

Trace Metal Bioaccumulation by Soft-bottom Polychaetes (Annelida) of Sundarban Mangrove Wetland, India and Their Potential Use as Contamination Indicator

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Abstract Bioaccumulation of trace metals in four soft-bottom polychaetes along with the host sediments was recorded from the intertidal regions of Indian Sundarban mangrove wetland. The enrichment of Mn (maximum 730 mg kg^{-1}) and Zn (maximum 320 mg kg^{-1}) was recorded for all the polychaetes followed by Cr, Cu, As, Co, Ni and Pb, showing sharp variations between the species. However, concentrations of Sn and Hg were very low with small variations between the species. An overall high bioconcentration factor (BCF) was recorded in the capitellid worm *Mastobranchius indicus*. The results of the investigation indicate the possibility of developing a standard test protocol for bioaccumulation by polychaetous annelids.

Keywords Trace metals · Bioaccumulation · Polychaetes · Sundarban wetland

Polychaetes are one of the most significant groups of benthic macroinvertebrate fauna and since these species come in direct contact with the sediments (Hutchings 1998; Olsgard and Somerfield 2000), the potential effect of these toxicants on this group of organisms is of ecological importance (Sato and Nakashima 2003; Zhou et al. 2004). There has been dearth of studies on documenting sediment-based bioaccumulation of heavy metals by polychaetous annelids. Hence the present study was undertaken to assess the efficiency of this specific macrozoobenthos for bioaccumulation of heavy metals and also to evaluate the use of any species of this group as bioindicator species.

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Materials and Methods

The Indian Sundarban mangrove wetland ($21^{\circ}31'6''$ – $22^{\circ}12'14''$ N and $88^{\circ}11'28''$ – $89^{\circ}05'53''$) is one of the most dynamic, complex and vulnerable low-elevated coastal zone (LECZ) situated in a typical geographical locations in the northeastern part of Bay of Bengal. Depending on the availability of benthic polychaetes, five sites of different geomorphological characteristics were chosen, namely, Haribhanga (S_1), Gangasagar (S_2), Chemaguri (S_3), Mayagoalinir ghat (S_4) and Ghushighata (S_5). Description of the stations has been given by previous publications (Saha et al. 2006; Sarkar et al. 2005). The following four soft-bottom benthic polychaete species were collected from the above-referred sites: (i) *Namalycastis fauveli* (Family: Nereididae) (ii) *Dendronerides arborifera* (Family: Nereididae) (iii) *Perenereis cultrifera* (Family: Nereididae)

and (iv) *Mastobranchus indicus* (Family: Capitellidae). Regarding feeding guilds, the first three species are omnivorous while the last one is surface-deposit feeders.

Polychaete samples were collected from mean high water and mean low water lines. For trace metal analyses, 20 congeneric polychaete species of uniform size (Simpson 1979) were collected from each station, transported to the laboratory in acid-washed plastic containers (Moody and Lindstrom 1977), rinsed free of mucus and sediments with seawater, depurated properly for 2–3 days (defecation of sediments and any undigested materials), and dried to a constant weight at 60°C. Samples were pulverized and homogenized in a Teflon mortar. The adjacent sediment samples of the respective sites were simultaneously collected from all the sampling sites, using a Peterson grab and transported to the laboratory in acid-wash plastic containers (Moody and Lindstrom 1977). Then the sediment was wet sieved (63 µm) since this fraction contains more sorbed metal per gram of sediment due to its larger specific surface area and subsequently oven dried at 50°C. The fraction <63 µm has been recommended for pollutant studies especially with respect to trace metals (Foster 2004). Organic carbon (C_{org}) content of the soil was determined employing a rapid titration method (in chronic acid environment) (Walkey and Black 1934). pH was measured with the help of a deluxe pH meter (model no. 101E) using combination glass electrode manufactured by M.S. Electronics (India) Pvt. Ltd. Mechanical analysis of sediment were done by sieving in a Ro-Tap Shaker (Krumbein and Pettijohn 1938) manufactured by W.S. Tyler Company, Cleveland, Ohio, and statistical computation of texture parameters was done by using the formulae of Folk and Ward (1957) and following standards of Friedman and Sanders (1978). Salinity was measured at the study sites by Refractometer.

