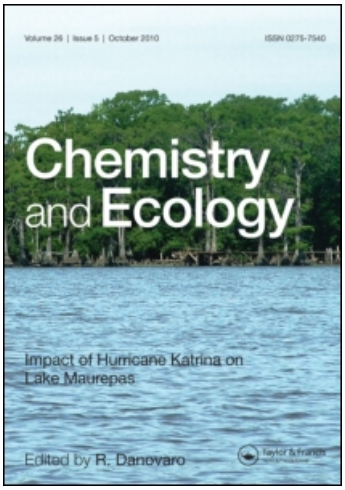

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METAL CONTENT IN SELECTED TISSUES AND SHELL OF *PERNA VIRIDIS* (L) FROM PONDICHERRY, EAST COAST OF INDIA

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Mussels are good bioaccumulators of metals and have been used as indicators for environmental monitoring. In this study on *P.viridis* from Pondicherry coast, metal content (aluminium, lead, cadmium, copper and zinc) were analysed in selected tissues and shell nacre for a period of one year. The metal content shows that digestive gland accumulates higher concentrations of metal ions followed by gill, mantle and shell. Of the different components (digestive gland, gill, mantle and shell nacre) of the organism, only shell nacre exhibited a significant relationship with ambient levels and therefore can be applied to temporal monitoring of metal contamination.

KEY WORDS: mussels, trace metals, shell, soft tissues, monitoring

INTRODUCTION

Bivalves, such as mussels and oysters, accumulate and concentrate metal contaminants within their tissues in significant levels and are therefore good 'sentinel' organisms for marine pollution monitoring programmes (Goldberg, 1975; Phillips, 1980). Studies have shown that accumulation of metals in tissues and shells of mussels can be related to their content in the surrounding medium (Gault *et al.*, 1983). However, tissue level elemental concentrations are influenced by factors of extrinsic and intrinsic nature, and also by an unexplained inherent variability (Cunningham, 1979; Lobel *et al.*, 1991). Alternatively, bivalve shells have been considered for monitoring heavy metal pollutants, as they are better recorders of environmental levels and also offer several advantages over tissues (Koide *et al.*, 1982). Among the different components of the shell, nacre incorporates biologically deposited metal ions and therefore could be an appropriate indicator of metals assimilated by the organism (Bourgoin, 1990).

This study reports metal concentrations (aluminium, lead, cadmium, copper and zinc) in selected tissues and shells of a bivalve mollusc, *Perna viridis* (L), from Pondicherry coastal waters observed over a single year. Along the coastline of Pondicherry, industries producing metal alloys, processed leather and analytical/industrial chemicals exist. This study analyzes the relationship between metal content in tissues of the organism and metals dissolved in ambient sea water.

Perna viridis (L) is a widely distributed and commercially valuable seafood, and has also been used as an 'indicator' organism for biomonitoring of environmental pollutants (Kuriakose, 1980; Phillips, 1984).

MATERIAL AND METHODS

I. Location of the Sampling Sites

The coast of Pondicherry is located in the southern east coast region of India (11° 59'N 79° 50'E). The sampling was done from fixed locations along the coast following the pollution gradient.

II. Duration of Study

Samples of sea water (salinity 34.7–36.6‰) and mussels were collected every alternate month through one year (January 1992–December 1992).

III. Collection and Analysis of Samples

i. Collection

Mussels (95–110 mm in length) were collected from the above sites during the study period. All specimens were collected from a monolayer bed with an assumption that exposure to ambient conditions is homogeneous. The mussels were kept for six hours in the laboratory for gut evacuation before dissection.

ii. Tissue Analysis

Soft tissues of the mussels were removed from their shells with a clean knife and rinsed with distilled water. The selected tissues were carefully dissected and separated into digestive gland, gills and mantle, and dried. Composite samples were prepared so as to reduce individual variability (Lobel *et al.*, 1991). Each composite sample and the replicate constituted 13 tissues of the same type from 26 individual mussels collected during each sampling. The samples were digested by boiling with Analar grade (0.2 N) nitric acid (approx. 0.5 g tissue in 12 ml) until the solution was clear. The acid digest was then diluted to a standard volume (100 ml) with double distilled water (Romeril, 1979). The metal concentrations (aluminium, lead, cadmium, copper and zinc) were determined by atomic absorption spectrophotometer (AAS; GBC Australia – model 908AA).

