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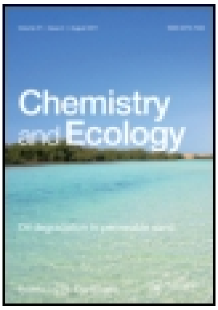


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# Heavy metals in the polychaete *Glycera longipinnis* from the southwest of India

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Metal concentrations in sediment and in whole tissue of the benthic polychaete *Glycera longipinnis* collected along the southwest coast of India were analysed. Relative seasonal accumulation of metals (Cu, Pb, Cr, Ni, Zn, Cd, Hg) was studied by categorising the habitat as less polluted or highly polluted based on metal contamination routed through industrial and urban sources. The metal content in tissues varied seasonally in the ranges, Cu: 2.21–27.08 $\mu g \cdot g^{-1}$, Pb: 0.06–4.92 $\mu g \cdot g^{-1}$, Cr: 1.73–29.20 $\mu g \cdot g^{-1}$, Ni: 1.60–4.61 $\mu g \cdot g^{-1}$, Zn: 14.72–82.30 $\mu g \cdot g^{-1}$, Cd: 0.04–1.38 $\mu g \cdot g^{-1}$and Hg: below decetable limits to 0.86 $\mu g \cdot g^{-1}$. Concentration of heavy metals was found to be high in the whole body of *G. longipinnis* pooled from the polluted transects. The results of this study suggest that *G. longipinnis* may act as a useful biological indicator for heavy metal pollution along the southwest coast of India.

**Keywords:** heavy metal; southwest coast; sediment; *Glycera longipinnis*; bioaccumulation

## 1. Introduction

The determination of heavy metals in benthic organisms has received added impetus owing to increased awareness of environmental pollution in aquatic realms. Generally, as a primary assessment tool, quantitative analysis of benthic communities is given precedence over the interpretation of contaminant effects, which are treated only as secondary, especially at sites where there is intense contamination. In the marine environment, sediments act as a sink for heavy metals which can be taken up by contaminant-tolerant benthic invertebrates like polychaetes [1]. Marine benthic invertebrates have been successfully employed in numerous biomonitoring studies because of their sessile nature, long and stable life span, moderately fast response to stress, and vulnerability to the effect of sediment contamination through their food and feeding [2]. Polychaetes are a species-rich component of marine benthic communities and are considered as a good anthropogenic pollutant indicator along the southwest coast of India [3]. Heavy metal pollution by untreated industrial discharge and domestic waste through rivers into the southwest coast of India

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is well documented [4,5]. Information on the bioavailability of heavy metals in sediments, and their accumulation in benthic-level organisms distributed along the Indian coasts is scanty. This is particularly true of polychaetes, which form one of the most important components of the benthic community as well as an important food source for demersal fish. A recent study conducted along the southwest coast of India recorded a high abundance of the polychaete *Glycera longipinnis* among the benthic community at all depths and in all seasons [6]. This study, therefore, was undertaken to determine temporal and spatial variability in the accumulation of heavy metals, viz. Cu, Pb, Cr, Ni, Zn, Cd and Hg in the polychaete *G. longipinnis*, and its usefulness as biological indicator of heavy metal pollution along the southwest coast of India.

## 2. Materials and methods

### 2.1. *Sample collection*

The location of the sampling transects (T1, T2, T3, and T4) along the southwest coast of India is presented in Figure 1. The selection of locations was based on possible anthropogenic activities. T1, Cochin (major port, fishing harbours, densely populated zone, busy waterways, tourist destination, major industrial centre); T2, Chettuva (agricultural region, river estuary, fishing village); T3, Calicut (commercial port, fishing harbour, densely populated area, industrial city); and T4,

