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# BIOACCUMULATION OF TRACE METALS BY MARINE FLORA AND FAUNA NEAR A CAUSTIC SODA PLANT (KARWAR, INDIA)

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## ABSTRACT

The concentration of Cd, Pb, Cu, Zn and Mn in fishes, shell fishes and seaweed near a caustic soda plant has been measured. Trace metal concentration in the oyster (*Crassostrea cucullata*), mussel (*Perna viridis*) and seaweed (*Sargassum tenerimum*) collected from the vicinity of discharge point were found to be comparatively high. It is found that oysters are more effective bioaccumulators for Zn, Cu, and Cd, while mussels and seaweeds for Pb and Mn.

There were no significant differences between the whole soft tissue for Pb and Cd concentration of *P. viridis* collected from a relatively clean and contaminated sites. The kidney and mantle showed significant differences for all the trace metals, gill for Zn, Cu, Pb and Cd and digestive gland for Zn, Cu and Cd. The kidney was found to be a major site of trace metal accumulation followed by digestive gland, from both the sites.

## INTRODUCTION

Several toxic pollutants, probably unknown to the biota before, have been introduced in large quantities into the marine environment. Trace metals, particularly mercury, cadmium and lead have received much attention from researchers because of their high toxic, transport, bioaccumulation and bioconcentration potentials. The capacity of some marine animals and plants to accumulate potentially toxic trace metals in their tissues, far in excess of ambient level is well known and has become the focus of increasing number of studies from the Indian waters (Zingde *et al.*, 1976; Sankaranarayanan *et al.*, 1978; Agadi *et al.*, 1978; Kureishy *et al.*, 1981, 1983; Lakshmanan and Nambisan, 1983; Patel *et al.*, 1985; Pillai *et al.*, 1986). The bivalve molluscs accumulate pollutants in their tissues and species of the genera *Mytilus* and *Crassostrea* have been used with considerable success in various monitoring programmes (Goldberg *et al.*, 1978; Martin, 1985; Philips, 1985).

A caustic soda factory, commissioned in 1975, is situated south of Karwar along the west coast of India (Fig. 1). The effluent resulting from the production of caustic soda and phosphoric acid, are discharged into the Binaga bay, which is well known for its mackerel fishery. Detailed study of the mercury levels in seawater, sediment and biota from this area has been carried out (Kureishy *et al.*, 1987; Krishnakumar and Pillai, 1990).

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The present study was to understand the distribution of trace metals in oysters, mussels and seaweeds in and around the impact area. In addition, varieties of pelagic fish and shell fish from commercial landings at Karwar were also monitored for trace metals. Concentrations of some toxic metals were found to be very high in certain tissues of bivalves and identification of such sites of metal accumulation may indicate the potential of a tissue for use in monitoring programmes (Mason and Simkiss, 1983; Lobel, 1987). Concentration of metal in the various tissues of the green mussel *Perna viridis*, collected from a relatively clean and contaminated sites were also studied for comparison.

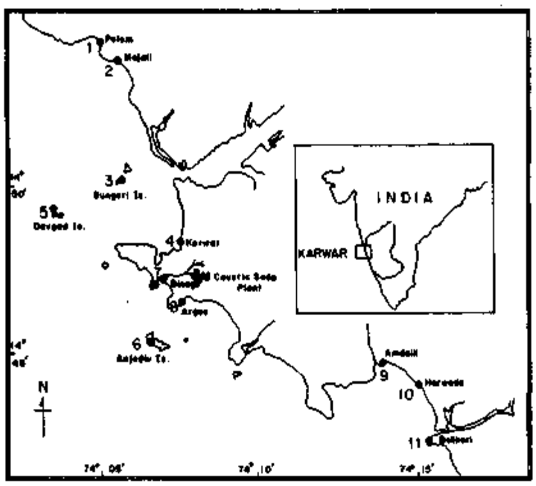

Fig. 1. Map of the study area showing sampling locations.

## Materials and Methods

Mussel (*Perna viridis*), oyster (*Crassostrea cucullata*) and seaweed (*Sargassum tenerimum*) were sampled from eleven sites along the Karwar coast (Fig. 1), as determined by the availability, during September, 1987 to February, 1989. Fish, prawn, crab and squid samples were collected from the commercial landings at Karwar. Time bulking method (Phillips and Segar, 1986) was followed for sampling the biota.

