

# Arsenic, Copper, Zinc & Manganese in the Marine Flora & Fauna of Coastal & Estuarine Waters around Goa

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Arsenic, copper, zinc and manganese are analysed in 24 species including seaweeds, Ashes, crustaceans and molluscs. Among seaweeds, *sargassum tenerimum* and *Padina tetrastromatica* are more efficient in accumulating metals. Levels of arsenic in fishes are low, and the values on the higher side for *Mugil parsia* and *Etroplus suratensis* are attributed to their feeding habits. Among the crustaceans highest levels (25 ppm) are observed in crabs, while prawns, mussels and bivalves have intermediate levels. Highest copper and zinc concentrations are exhibited by oysters and the concentration of the latter decreases from 2800 to 715 ppm from south to north of Goa. Oysters show little preference for arsenic and manganese. Levels of arsenic, copper, zinc and manganese in the coastal waters of Goa have also been investigated. The abnormally high levels of dissolved manganese in water (45.2  $\mu\text{g/litre}$ ) are attributed to the ore bearing land mass and the mining operations. High levels of manganese in water is not reflected in the accumulation of this element by marine organisms. Seaweeds however exhibit high concentrations of manganese.

**H**AZARDS arising from the discharge of toxic metals from urban and industrial sources have necessitated the evaluation of levels of metals in marine organisms. Capacity of organisms to accumulate metals is known to vary widely and, therefore, it is essential to study the chemical composition of sizable numbers of animals used for human consumption<sup>1</sup>. The present work is undertaken with the primary objective of obtaining the base-line data and to establish the distribution of some metals in different shore-animals of Goa which could serve as indices of pollution. This forms part of the project aimed at documenting the bioaccumulation of trace metals in flora and fauna of the west coast of India. To date little information is available on this subject. Somayajulu and Rama<sup>2</sup> have obtained data on mercury concentration in a variety of fishes collected from the coastal waters of Bombay. Tejam and Haldar<sup>3</sup> have found that the mercury concentrations in brain, muscles and bone of different fishes followed the order brain > muscle > bone for specimens collected from the Thana area and Bombay fish market.

## Materials and Methods

Biological specimens were collected from April to May 1975 by shore collecting, trawling and diving. The samples were cleaned and stored in deep-freeze until the time of analysis. Gut contents and other extraneous material were excluded wherever possible. In case of fishes the dorsal muscles were selected for analysis. In some cases, tissues from several small organisms of the same species in particular location were pooled for analysis.

Water samples were collected twice, during the months of April and May 1975 from stations shown in Fig. 1. Collections were made using Van Dorn

sampler and stored in deep-freeze in previously acid cleaned polyethylene bottles after filtering through 0.45  $\mu$  millipore membrane filter paper and acidifying to pH 4. The analyses were completed within a week of sampling.

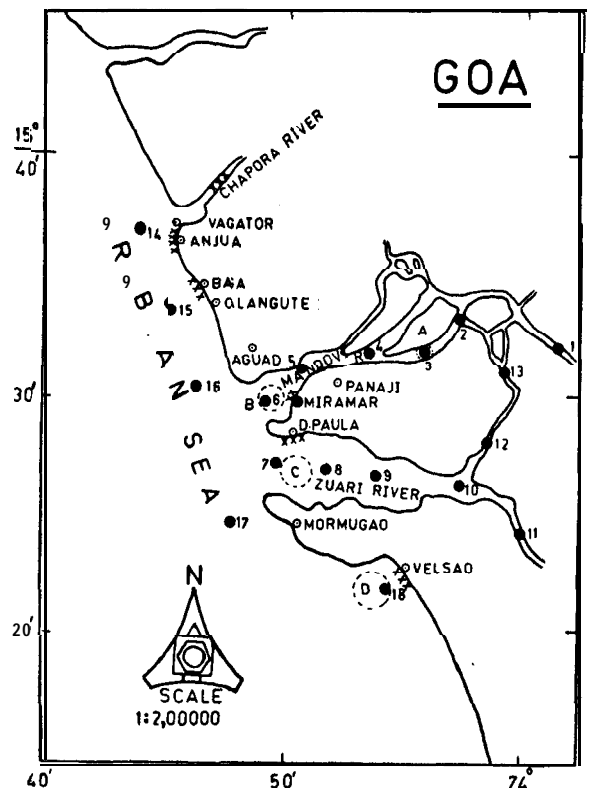


Fig. 1 — Station positions, trawling areas and places of biological collection

Sediment samples at stations 3, 6, 14, 15 and 18 were collected using modified Van Veen's grab and frozen.