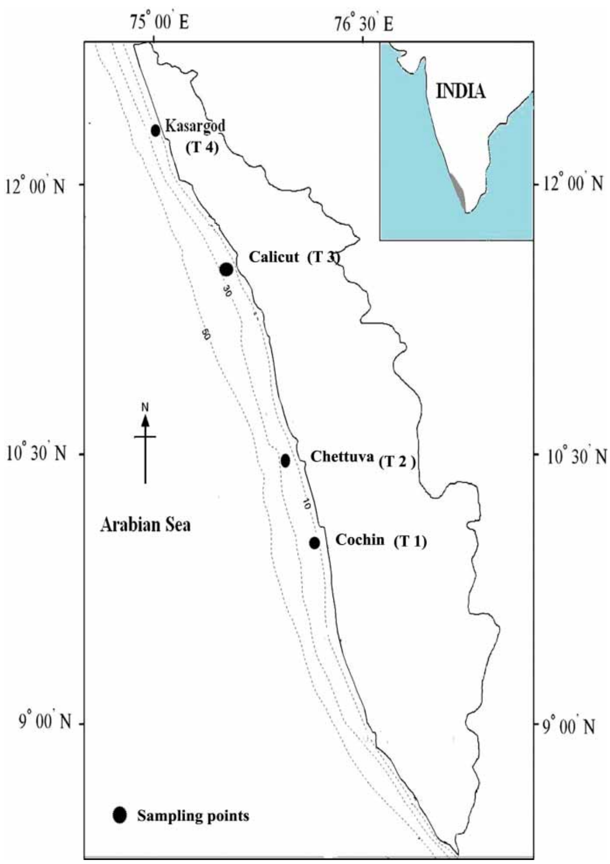


Figure 1. Location of sampling stations along the southwest coast of India.

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Kasargod (fishing harbour, small-scale industries, agricultural region). Sediment samples were collected seasonally, representing post monsoon, pre monsoon and monsoon of 2008 using van Veen Grab (0.04 $m^2$) operated onboard the Coastal Research Vessels (CRV) *Sagar Purvi* and *Sagar Paschimi* from a depth of 10–15 m. Nine replicate sediment samples were taken from each of the stations. The top 3–5 cm of sediment samples (composite) were placed in polyethylene plastic bags and kept frozen prior to further analysis. Macro polychaetes were separated by washing the sediments kept on 0.5mm sieves using a backwash technique with seawater. Polychaetes were carefully handpicked, depurated onboard with seawater collected from the same location on a clean plastic container for 48 h and kept frozen in polypropylene containers. The frozen specimens were transported to the laboratory for further analysis.

### 2.2. *Sample preparation*

#### 2.2.1. *Polychaetes*

In the laboratory, *G. longipinnis* specimens of uniform size (1.80–2.10 cm) were segregated according to sampling transect to attenuate the possible variation in metal concentration. Identification of the polychaete was carried out using standard identification manuals [7,8]. Specimens were then washed with deionised water to remove mucus and salt, macerated and air dried at 60°C for 48 h in a hot air oven before digestion. Dried tissue (0.250 g) was digested using 2 mL $HNO_3$ (65%) and 1 mL $H_2O_2$ (30%) in a Teflon vessel kept overnight and digested at 80°C for 2 h [9]. After digestion, the residue was transferred into an Erlenmeyer flask and the volume reduced to almost dryness. The processed sample was leached with 6 M HCl solutions and was made up to 25 mL with deionised water. All the glassware used was acid washed (diluted $HNO_3$) and subsequently rinsed in double-distilled water.