Mussels (45-50 mm) were sampled from a relatively clean (Sungeri Island) and a contaminated site (Harwada), on January 30, 1989, to study the tissue metal concentration. Twenty five animals were removed and dissected into six tissue fractions : kidney, digestive gland, gill, mantle, posterior adductor muscle and foot. Edge portion of the shells and the whole soft tissues were also collected. From each site, the respective tissue fractions were pooled from twenty five animals, and then divided into five samples for metal analysis.

Shell of the mussels, soft tissues of mussels and oysters and muscle portion of the fish and shell fish samples were analysed for trace metals. Epiphytes of seaweeds were removed and the entire green portion above the basal part was taken for analysis. Samples were wet digested using $HNO_3$ and $H_2O_2$ mixture (Dalziel and Baker, 1983). All the digested samples were analysed for Cd, Pb, Cu, Zn and Mn using Perkin Elmer AAS (model 2380) in an air-acetylene flame.

The accuracy of the analytical procedure was checked using standard reference materials supplied by IAEA, Monaco (fish tissue) and NIES, Japan (*Sargasso* sample) and found to be within ± 10%. Repeated digestion and analysis of same samples were carried out and the precision of analysis was found to be within 10%. The percentage recovery of metals from the samples was found to be >90%.

## Results

Trace metal concentration in the muscles of fish, prawn, crab and squid is given in Table 1 and in the seaweed, mussels and oysters, collected from the coastal waters of Karwar is given in Table 2. In the bivalves and seaweed collected from the vicinity of discharge point (Stations 4 - 7), trace metal levels were found to be comparatively high.

The mean metal concentration in the oyster, mussel and seaweed and the results of their comparison (t-test) are given in Table 3. The maximum concentration of Zn, Cu and Cd was observed in oysters while maximum concentration of Pb and Mn was in mussels and seaweed. Comparison of metal levels in the bivalves shows that oysters accumulate about 6.5 fold higher quantities of Zn than the mussels, 17 fold higher for Cu and 6.8 fold for Cd. Compared to oysters, mussels accumulated 1.7 fold higher quantities of Pb and 1.6 fold higher of Mn.

Trace metal concentration in the various tissues of *P. viridis*, collected from a relatively clean and contaminated sites are given in Tables 4 and 5 respectively. The results of the t-test to estimate the significance of observed differences in the concentration of the metals in the tissues of mussels from the two sites are given in Table 6. Concentration of all the trace metals in the kidney and mantle from the two sites were significantly different. Difference in metal levels of the shells was not significant.

The order of metal concentration in the selected tissues of mussels collected from the two sites, as determined by t-test is given in Table 7. Higher concentration of Zn and Cd was observed in the kidney, while the concentration of Cu was maximum in the digestive gland. Concentration of Pb and Mn was maximum in the whole soft tissue, followed by digestive gland.

## Discussion

Concentration of trace metals in the pelagic species like sardine, mackerel and squid was found to be lower than that of benthic animals like crab and prawn (Table 1). In general, metal levels in the sardine and mackerel are very low and comparable with the values reported for the above species from Andaman Sea (Kureishy *et al.*, 1981; 1983).

Table 1. *Trace metal concentration (μg⁻¹; wet weight) in the muscles of fish and shell fish sampled from the commercial landings at Karwar ($\bar{x} \pm SD$, N = 3)*

| Species | Zn | Cu | Pb | Cd | Mn |
|---|---|---|---|---|---|
| Sardine | | | | | |
| *Sardinella longiceps* | 5.28 ± 1.07 | 1.06 ± 0.16 | ND | 0.76 ± 0.18 | 1.21 ± 0.40 |
| Mackerel | | | | | |
| *Rastrelliger kanagurta* | 6.66 ± 0.75 | 1.16 ± 0.16 | 0.006 ± 0.002 | 0.66 ± 0.08 | 0.42 ± 0.44 |
| Squid | | | | | |
| *Loligo duvaucellii* | 12.54 ± 2.20 | 7.08 ± 0.99 | 0.142 ± 0.04 | 0.66 ± 0.08 | 1.73 ± 0.10 |
| Prawn | | | | | |
| *Penaeus merguiensis* | 11.52 ± 2.08 | 14.63 ± 2.50 | 0.208 ± 0.01 | 0.38 ± 0.09 | 0.98 ± 0.10 |
| *Parapenaeopsis stylifera* | 26.61 ± 3.90 | 18.63 ± 2.21 | 0.092 ± 0.01 | 0.83 ± 0.13 | 0.23 ± 0.07 |
| Crab | | | | | |
| *Portunus pelagicus* | 21.55 ± 2.95 | 20.06 ± 2.04 | 0.56 ± 0.14 | 1.29 ± 0.48 | 1.81 ± 0.23 |

