45.65 µg/g; Zn, 28.02 - 387.2 µg/g; Cu, 11.56 - 58.88 µg/g; Cr, 1.38 - 30.17µg/g; Mn, 13.76 - 86.9 µg/g; Cd, 0.9 – 2.45 µg/g; Pb, 1 - 24.06 µg/g **(Table 1)**. Higher concentration of Fe and Ni in copepods was found at station 2 during monsoon period and lowest at station 5 during pre monsoon period **(Table 1)**. A higher value of Zn was observed at station 1 during monsoon period while lowest during pre monsoon at station 5 **(Table 1)**. Higher concentrations of Cu and Cr in copepods were found at station 2 during post monsoon period and lowest at station 5 during pre monsoon period **(Table 1)**. Higher and lower concentration of Mn in copepods was observed during monsoon at station 5 and pre monsoon at station 3 respectively **(Table 1)**. Lower concentration of Cd and Pb in copepods were observed during pre monsoon period at station 5, while highest values of Cd and Pb at station 1 during monsoon and at station 2 during post monsoon period respectively **(Table 1)**.

Most of the metals showed significant seasonal and spatial differences ($p \leq 0.05$), during the study period **(Table 2)**. In copepods, monsoon period showed highest average values of Fe (1031.32 ± 635.55 µg/g), Zn (235.41± 93.50 µg/g), Cr (26.43 ± 1.06 µg/g) and Mn (62.603 ± 25.78 µg/g) when compared to pre monsoon and post monsoon, while comparatively highest average values of Cu (45.836 ± 9.66 µg/g), Cd (1.85 ± 0.59 µg/g) and Pb (17.312 ± 5.95 µg/g) were observed during post monsoon period than other seasons.

Possible association among the elements in Copepods was assessed using Pearson Correlation Coefficients. Significant correlations noted between essential (Fe, Mn, Co, Ni, Cu and Zn) and non-essential elements (Cr, Cd and Pb) **(Table 3)**. The order of trace metal occurrence in Copepods follows a decreasing trend: Fe > Zn > Mn > Cu > Ni > Cr > Pb > Cd. This ranking shows that iron is much more efficiently bio-accumulated than any other metal whereas cadmium is least bio-accumulated.

Table 1: Average trace metal concentrations in Copepods during different seasons from the Cochin backwaters

Seasons	Stations	Trace metals (µg/g, dry weight)							
		Fe	Ni	Zn	Cu	Cr	Mn	Cd	Pb
Pre- monsoon	1	1548 ± 381	30.25 ± 3.18	235 ± 7.07	20.65 ± 2.9	18.45 ± 8.5	19.15 ± 10.96	1.35 ± 0.07	5.81 ± 3.40
	2	1645 ± 429	30.94 ± 2.7	182 ± 9.8	33.7 ± 6.7	17± 2.8	16.8± 1.6	1.4 ± 0.14	4.75 ± 3.1
	3	1250 ± 212	21.2 ± 5.3	69.62 ± 35.88	17.33 ± 2.9	5.19 ± 4.1	13.76 ± 1.6	1.24 ± 0.62	3.25 ± 1.7
	4	441.6 ± 295	20.75 ± 4.5	54.3 ± 20.9	18.85± 1.6	3.7 ± 3	21.75 ± 20.15	1.05 ± 0.35	1.52 ± 0.67
	5	272 ± 36.5	3.55 ± 0.21	28.02 ± 13.4	11.56 ± 0.61	1.38 ± 0.16	17.85 ± 5.8	0.9 ± 0.141	1± 0.14
Monsoon	1	5390 ± 719	33.5 ± 0.77	387.2 ± 49	45.21± 0.69	27.45 ± 13.6	30.8 ± 2.8	2.45 ± 0.21	16.76 ± 2.06
	2	5673 ± 646	45.65 ± 7.9	252 ± 9.4	56 ± 9.4	29.55 ± 0.9	39.46 ± 4.9	2.35 ± 0.35	17.95 ± 5.1
	3	4807 ± 23	32.69 ± 14.29	209 ± 125	30.9 ± 8.4	26.65 ± 5.02	72.88 ± 9.8	1.65 ± 0.21	10.5 ± 0.70
	4	4255 ± 664	26.5 ± 4.94	184 ± 63	30.5 ± 0.7	25.5 ± 4.8	83 ± 56.5	0.99 ± 0.43	12.75 ± 2.05
	5	2211 ± 760	24.25 ± 6.01	144.9 ± 59	34.73± 19.41	23 ± 12.3	86.9 ± 11.5	1.65 ± 0.21	14.32 ± 4.3
Post- monsoon	1	1070 ± 182	12.08 ± 7.65	260 ± 14	35.3 ± 3.9	21.03 ± 3.7	22.5 ± 6.7	2.35 ± 0.35	20.4 ± 2.6
	2	2767 ± 910	27.13 ± 5.4	297.3 ± 42	58.88 ± 9.7	30.17 ± 14.1	30 ± 14.4	2.45 ± 1.4	24.06 ± 3.06
	3	1941 ± 483	18.7 ± 1.8	285.1± 14	52.16 ± 6.8	28.5 ± 3.5	48.35 ± 10.8	2.025 ± 0.88	18.55 ± 2.7
	4	1045 ± 416	15.345 ± 4.1	193.3 ± 61	44.05 ± 6.2	21 ± 5.6	63.5 ± 6.15	1.205 ± 0.17	15.25 ± 2.4
	5	1024 ± 107	10.38 ± 1.3	124.35 ± 27	38.79 ± 1.13	16.1 ± 2.6	55.33 ± 6.9	1.25 ± 0.63	8.3 ± 3.0