#### 2.2.2. *Sediment*

For the analysis of total heavy metal in sediment, 1.0 g of finely powdered and dried (70°C) sediment sample was digested in a mixture of $HF–HClO_4–HNO_3$ [10]. Samples were evaporated in a platinum crucible. The step was repeated until a clear solution was obtained, which ensured complete digestion. For the quantification of Hg, wet sediment samples were digested in Bethge apparatus in a mixture of nitric and sulfuric acid (3 : 1) [11]. A flame atomic absorption spectrometer (Perkin–Elmer AA 200) was used to quantify the heavy metals (Cu, Pb, Cr, Ni, Zn and Cd). Total mercury was determined using cold vapour atomic absorption spectrometry (Mercury Analyzer MA 5840). Organic carbon in the samples was determined using wet digestion (chromic acid) followed by back titration with ferrous ammonium sulfate [12]. An assessment based on the pollution load index (PLI) was employed to find the extent of pollution by metals in sediments [13]. PLI was evaluated using the equation PLI = (product of $n$ number of CF values)$^{1/n}$, where CF is the contamination factor and $n$ is the number of metals. CF was obtained as a concentration of each metal with respect to the background value of the metal constituent in the sediment. The world average concentration of the metals reported for shale was used as the uncontaminated natural background value for a specific metal in the present study [14]. The transects were partitioned into polluted (PLI $>$ 1) and unpolluted (PLI $<$ 1) due to heavy metals on the basis of PLI formulated at each sampled transect.

The bioconcentration factors (BCF) of the heavy metals in the polychaete samples were obtained using equation given by Falusi and Olanipekun [15].

$$\mathrm{BCF} = \frac{\mathrm{C_{org}}}{\mathrm{C_{sed}}},$$

Table 1. Recoveries of heavy metals from certified reference materials ($\mu g \cdot g^{-1}$).

| Metal | Cd | Cr | Cu | Pb | Hg | Ni | Zn |
|---|---|---|---|---|---|---|---|
| NRCC DORM-2 Dogfish muscle | | | | | | | |
| Certified | 0.043 ± 0.008 | 34.7 ± 5.5 | 2.34 ± 0.16 | 0.065 ± 0.007 | 4.64 ± 0.26 | 19.4 ± 3.1 | 25.6 ± 2.3 |
| Measured ($n = 3$) | 0.039 ± 0.009 | 32.7 ± 2.8 | 2.17 ± 0.28 | 0.058 ± 0.003 | 4.44 ± 0.18 | 17.8 ± 2.8 | 25.4 ± 1.9 |
| BCSS-1 | | | | | | | |
| Certified | 0.25 ± 0.04 | 123 ± 14 | 18.5 ± 2.7 | 22.7 ± 3.4 | – | 55.3 ± 3.6 | 119 ± 12 |
| Measured ($n = 5$) | 0.23 ± 0.06 | 111 ± 16 | 17.9 ± 1.8 | 21.3 ± 3.2 | BDL | 52.7 ± 3.4 | 106 ± 22 |

Notes: Certified materials: mean ± standard deviation; Measured: mean ± standard deviation. BDL, below detectable limit.

where $C_{org}$ is the concentration of metal in the organism ($\mu g \cdot g^{-1}$ dry wt) and $C_{sed}$ is the concentration of the same metal in the sediment ($\mu g \cdot g^{-1}$ dry wt).

Quality assurance was established using Certified Biological Reference Material (dogfish muscle, DORM-2) and a Certified Marine Sediment Reference Material (BCSS-1) from the National Research Council of Canada and the recovery was above 90% (Table 1). The precision of analysis was ascertained by triplicate analysis and the results are expressed as $\mu g \cdot g^{-1}$ on dry weight basis.

### 2.3. *Statistical analysis*

Pearson's correlation was performed to find out the significant correlation among the metals in sediment and the polychaete from both less and highly polluted transects, and the analysis was carried out using SPSS (Version 10.0).