iii. Shell Analysis

The shells were cleaned of extraneous materials such as byssus threads and dried at 70°C for two hours. The dried shells were then exposed to 300°C for four hours to separate calcite and the nacreous layer (Bourgoin, 1988). Nacre was lightly scraped from the inner growth surface of the nacre shell at a depth no deeper than 0.1 mm so that only recently deposited material was collected (Bourgoin, 1990). The nacre samples were pooled and 50 mg of the sample was digested in Analar grade nitric acid. The samples were diluted to a standard volume (100 ml) with double distilled water for analysis of metals by AAS.

iv. Sea Water Analysis

Sea water samples were collected in polythene bottles, acidified with 10% nitric acid and tightly closed at the collection sites. The samples were later brought to the laboratory and filtered through millipore filters (0.45 μm) to separate the particulate fraction. Dissolved metals were pre-concentrated by APDC-MIBK (ammonium

pyrrolidine dithiocarbamate – methyl isobutyl ketone) extraction step (Jan and Young, 1978). The extracted samples were analysed using AAS.

Appropriate standard graphs were drawn for calibration and the metal concentrations were determined from AAS. Spearman rank correlation (Sokal and Rohlf, 1969) was done between metal concentrations in different tissues and the shell of the organism and dissolved metal concentrations in sea water.

RESULTS

Table I gives mean, range and standard deviation of metal concentrations in digestive gland, gill, mantle and shell of *P. viridis* and dissolved metal concentrations in sea water. The observations of relative concentrations are as follows:

a. Between Tissues

Aluminium: Digestive gland> shell> gill> sea water> mantle
Lead : Sea water> digestive gland> gill> shell> mantle
Cadmium : Digestive gland> gill> sea water> mantle≈ shell
Copper : Digestive gland> gill> sea water> shell> mantle
Zinc : Digestive gland> gill> sea water> mantle> shell

Table I Mean, standard deviation (SD) and range of metal concentration in tissues (μg/g dry wt.) and shell (μg/g) of *P. viridis* and dissolved metal concentration in sea water (μg/l). [N = 26]

		Al	Pb	Cd	Cu	Zn
Shell	Mean	409.8	1.4	0.3	7.7	45.2
	SD	119.4	0.3	0.07	1.9	18.6
	Min.	274.4	1.1	ND*	5.2	13.0
	Max.	589.6	1.7	0.4	10.7	71.8
Digestive gland	Mean	1587.0	1.9	2.8	186.6	502.8
	SD	913.0	0.5	2.0	37.7	101.7
	Min.	1005.4	1.4	1.0	145.0	386.0
	Max.	3363.3	2.7	6.8	240.4	636.7
Gill	Mean	408.3	1.6	2.2	58.3	432.3
	SD	172.8	0.8	2.2	27.1	106.6
	Min.	226.4	0.9	0.5	36.2	306.5
	Max.	630.5	3.1	6.5	109.4	615.0
Mantle	Mean	127.1	0.3	0.3	2.3	102.1
	SD	9.2	0.2	0.1	0.6	12.6
	Min.	120.0	ND*	ND*	1.6	91.8
	Max.	142.8	0.5	0.5	3.1	124.0
Sea water	Mean	159.0	3.1	0.8	31.0	333.0
	SD	46.8	0.8	0.2	4.5	21.9
	Min.	106.4	2.0	0.5	23.0	300.4
	Max.	223.0	4.1	1.0	35.2	354.4

* ND – Non-detectable

Table II Spearman rank correlation between dissolved metal content in sea water and different tissues of the *P.viridis* (SW – sea water; SH – shell; DG – digestive gland; GL – gill; MAN – mantle)

	SW/SH	SW/DG	SW/GL	SW/MAN
Al	1.00**	NS	NS	NS
Pb	0.77**	0.80***	0.76**	0.67*
Cd	0.87***	0.88****	0.79**	0.68*
Cu	0.89****	NS	NS	NS
Zn	NS	NS	NS	NS

