Bull. Environ. Contam. Toxicol. (2006) 76:293–300

DOI: 10.1007/s00128-006-0920-3

Bulletin of Environmental Contamination and Toxicology

Spatial and Temporal Variability of Copper, Zinc, and Cobalt in Marine Macroalgae from the Southwest Coast of India

N. S. Kalesh, S. M. Nair

Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Kochi-682 016, Kerala, India

Received: 22 June 2005/Accepted: 19 December 2005

The use of marine macroalgae as human food, livestock feed and agricultural fertilizer is a common practice in many parts of the world. The principal value of algae in such uses lies in their richness of minerals, especially trace elements. Marine algae have been found to contain almost all the essential elements required for the development of plants, animals and humans including trace metals such as Fe, Mn, Cu, Zn, Co, Ni etc. These are usually taken up by algae from the surrounding seawater in excess of their metabolic requirements and are accumulated in the tissues to concentrations above the levels in seawater giving rise to concentration factors of several orders of magnitude. Since the metal concentrations of algae often reflect the concentration levels in surrounding waters, algae have frequently been used as biological monitors of metal contamination in coastal environments (Lobban and Harrison 1997).

The utilization of marine algae as a source of essential trace metals or as bioindicator of metal pollution requires information on their metal content. Although several studies have been carried out on the metal distribution in Indian marine algae little information is available on the trace metal content of marine algae of southwest coast of India. The present study reports the distribution of three trace metals, viz., Zn, Cu and Co in different species of marine macroalgae from Kerala on the southwest coast of India. This study provides information on the interspecies and interclass variations in the levels of Zn, Cu and Co on a temporal and spatial scale. It also discusses the possible usefulness of marine algae from Kerala as biological indicators of these metals in coastal waters.

MATERIALS AND METHODS

Six species of marine macroalgae belonging to two different systematic groups (Chlorophyta and Rhodophyta) were chosen for the present study. They were: *Ulva lactuca* Linnaeus (Ulvales, Chlorophyta), *Enteromorpha intestinalis* (Linnaeus) Nees (Ulvales, Chlorophyta), *Chaetomorpha antennina* (Bory) Kuetz (Cladophorales, Chlorophyta), *Gracilaria corticata* J. Agardh (Gracilariales, Rhodophyta), *Centroceras clavulatum* (C. Agardh) Montagne (Ceramiales, Rhodophyta) and *Grateloupia filicina* (Wulfen) J. Agardh (Cryptonemiales,

Correspondence to: S. M. Nair

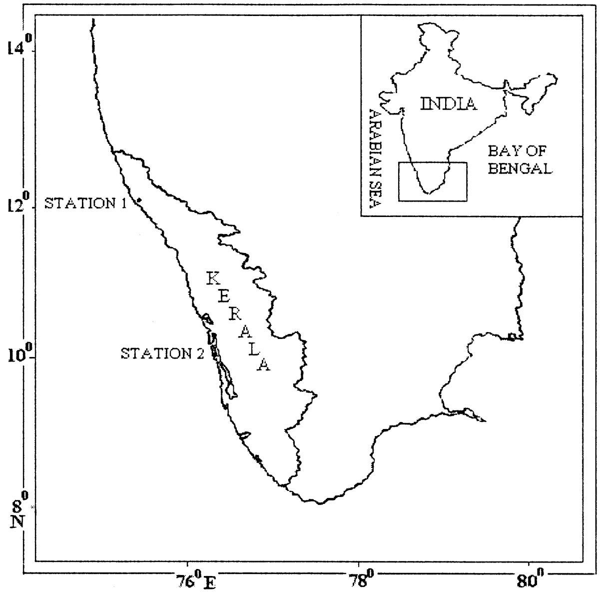

Figure 1. Location map of sampling sites.

