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A Study on Heavy Metal Composition in Shells of Foraminiferal Species; *Rotalidium annectans* from Mangrove Core Sediments of West coast of India

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A B S T R A C T

Benthic foraminifera were found to be very effective in understanding the impact of heavy metal pollution in coastal environments. They have the ability to bioaccumulate heavy metals in a species dependent pattern. In the present study, deposited tests of *Rotalidium annectans* were selected to understand their species specific response to heavy metal levels in mangrove sediments. The tests were recovered from one meter sediment cores collected from two mangrove areas in West coast of India, Chithrapu and Kumbla. The tests were subjected to acid digestion and AAS analyses of five heavy metals; Pb, Ni, Zn, Cu and Fe were performed. The heavy metal levels in foraminiferal tests from mangrove sediment cores of Chithrapu and Kumbla followed the order; Fe > Zn > Pb > Ni > Cu and Fe > Zn > Ni > Cu respectively. The concentrations of Zn and Fe in foraminiferal tests found to be higher, but Pb, Cu and Ni were found to be very less. Fe and Zn concentration in tests were in relation with that in sediments showing that the Fe and Zn levels of sediments directly affect its concentration in the calcium carbonate tests of foraminifera. Lower concentrations of Pb, Ni and Cu despite their higher concentrations in sediments suggest that the bioaccumulation of these heavy metals in to *Rotalidium annectans* might be probably from water from which they calcified than from sediments. Good correlations were found between heavy metals in tests, sediments and physicochemical parameters in cores. The incorporation of metals in to calcite shells of *Rotalidium annectans* was found to be greatly influenced by the uptake of Fe which suggests the mechanism of co-precipitation of metals. Being the best preserved species in a heavy depositional environment like mangroves, our study suggest the use of *Rotalidium annectans* as possible indicator of heavy metal pollution.

Introduction

Mangroves are unique ecosystem with great ecological and economic significance. They keep up with the sea level changes by the process of sedimentation and the sediment dynamics is influenced by many factors. Several properties of mangrove sediments like high sulphide content and organic matter, highly reduced and anaerobic state etc helps in the retention of water-borne heavy metals and other chemicals which makes them heavy metal sinks (Karbassi and Amirnezhad, 2004). The level of heavy metals in sediments is higher compared to that of underlying water due to the absorption of heavy metals in to the sediments (Zabetoglou *et al.*, 2002). Hence, the bioaccumulation of these metals in to the biota of the ecosystem especially those in the sediments are of considerable importance.

The mangrove sediments show assemblage of many organisms among which foraminifera are of great importance (Lezine *et al.*, 2002). The foraminifera form an order Foraminiferida that belongs to the phylum Protista. They typically produce a test or shell which is made up of calcium carbonate or agglutinated sediment particles which are well preserved following their death. The evolutionary significance of foraminifera and the exceptional quality of fossilization renders them an excellent proxy (Murray, 2006). Mangrove foraminifera have been found very useful in deducing past climatic conditions and in reconstructing sea level changes that had occurred over a period of time (Gehrels and Newman, 2004; Woodroffe *et al.*, 2005). Like other biota, foraminifera also bioaccumulate the metals from sediments and water and they will be deposited on their calcium carbonate shells. Assessing the heavy metal content in these shells is useful in understanding the role of foraminifera as indicators of pollution. The study of shell chemistry also gives an account of the environmental and geochemical parameters influencing the bioaccumulation mechanisms and the ecology of mangrove foraminifera.