ND = Not detectable

TABLE 2. *Trace metal levels in mussels, oysters and seaweeds collected from the coastal waters of Karwar, near a caustic soda plant ($\mu g^{-1}$, wet weight), $\bar{X} \pm SD$, N = 3)*

| Site | Species | Zn | Cu | Pb | Cd | Mn |
|---|---|---|---|---|---|---|
| Polem | *Crassostrea cucullata* | 103. 1 ± 11.6 | 21.55 ± 1.00 | 0.20 ± 0.04 | 1.56 ± 0.10 | 1.97 ± 0.11 |
| | *Sargassum tenerimum* | 0.32 ± 0.11 | 3.86 ± 0.15 | 1.34 ± 0.57 | 0.76 ± 0.10 | 7.24 ± 0.57 |
| Majali | *Perna viridis* | 11.06 ± 0.23 | 1.77 ± 0.21 | 0.31 ± 0.03 | 0.16 ± 0.02 | 5.24 ± 0.33 |
| Sungeri Is. | *Perna viridis* | 11.09 ± 1.11 | 0.86 ± 0.19 | 0.32 ± 0.03 | 0.15 ± 0.03 | 3.62 ± 0.69 |
| Karwar | *Crassostrea cucullata* | 154. 90 ± 7.63 | 37.33 ± 4.67 | 0.38 ± 0.03 | 2.06 ± 0.49 | 6.05 ± 0.87 |
| | *Perna viridis* | 14.39 ± 0.47 | 1.50 ± 0.50 | 0.33 ± 0.10 | 0.31 ± 0.08 | 6.80 ± 0.76 |
| Devgad Is. | *Crassostrea cucullata* | 104.18 ± 4.49 | 36.09 ± 2.33 | 0.41 ± 0.07 | 2.24 ± 0.06 | 6.41 ± 0.16 |
| | *Perna viridis* | 14.54 ± 0.52 | 2.25 ± 0.32 | 0.50 ± 0.07 | 0.34 ± 0.08 | 6.98 ± 0.14 |
| Anjdiv Is. | *Crassostrea cucullata* | 154.50 ± 10.20 | 37.48 ± 1.11 | 1.43 ± 0.16 | 3.19 ± 0.16 | 6.85 ± 0.28 |
| | *Sargassum tenerimum* | 2.28 ± 0.21 | 6.00 ± 0.66 | 1.34 ± 0.18 | 0.78 ± 0.19 | 6.89 ± 0.09 |
| Binage | *Crassostrea cucullata* | 156.90 ± 17.40 | 41.92 ± 4.64 | 2.02 ± 0.73 | 2.67 ± 0.59 | 6.32 ± 1.10 |
| | *Sargassum tenerimum* | 1.23 ± 0.19 | 4.98 ± 0.21 | 1.02 ± 0.12 | 0.39 ± 0.05 | 15.86 ± 0.59 |
| Arga | *Crassostrea cucullata* | 31.13 ± 1.90 | 31.97 ± 1.40 | 0.27 ± 0.04 | 1.47 ± 0.09 | 3.22 ± 0.25 |
| | *Perna viridis* | 13.97 ± 2.89 | 1.57 ± 0.63 | 0.80 ± 0.08 | 0.22 ± 0.08 | 8.86 ± 0.20 |
| | *Sargassum tenerimum* | 1.72 ± 0.16 | 2.03 ± 0.73 | 0.11 ± 0.01 | 0.35 ± 0.03 | 3.30 ± 0.51 |
| Amdalli | *Crassostrea cucullata* | 68.04 ± 2.60 | 41.42 ± 0.96 | 0.26 ± 0.02 | 1.68 ± 0.15 | 3.21 ± 0.52 |
| | *Perna viridis* | 13.93 ± 0.25 | 2.89 ± 0.67 | 0.22 ± 0.06 | 0.28 ± 0.04 | 6.18 ± 0.74 |
| Harwada | *Crassostrea cucullata* | 52.47 ± 2.64 | 46.07 ± 0.41 | 0.28 ± 0.06 | 1.84 ± 0.06 | 4.37 ± 0.58 |
| | *Perna viridis* | 14.16 ± 1.10 | 2.62 ± 0.19 | 1.03 ± 0.42 | 0.24 ± 0.03 | 8.10 ± 0.73 |
| Belikeri | *Crassostrea cucullata* | 105.80 ± 2.40 | 48.78 ± 1.16 | 0.26 ± 0.02 | 1.25 ± 0.22 | 4.33 ± 0.30 |
| | *Perna viridis* | 11.62 ± 0.98 | 1.86 ± 0.21 | 0.26 ± 0.03 | 0.12 ± 0.06 | 6.67 ± 0.24 |