Biological samples were dried and oxidized with  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}_2$ . Sediment samples after drying were leached with  $\text{HCl}$  and  $\text{HNO}_3$ , and the soluble fraction was analysed only for copper. After the wet oxidation the trace metals were analysed spectrophotometrically employing the procedures given below. CZ Spekol was used for analysis.

Arsenic was determined<sup>4</sup> by the absorption of liberated arsine in pyridine solution of silver diethyldithiocarbamate. The brown colour was measured at 560 nm. Zinc<sup>5</sup> was extracted by dithizone in carbon tetrachloride after the preliminary separation of interfering elements and measured at 535 m $\mu$ . For the analysis of water samples preliminary separation was not desirable. Copper was determined by the extraction of its diethyldithiocarbamate complex in carbon tetrachloride and the absorbance measured at 440 nm. Preconcentration was required for water samples. Sea water (500 ml) was acidified to pH 2 with  $\text{HCl}$  and extracted twice with 5 ml portions of dithizone in carbon tetrachloride (50 mg/litre). The combined extracts were evaporated twice with 1 ml portions of conc.  $\text{HNO}_3$ , and the residue dissolved in 0.05N  $\text{HCl}$ . Copper was then estimated as its diethyldithiocarbamate complex.

Manganese was determined by formaldoxime method. A slightly modified procedure was employed for water samples<sup>6</sup>. Prior to use all reagents were checked for impurities. The blanks were low — of the order of 0.005-0.03 absorbance value, except for zinc for which the blank was around 0.1 due to self absorbance of dithizone at 535 nm. The blanks, standards and samples were treated similarly starting from wet oxidation to avoid the errors due to contamination. Analytical procedures were checked for accuracy and reproducibility by replicate analysis and by the recovery of the added standards to a sample of fish tissue. The per cent errors in the values for As, Cu, Zn and Mn were found to be 10, 8, 12 and 7% respectively.

### Results and Discussion

**Concentration of trace metals in water** — Sampling stations selected in the present investigation are around the shores of Goa and the two estuaries — Mandovi and Zuari — as shown in Fig. 1. These are the two important rivers on the central west coast of India and forms the navigational network for transportation of mainly iron and manganese ores to be shipped from the Mormugao harbour. For over 30 yr the mining operations caused discharge of large amounts of waste containing high levels of metals into these rivers. The water samples are analysed for zinc, manganese and copper in April and May 1975. No significant changes are observed and hence the values obtained for April collection are reported in Table 1. Values of copper for stations 14 to 18 are taken from reported values<sup>7</sup>, so also the values for arsenic for all stations<sup>8,9</sup>. Manganese concentration in the estuarine region falls in 6.2 to 102.2  $\mu\text{g/litre}$  with an average of 45.2  $\mu\text{g/litre}$ . This concentration which is much

higher than the reported<sup>10,11</sup> is related to high levels of manganese of the surrounding ore bearing land mass. The average concentration of zinc (18.7  $\mu\text{g/litre}$ ) is of the order generally observed<sup>7</sup> for estuarine environment. Occurrence of copper at levels reported by Sankaranarayanan and Reddy<sup>7</sup> in the inshore waters are quite high (30  $\mu\text{g/litre}$ ). The present study however, reveals (Table 1) that an average concentration of copper in the estuarine waters is low (4.5  $\mu\text{g/litre}$ ) and indicates only natural levels. Another observation which is apparent from Table 1 is the gradual decrease of dissolved metals as the river approaches the Arabian Sea. This is particularly recognizable for zinc and manganese. At station 1 for example the average manganese concentration is 88.9  $\mu\text{g/litre}$  while at station 6 it is only 14.9  $\mu\text{g/litre}$ . The similar trend is observable for Zuari estuary also. Table 1 also reveals the occurrence of higher levels of trace metals in surface water than the bottom water. The same trend is observed for samples collected in May and cannot be attributed to analytical errors.

