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МІКРОКОНЦЕНТРАЦІЇ МЕТАЛІВ У *MESOPODOPSIS ORIENTALIS* (CRUSTACEA : MYSIDAE) З ОГЛЯДУ НА ЙОГО ЖИТТЄВІ СТАДІЇ ТА СТАТЕВУ ПРИНАЛЕЖНІСТЬ В ЕСТУАРІЇ КОЧІН, ІНДІЯ[1]

Мікроконцентрації металів у Mesopodopsis orientalis *з огляду на його життєві стадії та статеву приналежність вивчали в естуарії Кочін в сезон мусонів та у перед-мусонний період. Просторову неоднорідність розподілу концентрацій металів спостерігали серед статевозрілих самців, незрілих самців, самиць, що віднерестилися, статевозрілих самиць, незрілих самиць та ювенільних особин* M. orientalis. *Концентрації таких металів як Fe, Mn, Zn, Pb, Cu, Cd, Ni, Cr і Co у* M. orientalis *коливались у наступних межах: відповідно 590,5—1554,9 мг/кг, 4,1—15,1, 42,0—126,5, 2,5—17,0, 12,8—61,9, 0,2—2,3, 7,6—25,5, 8,8—33,4 та 0,2—2,2 мг/кг. Вищі концентрації металів у* M. orientalis *спостерігали в сезон мусонів порівняно з перед-мусонним періодом. Статевозрілі самці, незрілі самці, самиці, що віднерестилися, статевозрілі самиці, незрілі самиці та ювенільні особини* M. orientalis *значно відрізнялися за своєю здатністю до біоакумуляції певних металів, що очевидно пов'язано зі специфікою їхніх фізіологічних процесів. Ювенільні та незрілі особини* M. orientalis *характеризувалися вищою концентрацією металів порівняно зі статевозрілими особинами.*

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Ц и т у в а н н я: Біджу Абрахам, Джордж Реджомон, Аріа М.С., Беттіна П.А., Мохан Джотирмайє. Мікроконцентрації металів у *Mesopodopsis orientalis* (Crustacea : Mysidae) з огляду на його життєві стадії та статеву приналежність в естуарії Кочін, Індія. *Гідробіол. журн.* 2023. Т. 59, № 2. С. 71—89.

Здатність до біоакумуляції значних концентрацій металів та просторова і сезонна неоднорідність їхнього розподілу у M. orientalis в залежності від його життєвих стадій та статевої приналежності свідчать про можливість використання цього виду як біомонітора забруднення естуаріїв металами.

Ключові слова: *мікроконцентрації металів, біоакумуляція, життєві стадії, Mesopodopsis orientalis, естуарій Кочін.*

The role of zooplankton in the biogeochemical cycling of trace metals by bioaccumulation has been appreciated markedly in marine ecosystems of the northern Indian Ocean [1, 4, 7, 9, 11]. The use of mesozooplankton as a biomonitor for the assessment of the bioavailability of trace metals in the eastern Arabian Sea and the western Bay of Bengal differs widely over a variety of spatial and temporal scales [9, 11]. Since mesozooplankton acts as a secondary producer and represents the most abundant group of animals in marine ecosystems it plays a key intermediate role in the transfer of trace metals from lower to higher trophic levels due to its significant capacity to bio-accumulate metals from water, and also to assimilate them through food substances [9, 11, 22, 27]. Thus, zooplankton becomes one of the recommended groups of animals for baseline studies of trace metals in marine environments since bioaccumulation data for trace metals in zooplankton serves as an index to define the pollution status of a relevant water body [4, 9]. However, data on metal variability between sorted zooplankton samples that belong to different taxa or their life stages and their spatial heterogeneity within the coastal waters of India are very scarce [7, 22]. Nevertheless, information on metal concentrations at the species level of zooplankton (e.g. mysids) and their life stages are particularly relevant in basic physiological studies as it is used for describing the process of metal metabolism in zooplankton assemblages [4, 26].

Mysids are a major component of zooplankton and their ecological importance as a vital link between the pelagic and benthic food webs of the estuarine and brackish water bodies of India is becoming increasingly apparent [3]. *Mesopodopsis orientalis* (W. Tattersall, 1908) is an important component of the shallow coastal water crustacean community and is one of the most widespread and abundant mysids in the estuarine and brackish water bodies of both the east and the west coast of India [5, 8, 17, 24]. However, trace metal studies in mysids from the coastal waters of India are scarce and no previous studies were available on the distribution of trace metals depending on life stages and sex of mysids from the Cochin estuary [4, 22]. The present study was carried out to measure the concentrations of trace metals like iron, manganese, cobalt, nickel, copper, zinc, chromium, cadmium and lead with regard to certain life stages and sex of *M. orientalis* collected from the Cochin estuary during the monsoon and pre-monsoon seasons. The evaluated communities of zooplankton (e.g. mysids) demonstrated their suitability as biomonitors for tracing metal contamination in estuarine ecosystems.

Material and Methods

Study area. The Cochin backwater is a part of a long chain of lakes and canals, which runs parallel to the southwest coast of India, extending between Lat. 9°30'—10°10' N and Long. 76°15'—76°25' E. This coastal stretch has been rapidly developing and is a host of various industrial activities. The Eloor-Edayar-Ambalamugal area is the major industrial area located in the coastal zone of the city of Kochi in the Ernakulam District. Kochi, a port city located on the southwest coast of India, harbours a variety of industrial establishments, which includes chemical, engineering, food, drug, paper, rayon, rubber, textiles and plywood industries clustered at two zones — one at the Eloor area on the banks of the Periyar River and another — at the Ambalamughal area, by the side of the Chitrapuzha River, which is a tributary of the Periyar River. The effluents from industries at the Eloor (260 MLD) and Ambalamughal (80 MLD) areas are discharged into the lower parts of the Periyar and Chitrapuzha rivers. They are mixed into the northern and central parts of the estuary by tides and freshwater flows [23]. In addition to the release of waste oil, paints, metal and paint scrapings from the Cochin port, the city's domestic sewage (2550 MLD) also drains into the central part of the estuary. The indiscriminate discharge of industrial effluents and the weak flows have resulted in the northern estuary acting as a stagnant pool of metallic contaminants accumulated than from the more dynamic central zone [2]. Being non-biodegradable, trace metals discharged into estuarine environments through urban and industrial discharges will remain in solution or in suspension as colloids forming complexes with inorganic and organic ligands and particulate matter and settling onto the bottom sediments, thus creating a potential source of metals for bioaccumulation by aquatic organisms [13].