TABLE 3. *Trace metal concentrations ($\mu g\ g^{-1}$; wet weight; X ± SD) of the three taxonomic groups collected from the coastal waters of Karwar and the result of t-test*

| Taxonomic group | Zn | Cu | Pb | Cd | Mn |
|---|---|---|---|---|---|
| Oyster (0) | 91.1 ± 51.2 | 38.6 ± 13.6 | 0.308 ± 0.07 | 1.82 ± 0.4 | 4.60 ± 1.35 |
| (n = 18) | 0 > M*** | 0 > M*** | M > 0* | 0 > M*** | M > 0*** |
| Mussel (M) | 14.41 ± 1.9 | 2.28 ± 0.79 | 0.53 ± 0.35 | 0.27 ± 0.12 | 7.26 ± 1.05 |
| (n = 18) | M > S*** | S > M** | M = S** | S > M | M = S |
| Seaweed (S) | 1.38 ± 0.76 | 4.22 ± 1.7 | 0.95 ± 0.59 | 0.57 ± 0.23 | 8.57 ± 5.31 |
| (n = 12) | 0 > S*** | 0 > S*** | S > 0* | 0 > S** | S > 0*** |

* P< 0.05; ** P< 0.005; *** P< 0.001.

In bivalves and seaweeds collected from the vicinity of discharge point and south of discharge point, comparatively higher levels of trace metals were observed (Table 2). Similarly, high mercury levels were reported in the water, sediment, bivalves and seaweed collected from the vicinity of discharge point (Kureishy *et al.*, 1987; Krishnakumar and Pillai, 1990). Stations 1, 2 and 3 were found to be comparatively unpolluted areas.

Before the construction of the present submarine effluent pipeline, the discharge went directly into a stream which originates from the factory site and flows into the sea (Annigeri, 1977). The oysters collected from this stream (station 7) showed higher concentration of metals. Earlier workers reported high mercury levels in the water, sediment and oysters collected from this stream (Kureishy *et al.*, 1987; Krishnakumar and Pillai, 1990). The results of the present study show the same trend.

TABLE 4. *Trace metal levels (μg $g^{-1}$, wet weight) in various tissues of* Perna viridis *(n = 25) collected from a clean site (Sungeri Island) on January 30, 1989 ($\bar{X}$ ± SD, N = 5)*