Levels of significance:
* – $P > 0.1$
** – $P > 0.05$
*** – $P > 0.02$
**** – $P > 0.01$
NS – Not significant

b. Between Metals

Digestive gland : aluminium> zinc> copper> cadmium> lead
Gill : aluminium> zinc> copper> cadmium> lead
Mantle : aluminium> zinc> copper> cadmium≈ lead
Shell : aluminium> zinc> copper> lead> cadmium
Sea water : aluminium> zinc> copper> lead> cadmium

Spearman rank correlation values for metal content in sea water, shell and tissues are given in Table II. Significant relationships were found for concentrations of aluminium, lead, cadmium and copper between sea water and shell concentrations. However, the concentration of zinc in sea water and shell showed no significant relationship. In digestive gland, gill and mantle, there was no relationship with sea water concentrations of aluminium, zinc and copper. Lead and cadmium in these tissues, however, exhibited a significant relationship with their concentrations in sea water.

DISCUSSION

Metal Content in Tissues and Shell

The present study on the metal contents of selected tissue components and shell nacre of *P.viridis* has shown that digestive gland is the major bioaccumulator for all the metals studied, followed by gill, mantle or shell.

The ability of digestive gland to accumulate high concentrations of metals is facilitated by the presence of complexing mucus, which sequesters metal ions from solution (Brooks and Rumsby, 1985). Metal ingested with the food would be expected to bind first in digestive gland cells (Engels and Brouwer, 1989). This tissue plays an important role in metal storage (metallothioneins, lysosomal vacuolar system, metal-containing granules and membrane vesicles), and in metabolism and detoxification (lipid peroxidation mechanism) processes (Viarengo, 1989). The observation of higher concentrations in digestive gland in this study may be due to these various mechanisms.

Gills, functioning both as feeding and respiratory organs, absorb metal ions with food particles on the mucus laden surface (Fretter, 1953). Being in constant contact with particulate matter, the gills keep the mucosal surface free from absorbed or clinging materials in order to carry out filtration efficiently (Lobel *et al.*, 1991). In this process, the tissue quickly passes the particles to the digestive gland via mouth (Hobden, 1969). Lower concentrations in the gills in the present study indicate a shorter retention time of metal ions there than in the other tissues.

Metal concentrations in the mantle are due to endocytotic activity and/or transport of metals by amoebocytes. The mantle surface also sequesters metal ions as it is exposed to water drawn into the cavity (Cunningham, 1979). Metal loss from this tissue is continuous through elimination of metals from metal-containing amoebocytes and through the production of shell (Tripp, 1963; Galtsoff, 1964). Lower concentrations of metal ions in the mantle may be due to their continuous loss by these processes.

The bivalve shell, produced from extrapallial fluid secreted actively by the mantle epithelium, incorporates metal ions from the body tissues (Wilbur and Saleuddin, 1983). Of the different components of the shell, nacre is expected to deposit trace metals assimilated biologically by the organism (Bourgoin, 1990). Observations in this study, in general, have shown that the shell accumulates metal ions less than soft tissues.

Relationship Between Metal Content in Tissues/Shell and Sea Water

From the correlation coefficient values (Table II), it can be noted that essential metals, namely copper and zinc, in digestive gland and gill had little or no relationship with their concentrations in sea water. This may be ascribed to their retention/storage for metabolic activity (Simkiss and Mason, 1983).

Cadmium and lead, though retained or eliminated from tissues due to the presence of metal chelating ligands (Langston and Bebbianno, 1990) or toxicity (Brooks and Rumsby, 1965), may be in equilibrium with the surrounding medium because of their biological inactivity.

Bivalve shell as a better index of metal bioaccumulation has been reported by various authors (Babukutty and Chacko, 1992; Bourgoin, 1990; Koide *et al.*, 1982). The present study has shown that a stronger relationship exists for metal concentrations in shell nacre and the ambient medium. Metal ions are bound in various forms in shell lattices (Lingard *et al.*, 1992), and accumulation of aluminium and lead, in particular, may be facilitated by their chemical characteristics and structural similarity with crystalline components of the shell (Babukutty and Chacko, 1992).

The study reported here of metal content in tissues and shell nacre of *P.viridis* provides a baseline for monitoring temporal and spatial changes for this species.

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