The sampling stations were selected according to supposedly contrasting contamination status (Figure 1). Station 1 (Bolghatty) and station 2 (Thevara) are located at the confluence points of the Periyar and Chitrapuzha rivers, respectively. Of these two sampling stations, station 1 is situated in the northern limb of the estuary which receives effluents from the industries located on the banks of the Periyar River. Similarly, station 2 is situated in the central estuary which receives effluents from the industries located on the banks of the Chitrapuzha River.

Sampling procedure and data analysis. Zooplankton samples were collected from the Cochin estuary (Figure 1) using a Working Party (WP) net (mesh size 0.2 mm, mouth area 0.6 m^2) during the pre-monsoon (March 2013) and monsoon (August 2013) periods.

In the laboratory, mysids were sorted under a binocular microscope and classified according to the degree of development of secondary sexual characteristics; juveniles (J) — individuals without secondary sexual characteristics; adult/mature males (MM) — well-developed lobus masculin; immature males (IM) — lobus masculinus present but not yet setose; adult/brooding females (BF) — well-developed marsupium with eggs or young present in the marsupi-

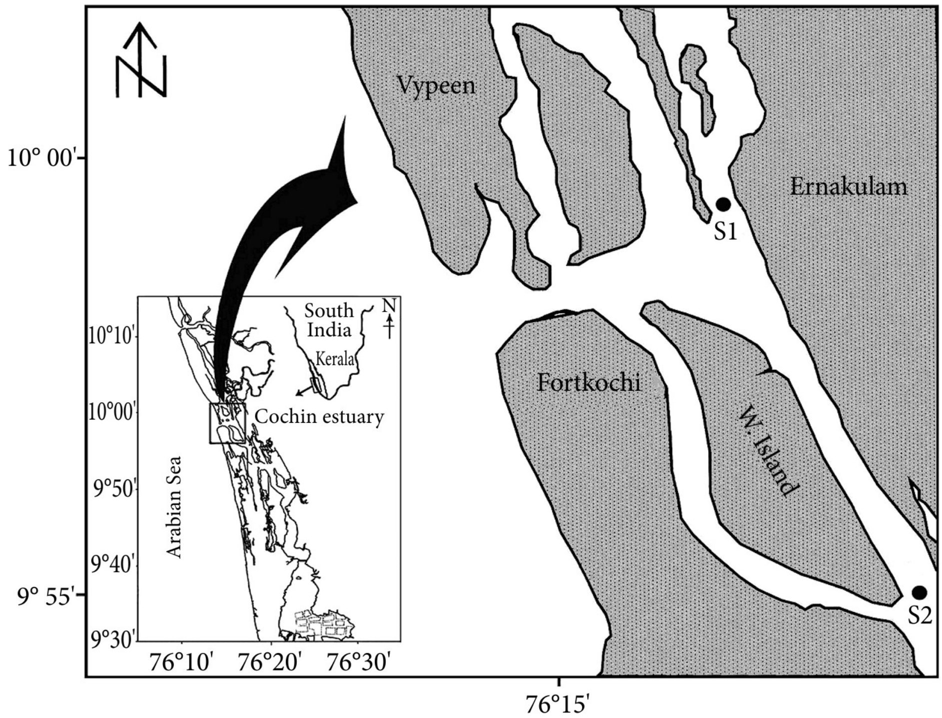

Fig. 1. Map of study area with station locations

um; immature females (IF) — incompletely developed marsupium, spent females (SF) — females with fully exposed marsupium [5].

M. orientalis occurred throughout the study period and contributed to 55.84 % and 44.16 % at stations 1 and 2 respectively (Table 1). The population structure of *M. orientalis* at station 1 during the pre-monsooon period consisted of 15.90 % mature males, 11.27 % immature males, 8.96 % spent females, 8.67 % brooding females, 18.50 % immature females, and 36.71 % juveniles. During the monsoon period, station 1 was comprised of 16.33 % mature males, 14.51 % immature males, 8.62 % spent females, 10.20 % brooding females, 17.46 % immature females and 32.88 % juveniles. During the pre-monsoon period, station 2 was embraced with 11.57 % mature males, 14.05 % immature males, 7.44 % spent females, 8.68 % brooding females, 15.70 % immature females, and 42.56 % juveniles. Similarly, during the monsoon period, station 2 was constituted by 12.70 % mature males, 15.08 % immature males, 8.47 % spent females, 9.79 % brooding females, 14.55 % immature females, and 39.42 % juveniles, respectively.

The sorted samples were placed in small nylon sieves and thoroughly rinsed with Milli-Q water to remove salts. Water adhering to the samples was removed by placing the sieve on the laboratory filter paper, which introduced no contamination. Subsequently, the samples were dried in an oven at 65 °C and stored in a vacuum desiccator. The dried samples were powdered and aliquots of ~ 300 mg were digested for 3 hours at 80 °C with 3 ml of HNO_3 and 1 ml of $HClO_4$ (65 %, Merck, Suprapure) in tightly closed Eppendorf reaction tubes [4, 15, 16]. The digests were diluted to 25 ml with Milli-Q water and metals were analyzed using a Flame Atomic Absorption Spectrophotometer (FAAS, Perkin Elmer) after calibration with suitable E-Merck elemental standards. All metal concentrations in *M. orientalis* are reported in mg/kg of dry weight.

Non-Parametric *Kruskal-Wallis Analysis of Variance* (ANOVA) was used for comparing the distribution of trace metals with regard to the life stages of *M. orientalis*. Dunns Multiple Post hoc Pair wise Comparison test was performed to find the pair wise significance. Pearson correlation analysis was performed to find the inter-relationships of the trace metals concentrations in *M. orientalis*. The Kruskal Wallis test and Pearson correlation analysis were performed in the XL-STAT pro software package.

Results

Trace metal concentrations in M. orientalis. The variations of trace metals like iron (Fe), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), chromium (Cr), cadmium (Cd), and lead (Pb) in *M. orientalis* with regard to certain life-stages and sex during the monsoon and pre-monsoon seasons are given in Table 2.