| Tissue | Zn | Cu | Pb | Cd | Mn |
|---|---|---|---|---|---|
| Whole soft tissue | 11.4 ± 0.99 | 0.85 ± 0.15 | 0.33 ± 0.05 | 0.15 ± 0.03 | 3.99 ± 0.71 |
| Kidney | 27.9 ± 2.79 | 1.47 ± 0.31 | 0.12 ± 0.02 | 2.16 ± 0.51 | 1.98 ± 0.29 |
| Digestive gland | 8.1 ± 1.21 | 1.79 ± 0.42 | 0.19 ± 0.08 | 0.85 ± 0.17 | 5.65 ± 0.75 |
| Gill | 13.89 ± 2.98 | 0.38 ± 0.10 | 0.12 ± 0.05 | 0.30 ± 0.06 | 4.74 ± 1.39 |
| Mantle | 6.76 ± 1.12 | 0.80 ± 0.09 | Trace | 0.58 ± 0.09 | 2.99 ± 0.30 |
| Posterior adductor muscle | 11.39 ± 0.86 | 0.42 ± 0.05 | Trace | 0.42 ± 0.17 | 2.15 ± 0.50 |
| Foot | 3.55 ± 0.59 | 0.16 ± 0.03 | Trace | 0.19 ± 0.06 | 3.71 ± 0.86 |
| Shell | 0.10 ± 0.02 | 5.41 ± 0.69 | 4.24 ± 0.99 | Trace | 8.30 ± 0.70 |

TABLE 5. *Trace metal levels (μg $g^{-1}$, wet weight) in various tissues of* Perna viridis *(n = 25) collected from a contaminated site (Harwada) on January 30, 1989 ($\bar{X}$ ± SD, N = 5)*

| Tissue | Zn | Cu | Pb | Cd | Mn |
|---|---|---|---|---|---|
| Whole soft tissue | 14.61 ± 1.01 | 2.25 ± 0.54 | 0.74 ± 0.5 | 0.24 ± 0.05 | 7.67 ± 0.77 |
| Kidney | 48.09 ± 4.24 | 2.32 ± 0.22 | 0.47 ± 0.12 | 3.85 ± 0.59 | 2.72 ± 0.53 |
| Digestive gland | 15.21 ± 1.46 | 4.35 ± 0.83 | 0.32 ± 0.14 | 2.17 ± 0.44 | 4.76 ± 1.17 |
| Gill | 14.05 ± 2.05 | 1.14 ± 0.24 | 0.33 ± 0.09 | 0.39 ± 0.06 | 6.27 ± 0.70 |
| Mantle | 13.88 ± 1.60 | 1.92 ± 0.23 | 0.21 ± 0.06 | 0.76 ± 0.11 | 4.92 ± 1.16 |
| Posterior adductor muscle | 11.56 ± 1.57 | 0.39 ± 0.08 | 0.24 ± 0.13 | 0.35 ± 0.13 | 2.96 ± 0.47 |
| Foot | 7.04 ± 0.78 | 0.43 ± 0.07 | Trace | 0.26 ± 0.07 | 4.62 ± 0.49 |
| Shell | 0.14 ± 0.04 | 6.22 ± 0.49 | 4.66 ± 0.74 | Trace | 7.91 ± 1.34 |

If mussels and oysters grow at the same location, it is observed that oysters are more effective bioaccumulators for Zn, Cu and Cd (Table 3). In the published literature

TABLE 6. *t-test values to estimate the significance of the observed differences in the concentration of the metals in the tissue of* Perna viridis *collected from a clean (Sungeri Island) and contaminated (Harwada) sites*

| Tissue | Zn | Cu | Pb | Cd | Mn |
|---|---|---|---|---|---|
| Whole soft tissue | *** | *** | NS | NS | *** |
| Kidney | *** | *** | *** | ** | * |
| Digestive gland | *** | ** | NS | *** | NS |
| Gill | NS | *** | ** | * | NS |
| Mantle | *** | *** | *** | * | *** |
| Posterior adductor muscle | NS | NS | ** | NS | NS |
| Foot | *** | *** | — | NS | NS |
| Shell | NS | NS | NS | — | NS |

*P< 0.05, **P< 0.005, ***P< 0.001.
NS = Not significant.

TABLE 7. *The order of trace metal concentration in the selected tissues of* Perna viridis, *collected from a clean (Sungeri Island) and contaminated (Harwada) sites, determined using t-test. (Ws. tissue = Whole soft tissue; D. gland = Digestive gland)*