Table 2: Results of ANOVA for testing the spatial and seasonal variations of trace metals in Copepods

Metal	Sources of Variation	F ratio	P value
Fe	Spatial	5.37	0.02
	Seasonal	43.5	0.00005
Ni	Spatial	7.68	0.007
	Seasonal	13.5	0.002
Zn	Spatial	9.11	0.004
	Seasonal	12.1	0.003
Cu	Spatial	5.43	0.020
	Seasonal	23.1	0.0004
Cr	Spatial	3.6	0.057
	Seasonal	23.07	0.0004
Mn	Spatial	3.57	0.05
	Seasonal	14.5	0.002
Cd	Spatial	7.75	0.007
	Seasonal	9.10	0.008
Pb	Spatial	3.4	0.065
	Seasonal	30.5	0.0001

Table 3: Pearson's Correlation matrix showing interrelationship between different trace metals accumulated in Copepods

	Fe	Ni	Zn	Cu	Cr	Mn	Cd	Pb
Fe	1.00							
Ni	0.802*	1.00						
Zn	0.619*	0.512	1.00					
Cu	0.495	0.391	0.746*	1.00				
Cr	0.720*	0.569*	0.858*	0.839*	1.00			
Mn	0.365	0.088	0.068	0.262	0.488	1.00		
Cd	0.525*	0.442	0.829*	0.757*	0.710*	-0.095	1.00	
Pb	0.468	0.257	0.793*	0.871*	0.859*	0.318	0.821*	1.00

*** significant at 0.05 level**

DISCUSSION

Trace metal concentrations noted in Copepods **(Table 2)** showed considerable spatial and seasonal variability and were statistically significant ($p \leq 0.05$). Since very little work has been published on the trace metal distribution in zooplankton from the Cochin backwaters, the data obtained for trace metals in copepods were compared with those reported for similar geographical areas **[4, 11, 12]**.