Table 1

Details of life stages/sex groups of *M. orientalis* analysed

Life stages	Characters	Number of individuals analysed				Size range (mm)
		Bolghatty (station 1)		Thevara (station 2)		
		Pre-monsoon	Monsoon	Pre-monsoon	monsoon	
MM	Well-developed lobus masculin	55	72	28	48	5.2—7.6
IM	Lobus masculinus present but not yet setose	39	64	34	57	4.2—5.8
SF	Females with fully exposed marsupium	31	38	18	32	4.9—8.3
BF	Well-developed marsupium with eggs or young present in the marsupium	30	45	21	37	4.7—7.4
IF	Incompletely developed marsupium	64	77	38	55	4.3—6.5
J	Individuals without secondary sexual characteristics	127	145	103	149	1.2—4.6

Note. Here and in the Tables 2—11, 13: MM — mature males; IM — immature males; SF — spent females; BF — brooding females; IF — immature females; J — juveniles.

During the monsoon and pre-monsoon seasons trace metal concentrations of *M. orientalis* were found to be relatively higher at station 1 of Bolgatty than at station 2 of Thevara (Figure 2). Trace metal content of *M. orientalis* increased from pre-monsoon to monsoon season at stations 1 and 2.

Iron (Fe) was the most abundant element examined in *M. orientalis*. Fe concentrations in *M. orientalis* showed wide variations depending on its life stages. During the monsoon and pre-monsoon seasons, average concentrations of Fe with regard to certain life stages of *M. orientalis* ranged from 715.3 to 1554.9 mg/kg and 590.5 to 1155.0 mg/kg, respectively. Fe concentrations were found to be comparatively higher for immature males, immature females and juveniles compared to other life stages of *M. orientalis*.

Mn concentrations showed wide fluctuations between different age groups of *M. orientalis*. The average concentrations of Mn in relation to certain life stages of *M. orientalis* ranged from 6.1 to 15.1 mg/kg and 4.1 to 12.1 mg/kg, respectively, for the monsoon and pre-monsoon seasons. Similar to Fe, Mn con-

Table 2

Average concentration of trace metals (mg/kg) **with regard to certain life stages and sex of *M. orientalis* during the monsoon** (M) **and pre monsoon** (P) **seasons**

Life stages		Fe	Mn	Zn	Pb	Cu	Cd	Ni	Cr	Co
MM	P	590.5± 70.24	4.1± 0.18	42.0± 7.18	4.4± 1.37	12.8± 4.21	0.3± 0.01	7.6± 1.31	8.8± 1.86	0.2± 0.04
	M	715.3± 151.97	6.1± 1.35	55.0± 11.31	6.2± 1.35	18.2± 4.09	0.6± 0.16	9.1± 1.63	10.9± 3.68	0.4± 0.31
IM	P	1003.5± 216.74	11.0± 1.41	83.2± 5.98	9.1± 1.24	29.1± 5.16	0.7± 0.08	17.3± 2.83	24.4± 5.13	1.8± 0.62
	M	1054.7± 268.53	12.2± 2.95	92.2± 5.86	11.2± 1.48	42.9± 17.98	1.2± 0.11	20.9± 3.24	30.0± 10.25	2.2± 0.47
SF	P	682.0± 172.02	5.1± 1.46	48.1± 4.32	4.0± 0.78	15.5± 2.52	0.4± 0.04	9.8± 2.43	16.7± 5.03	0.55± 0.16
	M	767.2± 190.09	6.7± 1.13	56.0± 2.83	5.2± 1.48	18.9± 3.61	0.6± 0.05	12.7± 2.72	23.6± 2.69	0.70± 0.22
BF	P	805.9± 163.92	7.8± 0.73	61.8± 11.00	2.5± 0.48	19.4± 6.02	0.2± 0.09	9.0± 1.57	11.3± 1.61	0.43± 0.11
	M	833.2± 63.76	8.0± 1.55	71.5± 7.78	3.8± 0.57	21.9± 5.24	0.4± 0.17	10.8± 1.89	18.1± 2.43	0.56± 0.01
IF	P	1155.0± 177.99	12.1± 1.54	110.7± 13.72	12.8± 2.58	32.3± 4.56	0.6± 0.13	13.2± 0.74	24.5± 9.65	1.38± 0.46
	M	1554.9± 330.53	15.1± 1.91	126.5± 21.92	16.8± 2.27	61.9± 19.89	1.0± 0.15	16.6± 1.47	33.4± 7.06	1.80± 0.35
J	P	1021.6± 53.23	11.5± 0.95	91.7±9. 50	15.7± 1.51	30.2± 4.83	1.4± 0.27	18.6± 2.83	17.8± 1.73	1.4± 0.21
	M	1239.6± 115.39	13.7± 2.97	124.0± 11.31	17.0± 2.72	42.1± 8.21	2.3± 0.25	25.5± 2.52	23.5± 7.07	2.0± 0.42

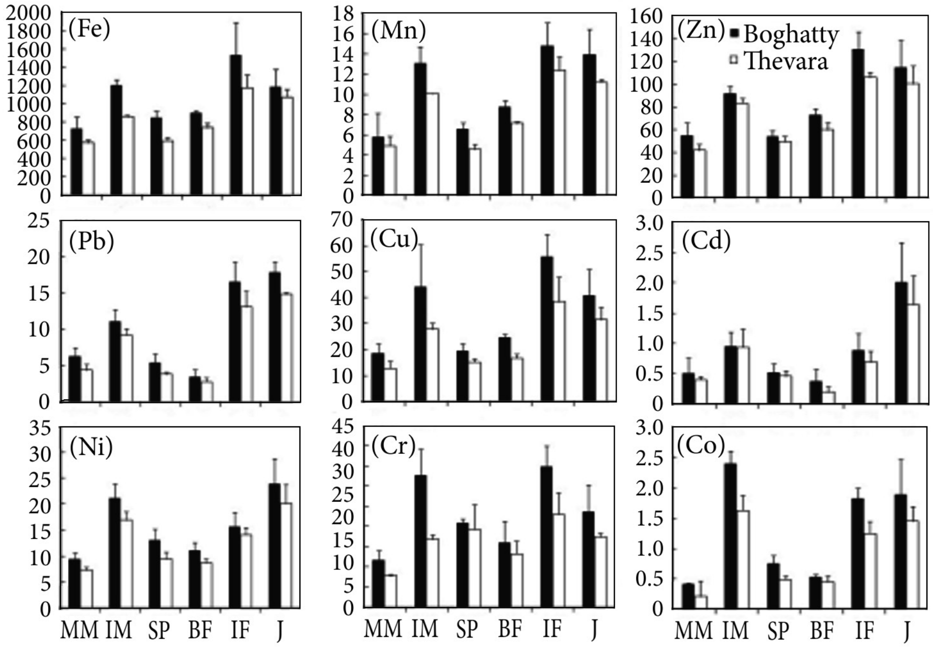

Fig. 2. Average concentration of trace metals (mg/kg) with regard to certain life stages and sex of *M. orientalis*: MM — mature males; IM — immature males; SF — spent females; BF — brooding females; IF — immature females; J — juveniles

centrations were also found to be somewhat higher for immature males, immature females and juveniles than in the other life stages of *M. orientalis.*