## 3. Results

### 3.1. *Seasonal variations of heavy metals in sediment and polychaete*

Seasonal variations in the heavy metal concentration in sediment and in *G. longipinnis* pooled from the sampling transects are summarised in Figure 2. The distribution of metals in sediment from the sampled transects showed wide seasonal variation. Concentrations in sediments varied as follows: Cu, 19.08–62.70 $\mu g \cdot g^{-1}$; Pb, 13.79–40.97 $\mu g \cdot g^{-1}$; Cr, 80.79–282.80 $\mu g \cdot g^{-1}$; Ni, 28.10–121.12 $\mu g \cdot g^{-1}$; Zn, 42.24–148.21 $\mu g \cdot g^{-1}$; Cd, 0.18–2.80 $\mu g \cdot g^{-1}$; and Hg, 0.08–0.56 $\mu g \cdot g^{-1}$. Organic carbon in sediments varied from 1.37 to 5.35% (Figure 3). Seasonal variations in heavy metal concentrations were as follows: Cu, pre-monsoon > post monsoon > monsoon; Pb and Zn, monsoon > pre monsoon > post monsoon; Ni, monsoon > pre monsoon > post monsoon; Cr and Hg, monsoon > post monsoon > pre monsoon; and Cd, pre monsoon > monsoon > post monsoon. Accumulation of heavy metals in *G. longipinnis* was in the range: Cu, 2.21–27.08 $\mu g \cdot g^{-1}$; Pb, 0.06–4.92 $\mu g \cdot g^{-1}$; Cr, 1.73–29.20 $\mu g \cdot g^{-1}$; Ni, 1.60–4.61 $\mu g \cdot g^{-1}$; Zn, 42.84–82.30 $\mu g \cdot g^{-1}$; Cd, 0.04–1.38 $\mu g \cdot g^{-1}$; and Hg, below decetable limits to 0.86 $\mu g \cdot g^{-1}$.

Seasonally, it was noted that the accumulation levels in *G. longipinnis* for Cd, Ni, Cu, Zn were higher during pre monsoon than in monsoon in all transects. During post monsoon, however, the accumulation levels were found to be intermediate in all transects. In the case of Cr, the accumulation level was high in the monsoon season, followed by post and pre monsoon seasons at all transects. The concentration of Pb, however, showed a consistent pattern, and remained high at transects T1 and T3 during pre monsoon and monsoon periods. The accumulation level of Hg showed high values during the monsoon followed by pre monsoon and post monsoon periods. The concentrations of Cd and Hg were high in the majority of polychaetes compared to that in sediment.

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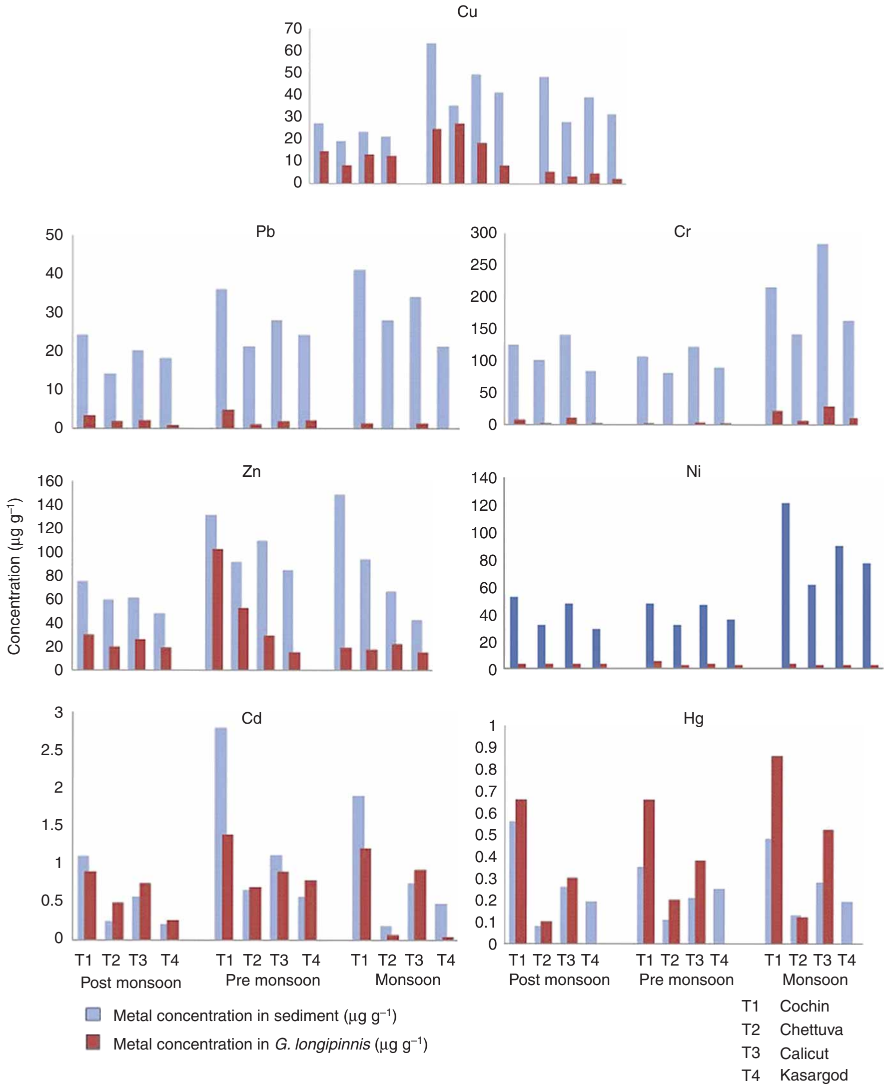


