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Accumulation of trace metals in green mussel *Perna viridis* in the shellfish harvesting environment along southern Karnataka coast

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ABSTRACT

The levels of trace metals, Cd, Pb, Cu, Zn, Ni and Fe in seawater and their bioaccumulation in green mussel *Perna viridis* were examined in two shellfish harvesting environments, Someshwara and Surathkal, along the southern Karnataka coast. In seawater, the concentration of trace metals analysed were below the levels causing harmful effects on the larvae and adult mussels, set by the European Council Directive 79/923/EEC. The Fe, Ni, Cd and Zn levels in mussel tissue from Surathkal were relatively higher when compared to that of mussel tissue samples from Someshwara while Cu and Pb levels were lower. The mean tissue concentrations of trace metals in the soft tissue of *P. viridis* from shellfish waters were found to be safe and below the permissible concentrations for seafood (US FDA and WHO) as well as EU limits in marine products.

Keywords: Bioaccumulation, Karnataka, *Perna viridis*, Trace metals

Introduction

Trace metals are derived from a variety of natural and anthropogenic sources in coastal waters. The marine mussels of the genus *Perna* have tremendous potential to accumulate potentially toxic trace metals in their tissues far in excess of the environmental levels particularly in warm waters (Rainbow, 1995). Subsequent to the incidence of health problems associated with shellfish consumption and detection of contaminants in the products, strict quality control measures are implemented by different countries and international bodies controlling the seafood trade. These standards pertain to the quality of final product as well as the quality of the shellfish growing/ harvesting waters.

In India, quality control guidelines for shellfish are mainly applicable to exports since it is made mandatory by the importing countries whereas strict quality control measures are not applicable to domestic marketing. Dakshina Kannada (DK) District is one of the most industrialised areas in the State of Karnataka. Consequently, contamination of marine ecosystems along the DK coast with trace metals and xenobiotics receives continued attention in the scientific literature and environmental monitoring programmes (Krishnakumar *et al.*, 1990 a,b; 1998, 2004 a,b; Mohankumar *et al.*, 2003; Sasikumar *et al.*, 2006 and Verlecar, 2006). More than five major industries are functioning between Someshwara and Surathkal and the mussel fishing activity in the state is mainly concentrated off this stretch. The present study was carried out to assess the levels of trace metals in seawater and their bioaccumulation in the soft tissues of green mussels in the shellfish harvesting environment of southern Karnataka.

Materials and methods

Study area

In Karnataka, rich natural beds of the green mussel, *P. viridis* exist in the intertidal and subtidal rocky areas along the coast. Mussel production of the state was estimated at 5,324 t during 2003-04. Among the three coastal districts of the State, shellfish waters of DK District contributed 36% (1,905 t) to the mussel production of Karnataka. In DK, dense settlements of green mussels are seen in the littoral and sublittoral rocky stretches off Someshwara (12.79^{0}N; 74.84^{0}E) and Surathkal (13.00^{0}N; 74.79^{0}E) up to a depth of 6-10 m from the shoreline. The mussel beds off Someshwara is located at 5.5 km south of Nethravati-Gurpur estuary whereas, Surathkal mussel bed is located at 18 km north of Nethravati-Gurpur estuary.

Sampling

Water and tissue samples were collected from Someshwara and Surathkal shellfish waters during February-March, June-July and September-October 2003. The mussels were depurated overnight in filtered seawater and stored frozen until analysis. Seawater samples for trace metal analysis were collected in acid washed polyethelene flasks. Immediately on arrival at the laboratory, the water samples were filtered through a 2.5 µm cellulose acetate filter and the organic matter was digested by adding HNO_3 to a final pH of < 2 and kept frozen for analysis.

Analytical methods

Trace metals in seawater

Trace metals were analysed using Stripping Voltammetry in a 757 VA Computrace attached to 765 Dosimat (Metrohm, Switzerland). A hanging mercury drop electrode was used as the working electrode and potentials were measured against KCl, 3 mol l^{-1} reference electrode and an auxiliary platinum electrode. Total dissolved Zn, Cd, Pb and Cu were estimated by adding 10 ml sample and 1 ml acetate buffer in a Teflon cell, using Differential Pulse Anodic Stripping Voltammetry (DPASV). The concentrations of these metals were simultaneously measured by addition of mixed metal standards using Dosimat (Florence, 1972). Total dissolved Ni was estimated using Cathodic Stripping Voltammetry (CSV) by adding 10 ml sample, 0.05 ml dimethyl glycoxime (DMG) and 0.5 ml NH_3 buffer into the Teflon cell and analysed using the standard (Meyer and Neeb, 1983). The accuracy of the analytical procedure was checked using certified reference material (BCR, CRM 403 Seawater). The recovery estimated by measuring standard spiked samples was 97% for all the metals studied.