Not many studies have been done on the heavy metal composition in the shells of foraminifera before. Infact, there is a lacunae in the research on heavy metal accumulation by small organisms like foraminifera that reside in the mangrove sediments which possibly are one of the best ecological indicators. The response of foraminifera in core samples from heavy metal polluted areas was studied by Alve (1991) which showed the dominance of some tolerant species in the cores with frequent occurance of abnormal specimens. Study on benthic foraminifera those belonging to the family Rotaliidea (*Elphidium sps*, *Ammonia beccarii*, etc) and others were studied by Madkour and Ali (2009) from the coastal lagoons of Red sea, Egypt to understand the heavy metal concentrations in their shells. Since the heavy metal accumulation and its concentration in foraminiferal tests are species dependent, this study on *Rotalidium annectans* which is the most abundant and prominent species in West coast of India is of significant importance. In addition to that, the present study used the deposited tests from mangrove sediment cores which will help us to understand the heavy metal accumulation patterns over a period of time in mangroves which are referred as 'heavy metal sinks'.

Materials and Methods

The foraminiferal tests used in this study were that of *Rotalidium annectans*, due to its abundance in Indian coasts (Figure 1). The tests were from mangrove sediment cores of

Chitrapu (13°04.606'N and 074°46'49.830'E), Karnataka and Kumbla (12°35.995'N and 074°56.255'E), Kerala, along the West coast of India (Figure 2). Parallel sediment cores were taken from each of these sites during periods of low tides. The cores were brought to lab, cut and sub sampled by slicing at every 2.5 cm intervals (Figure 3).

The sediment subsamples from identical depths of replicate cores were pooled together and homogenized for further procedures. The subsamples at every 5 cm depth intervals were taken for analyses. The sediment samples for foraminiferal assemblage study were oven dried at 60◦C. The foraminiferal tests in the cores were easily susceptible to breakage and dissolution due to the long time deposition. Hence, chemical treatments were avoided and samples were repeatedly washed through 63µm sieve under low water pressure.

The sand fractions were collected over Whatman filter paper and oven dried at 60◦C. Twenty non- grinded, dried tests of *Rotalidium annectans* (~250 µm) were used for the analyses. These tests were completely digested overnight in a Teflon cup by slowly adding a mixture of con HNO3, HClO4 and HF in 3:2:1 ratio. The digested samples were then heated for 2 hours on a hot plate at ~ 200°C.

The cooled samples were then filtered using Whatman filter paper to get rid of non digested parts. The solution was made up to 25 ml with metal free Milli Q water for further analyses (Madkour and Ali, 2009; Frontalini *et al.*, 2009). Along with reagent blanks and suitable standards, the digested samples were analysed. Pb, Ni, Zn, Cu and Fe were analyzed using Flame Atomic Absorption Spectrophotometry (Thermo, M Series AAS). The concentrations of heavy metals in tests were expressed as mg/Kg weight of the sediment.

Results and Discussion

The tests of *Rotalidium annectans* were well preserved in the mangrove sediments with minimal deformities despite post depositional pressures and taphonomic losses. Foraminifera have the ability to incorporate metals from water and sediments in to their calcium carbonate shells during calcification and growth. The concentration of heavy metals in the shells suggests the heavy metal status of the environment in which they lived.

The distribution of Pb, Ni, Zn, Cu and Fe in foraminiferal tests at various depths of mangrove sediment cores of Chithrapu and Kumbla including minimum – maximum values, mean and standard deviations are presented in tables 1 and 2. Figures 4 and 5 show the graphical representation of the depth profiles of heavy metals in foraminiferal tests. Pearson correlation analyses of Total foraminiferal number (TFN), physico – chemical parameters and heavy metals in sediments and foraminifera shells within the cores of Chithrapu and Kumbla were studied and the correlation statistics are summarized in tables 3 and 4, respectively.