The concentration of Zn with regard to certain life stages of *M. orientalis* showed wide fluctuations and ranged from 55.0 to 126.5 mg/kg and 42.0 to 110.7 mg/kg, respectively, during the monsoon and pre-monsoon seasons. Immature females and juveniles showed reasonably higher Zn concentrations compared to other life stages of *M. orientalis*.

The concentration of Pb with reference to certain life stages of *M. orientalis* also showed wide fluctuations and ranged from 3.8 to 17.0 mg/kg and 2.5 to 15.7 mg/kg, respectively, for the monsoon and pre-monsoon seasons. Pb concentrations of *M. orientalis* were found to be relatively higher for immature females and juveniles than for other life stages.

Cu concentrations of *M. orientalis* with regard to noted life stages showed wide fluctuations and ranged from 18.2 to 61.9 mg/kg and 12.8 to 32.3 mg/kg, respectively, during the monsoon and pre-monsoon seasons. The concentrations of Cu were found to be quite higher in immature males, immature females and juveniles than the other life stages of *M. orientalis*.

The concentration of Cd with regard to certain life stages of *M. orientalis* also showed wide variations and ranged from 0.4 to 2.3 mg/kg and 0.2 to 1.4 mg/kg, respectively, for the monsoon and pre-monsoon seasons. Cd concentrations were found to be higher in juveniles than in the other life stages of *M. orientalis*.

Ni content with regard to different age groups of *M. orientalis* showed wide differences and ranged from 9.1 to 25.5 mg/kg and 7.6 to 18.6 mg/kg, respectively, during the monsoon and pre-monsoon seasons. Ni content was found to be reasonably higher in immature females and juveniles than in the other life stages of *M. orientalis*.

Chromium (Cr) content depending on certain life stages of *M. orientalis* showed large variations and ranged from 10.9 to 33.4 mg/kg and 8.8 to 24.5 mg/kg, respectively, during the monsoon and pre-monsoon seasons. Cr concentrations were found to be moderately higher in immature males and immature females than in the other life stages of *M. orientalis*.

Co concentrations with regard to certain life stages of *M. orientalis* also showed wide variations and ranged from 0.4 to 2.2 mg/kg and 0.2 to 1.8 mg/kg, respectively, during the monsoon and pre-monsoon seasons. Co concentrations were found to be relatively higher in immature males, immature females and juveniles than in the other life stages of *M. orientalis*.

Metal-specific differences were found to be apparent (Dunn's Multiple Comparison Test, Tables 3 to 12) for all studied mysid individuals representing certain life stages and sex. A significant difference ($p<0.05$) in metal content was observed as follows: between mature males and immature males for Fe, Mn, Zn, Pb, Cu, Ni, Cr and Co. Likewise significant difference ($p<0.05$) in metal content was noted as follows: between mature males and immature females for Fe, Mn, Zn, Pb, Cu, Ni, and Co; between mature males and spent females for Cr; between mature males and immature females for Cr and Co; between mature males and juveniles for Zn, Pb, Cu, Cd, Ni, Cr and Co; between immature males and juveniles for Fe and Mn; between immature males and spent females for Fe, Mn, Cu and Co; between immature males and spent females for Fe, Mn and Zn; between immature males and brooding females for Mn, Pb, Cu, Cd, Ni, Cr and Co; between spent females and immature females for Fe, Mn, Zn, Pb and Cu; between spent females and juveniles for Fe, Mn, Zn, Pb, Cu, Cd

Table 3

Dunn's multiple pair wise comparison test for Fe concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	**0.040**	1.000				
SF	0.653	**0.046**	1.000			
BF	0.294	0.317	0.548	1.000		
IF	**0.001**	0.220	**0.005**	**0.026**	1.000	
J	**0.006**	0.468	**0.020**	0.084	0.617	1.000

Note. Here and in the Tables 4—12: bold represents the significant relationships at $p<0.05$.

and Ni; between brooding females and immature females for Fe, Mn, Zn, Pb, Cu, Cd, Cr and Co; and between brooding females and juveniles for Mn, Pb, Cu, Cd, Ni and Co, respectively.

Inter-elemental relationships. Possible associations among the elements with reference to the life stages of *M. orientalis* were assessed using Pearson's correlation coefficients. Accumulation behaviours of trace metals in mysids re-

Table 4

Dunn's multiple pair wise comparison test for Mn concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	**0.016**	1.000				
SF	1.000	**0.016**	1.000			
BF	0.294	**0.047**	0.294	1.000		
IF	**0.002**	0.484	**0.002**	**0.040**	1.000	
J	**0.006**	0.726	**0.006**	**0.049**	0.726	1.000

Table 5

Dunn's multiple pair wise comparison test for Zn concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	**0.028**	1.000				
SF	0.822	**0.048**	1.000			
BF	0.260	0.282	0.368	1.000		
IF	**0.001**	0.230	**0.001**	**0.023**	1.000	
J	**0.003**	0.453	**0.006**	0.068	0.653	1.000

Table 6

Dunn's multiple pair wise comparison test for Pb concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	**0.420**	1.000				
SF	0.726	0.134	1.000			
BF	0.250	**0.021**	0.424	1.000		
IF	**0.036**	0.342	**0.014**	**0.001**	1.000	
J	**0.014**	0.194	**0.005**	**0.001**	0.726	1.000

sult in part from their interaction with the habitat [11]. Trace metals originating from industrial and domestic effluents in a flow-restricted estuary can undergo metal-metal interactions synergistically at the exposure concentrations in the habitat and may perhaps undergo bioaccumulation in individuals of *M. orientalis* representing certain life-stages and sex [4]. All the studied metals were found to be significantly correlated with each other (p<0.05, Table 12).

