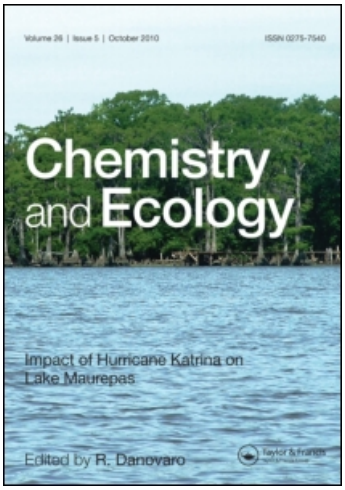

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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P. Shahul Hameed[a]; A. Isaac Mohan Raj[a]
[a] Post-Graduate Department of Zoology, Jamal Mohamed College, Tamil Nadu, India

To cite this Article Hameed, P. Shahul and Raj, A. Isaac Mohan(1990) 'Freshwater Mussel, *Lamellidens Marginalis* (Lamarck) (Mollusca: Bivalvia: Unionidae) As an Indicator of River Pollution', Chemistry and Ecology, 4: 2, 57 — 64
To link to this Article: DOI: 10.1080/02757549008035965
URL: http://dx.doi.org/10.1080/02757549008035965

Chemistry and Ecology, 1990, Vol. 4, pp. 57–64
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FRESHWATER MUSSEL, *LAMELLIDENS MARGINALIS* (LAMARCK) (MOLLUSCA: BIVALVIA: UNIONIDAE) AS AN INDICATOR OF RIVER POLLUTION

P. SHAHUL HAMEED and A. ISAAC MOHAN RAJ

Post-Graduate Department of Zoology, Jamal Mohamed College, Tiruchirapalli 620 020, Tamil Nadu, India

(Received September 26, 1988; June 22, 1989 in final form)

Analyses were made of heavy metals, manganese, nickel, copper, zinc and lead in water samples and soft body, shell and different tissues (gills, digestive glands, mantle and viscera) of the Unionid mussel, *Lamellidens marginalis*, collected from two tributaries of the Cauvery river. Water samples from Station I contained higher concentrations of the metals than those from Station II. The concentration of metals in water at both stations were in the descending order: Mn > Zn > Pb > Ni > Cu. However, the concentrations of metals in the soft body were in the descending order: Zn > Mn > Pb > Ni > Cu at both stations in all size groups of mussels tested. The concentration of zinc maintained a linear relationship with the size of the mussels, but manganese showed a reverse trend. Small size (4–5 cm) mussels accumulated more manganese (105.5 $\mu g.g^{-1}$ dry wt.) than larger ones (7–8 cm; 6.5 $\mu g.g^{-1}$ dry wt.). Both young and old mussels accumulate the same level of lead, copper and nickel in the soft body. The order of concentrations of metals (Mn, Pb, Zn, Ni and Cu) in the shell of mussels from both stations coincided with the order of concentrations of background water except for lead. The accumulation of lead was higher in shell (30.4–36.2 $\mu g.g^{-1}$ dry wt.) than in soft body (6.4–12.0 $\mu g.g^{-1}$ dry wt.). The pattern of concentration of metals in the various tissues reveal that the digestive glands have greater ability than other tissues to concentrate most metals under study. The concentration factors for soft body, shell and different tissues are presented. The advantages in using the common mussel for biomonitoring of contaminants in water is also discussed.

INTRODUCTION

Monitoring the levels of contaminants in the natural waters at an early stage is rather difficult due to the low concentrations encountered. However, some bivalve molluscs such as marine mussels (Goldberg, 1975; Phillips, 1976; Thomson, 1979; Farrington *et al.*, 1983) and oysters (Brooks and Rumsby, 1965; Cunningham and Tripp, 1975; Zumuda and Sunda, 1982) have been used extensively as biological indicators of heavy metal contamination of the marine environment. Similarly, freshwater bivalves belonging to Unionidae (Naiades) are generally viewed as reliable indicators of contamination, because they are sedentary and easy for repeated observations, occupy the pivotal position as a primary consumer in the food chain and withstand a high rate of accumulation of contaminants (Fuller, 1974; Forester, 1980; McCleneghan *et al.*, 1981; Havlik and Marking, 1987). The metals of zinc, manganese, copper and cadmium have been the most frequently studied in the Unionid bivalves (Johnson *et al.*, 1966;

Merlini, 1966; Patel and Doshi, 1971; Anderson, 1977; Forester, 1980; Schmitt and Finger, 1982). The biomonitoring of metal contamination has become necessary in the river systems owing to increasing loads of metal discharges. The Unionid mussel *Lamellidens marginalis,* inhabiting the Cauvery river system, has the potential to serve as a good indicator. The present investigation was launched to collect base line data on the bioaccumulation of the metals in the divisions of the river in relation to potentially polluting discharges.