The concentrations of heavy metals in foraminiferal tests from mangrove sediment cores of Chithrapu and Kumbla were in the order Fe > Zn > Pb > Ni > Cu and Fe > Zn > Ni > Cu respectively. Pb was not detected throughout in the tests from Kumbla cores while it was not detected in Chithrapu cores after 30cm depths. Ni and Cu were not detected in the tests from bottom most depth (95–97.5cm, Ni not detected at depth 30–32.5 cm) in Chithrapu cores. In cores from Kumbla, Ni was detected only in tests from depths 7.5–17.5cm while Cu was not detected after 30 cm. The concentration of heavy metals in the sediments from these cores were in the order Fe > Pb > Zn > Ni > Cu. The sediments from Kumbla cores showed higher concentrations of Pb, Ni, Zn and Cu while Fe was higher in Chithrapu core sediments. Foraminiferal tests from Chithrapu cores had the higher concentrations of metals Pb, Ni, Cu and Fe compared to that from Kumbla which had higher amount of Zn. The Concentrations of heavy metals in sediments Chithrapu cores were 3–5 times higher than that of foraminiferal tets with Pb as an exception having around 20 times more concentration in sediments. In Kumbla cores, there was huge difference between the concentrations of heavy metals like Pb, Ni and Fe in sediments (>50 times) and tests, Zn and Cu were around 2 and 10 times respectively higher in sediments.

The bioaccumulation of heavy metals by invertebrates is affected by their feeding behaviour and the metal source (Rainbow, 2002; Simpson *et al.*, 2005). In both the cores, the concentrations of Zn and Fe in foraminiferal tests found to be higher, but Pb, Cu and Ni were found very less. The bioaccumulation of heavy metals by foraminiferal tests is generally species dependant as different species has different patterns of bioaccumulation mechanisms.

The mangrove sediments in the cores had shown very high concentrations of Fe and Pb which might be due to diagenetic or anthropogenic activities. The bioaccumulation of Fe by foraminiferal tests from theses sediments could be the reason for its high concentration in tests. Iron from terrigenous sediments normally precipitates around centres of organic material such as foraminiferal tests (Madkour and Ali, 2009). Similar trend was seen between the concentration of Zn in foraminiferal tests and sediments from these cores which show the accumulation of Zn from the sediments by foraminifera. Zn is mainly co-precipitated with CaCO3 and substitutes Ca2+ to form isomorphous Zinc carbonate. The increased concentrations of Zn can be due to ecological damage as it has long residence time or due to human impact (Mansour et al, 2005). Even though the Pb concentrations in sediments were extremely high, the Pb in the tests were low Chithrapu and not detectable in Kumbla cores. The Ni and Cu concentrations were also very low in tests compared to that in sediments. Other than difference in accumulation patterns, various post depositional changes also can be responsible for the differences in the heavy metal concentrations between sediments and foraminiferal tests in the mangrove sediment cores.

Pearson correlation analyses of physico – chemical parameters and heavy metals in sediments and foraminiferal tests within the cores were studied. Extensive correlations between heavy metals in tests with each other and with other parameters studied were found in Chithrapu cores. Fe concentration in tests showed statistically significant positive correlation with all the other 4 test metal concentrations and also with the sediment Zn concentrations.

This shows the role of iron in the uptake of other heavy metals in to foraminiferal shells by the process of co-precipitation. Cu in tests was positively correlated to Pb, Ni, and Zn in tests and to Cu in sediments. The Zn concentration in tests showed positive correlations with Cu in sediment and also Pb and Ni in tests. Ni in foraminiferal tests was showing positive correlation with pH and Pb in tests while a significant negative correlation with the Total Foraminferal Number (TFN) ($r = -0.674$; $P > 0.05$).

Table.1 Concentration of heavy metals in tests of *Rotalidium annectans* from mangrove core sediments of Chithrapu (ND – Not Detectable)

