

Quantization of specific trace metals in bivalve, *Villorita Cyprinoides Var Cochinesis* in the Cochin estuary

Ranjitha Raveenderan & Sujatha C.H.*

Department of Chemical Oceanography, CUSAT, Kochi-682016, Kerala, India.

[E.mail: drchsujatha@yahoo.co.in]

Received 10 November 2009; revised 04 March 2010

Present study consists the quantization of specific metals-- Cr, Cd, Pb, Zn and Cu observed in the experimental bivalve, *Villorita* species. Bivalve specimens were collected seasonally from the identified three hot spots of Vembanad Lake. Soft tissue concentrations of metals are very sensitive in reflecting changes in the ambient environment and hence important in assessing the environmental quality. Concentrations of Zn in bivalves were fairly high compared to other metals. All the stations showed a maximum concentration during premonsoon and minimum during the other two seasons. Levels of Pb, Cu, Zn, Cd and Cr are between 0-6.17mg/kg, 0-17.224mg/kg, 1.916-255.163mg/kg, 0.325-4.133mg/kg, and 0-15.233mg/kg respectively.

[**Keywords:** Clams, Vembanad, Lake, Environment, Contaminants, Sentinel organisms, Pollution, Monitors.]

Introduction

Vembanad lake had brought to light on various environmental factors related to both "white clam" and black clam fishery^{1,2}. Trace metals, the persistent inorganic chemicals create environmental disturbances. These metals comprise of non-essential-Cadmium and Lead that can be toxic even at trace levels; biologically essential elements-Copper and Zinc, which might cause toxic effects at elevated concentrations. Under certain environmental condition these could accumulate to a toxic level and cause ecological damage³. Generally, the concentration of metal in marine organism is many times greater than the concentration of metal in the surrounding seawater. Several monitoring programmes were recommended the use of filter feeder mollusk, as indicator of pollution and D.J.H.Philip (1980) strongly supports this finding and conducted a number of similar studies choosing mollusk as a biological indicator of metal pollution⁴.

An attempt had been made in the present study to quantify the biologically available levels of conservative contaminants - the heavy metals in the aquatic ecosystem by the indigenous black clam-*Villorita Cyprinoides Var Cochinesis* (Fig-1). The clam, *Villorita*^{5,6} exclusively sustained the molluscan

fishery of the Cochin backwaters. Similar studies were also carried out by several workers in different countries on the levels of trace metals in seawater by choosing marine organisms as the sentinel species to find out their effects on human beings^{7,8,9,10}.

Major production centers of clam in Kerala are the Vembanad and Ashtamudi Lakes. The total landing of *Villorita* species from estuary has nearly 1300 tones⁶. This is a cheap source of protein rich food. Thousands of families are depending on this species for their living. This species has been found to offer most of the features of a biological indicator such as non-migrant, long life, reasonable size, easy sampling, fairly abundant distribution, tolerance to brackish waters and ability to concentrate numerous pollutants^{7,11}. By understanding the concentration levels of these trace metals by the indicator species we can describe the nature and the extent of pollution status at these sampling sites.

Villorita cyprinoids Var Cochinesis occurs abundantly in the upper reaches of the estuarine backwaters of Kerala. They form vast beds near the farthest end of these backwaters. Griffith and Pidgeon introduced the name *Villorita* for the first time in 1833 and Hanley named *Villorita Cyprinoides Var Cochinesis* in 1866¹². Shell is sculptured with concentric ridges. The animal is commonly seen in the fresh water end of the estuarine waters. Since these animals inhabit unstable saline environments,