MATERIALS AND METHODS

Freshwater mussels, *Lamellidens marginalis,* were collected from the two tributaries (at Station I & II) of the Cauvery river at Tiruchirapalli, India (10°48′ Lat. N 78°42′ Long E.) Station I is located on the Kudamurutti river (agriculture drainage canal) which receives domestic sewage discharge and effluents from the nearby textile dyeing industries from the upstream Woriyur area before it joins the river Cauvery at Tiruchirapalli. Station II is located in Thirumanjana river at Srirangam, 5 km downstream from Tiruchirapalli. The water is clean and unpolluted here and is used by the local population largely for household purposes.

Estimation of Heavy Metal Concentrations in Natural Water Samples

For estimating the heavy metal concentrations, a 5 litre sample of water from each station was filtered and evaporated to dryness. A few drops of concentrated NHO_3 was added to dissolve the residue and this was filtered through Whatman No. 42 filter paper and made up to 50 ml with glass distilled water. The normality of the final solution was maintained between 0.1 N and 0.5 N.

Estimation of the Concentrations of Heavy Metals in the Soft Part and Shell of L. marginalis

In the whole soft body. The mussels collected from each station were sorted into four groups based on the length of the shells namely (1) 4 to 5 cm, (2) 5 to 6 cm, (3) 6 to 7 cm and (4) 7 to 8 cm. Mussels from each size group were used for ashing. The shells of the mussels were removed and the water contained in the mantle was removed with filter paper. The soft body of the mussels was blotted dry repeatedly. The weight of soft body was determined. The wet tissues were dried in the electrical oven at 105°C until constancy in weight was obtained. The dry weight of the tissue was determined. The dried tissue was ashed at 450°C. The ash was weighed in an electronic micro-balance. It was digested by adding equal parts of concentrated HNO_3 and H_2O_2 filtered through Whatman No. 42 filter paper and made up to 50 ml with glass distilled water, keeping the normality of the final solution in the range 0.1 N and 0.5 N (IAEA, 1967).

In the shell. One of the two valves of mussels (7.0 cm) collected from station I was dried and weighed. It was wet ashed by adding concentrated HNO_3 and H_2O_2 in equal parts (1:1) until a white residue was obtained (IAEA, 1967). The white residue was dissolved in 5% HNO_3.

In the different tissues. Mussels having shell length between 7.0 and 8.0 cm from station I were used for this study. Twenty mussels were dissected out and the organs such as gills, mantle, digestive glands and viscera (the visceral mass including foot) were removed. Each such 'organ' was pooled, weighed, dried (at 105°C) and reweighed. The dried tissues were ashed in a muffle furnance at 450°C. The ash was weighed in an electronic micro-balance and the same subsequent procedure was repeated as stated above.

The metal concentrations in water, soft body, shell and different tissues were measured using a Varian Techron model 1100 Atomic Absorption Spectrophotometer.

RESULTS

The concentrations of heavy metals: manganese, nickel, copper, zinc and lead in water samples and the soft body tissues and shells of *L. marginalis* from the two stations are presented in Table I. When heavy metals estimated in water samples are arranged in the order of descending concentration, they form the series

Table I Concentrations of metals in soft-body and shell of *L. marginalis* and water samples collected from two sations located in the tributaries of the Cauvery river.