Figure 2. Variations in the concentrations of heavy metals in sediment and *G. longipinnis* among different seasons in the sampled transects.

### 3.2. *Relationship of heavy metal concentration in sediment and polycheate between polluted and non-polluted transects*

An evaluation based on PLI to assess the extent of pollution by heavy metals in sediments among the transects revealed values $>1$ at T1 during all the seasons and T3 during the post monsoon and monsoon seasons compared with transects T2 and T4 where the values remained $<1$ in all seasons (Figure 4). Transects T1 & T3 and T2 & T4, therefore, were categorised as polluted and unpolluted zones respectively.

Results of analysis using Pearson's correlation revealed significant correlation between the Cr and Cd content in polychaetes and that found in sediment in the polluted zone (Table 2). In the unpolluted zone (Table 3), significant correlations were observed between bioaccumulated Cr and Cr in sediment.

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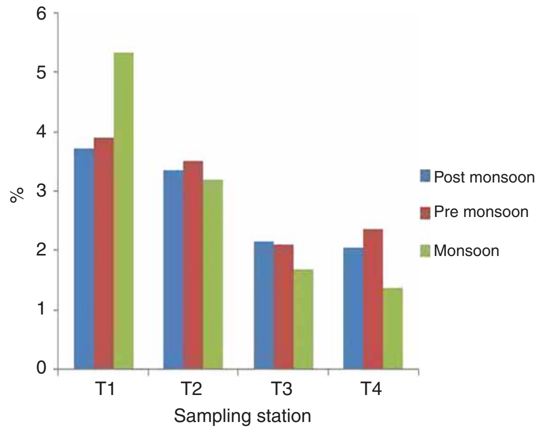


Figure 3. Seasonal variations in organic carbon for sampling stations along the southwest coast of India.

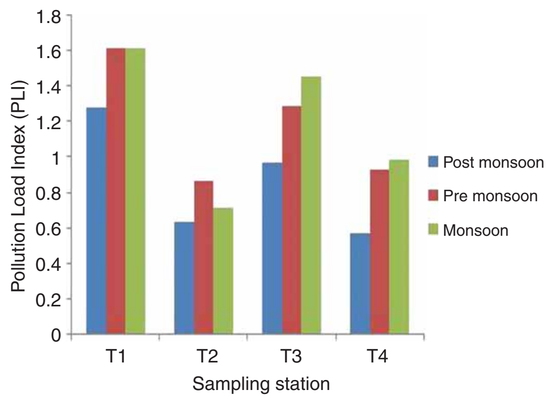


Figure 4. Seasonal variations in Pollution Load Index (PLI) for sampling stations along the southwest coast of India.