**Biological specimens** — Arsenic: Majority of species studied contained detectable concentrations. In marine algae (Table 2) it ranged from 1 to 13.6 ppm. No marked differences were observed between

TABLE 1 — LEVELS OF TRACE METALS IN MANDOVI-ZUARI ESTUARIES AND ALONG THE COAST OF GOA

Station No.	Depth (m)	Conc. of metals ( $\mu\text{g/litre}$ )			
		Zn	Mn	Cu*	As†
1	0	28.1	85.6	5.8	—
	4	12.5	92.3	4.2	—
2	0	20.4	74.8	4.9	9
	3.5	18.7	80.1	3.3	9
3	0	16.9	50	3.6	1.5
	5	20	35	3.3	—
4	0	18.3	50.2	4	8.25
	5	12.1	46.7	3.1	10.5
5	0	14.1	22.5	2.5	3.75
	5.5	6.3	23.4	4.8	6
6	0	8	11.4	3.6	—
	7	6.2	10.5	4.5	—
7	0	12.1	10.3	4.8	16.11
	4	8.8	6.2	2.7	30.53
8	0	12.6	51.4	3.2	2.97
	5	5.2	22.8	3.5	18.65
9	0	10.3	21.5	—	66.57
	5	19.8	45.6	—	30.1
10	0	28.2	37.1	5.4	47.91
	3.5	28.1	28.3	6	43.25
11	0	35.5	40.3	2.6	—
	3	28.4	72.5	6.8	—
12	0	35.8	56.6	7.1	—
	0	42.3	102.2	8.2	—
14	0	8.4	15.9	38.87	—
	10	6.3	20	69.77	—
15	0	4.6	12.5	—	—
	10	2.8	20.7	—	—
16	0	12	15	3434	3
	9	3	2.1	42.06	5.25
17	0	7.3	21.5	3.41	—
	9	6.2	25	17.66	—
18	0	7.6	24.4	—	—
	10	5.1	21.5	—	—

Values for \*Cu for stations 14 to 18 are from reported values and for †arsenic for all stations from reported values<sup>7-9</sup>.

TABLE 2 -ARSENIC, COPPER, ZINC AND MANGANESE IN MARINE ALGAE, FISHES AND CRUSTACEANS AND MOLLUSCS

Location	Conc. of metals (ppm dry wt)			
	AS	cu	Zn	Mn
MARINE ALGAE				
<i>Ulva fasciata</i>				
Vagator	2.5	6.8	25.2	167
Anjuna	2.8	10.1	17.8	203
Baga	1		19.3	37
<i>Caulerpa sertularioides</i>				
Vagator	2.9	12.6	6.4	115
Anjuna	3.2			175
Baga	3.5	87.32	18.5	216
<i>Sargassum tenerimum</i>				
Vagator	10.4	20.1	35.7	302
Baga	8.7			270
Dona Paula	13.6	10.3	60.2 40.1	586
<i>Padina tetrastromatica</i>				
Vagator	12.6	8.7	20.2	456
Baga	4.8	12.5	31.5	233
Dona Paula	7	20.1	28.8	388
<i>Dictyota dumosa</i>				
Dona Paula	6.7	12.9	37.9	560
<i>Hypnea musciformis</i>				
Vagator	2.5	8.6	50.8	193
Baga	4	22.5	19.4	63
Dona Paula	3.9	15	22.1	361
FISHES				
<i>Leiognathus splendens</i> (Silver bellies)				
B	0.5	5.3	30.6	5.3
C	1.6	6.1	27.1	9.8
D	1.2		21	12.9
<i>Otolithus ruber</i> (Rosy Jew fish)				
B	3.3	4.9	30.1	20
C	1.8	12.6	11.5	12.5
D	1.3	5.2	15.7	9.7
<i>Pellona ditchela</i> (Shad)				
C	2.1	3.6	---	---
D	4	8.1	---	---
<i>Pampus argenteus</i> (Pamprct)				
C	0.5	3.5	40.2	3.9
D	1.7	12	21.8	12.6
<i>Lactarius lactarius</i> (Butter fish)				
C	0.3	2.6	15.4	15.9
D	0.8	5.4	11.5	8.2
<i>Sardinella fimbriata</i> (Sardine)				
B	23.3	12.2	176 13.1	---
C				---
D	7.6	5.9	7.5	3.8