Trace metals in mussel tissue

Mussels (size >50 mm) were rinsed with deionised water and wet shucked. The composite whole tissue mass from 10 mussels were pooled, homogenised and used for the analysis separately for the three sampling. Approximately 1-2 g of tissue was digested with 10 ml mixture of HNO_3 and H_2O_2 (1:1 ratio) in replicates following standard procedures (Robisch and Clark, 1993). The trace metals in the digested samples were determined by Atomic Absorption Spectrophotometry with either airacetylene flame (zinc) or graphite furnace (copper, cadmium, nickel, lead). The results were expressed in ppm wet tissue weight. Blanks and standards were digested with each sample set to provide quality control. The accuracy of the method was verified (10 replicates) using standard reference material (fish tissue, MA-B3/TM) obtained from the International Laboratory of Marine Radioactivity, IAEA. Recoveries were above 90% for all the trace metals measured.

Trace metals in mussel shell

The shells after wet shucking were cleaned and rinsed with deionised water. The periostracum and the calcite section of the recently formed shell layers were scrapped off leaving only the nacreous part of the shell (Bourgoin, 1990). Strips of the recently formed nacreous layer from the shells were used for analysis. Approximately 1-2 g of the sample was digested following standard procedures as detailed above (Robisch and Clark, 1993). The results were expressed in ppm shell weight.

Bioaccumulation factor

Bioaccumulation factor (BF) was calculated as: 1) BF-Tissue/water = Concentration of metal in the tissue/ Concentration of metal in the seawater 2) BF-Shell/water = Concentration of metal in the shell/Concentration of metal in the seawater; 3) BF-Shell/tissue = Concentration of metal in the shell/ Concentration of metal in the tissue (Nair, 1984).

Results

The trace metal concentrations in environmental matrices of the shellfish harvesting area showed no statistically significant difference (ANOVA) between the three sampling periods therefore, the data was pooled for further analysis.

Trace metals in seawater

Mean trace metal concentrations of Cd, Pb, Cu, Zn, Ni and Fe in seawater (ppm) from Someshwara and Surathkal shellfish waters are presented in Table 1. Cd showed relatively very low concentrations in the seawater and the distribution pattern being in the order of Fe>Zn>Cu>Ni>Pb>Cd at Someshwara and Fe>Zn>Cu>Pb>Ni>Cd at Surathkal. Analysis of variance (ANOVA) of trace metal concentrations in seawater between the two shellfish waters showed significantly higher (1.5 times) Pb concentrations ($p<0.05$) in Surathkal (0.0019±0.0013 ppm) than in Someshwara (0.0014±0.0010 ppm). Cd concentrations in seawater were 2.8 times higher in Surathkal (0.0005±0.0007 ppm) than the levels in Someshwara (0.0002±0.0003 ppm). Similarly the Cu and Zn concentrations in seawater of the Surathkal waters were 1.2 and 1.7 times higher than that of Someshwara. Between the shellfish waters, Ni and Fe levels in seawater presented the least spatial trends during the study.

Trace metals in mussel tissue

The concentrations of trace metals analysed in tissue and shell were higher than the concentrations in seawater (Table 1). The pattern of distribution of trace metals in the mussel tissue was Fe>Zn>Cu>Pb>Ni>Cd at Someshwara and at Surathkal it was in the order of

Fe>Zn>Cu>Ni>Cd>Pb. The concentration of Cd in mussel tissue was 3.1 times higher at Surathkal but the spatial differences were not significant. Fe, Ni, Cd and Zn levels of mussel tissue samples originating from Surathkal were higher compared to Someshwara while Cu and Pb levels were lower. The concentration of metals showed no significant difference between the two shellfish waters stastically.

Trace metals in mussel shell

The distribution pattern of trace metals at Someshwara was in the order of Fe>Zn>Cu>Cd>Pb>Ni where as at Surathkal it was in the order of Zn>Cu>Fe>Cd>Pb>Ni (Table 1). The Cd, Cu and Pb concentrations were higher in mussel shell than in the soft tissues; conversely Fe and Zn concentrations were noticeably higher in the soft tissues than in the shell. The concentration of Cd in mussel shell (1.4 times) was higher at Surathkal than at Someshwara whereas, the concentration of all the other metals studied was found to be low at Surathkal. Analysis of variance indicated significant difference in the levels of Cd, Cu and Fe between the two shellfish waters ($p<0.05$).