Table 2. Results of Pearson's correlation coefficient among heavy metals in sediment and *G. longipinnis* in a polluted zone.

| | P#Cu | P#Pb | P#Cr | P#Ni | P#Zn | P#Cd | P#Hg |
|---|---|---|---|---|---|---|---|
| S#Cu | 0.517 | 0.319 | −0.325 | 0.655 | 0.704 | 0.792 | 0.038 |
| S#Pb | −0.309 | −0.2 | 0.476 | 0.135 | 0.158 | 0.711 | 0.578 |
| S#Cr | −0.903* | −0.707 | 0.985** | −0.718 | −0.564 | −0.222 | 0.123 |
| S#Ni | −0.855 | −0.673 | 0.862 | −0.49 | −0.523 | 0.127 | 0.604 |
| S#Zn | 0.242 | 0.088 | −0.218 | 0.508 | 0.344 | 0.775 | 0.52 |
| S#Cd | 0.566 | 0.644 | −0.426 | 0.898* | 0.82 | 0.970** | 0.507 |
| S#Hg | −0.185 | 0.217 | 0.051 | 0.12 | −0.107 | 0.134 | 0.786 |
| S#OC | −0.009 | 0.198 | −0.052 | 0.412 | 0.157 | 0.637 | 0.926(*) |

Notes: *Correlation significant at $p < 0.05$ (two-tailed). **Correlation is significant at $p < 0.01$ (two-tailed). S#, Concentration of heavy metal in sediment; P#, concentration of heavy metal in *G. longipinnis*.

The bioconcentration factor (BCF) was calculated for the metals studied at each transect to determine bioaccumulation in *G. longipinnis* (Table 4). Bioaccumulation of metals by organisms occurs if the BCF $> 1$ [15]. In this study, the BCF generally showed high values for Cd at transects T2, T3, and Hg at T1, T2 and T3. The BCF for all the other metals under study was $<1$.

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Table 3. Results of Pearson's correlation coefficient among heavy metals in sediment and *G. longipinnis* in an unpolluted zone.

| | P#Cu | P#Pb | P#Cr | P#Ni | P#Zn | P#Cd | P#Hg |
|---|---|---|---|---|---|---|---|
| S#Cu | 0.155 | 0.012 | −0.12 | −0.749 | 0.206 | 0.295 | −0.068 |
| S#Pb | −0.222 | −0.388 | 0.236 | −0.756* | −0.083 | −0.208 | 0.019 |
| S#Cr | −0.643 | −0.433 | 0.925** | −0.301 | −0.412 | −0.531 | 0.16 |
| S#Ni | −0.617 | −0.64 | 0.806* | −0.499 | −0.357 | −0.641 | −0.044 |
| S#Zn | 0.321 | 0.105 | −0.342 | −0.661 | 0.419 | 0.316 | 0.192 |
| S#Cd | 0.536 | 0.373 | 0.175 | −0.338 | 0.566 | 0.668 | 0.575 |
| S#Hg | −0.171 | 0.287 | 0.471 | 0.084 | −0.331 | 0.292 | 0.293 |
| S#OC | 0.447 | 0.208 | −0.591 | −0.214 | 0.529 | 0.275 | 0.117 |

Notes: *Correlation significant at $p < 0.05$ (two-tailed). **Correlation significant at $p < 0.01$ (two-tailed). S#, Concentration of heavy metal in sediment; P#, concentration of heavy metal in *G. longipinnis*.