Table 7

Dunn's multiple pair wise comparison test for Cu concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	**0.014**	1.000				
SF	0.764	**0.032**	1.000			
BF	0.424	**0.049**	0.617	1.000		
IF	**0.002**	0.565	**0.006**	**0.026**	1.000	
J	**0.006**	0.783	**0.015**	**0.044**	0.764	1.000

Table 8

Dunn's multiple pair wise comparison test for Cd concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	0.057	1.000				
SF	0.803	0.099	1.000			
BF	0.438	**0.007**	0.305	1.000		
IF	0.154	0.635	0.240	**0.028**	1.000	
J	**0.004**	0.317	**0.008**	**0.001**	0.140	1.000

Table 9

Dunn's multiple pair wise comparison test for Ni concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	**0.003**	1.000				
SF	0.317	0.051	1.000			
BF	0.653	**0.012**	0.582	1.000		
IF	**0.036**	0.395	0.271	0.099	1.000	
J	**0.001**	0.653	**0.016**	**0.003**	0.194	1.000

These strong correlations were possible because of efficient uptake and low (or limited) elimination rate of these metals in *M. orientalis* [4, 25]. Further, the strong correlations between essential (Fe, Mn, Co, Ni, Cu and Zn) and non-essential (Cr, Cd and Pb) metals indicate synergistic interactive effects with similar polluting sources (industrial effluents) suggesting common routes of uptake in *M. orientalis* by interaction with the habitat [21].

Discussion

Trace metal levels detected within certain life stages and sex of *M. orientalis* varied spatially and seasonally with higher concentrations during the monsoon period than during the pre-monsoon period. The difference in elemental bioaccumulation patterns between mature males, immature males, spent females, brooding females, immature females and juveniles will be discussed according to specific accumulation strategies and to potential metabolic requirements [4].

Order of metal occurrence with regard to life stages and sex of M. orientalis. Trace metal content in mature males can be arranged in the following order: Fe > Zn > Cu > Cr > Ni > Pb > Mn > Cd > Co, whereas in immature males and brooding females except for Fe, Zn, Cu and Cr it follows another order: Fe > Zn >

Table 10

Dunn's multiple pair wise comparison test for Cr concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	**0.002**	1.000				
SF	**0.040**	0.317	1.000			
BF	0.395	**0.028**	0.230	1.000		
IF	**0.001**	0.901	0.260	**0.020**	1.000	
J	**0.048**	0.282	0.940	0.260	0.230	1.000

Table 11

Dunn's multiple pair wise comparison test for Co concentration relating to life stages and sex of *M. orientalis*

	MM	IM	SF	BF	IF	J
MM	1.000					
IM	**0.001**	1.000				
SF	0.368	**0.020**	1.000			
BF	0.599	**0.007**	0.708	1.000		
IF	**0.009**	0.532	0.089	**0.038**	1.000	
J	**0.005**	0.671	0.057	**0.023**	0.841	1.000

Cu > Cr > Ni > Mn > Pb > Co > Cd. Similarly, trace metal content in the spent females when compared with mature males, immature males and brooding females except for Fe, Zn and Ni it follows a different order: Fe > Zn > Cr > Cu > Ni > Mn > Pb > Co > Cd. Likewise, trace metal content in immature females follows an order: Fe > Zn > Cu > Cr > Ni > Pb > Mn > Co > Cd, whereas in juveniles except for Cr and Ni, it follows a similar order: Fe > Zn > Cu > Ni > Cr > Pb > Mn > Cd > Co. Thus, the hierarchy of metal concentrations with regard to life stages of *M. orientalis* was found to vary for most elements except for Fe and Zn.

A comparison of metal bioaccumulation relating to the life stages of *M. orientalis* reveals that Fe is bioaccumulated more than any other metal; Zn showed a similar enrichment and least for Cd (except for mature males and juveniles). The non-essential element Cr is taken up at a higher rate than the essential element Mn under study for most life stages of *M. orientalis*. Similarly, the non-essential element Pb is taken up at a higher rate than the essential element Mn under study in immature males and females, brooding females and juveniles of *M. orientalis*. The occurrence of high concentrations of the non-essential element Cd in the estuarine environment of Cochin from industrial effluents is immediately reflected in the Cd levels of mature males and juveniles of *M. orientalis*, which in fact is concentrated more effectively than the essential element Co under study [13]. Similarly, the occurrence of high concentrations of Zn in the estuarine environment of Cochin from industrial effluents is immediately reflected in the Zn levels at certain life stages of *M. orientalis*, which in fact is concentrated more effectively than Cu [13, 22]. High accumulation of Zn noted in certain life stages of *M. orientalis* may be due to co-precipitation of Zn along with calcium carbonate minerals in the estuarine environment of Cochin [9, 25].

Table 12

Pearson correlation coefficients between trace metals relating to life stages and sex of *M. orientalis*

	Fe	Mn	Zn	Pb	Cu	Cd	Ni	Cr	Co
Fe	**1.000**								
Mn	**0.952**	**1.000**							
Zn	**0.946**	**0.961**	**1.000**						
Pb	**0.784**	**0.831**	**0.854**	**1.000**					
Cu	**0.969**	**0.967**	**0.964**	**0.849**	**1.000**				
Cd	**0.672**	**0.658**	**0.609**	**0.896**	**0.680**	**1.000**			
Ni	**0.776**	**0.835**	**0.734**	**0.833**	**0.855**	**0.873**	**1.000**		
Cr	**0.849**	**0.774**	**0.775**	**0.638**	**0.814**	**0.646**	**0.697**	**1.000**	
Co	**0.869**	**0.884**	**0.816**	**0.762**	**0.898**	**0.659**	**0.896**	**0.896**	**1.000**

Assessment of trace metal concentrations with regard to life stages and sex of M. orientalis. The statistical significance of the spatial and seasonal differences of trace metal concentrations noted with regard to certain life stages and sex of *M. orientalis* was tested using the *Kruskal-Wallis Analysis of Variance*. Results indicate that the spatial and seasonal variability in the concentrations of metals relating to the life stages of *M. orientalis* was found to be statistically significant ($p<0.05$, Table 13).