Rhodophyta). They were collected from two localities along the Kerala coast – Station 1 on the northern coast and Station 2 on the central coast (Fig.1). Station 1 is a rocky beach, which is bordered by the Arabian Sea on the west and seven large hills on the other sides. The area in between the slope of the hills and the beach is under marginal cultivation. There is the influence of back-land drainage in the area. Station 2 is a sandy beach. Here, the substrata for macroalgae are the artificial dykes of rubbles constructed as a preventive measure against coastal erosion during monsoon months. This site is proximate to the Cochin barmouth through which the Cochin backwaters open out into the Arabian Sea. This backwater is intensely polluted due to the inputs from several major and minor industries situated in the upstream region. Several rivers, irrigation channels and sewers open into this backwater making it polluted.

A total of six collections were made from each location: August and December 1999, May and October 2000 and January and April 2001. The collection dates fall in different seasons of the year as follows: August – summer monsoon (southwest monsoon) season, October – retreating monsoon season, December – early winter monsoon (northeast monsoon) season, January – mid winter monsoon season, April – mid hot season (pre-monsoon season) and May – late hot season.

Sample collection was done during the period of low tides. The algae were hand picked from their natural habitats, sorted out and washed thoroughly in seawater followed by rinsing with tap water and then with deionised water (milli-Q grade). After air-drying, they were dried to constant weight at 60 ^{0}C in an air oven. They were powdered and stored in sealed polythene bags until the analysis.

Table 1. Trace metal contents (µg g^{-1} dry weight) of different species of marine algae from Kerala in different periods

	Zn						Cu						Co					
	SM[a]	RM[b]	WM1[c]	WM2[d]	HS1[e]	HS2[f]	SM	RM	WM1	WM2	HS1	HS2	SM	RM	WM1	WM2	HS1	HS2
Station 1																		
Chlorophyceae																		
C. antennina	24.08	15.87	20.24	12.26	18.91	17.30	4.30	7.34	7.78	5.63	7.56	5.23	5.95	3.39	6.78	3.19	1.29	9.37
U. lactuca	14.67	7.89	15.18	12.41	12.83	14.03	3.88	2.39	6.32	7.83	7.51	5.87	8.41	5.29	2.14	2.89	4.77	4.13
E. intestinalis	15.00	*	*	*	*	*	4.22	*	*	*	*	*	8.24	*	*	*	*	*
Rhodophyceae																		
G. corticata	12.40	13.08	25.13	5.49	12.46	16.70	3.84	0.60	5.92	2.09	4.42	3.77	6.20	4.13	4.28	5.03	2.38	5.79
C. clavulatum	*	22.20	*	22.84	*	35.95	*	11.82	*	12.56	*	8.99	*	4.49	*	8.88	*	9.11
G. filicina	*	34.03	*	*	*	*	*	9.03	*	*	*	*	*	3.14	*	*	*	*
Station 2																		
Chlorophyceae																		
C. antennina	39.31	23.19	*	*	*	*	6.23	7.03	*	*	*	*	6.77	0.8	*	*	*	*
E. intestinalis	20.71	*	*	*	*	27.27	4.89	*	*	*	*	9.23	10.08	*	*	*	*	3.62
Rhodophyceae																		
C. clavulatum	33.91	29.62	51.48	30.90	35.83	25.09	9.08	6.43	14.59	9.63	11.84	6.87	7.68	3.34	7.50	5.09	6.39	5.48
G. filicina	83.88	62.06	80.09	*	44.72	35.36	4.87	3.45	8.00	*	5.89	3.20	5.72	ND[g]	6.27	*	9.74	9.19

* Species were absent [a] Summer monsoon [b] Retreating monsoon [c] Early winter monsoon [d] Mid-winter monsoon [e] Mid-hot season [f] Late hot season [g] Not detectable

Trace metal contents of algal samples were determined by atomic absorption spectrophotometry after digestion of the samples in triple acid mixture (HNO_3: $HClO_4$: H_2SO_4 5:1:1) (Rao et al. 1995). Metal concentrations were measured using Perkin Elmer 3110 atomic absorption spectrophotometer.