Table 4. Bioconcentration factors for heavy metals ($n = 3$, mean ± SD) at the sampled transect.

| Transects | Cu | Pb | Cr | Ni | Zn | Cd | Hg |
|---|---|---|---|---|---|---|---|
| T1 | 0.34 ± 0.21 | 0.10 ± 0.06 | 0.07 ± 0.04 | 0.06 ± 0.04 | 0.43 ± 0.33 | 0.65 ± 0.17 | 1.62 ± 0.38 |
| T2 | 0.44 ± 0.33 | 0.07 ± 0.07 | 0.03 ± 0.02 | 0.06 ± 0.03 | 0.36 ± 0.20 | 1.14 ± 0.86 | 1.33 ± 0.46 |
| T3 | 0.35 ± 0.23 | 0.07 ± 0.04 | 0.07 ± 0.03 | 0.05 ± 0.02 | 0.34 ± 0.08 | 1.11 ± 0.27 | 1.61 ± 0.40 |
| T4 | 0.29 ± 0.27 | 0.04 ± 0.05 | 0.04 ± 0.02 | 0.07 ± 0.05 | 0.35 ± 0.03 | 0.93 ± 73 | 0 |

## 4. Discussion

The results of this study clearly show that the order of heavy metal accumulation in sediment and *G. longipinnis* was not consistent throughout the seasons. These observations are more or less comparable with earlier findings carried out in polychaetes along the east coast of India [9]. Analogous observations were recorded in benthic bivalves and other bottom-dwelling organisms owing to increased feeding rate, warming of seawater, particulate material run-off into the environment and reproductive development [16,17]. In our study, the seasonal variations can be attributed to the different inputs of metals for accumulation and the feeding habits of the polychaete. The results revealed high concentrations for most of the heavy metals (Cu, Pb, Zn, Cd, and Hg) in sediments as well as *G. longipinnis* in the pre monsoon season during which a corresponding increase in organic carbon concentrations was also noted. Obviously this could facilitate scavenging of heavy metals, which can be accumulated in the polychaete through dietary intake.The sediments collected during post monsoon reflected low metal enrichment throughout the study sites. The influence of northerly currents that are capable of dispersing their deposition along the southwest coast of India has been described previously [18]. Heavy metals were found to accumulate at noticeable levels in *G. longipinnis* from the southwest coast of India. Table 5 gives a comparison of heavy metal content of polychaete species from this coast with those of other coastal areas. The Cr content recorded in the polychaete species during this study was considerably higher than that reported from other coastal regions of the world. The increased Cr content was reported from transects T1 and T3 where there is a heavy influence of industrial drainage. All the other heavy metal content was, in general, of a similar magnitude to values reported for other polychaete species.

The concentration of heavy metals in *G. longipinnis* varied considerably among the different transects. This may be due to feeding on subtly different available food sources with consequently different inputs of metals for accumulation, which are diverse at each transect. The effectiveness of metal uptake from the same sources varying in relation to ecological needs, metabolism, environmental contamination and various abiotic factors has been reported [23]. In this study, accumulation was high in regions T1 and T3 where there is a heavy influence of industrial and

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Table 5. Average metal concentrations ($\mu g \cdot g^{-1}$ dry wt) found in polychaete sp. from various regions of the world.

| Location | Cd | Cr | Cu | Ni | Pb | Zn | Hg | Ref. |
|---|---|---|---|---|---|---|---|---|
| Southwest coast of India | 0.043–1.38 | 1.73–29.2 | 2.21–27.08 | 1.6–4.61 | 0.06–4.92 | 14.72–82.3 | BDL–0.86 | This study |
| Oualidia lagoon | 0.09 | 2.0 | 6.8 | 1.7 | 1.0 | 115 | – | [19] |
| UK estuaries | 0.03–10 | 0.1–10 | 10–1430 | 0.6–15 | 0–1190 | 91–510 | – | [20] |
| Urdaibai estuary | 0.1–1.7 | 0.1–1.5 | 6.3–39 | 1.3–7 | 0–10 | 25–300 | – | [21] |
| Australian coast | 0.07–17 | – | 3.4–26 | – | 0.09–3.2 | 47–225 | 0.08–0.88 | [1] |
| Barents Sea | 0.34 | – | 6.8 | 11 | 0.8 | 47 | – | [22] |
| Hugli estuary, NE India | – | 11.10–54.05 | 8.15–30.66 | – | – | 18.28–102.25 | BDL–0.44 | [9] |

Note: BDL, below detectable limit.