TABLE --ARSENIC, COPPER, ZINC AND MANGANESE IN MARINE ALGAE, FISHES AND CRUSTACEANS AND MOLLUSCS -- Contd

Location	Conc. of metals (ppm dry wt)			
	AS	cu	Zn	Mn
FISHES				
<i>Carcharias sorrahawah</i> (Dog fish)				
C	6.3	23.2	17.6	10.6
D	10.8	15.8	18	6.6
<i>Mugil parsia</i> (Grey mullet)				
	8.3			
A	12.6	28.6	47.8	17.9
B		32.5	63	26.3
<i>Etroplus suratensis</i> (Pearl spot)				
A				---
C	11.2	14.9 17	76.5 51.8	---
<i>Rastrelliger kanagurta</i> (Mackerel)				
B	3.2	15.8	23.4	4.2
CRUSTACEANS & MOLLUSCS				
<i>Crassostrea cucullata</i> (Small oysters)				
Vagator	5.6	433	715	3.2
Baga	4.3	728	1398	---
Miramar	3.5	251	446	---
Dona Paula		356	2618	---
Shiridao	3.1	480	2232	11.7
Velsao	6.3	325	2800	17.5
<i>Crassostrea gryphoides</i> (Edible oysters)				
Chapora	3.2	210	323	---
Verem	5.8	175	550	---
<i>Penaeus monodon</i> (Tiger prawn)				
A				21.3
C	11.2	483 215	700 215	14.9
<i>Metapenaeus affinis</i> (Prawn)				
D	13.6	28.9	58.3	
<i>Portunus pelagicus</i> (Blue swimming crab)				
Chapora	24.8	45.3	78.5	---
C	11.3	29.0	80.2	---
D	25.2	94.4	73.7	---
<i>Mytilus viridis</i> (Common mussel)				
Baga	9.6	12.9	15.3	---
Miramar	8.2	25.0	63.0	---
Velsao	8.9	8.6	21.2	---
<i>Meretrix casta</i> (Bivalves)				
Chapora	10.9	16.1	50.2	---

A, B, C, D are trawling areas (Fig. 1).

different species of algae studied, although, somewhat higher concentrations were observed in *Sargassum tenerimum* and *Padina tetrastromatica*; these species also exhibited higher concentrations of other metals. Generally it was observed that

the brown seaweeds concentrate trace metals to a greater extent and the concentration depends on the relative absolute concentrations in the surrounding water. Because of this property these algae perhaps could serve as better pollution indicators among seaweeds. The average background levels of arsenic in seaweeds, presently studied, were lower than the comparable levels generally reported from other places. For marine algae from the Southampton water and the Solent region it varied from 11 to 47 ppm<sup>12</sup>. In the intertidal rockweed samples from Greenland concentration of 35 ppm was reported<sup>13</sup> while in *Laminaria hyperborea* from Trondheimfjord, levels as high as 142 ppm were observed<sup>14</sup>.

Levels of arsenic in fishes were of the same order as in the marine algae (0.3 to 12.6 ppm; Table 2). While the reported arsenic levels in marine fishes were generally from less than 1 to 20 ppm (wet weight)<sup>12</sup>, a few abnormal cases of arsenic accumulation were also known. As high as 540 ppm of arsenic was reported by Robertson *et al.*<sup>15</sup> in samples of cod muscle. In some cases, the muscles of American plaice, Greenland halibut and spotted wolffish were reported to contain arsenic of the order of 200 ppm<sup>13</sup>.

During the present study *Carcharias sorrakowah* was investigated in more detail to obtain information about the distribution in various organs. Four animals of varying length obtained during the trawling operation in area (D) were chosen for analysis. The values (ppm) obtained were: muscle, 10.8; gills, 15.2; liver, 23.2; bone, 10; and eyes, 1.6 (av. of 4 determinations). It appears, therefore, that the liver and gills have more arsenic concentrating ability than the other organs. The capacity of liver to accumulate arsenic has been noted in the Greenland halibut, the Shorthorn sculpin and the American plaice. However, in the spotted wolffish, the Greenland cod and *Chondrichthys* the muscles contained higher concentrations than the liver<sup>13</sup>. It, therefore, appears that the arsenic concentrating ability of liver is species specific. The study of individuals of varying sizes did not reveal any definite relation between the arsenic concentration and the size of the animal. Relationship between the length of certain species of shark and the mercury content of the muscle was established and was often used by fishing authorities of some countries to restrict sharks for human consumption<sup>16</sup> on the basis of mercury content. Such a relationship do not seem to be valid for arsenic as evidenced by the present investigation. Eustace<sup>16</sup> attempted the correlation of zinc and copper concentrations versus length of fishes from the Derwent estuary, Tasmania, but failed to get any correlation.