The instantaneous metal content in zooplankton (e.g. mysid species, *M. orientalis*) seems to depend on the degree of imbalance between the ingoing and outgoing fluxes of metals from anthropogenic sources on land that were discharged through the rivers that flow into the Cochin backwaters [4, 13]. One characteristic feature noted from the investigated data of life stages of *M. orientalis* for the monsoon and pre-monsoon seasons, is that the concentrations of essential (Fe, Mn, Co, Ni, Cu and Zn) and non-essential (Cr, Cd and Pb) metals detected are high and increase from station 2 of Thevara to station 1 of Bolghatty (Fig. 2). Hence, the spatial variations of trace metal levels in the life-cycle of *M. orientalis* from this restricted area of Cochin backwaters are associated with the evolving gradients in metal concentrations that are triggered by environmental pollution [15]. In the booming city of Cochin, heavy industrialization, rapid urbanization and agricultural expansion lead to trace metal enrichment of the estuarine environment [13, 15]. The elevated concentrations of metals detected in the life stages of *M. orientalis*, at stations 1 and 2, are probably related to the high influx of metals as a result of pollution from the industries located on the banks of the Periyar and Chitrapuzha rivers and consequently increased bioavailability of metals to the mysids there [4]. Thus, the spatial variability of metal concentrations noted with regard to life stages of *M. orientalis* is associated with its enrichment due to pollution by the discharge of industrial effluents impregnated with variable loads of trace metals to stations 1 and 2, respectively, of the Cochin backwaters through the nearby Periyar and Chithrapuzha rivers [4, 13, 15, 23].

Another characteristic feature that was noted is the wide fluctuations in the concentration of metals like Fe, Zn, Cu, Cr, Ni, Pb, Mn, Cd and Co with reference to life stages of the mysid species, *M. orientalis* and metal concentrations increased from the pre-monsoon to monsoon period. This can be attributed to the capacity of each age group (life-stage) to concentrate a particular element by different means like direct uptake from water or assimilation of metals through ingesting food [22]. A possible explanation for the seasonal variations of metal concentrations detected with regard to life stages of *M. orientalis* might be due to the changing accumulation strategies of *M. orientalis* that are related to variable feeding habits involved during the pre-monsoon and monsoon seasons [4]. Thus, the seasonal differences of metal concentrations detected in various life stages of the mysid species, *M. orientalis* is probably related to the seasonally changing metal absorption in individuals of *M. orientalis* from the variable competence for food items during the pre-monsoon and monsoon seasons. Hence, during the pre-monsoon and monsoon seasons, the decreased and increased concentrations of metals detected for life stages of *M. orientalis*

Table 13

Results of *Kruskal-Wallis Analysis of Variance* for testing the spatial and seasonal variations of trace metals with regard to certain life stages and sex of *M. orientalis*

Trace metals	Pattern of variation	MM		IM		SF		BF		IF		J	
		F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value
Fe	Spatial	308.88	0.04	613.88	0.03	208.50	0.04	234.92	0.04	189.72	0.05	157.90	0.05
	Seasonal	549.21	0.03	570.30	0.03	421.49	0.03	830.90	0.02	278.33	0.04	555.81	0.03
Mn	Spatial	198.16	0.05	156.73	0.05	618.77	0.03	213.89	0.04	434.17	0.03	184.18	0.05
	Seasonal	617.33	0.03	432.16	0.03	150.06	0.05	200.22	0.04	610.80	0.03	225.00	0.04
Zn	Spatial	273.22	0.04	826.91	0.02	517.56	0.03	690.26	0.02	269.60	0.04	132.25	0.06
	Seasonal	793.91	0.02	418.75	0.03	441.00	0.03	403.64	0.03	167.33	0.05	635.99	0.03
Pb	Spatial	154.47	0.05	441.00	0.03	155.83	0.05	295.21	0.04	243.08	0.04	142.59	0.05
	Seasonal	857.65	0.02	514.57	0.03	322.79	0.04	131.21	0.06	340.57	0.03	767.62	0.02
Cu	Spatial	163.42	0.05	252.43	0.04	250.01	0.04	197.09	0.05	174.22	0.05	146.86	0.05
	Seasonal	435.67	0.03	168.83	0.05	386.78	0.03	334.41	0.03	308.00	0.04	228.74	0.04
Cd	Spatial	488.90	0.03	169.00	0.05	169.00	0.05	209.28	0.04	169.00	0.05	196.00	0.05
	Seasonal	165.17	0.05	400.00	0.03	136.00	0.02	553.82	0.03	592.11	0.03	676.00	0.02
Ni	Spatial	201.59	0.04	218.84	0.04	316.15	0.04	232.56	0.04	595.18	0.03	309.92	0.04
	Seasonal	482.86	0.03	154.96	0.05	191.25	0.05	392.04	0.03	989.28	0.02	105.47	0.02
Cr	Spatial	205.44	0.04	158.76	0.05	218.73	0.04	240.25	0.04	41.65	0.10	576.00	0.03
	Seasonal	765.44	0.02	190.44	0.05	312.11	0.04	930.25	0.02	23.81	0.13	511.89	0.03
Co	Spatial	174.83	0.05	635.39	0.03	625.00	0.03	225.00	0.04	215.11	0.04	245.44	0.04
	Seasonal	777.79	0.02	387.68	0.03	413.44	0.03	529.00	0.03	160.44	0.05	152.11	0.05

are associated with low and high primary production, where *M. orientalis* feebly and intensively feeds on phytoplankton, respectively, in the Cochin backwaters [4, 19].

The influence of life-history status on the accumulated metal levels of the individuals of *M. orientalis* during both the monsoon and pre-monsoon periods is remarkable (Fig. 2). For instance, Fe showed 1.5 and 2.2 times higher accumulation in immature males and immature females, respectively, when compared to mature males. Similarly, Mn showed 1.3, 2.0, 2.2 and 2.5 times higher accumulation in brooding females, immature males, juveniles, and immature females, respectively, when compared to mature males. Zn also showed 1.3, 1.7, 2.2 and 2.3 times higher accumulation in brooding females, immature males, juveniles, and immature females, respectively, when compared to mature males. Pb showed 1.4, 1.6, 2.9, 4.4 and 4.5 times higher accumulation in spent females, mature males, immature males, immature females and juveniles, respectively, when compared to brooding females. Cu also showed 1.2, 2.3, 2.4 and 3.4 times higher accumulation in brooding females, juveniles, immature males and immature females, respectively, when compared to mature males. Similarly, Cd also showed 1.4, 1.5, 2.5, 3.0 and 5.8 times higher accumulation in mature males, spent females, immature females, immature males and juveniles, respectively, when compared to brooding females. Ni showed 1.4, 1.8, 2.2 and 2.8 times higher accumulation in spent females, immature females, immature males and juveniles, respectively, when compared to mature males. Cr also showed 1.7, 2.1, 2.2, 2.8 and 3.1 times higher accumulation in brooding females, juveniles, spent females, immature males and immature females, respectively, when compared to mature males. Likewise, Co showed 1.4, 1.8, 4.5, 5.0 and 5.5 times higher accumulation in brooding females, spent females, immature females, juveniles and immature males, respectively, when compared to mature males.