Size group (cm)	*Manganese Mn*	*Nickel Ni*	*Copper Cu*	*Zinc Zn*	*Lead Pb*
			Kudamurutti river (Station I)		
			($\mu g.g^{-1}$ *dry*)		
Soft body					
4–5	118.2 ± 6.1	2.2 ± 0.6	4.6 ± 0.6	359.5 ± 12.2	12.0 ± 1.8
5–6	12.8 ± 2.2	2.4 ± 1.2	5.3 ± 2.1	476.1 ± 13.3	11.4 ± 2.3
6–7	16.4 ± 1.1	2.9 ± 1.2	4.3 ± 0.5	516.9 ± 12.4	11.3 ± 1.7
7–8	30.6 ± 3.5	2.3 ± 1.7	2.3 ± 0.6	544.2 ± 10.4	10.3 ± 1.1
Shell					
7–8	152.9 ± 6.1	6.4 ± 2.1	4.4 ± 1.0	14.7 ± 1.0	30.4 ± 1.9
			($\mu g.ml^{-1}$)		
Water					
	0.226 ± 0.0103	0.009 ± 0.0003	0.0079 ± 0.0006	0.0219 ± 0.0020	0.0157 ± 0.0009
			Thirumanjana river (Station II)		
			($\mu g.g^{-1}$ *dry*)		
Soft body					
4–5	105.4 ± 2.8	2.2 ± 1.1	4.7 ± 0.6	250.0 ± 0.6	6.4 ± 3.7
5–6	41.1 ± 2.1	2.1 ± 1.1	7.9 ± 0.6	327.0 ± 10.6	10.7 ± 2.1
6–7	16.2 ± 0.5	3.8 ± 0.8	6.7 ± 1.0	373.0 ± 8.9	10.8 ± 2.2
7–8	6.5 ± 0.7	2.3 ± 0.6	5.8 ± 0.6	383.0 ± 7.2	9.2 ± 5.1
Shell					
7–8	141.6 ± 3.9	6.5 ± 2.1	4.0 ± 2.1	16.3 ± 2.3	36.0 ± 3.4
			($\mu g.ml^{-1}$)		
Water					
	0.0310 ± 0.0004	0.0033 ± 0.0008	0.0031 ± 0.0003	0.0125 ± 0.0020	0.0097 ± 0.0004

Mn > Zn > Pb > Ni > Cu for both stations. Kudamurutti river water has a higher concentration for all heavy metals than Thirumanjana river water.

Table I shows the relationship between the size of the mussels and concentrations of metals in soft body and shells. It was observed that the relative concentrations of different metals in soft body was similar at both stations and the sequence of metals in descending concentrations was as follows: Zn > Mn > Pb > Cu > Ni. This pattern differs from the concentration series of metals found in the water samples. A positive correlation was evident between the size of mussels and the concentration of zinc in the soft body. However, manganese showed a reverse trend. Small sized (4–5 cm) mussels contained more manganese (105.4 $\mu g.g^{-1}$ dry wt.) than larger specimens 7–8 cm; 6.5 $\mu g.g^{-1}$ dry wt.).

The pattern of concentration of metals like nickel, copper and lead did not show any appreciable correlation with body size. Both young and old specimens tend to contain nickel, copper and lead at about the same level in their soft body. It is interesting to notice that, although Kudamurutti river water (Station I) revealed a higher concentration of metals than the Thirumanjana river water (Station II), the concentrations of the metals in mussel tissues from both stations were virtually identical. The shells of the organisms contained a larger quantity of

Table II Concentrations of metals in the various tissues of *L. marginalis* (7–8 cm) from Kudamurutti river (Station I).

Tissues	*Mn*	*Ni*	*Cu*	*Zn*	*Pb*
			($\mu g.g^{-1}$ *dry*)		
Digestive glands	286.0 ± 4.80	4.5 ± 2.63	5.9 ± 1.11	954.3 ± 7.40	23.6 ± 1.11
Gill	176.0 ± 6.57	3.8 ± 0.63	3.5 ± 0.70	682.6 ± 6.58	21.4 ± 1.30
Mantle	45.5 ± 9.94	1.9 ± 0.63	3.2 ± 0.64	575.9 ± 7.94	22.3 ± 1.31
Viscera	45.7 ± 0.59	2.5 ± 0.63	4.4 ± 0.55	434.4 ± 11.31	9.6 ± 3.00

Table III Concentration factors for heavy metals in soft-body and shell of *L. marginalis* (Dry weight basis).