Bioaccumulation factor

The bioaccumulation factor varied with different trace metals and it presented variation between the shell and soft tissue (Table 2). Among the trace metals, the highest bioaccumulation in tissue as well as shell was observed for Cu, Cd and Zn at Someshwara and Surathkal shellfish waters. In mussel tissue Cd showed a BF 10^2 times and Cu showed a BF 10^3 times as compared to the surrounding waters. Mussel shells showed highest ratios for Cd and Cu, accumulating 10^3 times more than the surrounding waters. Comparatively low BF was observed for Pb in tissue than in shells. The bioaccumulation indices of Fe in the tissue were higher than that of the shell from both the shellfish waters.

Influence of trace metal levels in ambient waters on the mussel tissue and shell concentrations

Correlation between concentrations of trace metals in seawater, mussel tissue and shell was examined to study their interrelationships (Table 3). Significant positive correlations were observed between metal levels in seawater and in mussel tissue.

Cd, Cu and Zn content in seawater exhibited significant positive correlation ($p<0.05$) with that in mussel shell. Significant positive correlation was observed between Cu, Fe and Zn in seawater ($p<0.05$) and levels of these metals in mussel tissue.

Discussion

In environmental monitoring studies, the interest in metals like Zn, Cu and Fe that are required for metabolic activity in organisms, lies in the narrow "window" between their essentiality and toxicity. Other trace metals like Cd and Pb may exhibit extreme toxicity even at low levels under certain conditions, thus, necessitating regular monitoring of sensitive aquatic environments (Peerzada *et al*., 1990). Along the DK coast, no spatial variation was observed between the shellfish waters of Someshwara and Surathkal in the trace metal concentration in mussel tissue.

Table 1. Trace metal concentrations (Mean ± SD) in seawater, soft tissue and shell of *P. viridis* in the shellfish waters of Someshwara and Surathkal

TraceMetals	Seawater (ppm)		Mussel tissue (ppm)		Mussel shell (ppm)	
	Someshwara	Surathkal	Someshwara	Surathkal	Someshwara	Surathkal
Cd	0.0002±0.0003	0.0005±0.0007	0.03±0.10	0.10±0.46	1.18±1.27	1.68±1.71
Pb	0.0014±0.0010	0.0019±0.0013	0.11±0.84	0.06±0.48	1.08±3.78	0.91±3.45
Cu	0.0023±0.0016	0.0027±0.0017	3.38±1.97	2.98±3.07	7.22±3.36	5.66±5.33
Zn	0.0090±0.0068	0.0149±0.0054	14.07±5.28	14.94±7.21	7.70±8.70	6.11±9.12
Ni	0.0019±0.0015	0.0019±0.0009	0.041±0.17	0.26±1.64	0.08±0.35	nd
Fe	0.6536±0.9078	0.4917±0.4414	30.56±42.4	33.6±46.6	9.85±17.22	4.01±6.79

nd - below detectable limits

Table 2. Bioaccumulation factor (BF) of trace metals in *P. viridis* from shellfish waters of Someshwara and Surathkal

Bioaccumulation factor	Mussel bed	Cd	Pb	Cu	Zn	Ni	Fe
BF-Tissue/water	Someshwara	200	73	1495	1555	21	47
	Surathkal	219	32	1083	1003	136	68
BF-Shell/water	Someshwara	7202	749	3196	852	41	15
	Surathkal	3590	463	2059	411	-	8
BF-Shell/tissue	Someshwara	36.02	10.28	2.14	0.55	1.95	0.32
	Surathkal	16.38	14.33	1.90	0.41	-	0.12

Table 3. Correlation coefficient (Pearson's) between concentrations of trace metals in seawater (water), mussel tissue (tissue) and shells of *P. viridis* from shellfish waters of Someshwara and Surathkal