The quality of the data was identified by parallel analysis of standard reference material (BCR 279, *Ulva lactuca*) following the same procedure as that used for samples. The measured values in reference material were 50.7 ± 1.6 μg g^{-1} for Zn (certified value = 51.3 ± 1.2 μg g^{-1}, recovery = 98.8%) and 12.82 ± 0.45 μg g^{-1} for Cu (certified value = 13.14 ± 0.37 μg g^{-1}, recovery = 97.6%). The precision of analysis was ascertained by triplicate analyses and the results were reported as mean values in μg g^{-1} on dry weight basis. Detection limit of analysis was 0.1 μg g^{-1}. Interspecies variations were tested by one-way ANOVA, while interclass and spatial comparisons were made by Student's t-test. All statistical tests were carried out using SPSS (Statsoft).

RESULTS AND DISCUSSION

Zn contents of the marine algae selected for the present study were found to vary in the range 5.49 – 83.88 μg g^{-1}, while Cu contents varied from 0.60 to 14.59 μg g^{-1} (Table 1). The lowest values of Zn and Cu were shown by the rhodophyte *Gracilaria corticata* collected from Station 1 in mid winter monsoon season and retreating monsoon season respectively. The rhodophyte *Grateloupia filicina* obtained from Station 2 in the summer monsoon season showed highest Zn content, while the rhodophyte *Centroceras clavulatum* collected from same location in the early winter monsoon season exhibited maximum Cu levels. The Co contents of algae varied from non-detectable level shown by *Grateloupia filicina* collected from Station 2 in the retreating monsoon season to 10.08 μg g^{-1} recorded by the chlorophyte *Enteromorpha intestinalis* collected from the same location in the summer monsoon season.

The marine algae were observed to show interspecies variability in the accumulation of trace metals (Fig. 2). Statistical analysis (One-way ANOVA) showed that the variations in the Zn levels were significant at 95% level at both the locations (Station 1: $F_{17,5} = 5.710$, $P = 0.003$; Station 2: $F_{11,3} = 4.724$, $P = 0.024$) while that in the Cu levels were significant at Station 1 only ($F_{17,5} = 7.819$, $P = 0.001$). Co contents of different species of algae were statistically indistinguishable ($P>0.05$).

The levels of Zn, Cu and Co in marine algae were found to vary considerably with season. Classwise comparison showed that rhodophyceae accumulated more Zn than chlorophyceae in all seasons except summer monsoon and mid hot season when chlorophyceae of Station 1 showed higher Zn content than rhodophyceae from that location (Fig. 3). Any general trend was not observed in the levels of Cu and Co. Interclass variations were statistically insignificant ($P>0.05$). Considering the temporal variations in the metal levels of individual species, it was observed

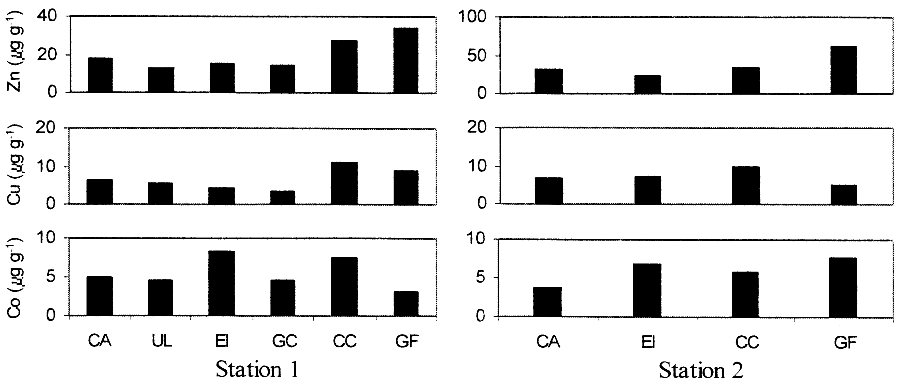

Figure 2. Interspecies variations in the trace metal levels of marine algae (CA: *Chaetomorpha antennina*, UL: *Ulva lactuca*, EI: *Enteromorpha intestinalis*, GC: *Gracilaria corticata*, CC: *Centroceras clavulatum*, GF: *Grateloupia filicina*)