Bioessential metals (Mn and Zn) and trace elements (Cr, Cu, As, Co, Ni and Pb) in solid sediments and polychaetes samples were determined by DCP-arc-Atomic-Emission Spectrometry (DCP-AES) using the instrument PGS-2 (Carl Zeiss Jena, Germany). The mercury Atomic Absorption (AA) analyzer RA-915⁺ of Lumex Ltd (St. Petersburg, Russia) with a pyrolytical attachment RP-91C was used for direct mercury determination in both sediments and polychaetes samples (Ganeev et al. 1995). The detection limits for Cd, Pb, As, Ni, Cr, Hg and Cu were 10, 10, 0.5, 10, 20, 0.5 and 10 µg g⁻¹, respectively. The non-selective absorption of the sample was controlled in the dynamic regime with Zeeman correction in the instrument. Samples preparation procedure for DCP-AES analysis included the following steps: (i) homogenization of the sample with graphite powder (1:1) in organic glass mortar; (ii) ashing the mixture at 450°C during 3 h and (iii) successive dilution of homogenized samples with graphite

powder and magnesium oxide MgO to prevent As loss at the ashing stage, providing its content in each sub-sample at the level of 10%. DCP-AES analysis: 20 mg of the sub-samples with dilution of 4 and 8 prepared according to above-mentioned procedure were placed into the cavity of graphite electrode. A unified series of reference samples was prepared on the base of graphite powder containing 10% of MgO. The average values of two results for each sub-sample with dilutions 4 and 8 were considered as ultimate ones. Quality assurance: certified reference materials (River Sediment BCR-320 as well as Dogfish Liver DOLT 2) were used to ensure the quality control and accuracy of the analyses. Certified reference materials (Sediment BCR-320 for sediment and Dogfish liver DOLT-2 for biota) were used to ensure the quality control and accuracy of the analyses, the recovery rates for heavy metals in the standard reference material ranged between 87 and 112% for sediments and 77–125% for biota.

Results and Discussion

The sediment quality parameters of five sampling sites of Sundarban wetland exhibit variations, as depicted in Table 1. Soil temperature showed minimum value of 20.5°C (during Monsoon 2008) to a maximum of 29.5°C (during Pre-monsoon 2008) resulting from seasonal effects in a tropical system. Values of pH range from slightly acidic to basic (range from 7.7 to 8.4) where the acidic nature is recorded as a solitary case for Ghusighata (during Monsoon 2008). This is partly due to the oxidation of FeS₂ and FeS to SO₄²⁻ and partly results from the decomposition of mangrove litter and hydrolysis of tannin in mangrove plants releasing various kinds of organic acids (Liao 1990). Organic carbon (OC) values were below 1% in all

Table 1 Sediment quality characteristics of the five sampling sites of Sundarban wetland during post-monsoon 2007–monsoon 2008

Sites	Seasons	Temp °C	pH	OC (%)	Sand (%)	Silt (%)	Clay (%)
S ₁	POM	21	8.1	0.4	23.65	62.05	14.3
	PRM	29	8.4	0.46	39.3	44.25	16.45
S ₂	PRM	28.5	8.4	0.22	32.85	58.45	8.7
S ₃	POM	24	8.1	0.55	22.8	67.7	9.5
	PRM	26	8	0.69	1.3	51.6	47.1
	M	20.5	8.2	0.89	0.8	84.74	14.46
S ₄	PRM	27	8	0.57	9.45	69.78	20.77
	M	20.5	8.3	0.73	0.35	49.68	49.97
S ₅	PRM	29.5	8	1.03	11.85	30.85	57.3
	M	24.5	7.7	1.44	2.7	47.25	50.05

POM postmonsoon; PRM premonsoon; M monsoon

Table 2 Trace metal concentrations in polychaete body tissues and adjacent sediments of five sites of Sundarban wetland (mg kg^{-1}) during Post-monsoon 2007–monsoon 2008