Size group (*cm*)	*Mn*	*Ni*	*Cu*	*Zn*	*Pb*
	Kudamuritti river (Station I)				
Soft-body					
4–5	523	246	580	16414	765
5–6	57	263	673	26307	723
6–7	72	332	547	23601	721
7–8	135	253	289	24850	658
Shell					
7–8	677	711	557	671	1936
	Thirumanjana river (Station II)				
Soft-body					
4–5	34004	649	1510	20055	662
5–6	13258	649	2563	26323	1104
6–7	52387	775	2158	29903	1116
7–8	2097	685	1879	30638	952
Shell					
7–8	4568	1970	1290	11328	3711

Table IV concentration factors for heavy metals in different tissues of *L. marginalis* (Dry weight basis).

Tissues	*Mn*	*Ni*	*Cu*	*Zn*	*Pb*
Digestive glands	1266	496	747	43574	1503
Gill	779	419	438	31170	1361
Mantle	2013	216	399	26297	1423
Viscera	202	275	562	19835	611

manganese, nickel and lead. However, more lead was found than manganese and nickel in shell. The descending series of concentration in shells is Mn > Pb > Zn > Ni > Cu.

The concentrations of heavy metals in the digestive glands, gills, mantle and viscera of *L. marginalis* for Station I are presented in Table II. Values for concentration factors i.e. $\frac{\mu g.g^{-1}\,dry}{\mu g.ml^{-1}}$ for the heavy metals in soft body and shell are shown in Table III and in various tissues in Table IV.

DISCUSSION

The results of this study provide baseline data on metal levels in two important tributaries of the Cauvery river. This monitoring of levels of contaminants will be useful in the assessment of future environmental contamination. Nair (1984) observed that metal concentrations were higher in river water than in ocean water and the metals intorduced into the sea by river discharge would be considerably reduced in concentration by precipitation, absorption and bioaccumulation. Concentrations in the water of the metals at both stations in the descending series would be: Mn > Zn > Pb > Ni > Cu. Station I exhibited higher concentrations of all these metals than did Station II. Location of the station I on Kudamurutti river is below the discharge of effluents from dyeing industries and of large quantities of domestic sewage.

In the soft body of the mussels from the two stations studied, the concentration of the metals was in the descending order of concentration: Zn > Mn > Pb > Cu > Ni for all size groups.

Zinc, manganese and lead were the metals concentrated in larger quantities. The reasons for this was the higher concentrations of these metals in the ambient water and also the greater ability of the *L. marginalis* to concentrate them in the soft body. Similar high concentrations of zinc, manganese and lead are reported by several workers (Harrison and Quinn, 1972; Watling and Watling, 1976; Forester, 1980; Schmitt and Finger, 1982). Havlik and Marking (1987) point out that manganese and lead are not known to be toxic to freshwater bivalves (Naiades or Unionidae). Zinc is toxic to these bivalves only at high concentrations: LC_{50} 66 $mg.l^{-1}$ in 336 h exposure (Millington and Walker, 1983). Hence the higher concentrations of zinc, manganese and lead found in the soft body of *L. marginalis* was not harmful. Among these 3 heavy metals the concentration of zinc was greatest. This could be partly due to the natural requirement for this metal by the organism since zinc is an essential metal acting as an enzyme

activator. It is a constituent of several important metalloprotein enzymes such as carbonic anhydrase and carboxypeptidase. The greater concentration of zinc in the soft body of the mussel from Station I would show that at this site organisms are more exposed to a variety of contaminating inputs such as effluents from dyeing works, municipal sewage discharges and the input of drainage from agricultural fields.

Manganese appears to be rapidly taken up in the soft tissues. A high concentration of manganese (>18000 ppm) in the gill did not affect the animal (Johnson *et al.*, 1966). Bradley (1910) pointed out that species of the group Naiades were found in beds where manganiferous materials were present, perhaps implying that manganese is an essential element for these organisms. However, no explanation has been given for the high levels of manganese found in tissues (Havlik and Marking, 1987). In the present study, the ambient water concentration of manganese was higher at Station I. However, a corresponding higher tissue concentration was not observed in samples taken at this station due to the presence of non-ionic manganese. Merlini *et al.*, (1965) and Merlini (1966) reported that the concentration of the manganese in an animal depends on the availability of manganese in the ionic form Mn^{++} which is an absorbable form in living systems. This would explain the higher concentration of manganese found at both stations.