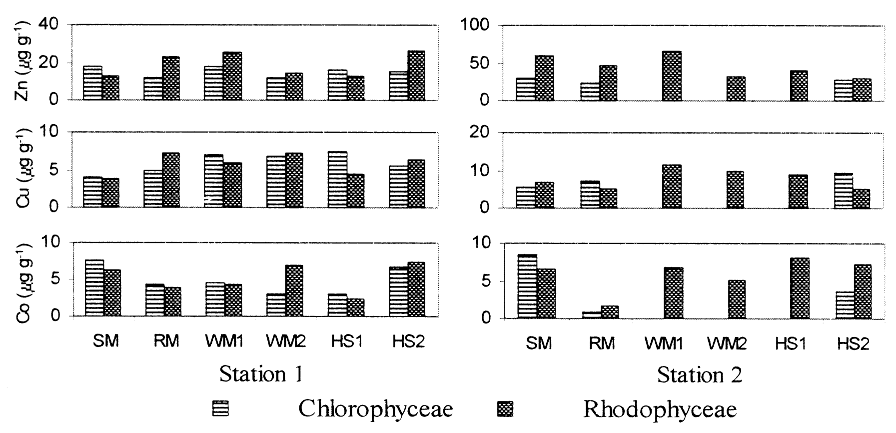

Figure 3. Interclass variations in the trace metal levels of marine algae (SM: Summer monsoon, RM: Retreating monsoon, WM1: Early winter monsoon, WM2: Mid-winter monsoon, HS1: Mid-hot season, HS2: Late hot season)

that in most cases, maximum values were more than twice the minimum values (Fig. 4). Most of the species showed highest accumulation of Zn in summer monsoon or early winter monsoon seasons. Maximum Cu accumulation by most algae occurred in the winter monsoon season, whereas maximum values of Co were recorded in the summer monsoon or hot seasons.

Spatial variations were also observed in the metal content of marine algae (Fig. 5). Both the chlorophyceae and rhodophyceae of Station 2 recorded significantly higher Zn levels than those from Station 1 in all the seasons (Chlorophyceae: df = 7, t = 5.739, P = 0.001; Rhodophyceae: df = 7, t = 4.036, P = 0.002). The chlorophyceae from Station 2 showed higher values for the average Cu content

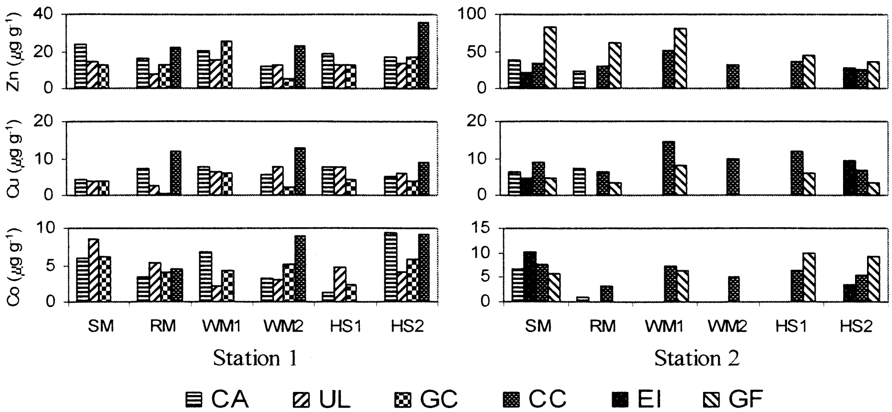

Figure 4. Temporal variations in the trace metal levels of different algal species