	Shell-Cd	Tissue-Cd	Water-Cd	Shell-Cu	Tissue-Cu	Water-Cu	Shell-Fe	Tissue-Fe	Water-Fe	Shell-Ni	Tissue-Ni	Water-Ni	Shell-Pb	Tissue-Pb	Water-Pb	Shell-Zn	Tissue-Zn	Water-Zn	Shell-Mn	Tissue-Mn	Water-Co
Shell-Cd	1.000	.459(**)	.408(*)	.769(**)	.645(**)	.645(**)	.689(**)	.369(*)	0.324	.653(**)	.422(*)	.386(*)	0.319	0.124	.524(**)	.758(**)	.793(**)	.489(**)	.519(**)	.481(**)	0.310
Tissue-Cd		1.000	0.023	.372(*)	.493(**)	.746(**)	0.295	.372(*)	0.123	0.115	0.095	0.143	-0.071	-0.088	.787(**)	0.236	0.228	0.239	0.062	0.074	0.048
Water-Cd			1.000	0.003	-0.052	0.327	0.220	.437(*)	0.299	0.308	0.196	0.288	.696(**)	.454(**)	.389(*)	0.134	.373(*)	.502(**)	0.051	0.093	.330(*)
Shell-Cu				1.000	.871(**)	.415(*)	.506(**)	0.037	0.167	.533(**)	0.310	0.190	-0.104	-0.169	0.236	.622(**)	.625(**)	0.138	.680(**)	.641(**)	0.128
Tissue-Cu					1.000	.387(*)	0.331	0.145	0.131	0.281	0.163	0.104	-0.182	-0.189	0.282	.439(*)	.435(*)	0.070	.685(**)	.648(**)	0.058
Water-Cu						1.000	.545(**)	.512(**)	.478(**)	0.211	0.303	.517(**)	0.158	0.119	.926(**)	.475(**)	.618(**)	.716(**)	0.103	0.066	.438(**)
Shell-Fe							1.000	.393(*)	0.307	0.296	0.164	.345(*)	.365(*)	.375(*)	.463(**)	.715(**)	.538(**)	.611(**)	0.200	0.144	0.262
Tissue-Fe								1.000	.539(**)	-0.094	-0.013	.419(*)	.643(**)	.660(**)	.643(**)	0.250	0.226	.542(**)	0.019	0.013	.464(**)
Water-Fe									1.000	0.018	0.008	.927(**)	0.133	0.128	.413(*)	0.274	.427(*)	.655(**)	0.138	0.106	.962(**)
Shell-Ni										1.000	0.335	0.240	0.264	-0.070	0.071	.549(**)	.588(**)	0.153	.370(*)	.446(**)	0.131
Tissue-Ni											1.000	-0.030	0.081	0.045	0.287	.618(**)	.646(**)	0.106	.430(*)	0.336	-0.001
Water-Ni												1.000	0.155	0.080	.397(*)	0.331	.471(**)	.711(**)	0.066	0.067	.956(**)
Shell-Pb													1.000	.871(**)	0.311	0.306	0.200	.456(**)	-0.052	-0.008	0.142
Tissue-Pb														1.000	0.307	0.297	0.049	.415(*)	-0.103	-0.109	0.076
Water-Pb															1.000	.408(*)	.475(**)	.688(**)	0.040	0.019	0.322
Shell-Zn																1.000	.775(**)	.500(**)	.550(**)	.452(**)	0.230
Tissue-Zn																	1.000	.605(**)	.580(**)	.517(**)	.410(*)
Water-Zn																		1.000	0.045	-0.006	.612(**)
Shell-Mn																			1.000	.957(**)	0.069
Tissue-Mn																				1.000	0.060
Water-Co																					1.000

*p<0.05 ** p<0.01

The Fe levels in the seawater and mussel tissue samples in the study area were the highest among the metals studied and of similar magnitude as reported from the region (Sasikumar *et al.,* 2006). When permissible levels of Fe are concerned, no guidelines are available regarding its limit in shellfish. Zinc levels in seawater were high in both the shellfish waters with significantly higher levels at Surathkal, but no significant difference in Zn concentrations in the soft tissue as well as mussel shells were noticed between the shellfish waters. Generally the effluent discharges from metallurgic industry, electroplating industry, petrochemical industry, run offs from agricultural areas, dredging of harbour and municipal wastes are the sources of Zinc in coastal waters. Relatively consistent tissue concentrations of Zn were observed in mussels in earlier studies with least spatial difference in its distribution throughout the Karnataka coast (Sasikumar *et al.,* 2006). Similar to Fe, Zn concentrations in the mussel were high and comparable with earlier reports by Krishnakumar *et al.* (1998). The high values for Zn were also reported in seawater (Krishnakumar *et al*., 2004a) and sediments along coastal waters of Karnataka (Krishnakumar *et al.,* 1998). However, mussels are known to regulate zinc uptake and are therefore reported as a variable indicator for zinc contamination (Lobel *et al*., 1982). Even then, significant positive correlations between Zn in seawater and tissue concentrations imply that Zn is accumulated in soft tissue and shell in green mussels in a proportion similar to its availability in ambient waters. Although Zn uptake is regulated, it was observed that the incorporated Zn remained in the soft tissue, suggesting the use of *P. viridis* as a potential biomonitoring agent for assessing Zn contamination over extended periods. Zn in aquatic environment predominantly binds to suspended material before settling in sediments.