Trace metal concentrations in zooplankton that reported from the India coastal waters were in the following ranges: Fe, 94 - 51118 µg/g ; Zn, 0.62 - 7546 µg/g ; Ni, 3 -110.3 µg/g ; Cu, 5 - 228 µg/g; Cr, 4.35 -194.3 µg/g; 7-1066.5 µg/g; Cd, 0.01 – 49.8 µg/g ; Pb, 3 - 97.5 µg/g [3, 13, 14, 15]. Trace metal concentrations reported in copepods from Farm Strait, the Greenland Sea and Antarctica were in the following ranges: Zn, 59 - 682 µg/g; Ni, 2.1 -18 µg/g; Cu, 3.8 - 51 µg/g; Cd, 0.27 - 14.4 µg/g; Pb, < 0.5 - 10.7 µg/g [16, 17]. Trace metal concentrations in copepods from the Weddell Sea were Zn, 518 µg/g, Cu 26, µg/g and Pb, 0.7 µg/g **[17]**. Similarly, **[18]** studied the trace metal concentrations in the copepod species of the Northern Taiwan Sea. The reported trace metal concentrations were ranged as follows: Fe, 921 - 4438 µg/g; Zn, 819 -2335 µg/g; Cu, 43.4 -129.2 µg/g; Cr, 43.6 -158.7µg/g; Mn, 28.4 - 64.7 µg/g; Cd, 0.54 - 1.48 µg/g; and Pb,10.7 -33.6 µg/g. Hsiao (2010) studied the trace metal concentration in Copepod from the same region during 2010. The reported ranges of values for metals were: Fe, 19.96 - 2886.9; Ni, 1.69 -954.9 µg/g; Zn, 59 - 682 µg/g; Cu, 4.36 - 195.5µg/g; Cr, 2.26 – 243.5 µg/g; Mn, 5.34 -735.8 µg/g; Cd, 0.39 - 141.78 µg/g; and Pb, 0.04 - 85.8 µg/g. Melisa et al. (2014) reported the metal concentrations in copepods from the Bahia Blanca estuary of Argentina. The concentration ranges of metals like Cu, Cd and Pb were: < 0.04 -148.9 µg/g, < 0.01 - 41.9 µg/g and < 0.5 - 71.8 µg/g, respectively. Metal concentrations in copepods of the Seine estuary from France was reported by **[19].** The accumulation ranges of metals like Cu, Cd and Pb were: 7 -134.7 µg/g, 0.13-1.1 µg/g and 8.5 - 48.8 µg/g, respectively.

A comparison of trace metal concentrations (Zn, Ni, Cu, Pb, and Cd) in copepods of Cochin estuary, with that reported for copepods of Farm Strait and the Greenland Sea showed higher Cd concentrations but Zn, Ni, Cu, and Pb concentrations are within the same ranges. Our findings in copepods of the Cochin estuary, agree well with the reported Fe and Zn concentrations in copepods of the Taiwan Sea but all other metals (Mn ,Cu, Ni, Cr, Pb and Cd) were higher in copepods of Taiwan sea. Similarly, when compared with our results in copepods of Cochin estuary, Bahia Blanca estuary in Argentina and of Seine estuary in France showed a higher metal concentration (Cu, Cd and Pb) in Copepods.

Copepods in marine environments generally showed a higher deposition of trace metals and were higher than that of other zooplankton species as metals become adsorbed to their exoskeletons at greater depths **[20].** The accumulated trace metal concentration in copepods depends on the rates of uptake from solution and food, excretion rate of the metal and the growth rate of the organism **[21].** Factors governing trace metal concentrations in copepods include different accumulation strategies, assimilation efficiencies, life stages, detoxification strategies, sources of pollution, etc. **[22].** High concentration of all metals noted in Copepods during the monsoon season could be attributed to heavy rainfall and subsequent river run off that carrying lot of industrial effluents, domestic and municipal sewage and also agricultural wastes (eg. fertilizer and pesticide) to the estuary all of which contain residues of trace metals **[4, 23].** Significant correlations between essential (Fe, Mn, Co, Ni, Cu and Zn) and non-essential elements (Cr, Cd and Pb) in copepods is due to efficient uptake, low elimination rates, and similar polluting sources of trace metals **[22]. Batuello *et al*., 2016, [21]** studied the influence of feeding strategies on trace element bioaccumulation in copepods and revealed that the essential elements like iron and copper were particularly higher in all Calanoida species when compared to other zooplankton species, reflecting the higher requirement of iron and copper in Calanoids. Iron was found to be the highest accumulated trace metal in Copepods of the Cochin backwaters. This was in agreement with the earlier study reports of trace metal concentrations noted in mysids and mesozooplankton from the Cochin backwaters **[4, 12].** It was reported from different parts of the world and that of Indian coastal waters that Fe concentration in zooplankton was higher than other metals **[3].**