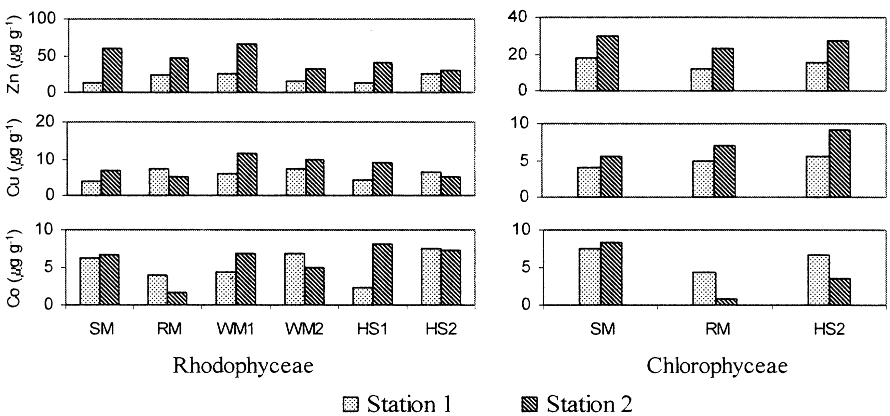

Figure 5. Spatial variations in the trace metal levels of different classes of algae

than that from Station 1. Rhodophyceae also showed a similar trend except in the late hot season and retreating monsoon season when reverse was true. Spatial variation in Co contents of marine algae did not show any general trend. Variations in the levels of Cu and Co were statistically insignificant ($P>0.05$).

The mean levels of Zn, Cu and Co in marine macroalgae from Kerala coast were found to be well within the ranges reported for Indian marine algae (Rao 1992). However, Zn and Cu levels were comparatively lower than those from many other Indian coasts. Co levels were comparable. The accumulation of all these metals were found to be of similar magnitude to those reported by various authors for algae from supposedly clean areas (Hagerhall 1973, Ho 1990). The Zn contents of all the species except *Grateloupia filicina* were below 50 μg g^{-1}, which is the maximum permissible limit of Zn prescribed for foods for human consumption (PFA 1955, FAO 1983). *G. filicina* recorded average Zn content of 56.69 μg g^{-1}

with a maximum of 83.88 μg g^{-1}. All the algal species showed Cu contents below 30 μg g^{-1}, which is the permitted maximum limit of Cu in foods in India (PFA 1955). FAO (1983) has prescribed a maximum Cu content of 10 μg g^{-1} for seafood for human consumption and only *Centroceras clavulatum* showed Cu contents greater than this value. This species recorded average Cu content of 10.20 μg g^{-1} with a maximum of 14.59 μg g^{-1}. This alga belongs to the order Ceramiales, members of which have been found to be good concentrators of metals (Saenko et al. 1978).

The trace metal distribution pattern in marine algae was found to be in the decreasing order Zn > Cu > Co. This pattern was observed in both chlorophyceae and rhodophyceae from the two locations selected for the present study. However, the extent of accumulation differed from species to species. The highest accumulations of Zn, Cu and Co were shown by *Grateloupia filicina*, *Centroceras clavulatum* and *Enteromorpha intestinalis* respectively. *Ulva lactuca* and *Gracilaria corticata* exhibited lower levels of metal accumulation. There may be different reasons for the specieswise variations in the accumulation of metals. The accumulation of metals by algae can occur by different mechanisms depending on the nature of alga, metal ion species, ambient solution conditions etc. (Greene and Bedell 1990). The accumulation mechanisms include active biological transport, intracellular chelation by biopolymers, accretion of the metals on the cell wall surface and adsorptive binding to chemical functional groups such as carboxylate, phosphate, sulphate, amine, thiol and imidazole associated with various biopolymers found in the cell walls. Since wide variations occur in the cell wall structures depending upon the algal division, genera, species and variety, in addition to the individual environmental adaptations, there can be species-dependent variation in biopolymers, side chains and functional groups that can bind metal ions. This may be the reason for the observed specieswise differences in metal accumulation. It could also originate from the specific environmental conditions surrounding each species. Different species of algae are susceptible to confounding environmental factors, which bring significant changes in metal content for a single species (Barreiro et al. 2002). Even under similar ambient conditions, variations in surface area available for absorption, permeability of cells/ tissues, number and nature of binding sites, and metabolic rate can result in difference in the metal uptake between species and even between individuals of the same species (Brown and Depledge 1998).