Sites	Seasons	Species	Mn	Zn	As	Cd	Co	Cr	Cu	Ni	Pb	Sn	Hg
S ₁	POM	<i>P. cultifera</i>	380	220	21	0.65	13.2	45	39	33	15	1.7	0.05
		Sediment	650	95	36.3	0.21	24.5	52.5	53.3	51.9	22.6	3.9	0.03
	PRM	<i>P. cultifera</i>	650	220	17	0.6	16.2	45	36.5	33	18.5	2.5	0.08
		Sediment	580	75	22.6	0.22	22.6	55	40	39.6	16.2	1.7	0.04
S ₂	PRM	<i>P. cultifera</i>	180	140	7	0.55	10.6	14.7	52	23	5.1	1.4	0.2
		Sediment	460	74	26.1	0.16	22	63	40.3	35.6	17.4	4.6	0.03
S ₃	POM	<i>M. indicus</i>	460	150	16.9	0.6	13	43	44.5	32	17	2.3	0.08
		Sediment	560	149	27	0.2	24.2	65	46	51	26.3	5.2	0.03
	PRM	<i>M. indicus</i>	420	180	13.5	0.6	10.2	38	35.5	25	22	0.8	0.07
		Sediment	600	190	22.2	0.16	22	57	41.5	39.2	28.2	2.1	0.02
	M	<i>M. indicus</i>	730	320	17.6	0.65	15	46	40	39	23	4	0.28
		Sediment	530	114	28.8	0.25	23.8	50	74.6	42.8	25.8	5.5	0.04
S ₄	PRM	<i>N. fauveli</i>	70	85	4.9	0.2	8.6	8.5	31	7.5	2.2	<0.5	0.2
		Sediment	400	75	14.8	0.16	23.4	65	46.4	40	23.4	1.6	0.03
	M	<i>M. indicus</i>	650	200	12.5	0.8	13.1	55	37	32.5	23	4.2	0.2
		Sediment	640	133	19.4	0.18	24.2	70.6	62.4	48.7	28	6.4	0.05
S ₅	PRM	<i>D. arborifera</i>	160	140	9.5	0.4	4.8	4.8	33	7.8	0.95	<0.5	0.15
		Sediment	350	95	29.4	0.5	23.8	143	37.5	43.3	25.3	4.7	0.05
	M	<i>M. indicus</i>	180	230	11	2	6.4	105	25	18	4.4	1	0.17
		Sediment	350	104	32.3	2.2	22.6	149	36.2	33.9	23.5	4.1	0.05

POM postmonsoon; PRM premonsoon; M monsoon

stations except at Gangasagar (S₂). One of the features of OC in the sediments is that the concentration increases as the particle size of the sediment decrease. The finer particles (silt + clay) showed an efficacious relationship with OC while the coarser fraction has no patent kinship. There exists strong positive correlations between organic carbon and clay particles ($p < 0.01$) which demonstrates adsorbability and efficacy of fine-grained particles for organic carbon. Regarding textural composition, the five stations also exhibit wide variations, such as, sandy silty clay at S₁, sandy clay at S₂ and silty clay for rest three stations.

Among the eleven trace metals examined, the maximum differences in metal concentration in the four polychaete species were found for Mn, Zn, Cu and As (as shown in Table 2) which are also well marked in concentration of metals in sediments (Table 2). Both the metals, Mn and Zn, showed their maximum values of 730 mg kg^{-1} and 320 mg kg^{-1} , respectively in *M. indicus* at the site Chemaguri (S₃) during Monsoon 2008. The elevated values of these bio-essential elements are expected as the play an important role in the metabolic processes of these organisms. Moreover, Cu has an important biochemical role as an enzyme activator as a consistent of flavoprotein (Mahler 1956). The elevated levels of Cu and Zn in the polychaete tissues are likely to be associated with antifouling paint residues arising from boat maintenance and vessel repair

activities by the coastal people. The consistent higher values of these two elements in all the studied polychaetes suggest that these two have a high potential for biomagnifications during their transfer to the top predators. The elevated level of Cu in *P. cultifera* of 52 mg kg^{-1} (at Gangasagar, pre-monsoon 2008) may also be involved in the deterrence of predators (Gibbs et al. 1981). Physiological differences can also result in marked differences in trace metal accumulation. For example, nereid worm can regulate Zn while Cu is accumulated and stored in membrane-bound vesicles rather than being excreted (Depledge and Rainbow 1990). The body Zn concentrations of all the four polychaete species are relatively independent of ambient concentrations and appear to be internally regulated. This might be attributed to the weak net accumulation through partial regulation of trace elements as observed in accumulation of Zn in the polychaete *Nereis (Hediste) diversicolor* (Amiard et al. 1987). The results of correlation coefficient matrix show that both Mn and Zn are positively and significantly correlated (either 5% or 1% level) with majority of the metals suggesting a common source or chemical similarity.