Depth(cms)	**Pb** (mg/ Kg)	**Ni** (mg/ Kg)	**Zn** (mg/ Kg)	**Cu** (mg/ Kg)	**Fe** (mg/ Kg)
0-2.5	10.75	10.25	38.75	3.00	2497.25
7.5-10.0	5.00	5.00	11.00	1.75	1375.50
15.0-17.5	9.75	9.00	74.25	3.50	1771.75
22.5-25.0	5.00	9.00	16.50	2.00	720.50
30.0-32.5	N.D	N.D	21.50	0.75	617.00
37.5-40.0	N.D	4.50	21.00	0.75	624.00
60.0-62.5	N.D	5.25	20.25	0.75	685.00
67.5-70.0	N.D	1.75	15.00	2.00	557.50
75.0-77.5	N.D	2.75	6.25	0.50	652.25
82.5-85.0	N.D	2.50	7.75	1.50	445.75
90.0-92.5	N.D	2.75	16.75	1.00	526.25
95.0-97.5	N.D	N.D	5.50	N.D	471.00
Mean	7.63	5.23	21.21	1.59	911.98
Max	10.75	10.25	74.25	3.50	2497.25
Min	5.00	1.75	5.50	0.50	445.75
S.D	3.058	3.092	18.970	0.983	638.108

Table.2 Concentration of heavy metals in tests of *Rotalidium annectans* from mangrove core sediments of Kumbla (ND – Not Detectable)

Depth (cms)	**Pb** (mg/Kg)	**Ni** (mg/ Kg)	**Zn**(mg/Kg)	**Cu**(mg/Kg)	**Fe** (mg/ Kg)
0-2.5	N.D	N.D	7.50	0.25	331.75
7.5-10.0	N.D	1.75	12.75	0.75	433.25
15.0-17.5	N.D	0.75	19.00	0.25	315.00
22.5-25.0	N.D	N.D	11.25	0.25	417.75
30.0-32.5	N.D	N.D	10.50	N.D	418.75
37.5-40.0	N.D	N.D	343.00	N.D	402.25
45.0-47.5	N.D	N.D	10.75	N.D	262.00
52.5-55.0	N.D	N.D	9.75	N.D	367.75
60.0-62.5	N.D	N.D	8.25	N.D	285.00
75.0-77.5	N.D	N.D	10.75	N.D	195.75
82.5-85.0	N.D	N.D	9.25	N.D	242.50
97.5-100	N.D	N.D	5.75	N.D	413.75
112.5-115.0	N.D	N.D	6.00	N.D	293.00
Mean	N.D	1.25	35.73	0.38	336.81
Max	N.D	1.75	343.00	0.75	433.25
Min	N.D	0.75	5.75	0.25	195.75
S.D	N.D	0.707	92.384	0.250	78.113

Table.3 Pearson correlation analyses of various parameters within core sediments of Chithrapu

	TFN/gm	pH	Organic Matter (%)	$CaCO_3$ (%)	Salinity (‰)	Pb(S)	Ni(S)	Zn(S)	Cu(S)	Fe(S)	Pb(F)	Ni(F)	Zn(F)	Cu(F)	Fe(F)
TFN / gm	1														
pH	-.527	1													
Organic Matter (%)	-.254	-.340	1												
$CaCO_3$ (%)	.278	.388	-.548	1											
Salinity (‰)	.606*	-.188	-.314	.673*	1										
Pb(S)	.107	-.132	.008	.084	.183	1									
Ni(S)	-.220	.240	.102	-.412	-.407	-.486	1								
Zn(S)	-.085	.184	-.043	.624*	.620*	.014	-.368	1							
Cu(S)	-.067	.129	-.094	-.148	-.073	.407	-.130	.089	1						
Fe(S)	.715**	-.128	-.313	.473	.668*	.365	-.454	.298	.293	1					
Pb(F)	-.392	**.606***	-.257	.543	.247	.337	-.453	**.673***	.474	.213	1				
Ni(F)	**-.674***	**.616***	-.185	.291	-.175	.149	-.263	.463	.429	-.214	**.860****	1			
Zn(F)	-.375	.509	-.120	.071	-.039	.260	-.084	.394	**.746****	.292	**.741****	**.650***	1		
Cu(F)	-.356	.386	-.004	.239	.100	.319	-.451	.498	**.644***	.165	**.868****	**.793****	**.761****	1	
Fe(F)	-.408	.503	-.201	.509	.354	.195	-.454	**.789****	.297	.197	**.935****	**.757****	**.682***	**.773****	1