Early life stages of *M. orientalis* bioaccumulated a higher amount of metals than the adult stages. For instance, the higher accumulation rates of Fe, Mn, Zn, Cu and Cr in immature females, Co in immature males, and Pb, Cd and Ni in juveniles, when compared to adults, suggest a net accumulation strategy for these metals during the early life span of *M. orientalis* for both the monsoon and pre-monsoon periods are noteworthy. The physiology of *M. orientalis* that is susceptible to specific trace metals during the growth periods of individual life stages may account for the differences in metal bioaccumulation patterns [26]. The large surface-to-body mass ratio of the younger individuals when compared to adults leads to a higher amount of metal accumulation through adsorption in *M. orientalis* [20]. The low amount of metal accumulation in adult species when compared to younger ones can be caused by a pronounced dilution effect produced by the increasing body mass during the growth of *M. orientalis* [20]. Metabolic activities in younger individuals of mysids are usually higher than in elder ones due to higher growth rates [28]. Since metal uptake rates in aquatic animals (e.g. fish, mysids, etc.) are positively correlated to metabolic rate the higher accumulation rates of Fe, Mn, Zn, Cu and Cr in immature females, Co in immature males and Pb, Cd and Ni in juveniles of *M. ori-*

entalis can be attributed to a high metabolism intensity associated with the enhanced feeding rates of younger ones when compared to adults [10, 14, 16, 20]. Consequently, the variation in accumulation behaviours noted for trace metals like Fe, Mn, Zn, Cu, Cr, Co, Pb, Cd, and Ni with regard to life stages and sex can be attributed to contrasting elemental regulations for these specific trace metals that are in relation with the different physiological requirements probably associated with the variations in metabolic rates that are linked with the disparity in feeding rates during its growth periods of *M. orientalis* [6, 18, 20, 28].

The net accumulation strategies shown for Fe, Mn, Zn, Cu and Cr in immature females, Co in immature males, and Pb, Cd and Ni in juveniles when compared to adults during the early life span of *M. orientalis* encourage us to recommend the use of these immature stages or juveniles as potential biomonitors for these trace metals. One of the implications of the high Zn and Cu concentrations (110.7 to 126.5 mg/kg and 32.3 to 61.9 mg/kg) noted in immature females, high Pb, Cd and Cr (15.7 to 17.0 mg/kg, 1.4 to 2.3 mg/kg and 24.5 to 33.4 mg/kg) concentrations noted in juveniles, of *M. orientalis* is that it may have an enormous potential for trophic transfer to fish inhabiting the Cochin backwaters [14]. The selective feeding of this metal-contaminated zooplankton (e.g. mysids) by fish may have toxic effects on human populations who consume them [10, 14, 16]. The question of whether these elements are stored in zooplankton (e.g. mysids) in a form that is bioavailable to fish must be directed to future studies. Future studies should also address the role of different metal uptake pathways in zooplankton (*via* dissolved fraction in water or *via* food items like particulates or phytoplankton etc.).

Conclusion

The present work reports the first study of trace metal concentrations with regard to certain life stages and sex of *Mesopodopsis orientalis* from a heavily industrialized estuary of India, the Cochin estuary. In conclusion, an enhanced variability of metal concentrations depending on its life-history status is noted for *M. orientalis*. High metal bioaccumulation and its spatial and seasonal variability with regard to its life stages and sex suggest the importance of *M. orientalis* as a potentially suitable biomonitor for detecting metal contamination events in estuarine ecosystems. Since immature stages and juveniles of *M. orientalis* showed well-enhanced metal bioaccumulation than mature (adult) organisms, these life stages of *M. orientalis* should be used in routine biomonitoring studies.

Literature Cited

1. Arunpandi N., Jyothibabu R., Jagadeesan L. et al. Impact of a large hydraulic barrage on the trace metals concentration in mesozooplankton in the Kochi backwaters, along the Southwest coast of India. *Mar. Poll. Bull.* 2020. Vol. 160. P. 111568.

2. Balachandran K.K., Lalu Raj C.M., Nair M. et al. Heavy metal accumulation in a flow restricted, tropical estuary. *Estuarine, Coastal and Shelf Science.* 2005. Vol. 65 (1—2). P. 361—370.

3. Biju A. Studies on taxonomy and ecology of Mysidacea from the EEZ of India : PhD thesis. Cochin University of Science and Technology, Kerala, India. 2009. 328 p.

4. Biju A., George R. Trace metal dynamics in mysids from the Cochin estuary. *Environ. Forensics.* 2021. Vol. 22 (1—2). P. 56—62.

5. Biju A., Gireesh R., Jayalakshmi K.V. et al. Seasonal abundance, ecology, reproductive biology and biochemical composition of *Mesopodopsis orientalis* Tattersall (Crustacea: Mysida) from a tropical estuary (Cochin backwater) in India. *Crustaceana.* 2009. Vol. 82 (8). P. 981—996.

6. Cripe G.M. Comparative acute toxicity of several pesticides and metals to *Mysidopsisbahia* and postlarval *Penaeus duorarum*. *Environ. Toxicol. Chem.* 1994. Vol. 12. P. 1867—1872.

7. Gajbhiye S.N, Vijayalakshmi R.N., Narvekar P.V., Desai B.N. Concentration and toxicity of some metals in zooplankton from near shore waters of Bombay. *Indian J. Mar. Sci.* 1985. Vol. 14. P. 181— 183.

8. George M.J. Observation on the plankton of Cochin backwaters. *Indian J.Fisheries.* 1958. Vol. 5. P. 373—401.

9. George R., Balachandran K.K., Nair M. et al. Trace metal concentrations in zooplankton from the Eastern Arabian Sea and Western Bay of Bengal. *Environ. Forensics.* 2008. Vol. 9. P. 22—32.