Regarding arsenic, interspecific variations are pronounced where the minimum value of 4.9 mg kg^{-1} in *N. fauveli* was almost four times less than that of *P. cultifera* where the maximum value of 21 mg kg^{-1} was

recorded. This elevated level of As in *P. cultrifera* may be involved in the deterrence of predators (Gibbs et al. 1983). Concentrations in polychaete species reflect total As loads in bottom sediments. Therefore, some species may serve as suitable biological indicators of the availability of sediment-bound As, ascertained by Bryan and Langston (1992). However, it is difficult to ascertain the differences in As bioaccumulation among the five sites of very different sediment chemistry as endorsed by Casado-Martinez et al. (2010). Concentration of As in sediments exceeded the sediment criterion (where ER-L value for As 8.2 mg kg^{-1}) for all the samples and thus expected to increase the incidence of adverse negative effects on living resources from rare to occasional. Cadmium is a non-essential metal which is not regulated metabolically or eliminated in organisms in comparison to essential metals (Cu and Zn) (Fernandes et al. 2007). Interestingly, the concentration of Cd has been found to be much greater in the majority of the polychaete samples in comparison to the sediment. Cadmium is present in the sediments at a trace level (ranges from 0.16 to 2.2 mg kg^{-1}), but it was readily accumulated in polychaetes much higher than adjacent sediments. This indicates their excellent accumulation capacity which might cause acute toxicity to other organisms and human being through marine food chain. Sun and Zhou (2007) has observed that Cd accumulation in the polychaete *H. japonica*, in terms of the amount and exposure time, suggesting that a passive regulation for Cd accumulation occurring in this species. The capitellid worm *M. indicus* at Chemagari (S_5) accumulated maximum concentration of Cr (105 mg kg^{-1}) and may be considered as potential Cr accumulators in comparison with rest of the polychaete species. The highly elevated chromium level at this site might be due to the mixing of tannery effluents in the sewage canal from the adjacent tanneries containing high toxic chromium compounds along with high organic matter and salt content. The elevated level of Cr in the body tissues of the same capitellid worm was recorded at this site (164 mg kg^{-1}) (Sarkar et al. 2005), which is in conformity with the present findings. It is worthwhile to mention that the chromium content in sediments was also high at this site (143 – 149 mg kg^{-1}) which is above the ER-L value and may possess some ecotoxicological risks to organisms living in sediments.

Copper is one of the most common contaminants found at high concentrations in aquatic environments. That is to say, aquatic organisms are being exposed to elevated levels of copper. In contrast to Cd, Cu is an essential element for the subsistence of many animals, is a component of many metalloenzymes and respiratory pigments and plays an important role in the activities of cellular metabolism (Cousins 1985). Accumulation of Pb in polychaete tissues showed an erratic pattern where the low values were

recorded in *P. cultrifera* (at S_2) (0.95 mg kg^{-1}) and *N. fauveli* (at S_4) (23 mg kg^{-1}) during pre-monsoon 2008. The accumulation of Pb in the individual polychaete species was found to be lower than those found in the adjacent sediments (Table 2) which endorse previous findings while studying gastropod, bivalve mollusks and fishes from Sundarban environment (Saha et al. 2006).

Concentrations of three potential pollutants, Cd, Sn and Hg, showed a lower degree of accumulation in all the polychaete species than rest eight metals. This might be related to the unique adaptive strategies of these worms by secreting mucus in response to these metals which help in reducing metal availability for uptake. This was demonstrated experimentally by Mouneyrac et al. (2003) on the polychaete *H. diversicolor*. Many parameters may affect the mercury accumulation, such as specimen size, sexual maturity, feeding habits, trophic position, water quality and environmental contamination (Kehrig et al. 1998). Even closely related species may be feeding on subtly different food sources with consequently different inputs of metals for accumulation (Rainbow 1995). It is, thus, not surprising that the polychaete species do not have similar metal body burdens even when present together at the same site. BAF was determined as a ratio between the concentrations of metal in the organism (mg kg^{-1} dry wt) and that in the surrounding medium (mg kg^{-1} dry wt). The maximum BAF value was recorded in *M. indicus* at Chemagari (Monsoon 2008) for Mn, Zn and Hg. Consistent higher values were also pronounced for all the polychaetes for Cd, Zn, and Hg.

The results presented provide important baseline information that can be used to assess future changes in contaminants in the study area and ultimately to begin to understand the changes in flux of potential contaminants on the polychaetous annelids as an approach toward sustainable management of this potential wetland.

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