Pearson correlation analyses of various parameters within core sediments of Chithrapu. Statistically significant correlations of interest are shown in bold fonts. * Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed). Parentheses (S) refers to that of sediment while (F) refers to that of foraminifera. TFN / gm – Total Foraminifera number per gram dry weight of sediment

Table.4 Pearson correlation analyses of various parameters within core sediments of Kumbla. Statistically significant correlations of interest are shown in bold fonts. * Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed). Parentheses (S) refers to that of sediment while (F) refers to that of foraminifera. TFN / gm – Total Foraminifera number per gram dry weight of sediment

	TFN / gm	pH	Organic Matter (%)	$CaCO_3$ (%)	Salinity (‰)	Pb(S)	Ni(S)	Zn(S)	Cu(S)	Fe(S)	Ni(F)	Zn(F)	Cu(F)	Fe(F)
TFN / gm	1													
pH	**.569***	1												
Organic Matter(%)	-.061	-.020	1											
$CaCO_3$(%)	**.807****	.280	.078	1										
Salinity(‰)	**.960****	.535	-.108	**.753****	1									
Pb(S)	**.586***	.481	.039	.457	**.646***	1								
Ni(S)	.032	.310	.493	.213	.017	.255	1							
Zn(S)	.092	.027	.392	.176	.179	.235	**.559***	1						
Cu(S)	.342	.539	-.015	.275	.393	.240	-.044	.167	1					
Fe(S)	-.106	-.234	.414	-.195	-.075	-.326	.048	.150	.064	1				
Ni(F)	-.112	.273	-.431	-.255	-.061	-.068	.033	-.276	-.200	-.124	1			
Zn(F)	-.099	.459	.042	-.086	-.091	.169	.057	-.156	**.753****	-.082	-.093	1		
Cu(F)	-.023	.242	-.508	-.042	.035	-.070	-.064	-.279	-.150	-.373	**.900****	-.142	1	
Fe(F)	.315	**.583***	-.124	.224	.201	.001	-.071	**-.564***	.092	-.402	.318	.252	.418	1

Figure.1 Preserved test of *Rotalidium annectans* from core sediments. **(A)** Stereo Microscopic image **(B)** Scanning Electron Microscopic image. Scale - 500µm

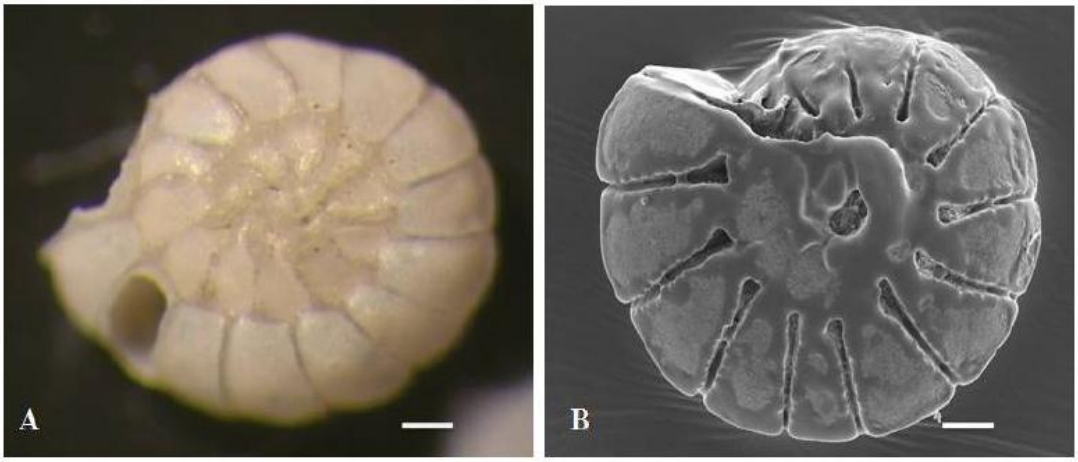


Figure.2 Images showing area of mangrove sediment core collection. **(A)** Map of West coast of India showing collection sites. Google earth images of mangroves of **(B)** Chithrapu (C) Kumbla. Scale bar – 1 Km