The concentration factors for the different metals in *L. marginalis* indicate a higher range of values (Table III) for all five metals in mussels collected from Thirumanjana river (Station II). This was due to lower concentrations of the metals in ambient water at Station II. The concentration factor for zinc ranged from 16,000 to 31,000, for manganese 52,000 to 57,000, and for lead from 600 to 1100. The concentration factor for copper is higher (300 to 2500) than for nickel (250 to 800).

The concentration of zinc in the soft body of *L. marginalis* increases with increase in the size of the mussel. This could be due to an increasing demand for zinc for cellular physiological functions. In the case of manganese, a reverse trend is seen. As stated earlier, young mussels were found to have manganese at a higher concentration than older individuals. Watling and Watling (1976), Rajendran and Kurian (1986) and Havlik and Marking (1987) reported that small-size organisms are often able to concentrate metals more than larger size individuals. No definite relationship could be established between mussel size and concentration rate of lead, copper and nickel.

The nature of accumulation of trace metals by bivalves is dependent on chemical speciation of the metals which can vary from site to site (Zamuda and Sunda, 1982). According to Galtsoff (1964), in addition metal concentrations in bivalves depend greatly on body weight and the reproductive stage of individuals.

It is interesting to note that there was a greater concentration of lead in shell (10.4–36.2 $\mu g.g^{-1}$ dry wt.) than in soft body (6.4–12.0 $\mu g.g^{-1}$ dry wt). Merlini *et al.*, (1965) reported a similar pattern in the freshwater mussel *Unio mancus* and Wesley and Sanjeevaraj (1983) in the marine mussel *Perna viridis*. The higher concentration of lead in shell may be linked to its capacity to replace calcium (Pillai, 1985). The results also indicate that the shell of *L. marginalis* tends to concentrate a large quantity of manganese, lead and nickel whereas the soft body concentrates more zinc and copper. Therefore, it is suggested that the shell of

mussels could serve as a good indicator of exposure to manganese, lead, and nickel, whereas the soft body could indicate exposure to zinc and copper. Concentrations of metals in soft body tissues are, however, subject to depuration and highly variable due to several endogenous and exogeneous factors. Such changes do not affect accumulation of metals in the shell. Hence it is suggested that residues in the soft body indicate recent or current exposure while residues in the shells indicate cumulative past exposure.

Measurement of metal concentrations in digestive glands, gills, mantle and viscera reveal a differential bio-accumulation ability of the organs. Invariably, digestive glands have the greatest ability to concentrate all the metals studied. After digestive glands, gills accumulate manganese, zinc and nickel to higher concentrations than other tissues, while mantle viscera showed a greater affinity for lead and copper. In a filter feeding organism like the mussel, the gills, and mucus secreted by them, play a vital role in the feeding process. The mucus has been suggested as the substance responsible for sequestering both particulate and soluble forms of metals from solution (Brooks and Rumsby, 1965; Romeril, 1971; Pentreath, 1973). Metallic ions may become adsorbed to the mucus and so may be passed to the mouth and then to the digestive glands. Digestive glands in bivalves play an active role in the intracellular digestion and adsorption of food (Morton, 1983). The physiological activity of the digestive glands also enhances the capacity for accumulation of metals from the water and sediment interface. In the literature a higher concentration of heavy metals is reported in benthic molluscs due to this intrinsic ability of digestive glands and gills (Segar *et al.*, 1971; Pentreath, 1973; Wesley and Sanjeevaraj, 1983). Since the mantle secretes the shell, a higher lead accumulation in mantle suggests a pathway of lead transfer from mantle to shell.

ACKNOWLEDGEMENTS

The authors express their gratitude and thanks to Dr. M. A. R. Iyengar, Head, Environmental Survey Laboratory, Department of Atomic Energy, Kalpakkam and Mr. V. Kannan for technical assistance for estimation of heavy metals by atomic absorption spectrophotometer and to Dr. K. Krishnamurthy, Professor of Marine Biology, Centre of Advanced Studies in Marine Biology, Parangipettai for critical review of the manuscript.

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