*Correspondence



Fig. 1—Villorita Cyprinoides Var Cochinesis

they are capable of tolerating wide salinity changes (2-10‰). They detect the presence of both known and unknown contaminants and also provide a temporally and spatially integrated measure of bioavailable pollutants. They can either detect exposure to and the toxic effects of parent compounds or integrate the toxicological interactions of mixtures of contaminants. The smaller size group has wider tolerance range than larger size groups; length range 10-42mm. Peak modes are 18-22mm and 24-28mm, of which the former is the dominant size group for the entire year¹³. They prefer sandy substratum with little amount of silt. These clams are used for human consumption apart from being used as an ingredient of prawn feed. The shell of this animal is widely used as a raw material for industries like cement and lime. This species have a peak spawning season in the monsoon i.e., from late May to August-September when salinity and temperature are considerably low. In addition to these characteristics they can act as short-term predators of long-term ecological effects through interactions into a suite of measurements and understanding at different levels of contaminations.

Materials and Methods

Villorita cyprinoides Var Cochinesis moderately large, thick, ovately triangular, inflated oblique shell, swollen in the umbonal region and in the middle region (Fig. 1). Present study were conducted between May 2007 and January 2008 at three prominent locations in the estuary. The following sites were identified as sampling locations where the experimental animals are widely distributed (Fig. 2). Besides, the general physico-chemical parameters were also analyzed. The chosen sites recorded both the physical and chemical parameters within the tolerance limit (Table 1). 1. Anchuthuruthu 2. Perumbalam 3. Champakara canal.

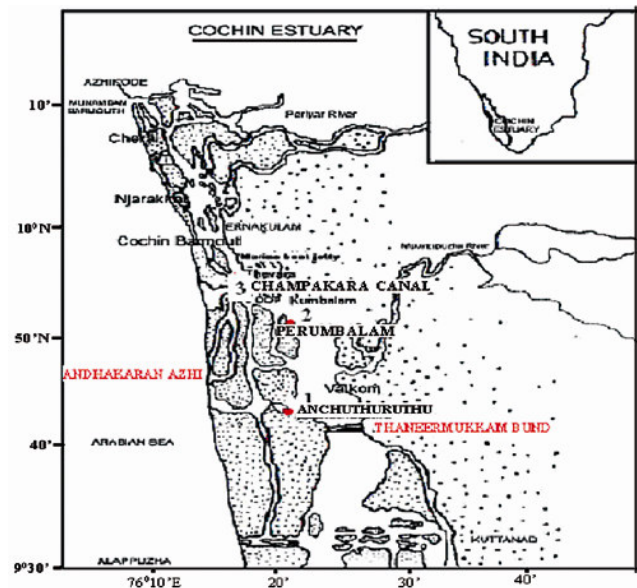


Fig. 2—Vembanad Lake

As a part of Vembanad lake stations 1 and 2 lies in the northwest part of Alappuzha District and station 3 in the Ernakulam District. During monsoon the stations exhibit riverine characteristics and as the season advances to post and premonsoon, estuarine condition prevails. Numerous irrigation channels, carrying discharges from various industrial and sewage outfalls are present in these areas.

The whole tissues from each location were carefully removed by shelling the bivalves with plastic knife adopted by the method of Chin *et al* 2000. The samples were dried and soft tissues were powdered. A definite weight of the powdered sample was weighed, digested with 15-20ml concentrated HNO₃ at 100°C till the sample become colourless. Additional HNO₃ was added if the sample was charred. Towards the end 1ml perchloric acid was added and brought near dryness. The solutions were then cooled and made up to 25ml using double distilled water. The blank were run with each set.

Table 1—Concentration of various Hydrographical Parameters

Site	pH			EC (mmho)			TDS (mg/l)			Alkalinity (mg/l)			Total Hardness (mg/l)		
	P	M	PO	P	M	PO	P	M	PO	P	M	PO	P	M	PO
S1	7.73	6.73	7.2	1700	9	1800	1020	5.4	1080	31.8	10.6	31.8	1880	20	1800
S2	8.09	6.6	7.1	1500	9	1900	900	5.4	1140	31.8	21.2	42.4	1620	20	2200
S3	7.03	6.8	7	180	12	2500	108	7.2	1500	31.8	21.2	53	235	25	2660
B1	7.70	6.9	7.3	2500	30	2300	1500	18	1380	53	10.6	53	2840	45	2440
B2	7.66	6.8	7.2	1800	12	2100	1080	7.2	1260	42.4	21.2	42.4	1900	20	2160
B3	7.15	6.9	7.0	300	15	2900	180	9	1740	42.4	10.6	63.6	340	25	3200