Grey mullet (*Mugil parsia*) and pearl spot (*Etroplus suratensis*) showed higher levels of metals than other bony fishes (Table 2). These small differences are probably attributable to the feeding habits. These animals are detritus feeders and feed either by sticking up the surface *layer* of the mud or by grazing on the rock surfaces leading to the transfer of mineral particles into the system along with food. The major contents of mullet stomach contain micro-algae including epiphytic and benthic forms,

decaying plant detritus and sediment particles<sup>17</sup>. The sediments are usually enriched with trace metals and as a result, detritus feeders are exposed to more quantities of metals than fishes with pelagic feeding habits. Distribution of arsenic and copper in the sediments collected at several locations from Mandovi-Zuari estuaries and the inshore regions were also investigated. At stations 3 to 9 the sediments contained 6-14 ppm of arsenic and at stations 14 to 18 it ranged from 10 to 13 ppm<sup>9</sup>. Concentration of copper in sediments from stations 3, 6, 14, 15 and 18 was 30-125 ppm<sup>18</sup>. These concentrations are much higher than those observed in water. All copper or arsenic in the sediments may not be available for the organism, however, it is likely that the fraction soluble in dilute acid could involve in bioaccumulation.

Decapod crustaceans, specially shrimps and crabs and some molluscs accumulate arsenic to high levels compared to other animals of the same environment. Blue swimming crab (*Portunus pelagicus*), obtained from Chapora and from region (D) showed concentration as high as 25 ppm. Results in Table 2 reveal higher levels of arsenic in prawns and mussels (*Mytilus viridis*) though the levels are well below the concentrations to cause any damage. In areas of high arsenic levels, shellfish contained 100 ppm arsenic<sup>19</sup>. A residue of 174 ppm (wet wt) was reported in prawn by Chapman<sup>20</sup>. LeBlanc and Jackson<sup>21</sup> reported 37.8 ppm (wet weight) in crab from the coast of British Columbia. In deep sea prawns of Greenland, 62-80 ppm of arsenic was reported<sup>13</sup>. High levels of arsenic found in some marine animals suggest that arsenic is likely to be accumulated in less toxic form since the quantities found in these animals would be highly toxic to the animal if arsenic is present in the oxide form or any other form of equivalent toxicity. In sea water arsenic occurs as arsenite [As(III)] as well as arsenate [As(V)]. Occurrence of thermodynamically less stable As(III) could result from biological reduction of As(V). Recently, it was shown that coral *Pocillopora verrucosa* is capable of such a reduction<sup>22</sup>. It has been suggested that since arsenate is chemically similar to phosphate, it may be transported into the cell along with phosphate. Arsenite formed from the arsenate in the cell might diffuse back again more easily than would arsenate avoiding the excessive accumulation of arsenic concentrations inside the cell.

The animals caught in the area (D) and collected from the Velsao bay were of particular interest from the levels of arsenic. For the last 3 yr this bay received effluents containing arsenic from a fertilizer factory. Levels of dissolved arsenic showed wide variation in this region and concentrations up to 500 µg/litre were observed<sup>23</sup>. There were occasional fish mortalities and arsenic was considered as the killing agent. The present study however, does not show any indication of serious arsenic accumulation and in general the animals studied showed levels in close proximity with the background levels. One algal species *Enteromorpha intestinalis* collected from Velsao had 5.2 ppm arsenic falling in line with the species from other localities (Table 2). Few fishes found dead on the

beach were also analysed. These did not show levels significantly higher than the background. It is to be noted that presently the fertilizer factory has modified the carbon dioxide separation process which largely eliminates the use of arsenic.