10. George R., Biju A., Martin G.D., Gerson V.J. Distribution and concentration of trace metals in tissues of pelagic and demersal fishes from the coastal waters of Cochin. *Ibid.* 2022. Vol. 23, Iss. 3—4. P. 371—388. DOI: 10.1080/15275922.2021.1907820

11. George R., Dineshkumar P.K., Nair M., Muraleedharan K.R. Trace metal dynamics in zooplankton from the Bay of Bengal during summer monsoon. *Environ. Toxicol.* 2010. Vol. 25 (6). P. 622—633.

12. George R., Martin G.D., Gerson V.J., Nair M. Biogeochemical cycling of phosphorus in the Cochin backwaters: southwest coast of India. *J. Environ. Analyt. Chem.* 2019. Vol. 6. P. 252.

13. George R., Martin G.D., Nair S.M. et al. Geochemical assessment of trace metal pollution in sediments of the Cochin Backwaters. *Environ. Forensics.* 2016. Vol. 17. P. 156—171.

14. George R., Martin G.D., Nair S.M., Chandramohanakumar N. Biomonitoring of trace metal pollution using fishes from the Cochin Backwaters. *Environ. Forensics.* 2012. Vol. 13. P. 272—283.

15. George R., Martin G.D., Nair S.M., Chandramohanakumar N. Biomonitoring of trace metal pollution using the bivalve molluscs, *Villorita cyprinoides*, from the Cochin backwaters. *Environ. Monitoring and Assessment.* 2013. Vol. 185. P. 10317—10331.

16. George R., Nair M., Joseph T. Trace metal dynamics in fishes from the southwest coast of India. *Ibid.* 2010. Vol. 167. P. 243—255.

17. Hanamura Y., Siow R., Chee P-E. Reproductive biology and seasonality of the Indo-Australasian mysid *Mesopodopsis orientalis* (Crustacea: Mysida) in a tropical mangrove estuary, Malaysia. *Estuarine, Coastal and Shelf Science.* 2008. Vol. 77. P. 467—474.

18. Jeckel W.H., Roth R.R., Ricci L. Patterns of trace-metal distribution in tissues of *Pleoticus muelleri* (Crustacea: Decapoda: Solenoceridae). *Marine Biol.* 1996. Vol. 125. P. 297—306.

19. Jyothibabu R., Madhu N.V., Jayalakshmi K.V. et al. Impact of fresh water influx on microzooplankton mediated food web in a tropical estuary (Cochin backwaters — India). *Estuarine, Coastal and Shelf Science.* 2006. Vol. 69. P. 505—518.

20. Kahle J., Zauke G.P. Bioaccumulation of trace metals in the Antarctic amphipod *Orchomene plebs*: evaluation of toxicokinetic models. *Marine Environ. Res.* 2003. Vol. 55. P. 359—384.

21. Luoma S.N. Bioavailability of trace metals to aquatic organisms. A review. *Sci. Total Environ.* 1983. Vol. 28. P. 1—22.

22. Nair R.V., Krishnamurti A.J., Gajbhiye S.H. Trophic structure and levels of selected metals in zooplankton community of Thane-Bassein creek, Bombay. *Indian J. Marine Sci.* 1999. Vol. 28. P. 173—183.

23. Ouseph P.P. Dissolved and particulate trace metals in the Cochin estuary. *Marine Poll. Bull.* 1992. Vol. 24 (4). P. 186—192.

24. Pillai N.K. A revision of the genus *Mesopodopsis* Czerniavsky (Crustacea: Mysidacea). *J. Zool. Society of India* 1968. Vol. Vol. 20 (1—2). P. 6—24.

25. Rainbow P.S. Physiology, physicochemistry and metal uptake — a crustacean perspective. *Marine Poll. Bull.* 1995. Vol. 31. P. 55—59.

26. Ritterhoff J., Zauke G.P. Bioaccumulation of trace metals in Greenland Sea copepod and amphipod collectives on board ship: verification of toxicokinetic model parameters. *Aquat. Toxicol.* 1997. Vol. 40. P. 63—78.

27. Wang W.X., Fisher N.S. Accumulation of trace elements in a marine copepod. *Limnol. Oceanogr.* 1998. Vol. 431. P. 273—283.

28. White S.L., Rainbow P.S. Regulation of Zn concentration by *Palaemon elegans*: zinc flux and effects of temperature, Zn concentration and moulting. *Marine Ecol. Progress Series.* 1984. Vol. 16. P. 145—147.

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TRACE METAL CONCENTRATIONS IN *MESOPODOPSIS ORIENTALIS* (CRUSTACEA: MYSIDAE) WITH REGARD TO ITS LIFE STAGES AND SEX IN THE COCHIN ESTUARY, INDIA

Trace metal concentrations with regard to certain life stages and sex of mysid species, *Mesopodopsis orientalis*, from the Cochin estuary were studied during monsoon and pre-monsoon seasons. Spatially varying heterogeneous patterns of trace metal loads were apparent within the mature males, immature males, spent females, brooding females, immature females and juveniles of *M. orientalis* for the region. The concentration ranges of trace metals like Fe, Mn, Zn, Pb, Cu, Cd, Ni, Cr and Co in *M. orientalis* were: 590.5 to 1554.9 mg/kg, 4.1 to 15.1 mg/kg, 42.0 to 126.5 mg/kg, 2.5 to 17.0 mg/kg, 12.8 to 61.9 mg/kg, 0.2 to 2.3 mg/kg, 7.6 to 25.5 mg/kg, 8.8 to 33.4 mg/kg and 0.2 to 2.2 mg/kg, respectively. Higher metal concentrations were noted for the life stages of *M. orientalis* during the monsoon season than in the pre-monsoon season. Mature males, immature males, spent females, brooding females, immature females and juveniles of *M. orientalis* showed large variability in bioaccumulation for specific metals probably associated with distinct physiological processes. Juveniles and immature stages of *M. orientalis* showed higher metal concentrations than adult organisms. High metal bioaccumulation and its spatial and seasonal variability with regard to animal life stages and sex suggest the importance of *M. orientalis* as a biomonitor for tracing metal contamination in estuarine environments.

***Keywords**: trace metal, bioaccumulation, life stages, Mesopodopsis orientalis, the Cochin estuary.*