The temporal variations observed in the levels of Zn, Cu and Co in marine algae may be due to different reasons including the environmental factors such as variations in the metal concentrations of the ambient seawater, interactions between metals and other elements, temperature, salinity, pH etc.; metabolic factors such as dilution of metal contents due to growth; or they may be due to interactions between both kinds of factors.

The present study supports the assumption on the selective ability of macroalgae to accumulate trace metals such as Zn, Cu and Co from seawater. *Enteromorpha* *intestinalis*, *Grateloupia filicina* and *Centroceras clavulatum* were found to be good concentrators of Co, while the latter two were observed to concentrate Zn to high levels. *Centroceras clavulatum* accumulated Cu to highest levels. Thus, the rhodophyte *Centroceras clavulatum*, which accumulates Zn, Cu and Co to high levels, can be considered for use as bioindicator of these metals in seawater. The use of *Enteromorpha intestinalis* as a bioindicator (for Co) is restricted due to its unavailability in the study area during most periods of the year. Although *Grateloupia filicina* can serve as bioindicator at Station 2, its use also is restricted at Station 1 due to its absence during most time of the year. Many authors have suggested that the chlorophyte *Ulva* is a good bioindicator of metal contamination in seawater (Ho 1990, Villares et al. 2001), but its use for this purpose is restricted in the study area because this species showed the low levels of accumulation of Zn, Cu and Co during the present study and hence may not act as a good indicator of the levels of these metals in seawater.

REFERENCES

Barreiro R, Picado L, Real C (2002) Biomonitoring heavy metals in estuaries: a field comparison of two brown algae species inhabiting upper estuarine reaches. Environ Monit Assess 75: 121 – 134

Brown MT, Depledge MH (1998) Determinants of trace metal concentrations in marine organisms. In: Langston WJ, Bebianno MJ (eds) Metal Metabolism in Aquatic Environments. Chapman & Hall Ltd., London, p 185 – 217

FAO (1983) Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products. FAO Fisheries Circular No. 764, Food and Agriculture Organization of the United Nations, Rome

Greene B, Bedell GW (1990) Algal gels or immobilized algae for metal recovery. In: Akatsuka I (ed) Introduction to Applied Phycology. SPB Academic publishing bv, The Hague, The Netherlands, p137 – 149

Hägerhäll B (1973) Marine botanical – hydrographical trace element studies in the Öresund area. Bot Mar 16: 53 – 64

Ho YB (1990) *Ulva lactuca* as bioindicator of metal contamination in intertidal waters in Hong Kong. Hydrobiologia 203: 73 – 81

Lobban CS, Harrison PJ (1997) Seaweed Ecology and Physiology. Cambridge University Press, Cambridge.

PFA (1955) The Prevention of Food Adulteration Rules Part XI. Poisonous Metals. Ministry of Health and Family Welfare, Govt. of India. World wide web electronic publication. http:/mohfw. nic.in.pfa.htm.

Rao ChK (1992) Elemental composition of Indian marine algae – A biogeochemical perspective. Indian J Mar Sci 21: 167 – 177

Rao IM, Murty MV, Satyanarayana D (1995) Trace metal distribution in marine algae of Visakhapatnam, east coast of India. Indian J Mar Sci 24: 142 – 146

Saenko GN, Kravtsova YY, Ivanenko VV, Sheludko SI (1978) Concentration of iodine and bromine by plants in the Seas of Japan and Okhotsk. Mar Biol 47: 243 – 250

Villares R, Puente X, Carballeira A (2001) *Ulva* and *Enteromorpha* as indicators of heavy metal pollution. Hydrobiologia 462: 221 – 232