Figure.3 (A) Collection of mangrove sediment cores **(B)** Sediment core showing the pattern of sedimentation

Figure.4 Down core depth profile of heavy metals in tests of *Rotalidium annectans* from mangrove core sediments of Chithrapu

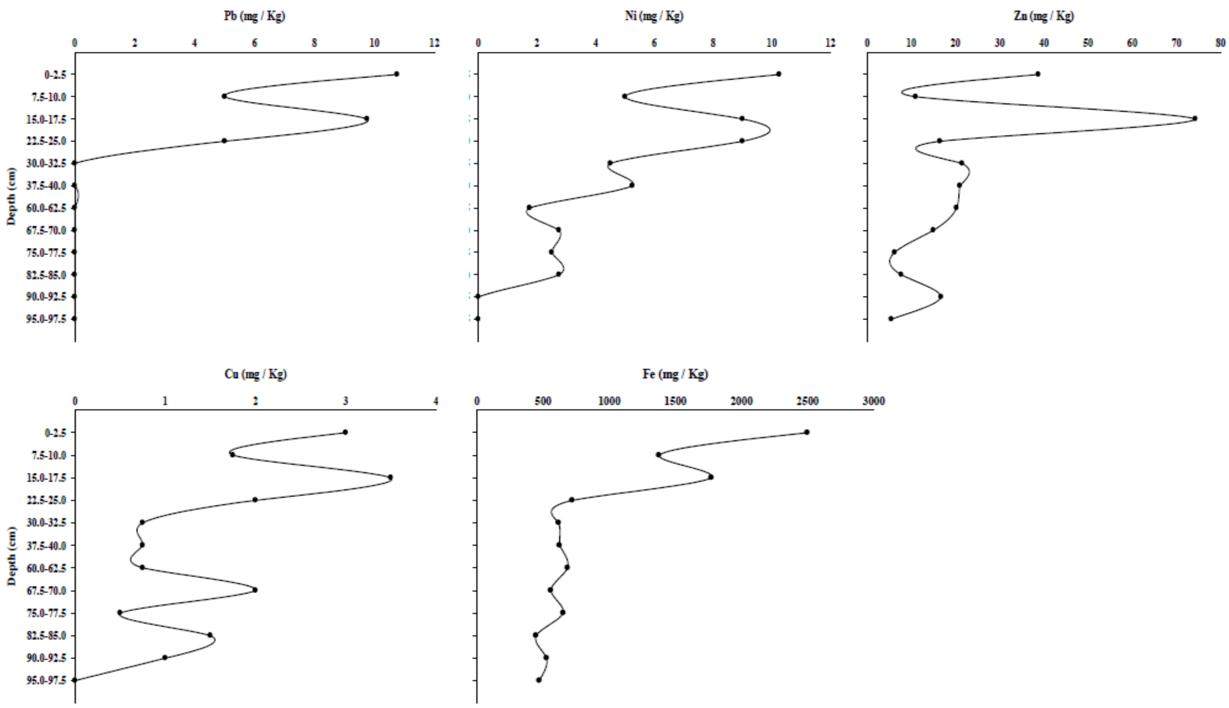
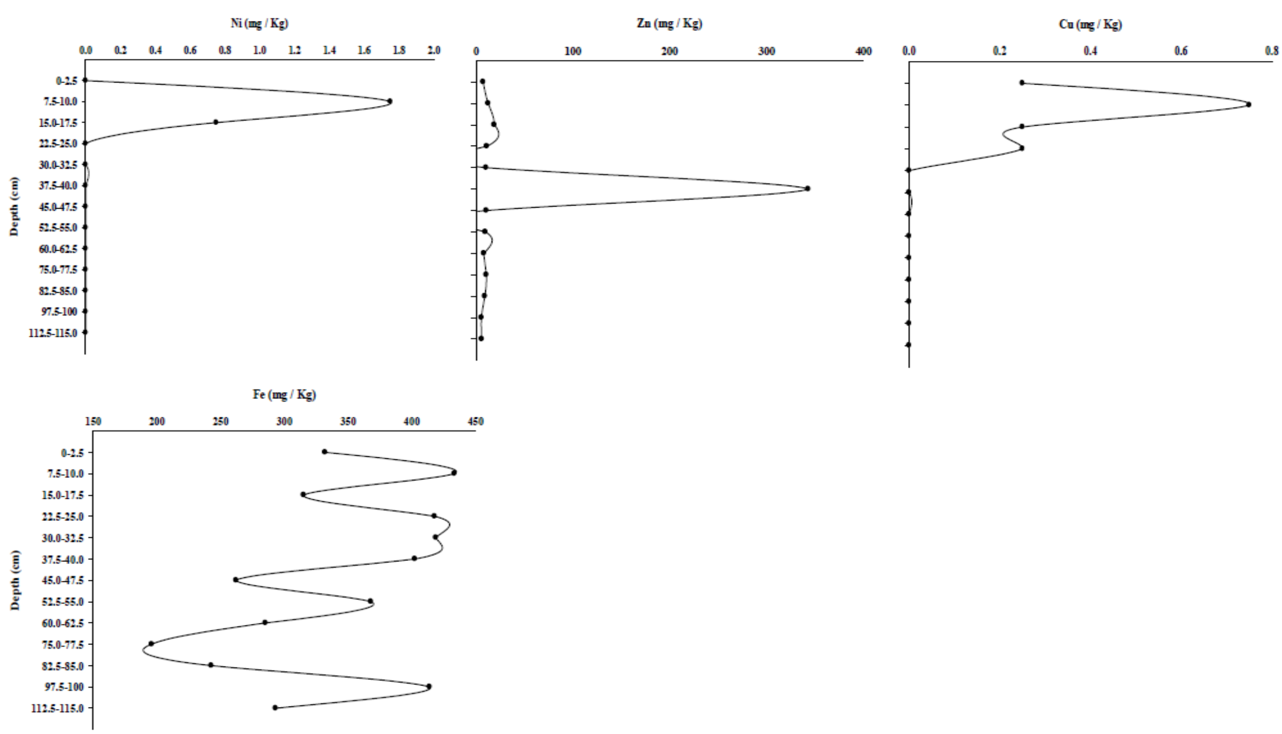


Figure.5 Down core depth profile of heavy metals in tests of *Rotalidium annectans* from mangrove core sediments of Kumbla



The Pb concentrations in foraminiferal tests were positively correlated with Zn in sediment and also with pH. In cores from Kumbla, Zn, cu and Fe in tests showed positive correlation with Cu in sediment, Ni in tests and Zn in sediment respectively, also Fe in tests were correlated positively to pH of the core. From the above shown significant correlations, similar patterns were seen within the foraminiferal test heavy metals and between concerned sediment characteristics in both the cores.

These correlations between concentration of metals with each other and with that in sediment suggest similar bioaccumulation mechanisms and sources. Apart from the sediment heavy metal content, physico chemical parameters also has role in the uptake of metals by foraminiferal tests (Harikumar *et al.*, 2009). Input of heavy metals from natural and human sources, environmental factors and species dependent responses might be the reasons for the variability in the concentrations of heavy metals in *Rotalidium annectans*. Considering all these factors, we suggest the use of certain species of foraminifera like *Rotalidium annectans*, which is able to resist most of the sediment post depositional pressures as suitable indicators for heavy metal pollution in mangroves.

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