| Metal | Tissue |
|---|---|
| Sungeri Is. | |
| Zn | Kidney $\overset{***}{>}$ Gill = Ws. tissue $\overset{**}{>}$ D.gland = Mantle |
| Cu | D. gland = Kidney $\overset{***}{>}$ Ws. tissue = mantle $\overset{***}{>}$ Gill |
| Pb | Ws. tissue $\overset{*}{>}$ D.gland = Gill = Kidney $\overset{*}{>}$ Mantle |
| Cd | Kidney $\overset{***}{>}$ D. gland $\overset{*}{>}$ Mantle $\overset{***}{>}$ Gill $\overset{***}{>}$ Ws. tissue |
| Mn | D. gland = Gill = Ws. tissue $\overset{***}{>}$ Mantle $\overset{**}{>}$ Kidney |
| Harwada | |
| Zn | Kidney $\overset{***}{>}$ D. Gland = Ws. tissue = Gill = Mantle |
| Cu | D. gland $\overset{**}{>}$ Kidney = Ws. tissue = Mantle $\overset{**}{>}$ Gill |
| Pb | Ws. tissue = Kidney = Gill = D. gland = Mantle |
| Cd | Kidney $\overset{***}{>}$ D. gland $\overset{***}{>}$ Mantle = Gill = Ws. tissue |
| Mn | Ws. tissue $\overset{*}{>}$ Gill = Mantle = D. gland $\overset{*}{>}$ Kidney |

*P< 0.05; **P< 0.005; ***P< 0.001.

also oyster has been described as an organism which has a very high ability to accumulate Zn and Cu (Goldberg *et al.*, 1978; Martincic *et al.*, 1984). It is reported that oysters are better accumulators of Cu, Zn and Cd, when compared to mussels and seaweeds (Ikuta, 1988). Mussels and seaweeds were found to be better accumulators of Pb and Mn than the oysters (Table 3). Similar results were reported by Goldberg *et al.* (1978). The green mussel *P. viridis* has been proposed as a biomonitor of trace metal in the tropical waters (Phillips, 1985). This study shows that one species alone will not serve as a good biomonitor for all the trace metals. The green mussel *P. viridis* and the oyster *C. cucullata* can be used simultaneously for monitoring trace metal levels along the west coast of India.

Zinc was found to be accumulated maximum in the tissues of bivalves (Table 3). It has been demonstrated by calculation that the observed Zn concentration in marine animals are greatly in excess of the theoretical biological requirements (Pequignat *et al.*, 1969; Coombs, 1972). From the Cu concentrations in the oysters (Table 3), it can be seen that Cu is the next favourable metal for accumulation in the tissue.

The various tissues of mussels (*P. viridis*), collected from a contaminated site were found to be having higher metal levels, compared to the clean site (Tables 4 & 5). There were no significant differences between the whole soft tissue Pb and Cd concentration of mussels collected from the two sites (Table 6). But kidney and mantle showed significant differences for all the trace metals, gill showed for Zn, Cu, Pb and Cd and the digestive gland for Zn, Cu and Cd. Mussel shell was found to be showing higher concentrations of Cu, Pb and Mn compared to the soft tissues. But differences between the shell metal concentrations from the two sites were found to be not significant (Table 6). Similarly foot and posterior adductor muscle were found to be poor reflectors of trace metals in the environment. It is reported that digestive and basophil cells of digestive gland and nephrocytes of kidney are involved in the metabolism of wide range of metals and the elemental composition of deposits inside these cells reflects the presence and availability of environmental pollutants (Mason *et al.*, 1984). It is therefore useful, in monitoring programme to select a specific tissue which shows maximal proportional response to changes in the environment rather than analyse the whole organism (Mason and Simkiss, 1983).

The kidney was found to be a major site of accumulation of Zn and Cd from both the sites (Table 7). Similar results were reported in *M. edulis* (George, 1983; Lobel, 1987). Since kidney is an excretory organ, it shows a particularly high affinity for metals relative to other organs, thus suggesting a prominent role in the metal metabolism. The localized differences in metal concentration presumably relate to the ability of the metal to penetrate the membrane of the cells in the tissues and then be metabolically retained at these sites (Mason and Simkiss, 1983). George (1983) has reported that most of the Zn and Cd in the mussels is stored in the kidney in membrane bound granules.

Next to the kidney, digestive gland accumulated high levels of trace metals from both the sites. Bivalves obtain metals through the food and by direct uptake from the seawater. The intracellular digestion and absorption of the food takes place in the digestive cells of the digestive gland. This may be attributed to the high concentration of metals observed in the digestive gland.

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