Cu concentrations in mussel samples from the study area indicated marginal increase when compared with previous studies (Krishnakumar *et al.,* 1998; Sasikumar *et al.,* 2006) from non-urban sites of Karnataka. On the other hand, the Cu levels in green mussel were lower when compared to industrial and urban sites of Karnataka (Krishnakumar *et al.,* 1998). A strong positive correlation was observed between the Cu concentration in seawater, mussel tissue and shells. The Cu concentrations in the shellfish waters may be related to inflows from non-point sources around the shellfish waters considering its proximity to fishing harbour. Being a biocide in antifoulant paints either as copper, copper oxide or copper sulphate, copper is the most common metal found at toxic concentrations in several harbours (NCDEM, 1991). Szefer *et al.* (2004) observed a positive correlation between Zn as well as Cu in blue mussel tissue and their levels in suspended matter and attributed it to their levels in suspended particulate matter, which constitute the potential food for the mussels as filter feeders.

At Someshwara mussel beds, Cd levels in seawater and mussel tissue were lower than at Surathkal. Significant positive correlations of Cd in seawater and mussel indicates that the increases in Cd concentrations in mussel tissue and shells at Surathkal corresponds to the higher Cd availability in the seawater. Relatively higher level of Cd in Surathkal mussel bed may indicate a higher input of Cd into the coastal waters. In general, the Cd levels in the mussel tissue were markedly lower than those previously reported from the area in green mussels (Krishnakumar *et al.,* 1998; Sasikumar *et al.,* 2006).

Concentration of Pb in seawater was significantly higher ($p<0.05$) at Surathkal but spatial difference in tissue bioaccumulation of lead was not observed between the shellfish waters. Pb concentrations observed in *P. viridis* in the present study was low compared to earlier reports of Krishnakumar *et al.* (1998), however, it was more than the levels reported in 2002 from the same area (Sasikumar *et al*., 2006). Lead is used as a fuel additive and may be released through incomplete fuel combustion and boat bilge discharges (NCDEM, 1991) therefore; relatively higher levels of Pb in seawater samples from Surathkal could be attributed to the input of lead from petroleum fuel in the vicinity of harbours and refineries.

The concentration of Ni recorded in the present study remained within the ranges previously reported in green mussel from the region (Sasikumar *et al.,* 2006). The accumulation of Ni indicated higher values for the mussel tissue than the shells and no significant correlations were detected between the levels of Ni in seawater, tissue and shells.

The mean tissue concentrations of trace metals in *P. viridis* from the shellfish waters were found to be within the permissible norms for seafood and marine products (US FDA; WHO 1972; 1987; EU) (Table 4). The overall picture emerged from the present study indicates that in the Someshwara and Surathkal shellfish waters, the trace metal contents in mussel tissue in general are close to background levels. In general, the mean tissue concentrations of trace metals in *P. viridis* collected from shellfish waters of the DK coast were found to be safe and below the permissible concentrations (FDA) for seafood (WHO, 1972; 1987) as well as EU limits in marine products. According to the European legislation (EC, 1979) the levels of pollutants in shellfish waters should not exceed those causing deleterious effects on the adult molluscs or their larvae. His *et al.* (2000) reviewed the LC_{50} values impairing bivalve embryogenesis in marine bivalve species and the geometric means are 40 µg^{-1} for Cu, 320 µg^{-1} for Zn, 968 µg^{-1} for Pb and 2219 µg^{-1} for Cd. The concentration of Cu, Zn, Pb and Cd in Someshwara and Surathkal shellfish waters are found to be below these values. Furthermore, the observed levels of trace metals in the shellfish waters were considerably lower than the LC_{50} values reported for green mussels (Yap *et al*., 2004).

Though the metal concentrations in water and mussel tissue were well below the permissible limits, the situation calls for regular monitoring to ensure that these safe limits are not exceeded. The pollution pressures in the region are likely to increase in future because of rapid industrialisation and urbanisation throughout the coastal zone. This necessitates well designed monitoring plans and concerted efforts to ensure that the pristine coastal ecosystems are protected from pollution effects and consumption of harvested mussels from these waters do not pose any public health hazard.

Table 4. Guidelines for metals and organochlorine pesticides in molluscs and seawater.

Trace metal	WHO (1987)	Tissue limits (ppm) FDA (2001) (for molluscan bivalves)	EC (2001) (for bivalves)	Seawater mg l^{-1} (ppm) EC
Cadmium	2	4	1.0	2.5 (0.002)
Copper	30	-	-	5 (0.005)
Lead	2	1.7	1.0	15 (0.015)
Nickel	2	80	-	-
Zinc	50	-	-	40 (0.04)

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