Copper, zinc and cadmium are a borderline group of metals which are frequently found together in aquatic environments due to their common occurrence by anthropogenic inputs **[24].** The elevated levels of these metals in copepods during the post monsoon season substantiate their common occurrence in the study region through anthropogenic processes. Stations 1 to 3 showed an elevated level of Zn accumulations in copepods. It could be attributed to the favourable adsorption and bioavailability of metals as a result of pollution from the industries located on the banks of Periyar, Chitrapuzha and Muvattupuzha rivers and the consequent higher bioavailability of metals to copepods there **[25].** The concentration of Fe and Zn were high in all stations when compared to other metals. This may be due to the release of zinc from the Zinc factory or nearby Shipyard areas. Copepods have a hard exoskeleton made of calcium carbonate. High accumulation of zinc in copepods may be due to coprecipitation of Zinc along with calcium carbonate **[3, 13].** Copepods assimilate Zn much more efficiently when their prey has obtained this metal primarily from food and may readily be transferred to higher trophic level through biomagnification **[26].** The use of agrochemicals in paddy fields, industrial effluents, metalliferous mining activities, ore processing and sewage sludge are the principal sources of zinc in the study sites.

Nickel (Ni) occurs in the aquatic system as soluble salts and plays an important role in algal growth. The toxic action of nickel was reported to be due to its ability to replace essential metals in metalloenzymes which results in the disruption of certain metabolic pathways **[27]**. Nickel concentrations in copepods at stations 1 to 3 showed the highest level in the study region which may be due to the release of nickel from the nearby oil loading terminals, municipal run-off, and rusted fishing boats **[28]**. Copper (Cu) is an essential metal which is required by crustacean as an essential component of haemocyanin. The copper requirement in copepods will depend on enzyme activities that related to the process of growth and egg production **[17]**. High copper concentrations were noted in copepods of Stations 1 to 5 from the Cochin backwaters. Copper influx through land runoff from agricultural areas and excessive use of copper in antifouling paints enrich the copper content in copepods of the Cochin backwaters. Chromium (Cr) concentrations were higher during the monsoon season when compared to post monsoon or pre monsoon seasons **(Table 1)**. Chromium concentrations in copepods of Cochin backwaters were quite comparable with the ranges of values 16.5-195µg/g reported for copepods in Ocean outfall area of the northern Taiwan region **[8]**. Manganese (Mn) is a naturally occurring essential trace metal. Mn in copepods during the present study varied between 13.76 and 86.9 µg/g. Negative correlations of Mn with Cd recorded during the present study **(Table 3)**, indicates biological effects and external inputs of these metals in the Cochin backwater system **[29]**.

Cadmium and lead are the two non-essential metals studied in copepods. They are highly toxic metals when concerned with human health. High concentrations of lead and cadmium were noted in copepods at all stations of the Cochin backwaters. Higher lead influxes at the study region were primarily from industrial and agricultural inputs and due to loading and unloading of bulk cargo at these localities **[28]**. When mixing with estuarine water Pb form colloids. These colloids get adsorbed onto planktonic debris which resulted in a higher concentration of lead in zooplankton **[28, 30]**. Similar to lead, high cadmium concentrations detected in copepods at all stations of the Cochin backwaters were due to industrial, municipal, domestic and agricultural waste discharges that are rich in cadmium.

CONCLUSION

Metal contamination in the Cochin estuary is a serious problem as it affects both ecosystems as well as human health. The present study investigated the trace metal concentrations in Copepods from the Cochin estuary. The average concentrations of trace metals in copepods followed a decreasing order: Fe > Zn > Mn > Cu > Ni > Cr > Pb > Cd. High metal concentrations noted in copepods indicate an increased bioavailability of metals to copepod and hence is useful for bio-monitoring the sources of metallic pollutants in estuarine environments. This study provides a novel insight into the metal distribution in Copepods and their possible role in metal transfer in the estuarine food chain.

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