**Copper:** Levels of copper in algae range from 2.7 to 35 ppm. *Sargassum tenerimum* from Baga contained highest concentration of 27.5 ppm. The levels were even less in fishes; the mackerel (*Rastrelliger kanagurta*) the grey mullet (*Mugil parsia*) and the pearl spot (*Etroplus suratensis*) showed the concentration range 14.9 to 32.5 ppm. Highest levels falling in the range 175 to 728 ppm were observed in oysters while prawns and crabs exhibited medium levels. Of the 2 species of oysters, *Crassostrea cucullata* accumulated copper more efficiently than *Crassostrea gryphoides*. The capacity of oysters to concentrate copper to a degree often more than reported here has been recognized and hence oysters occupy an unusual position among sea-foods from the point of toxicity studies with respect to human beings. These organisms may thus act as useful indicators of pollution. The levels of metals concentrated by oysters has been related to the levels in water. Around Dona Paula the average dissolved copper concentration was 4 µg/litre. This is magnified by at least 15000 times on weight to weight basis (on average the dry weight was found to be 5 times less than the wet weight). Oysters were reported to concentrate copper by 14000 times, zinc by 100000 times and cadmium by 300000 times; the accumulation factor was evaluated in relation to the concentration of the soluble metal ion in the water in which the oyster inhabits<sup>24</sup>. This concept of concentration factor should always be treated with caution. Oysters being filter feeders, concentration of metals in the mud and suspended particles which is often significant is also likely to contribute to the degree of bioaccumulation. Barnes<sup>25</sup> studied the passage of sediments through the gills of *Lamellibranchia molluscs* and found that the sediment particles get trapped by secretions of the hypobranchial glands. Ayling<sup>26</sup> observed that the concentration of metals in oysters is only 10-40 times the concentration in the mud. The mud analysis for copper were completed for the samples collected from stations 3, 6, 14, 15 and 18; the values ranged from 30 to 125 ppm. The contribution of such high levels of copper in sediments to bioaccumulation requires further study.

**Zinc:** Levels of dissolved zinc in the water around Goa are low. Hence relatively low concentrations were observed in the marine flora and fauna inhabiting the aquatic environment. Levels observed for algae were all comparable (6.4-60.2 ppm) with *Sargassum tenerimum* having the highest concentration. Levels of zinc in marine algae depend not only on the relative concentration in water but also vary according to their position from the shore as has been shown for *Fucus vesiculosus*<sup>27</sup>. At least in case of *Laminaria digitata* it was found<sup>28</sup> that the older parts contain higher concentration of zinc than the younger ones. Seasonal variations in the trace metals in *Fucus vesiculosus* were demonstrated<sup>29</sup>.

Levels of zinc in the fishes were also low and vary in the range 7.5 to 76.5 ppm. The occurrence of zinc in marine environment is immediately reflected on the zinc levels in oysters which in fact concentrate zinc more effectively than copper. Concentration factor on the weight to weight basis has been considered<sup>24</sup> to be of the order of 100000. It is, however, apparent from Table 2 that the accumulation by oysters does not depend only on the soluble fraction in water. Thus the levels in water at stations 6 and 7 were nearly same but the oyster from Dona Paula had zinc nearly 6 times more than those obtained from Miramar. Highest concentration of 2800 ppm was observed for oysters collected from Velsao and gradual reduction in levels was found along the coast towards the north, and samples from Vagator contained only 715 ppm (Table 2). These high levels are not indicative of pollution and happen to be the natural levels. In areas of zinc pollution the levels frequently exceed 10000 ppm. Thus oysters from several locations in Tasmania<sup>26</sup> were known to contain zinc levels as high as 21000 ppm (wet weight). The concentration of copper and zinc to such high levels in oysters than in other animals inhabiting the aquatic system is yet to be fully understood. It is likely that endogenous or exogenous ligands or chelating agents combine with the metal ions to form soluble complexes and are transferred into the cell<sup>30</sup>. Recently Coombs<sup>31</sup> has demonstrated that about 40% of total zinc and copper is weakly coordinated to the low molecular weight compounds, taurine, lysine, ATP and possibly homarine. Such mechanism however, fail to explain the specific concentration of zinc by the oyster.

**Manganese:** Determination of manganese was not possible in some cases because of analytical difficulties. The acid decomposition of the organic tissue, due to unknown reasons, sometimes rendered the digested solution yellow which also absorbed at 450 nm and uncertainty was, therefore, encountered in the value obtained for such samples. These values are, therefore, omitted from the tables.

Manganese is an element of low toxicity and has considerable significance biologically. Because of its non-toxic nature the data on bioaccumulating ability by marine flora and fauna is scarce. As noted earlier the waters around Goa have high levels of manganese and, therefore, it is interesting to evaluate the concentration by various species. It is evident from Table 2, that marine animals have little tendency to accumulate manganese. It is interesting to note that even oysters which concentrate copper and zinc many folds than fishes do not contain manganese in similar proportion. The seaweeds, however, have a tendency to concentrate manganese (37-586 ppm). Seaweeds, therefore, are better representatives of manganese in sea water than animals.

The high degree of variability in the bioaccumulation of different metals, exhibited by the marine organisms suggests the need for detailed studies involving more species of economic importance in evaluating the general background and toxic levels for utilizing them as indices of pollution.

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