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domestic wastes. Considering the spatial variation in heavy metal concentration in *G. longipinnis* in this study, it was found that higher accumulation occurred at T1 compared with the other transects. Assessing the species host sediment, however, checked this aberration. At transect T1, the enrichment of heavy metals in the sediment matrix is mainly due to quantum metal input from the contiguous industrial establishments situated along the coast. The industrial assortment includes fertiliser, pesticide, chemical and allied industries, radioactive mineral processing, petroleum refining, metal plating and fish-processing units. Results of Pearson's correlation showed the relevance of metal availability from sediments to the organisms. In polluted regions T1and T3, significant correlation occurred between Cd and Cr in sediment and that accumulated in *G. longipinnis*. The concentration of both metals in sediments was relatively high; because *G. longipinnis* are exposed to contaminants there is every possibility of accumulation via several different paths. Bioaccumulated Hg in *G. longipinnis* was positively correlated with organic carbon indicating the source of Hg through organic matter. According to Fauchland and Jumars [24], *Glycera* sp. feeding types include carnivorous/detritivore/omnivore. Although the polluted site showed increased concentrations of other toxic metals, its effect was not noticeable in *G. longipinnis*. Adsorption of many toxic substances onto the sediments and organic material has been recognised, rendering them less available to benthic organisms [25]. This may be the reason for these species thriving in sediments with greatly elevated levels of toxicants. In an unpolluted site, the increased concentration of Cr in the sediment facilitated a significant correlation with bioaccumulated Cr.

A BCF value $>1.0$ for Hg in *G. longipinnis* at the polluted transects T1, and for Cd and Hg at T3, indicates heavy bioaccumulation of these metals in the tissue due to the influence of industrial discharge. This also signifies their excellent accumulation capacity which might cause acute toxicity to other organisms and human beings via the marine food chain. A review of toxicological studies using polychaetes cited Cu and Hg as the most toxic metals tested, whereas the least toxic metals followed the order Cr, Cd, Zn and Pb [25]. Under captive conditions, Bryan [26] demonstrated that the polychaete *Capitella capitata* when exposed to polluted sediments took up Cd with little or no demonstrable effects. In contrast to this, at the unpolluted transect T2, where there is no industrial influence, the bioavailability of Hg and Cd can be attributed to the increased use of pesticides and fungicides containing these metals. Moreover, at this transect the percentage of organic carbon is higher, signifying increased productivity, which in turn can remove metals from solution and deposit them in sediment. A significant correlation of Hg with organic-rich sediments and its availability to polychaetes through feeding may be expected. The low bioaccumulation of metals Cr, Zn and Ni in *G. longipinnis* in contaminated sites T1 and T3 might be related to the capacity of polychaetes to control metal incorporation from contaminated sediments. The absence of additional metal bioaccumulation in the presence of very high metal concentration in sediments has also been reported [19].

## 5. Conclusion

Heavy metal accumulation in the polychaete *G. longipinnis* signified that it is being influenced by an increasing number of diverse sources such as urbanisation, industrial activities and agricultural run-off. Results of Pearson's correlation showed the relevance of Cd and Cr availability from sediments to the organisms. Cr content (1.73–29.20 $\mu g \cdot g^{-1}$) recorded for *G. longipinnis* during this study is considerably higher than that reported from other coastal regions of the world. The bioconcentration factor revealed the ability of this organism to accumulate Hg and Cd more than in sediments in both polluted and unpolluted transects. Organic carbon was found to play a pivotal role in the availability of mercury in sediments. Thus, the results of this study strongly suggest that the polychaete *G. longipinnis* is a useful indicator for contamination of heavy metals such as Cd, Hg and Cr (in a long run) due to anthropogenic sources along the southwest coast of India.

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