Table 2—Concentration of various Hydrographical Parameters

Site	DO (mg/l)			Salinity (psu)		
	P	M	PO	P	M	PO
S1	6.54	4.592	4.46	1.2774	0.0476	11.335
S2	7.448	4.704	4.896	0.863	0.0422	14.376
S3	3.04	2.24	3.024	1.0876	0.0301	16.001
B1	5.776	5.488	3.6	1.4684	0.192	14.0333
B2	7.752	5.376	4.464	1.0016	0.0487	13.194
B3	3.496	1.904	2.592	1.8887	0.0362	18.031

P-Premonsoon, M-Monsoon, PO-Post monsoon, S-Surface, B-Bottom.

The samples were analyzed using Atomic Absorption Spectrophotometer. The metal concentrations were expressed in mg/kg.

Results and Discussion

Previous experimental studies have showed that bivalves are selected as bioaccumulators of heavy metals in a considerable level of heavy metals. Factors known to influence metal concentration and accumulation in these organisms include metal availability, season of sampling, hydrodynamics of the environment, size, sex, change in tissue composition, reproductive cycle and anthropogenic activities like fish landing, tourism, fishing vessels etc¹⁴.

All the physico-chemical parameters lie within the tolerance limits, whereas salinity and DO show some variations (Table 2). Salinity ranged between 0.0301-18.031psu and the high values were found during post monsoon and the metal uptakes found to be less, whereas DO showed within the threshold limit 1.904-7.752mg/l. The distribution of DO is found to be inversely related to salinity. The lower DO values in the estuarine sites may be attributed to the low solubility of oxygen in saline waters and supports with the earlier studies conducted by Sankaranarayanan and Panampunnayil, 1979¹⁵.

Soft tissue concentrations of metals are very sensitive in reflecting changes in the ambient environment and hence important in assessing the environmental quality. The variations in concentration levels of metals are shown in (Fig. 3). The concentration of Pb, Cu, Zn, Cd and Cr is between 0-6.174mg/kg, 0-17.224mg/kg, 1.916-255.163mg/kg,

0.325-4.133mg/kg, and 0-15.233mg/kg respectively. Concentration observed in the tissue exhibited wide variation with respect to sampling site. High metal concentration observed at all stations during premonsoon especially in station 3. Among the metals Zn showed higher metal concentration. The major source for the metal contamination is the industrial effluents near Cochin. The increased monsoonal supply of materials together with previously deposited sediments through the Cochin backwaters is the reason for the increased metal contamination¹⁶.

The concentration of Pb shows maximum during premonsoon 6.174, 2.025 and 2.705mg/kg in Stations 1, 2 and 3 respectively. The presence of Pb is not detected in Stations 2 and 3 during post monsoon. Aquatic pollution of Pb can be mostly traced to its use in gasoline, in smelting, in refining, in recycling etc. It is a non-essential trace element whose presence even at low concentration in the biological system is extremely harmful. Toxicity of Pb is mainly due to its chemical nature, which makes it difficult to be removed once it enters the system. Similar accumulation and distribution studies of Pb in the aquatic environment have been widely studied^{17,18,19,20,21}.

Cu is essential trace element necessary for many metabolic processes. Despite being an essential element, Cu could provide to be toxic at elevated concentration. It shows maximum during premonsoon 17.224, 7.730 and 16.363mg/kg in stations 1, 2 and 3 respectively and minimum during post monsoon 0.013, 0 and 0.263mg/kg in stations 1, 2 and 3. These differences were not wholly explainable on the

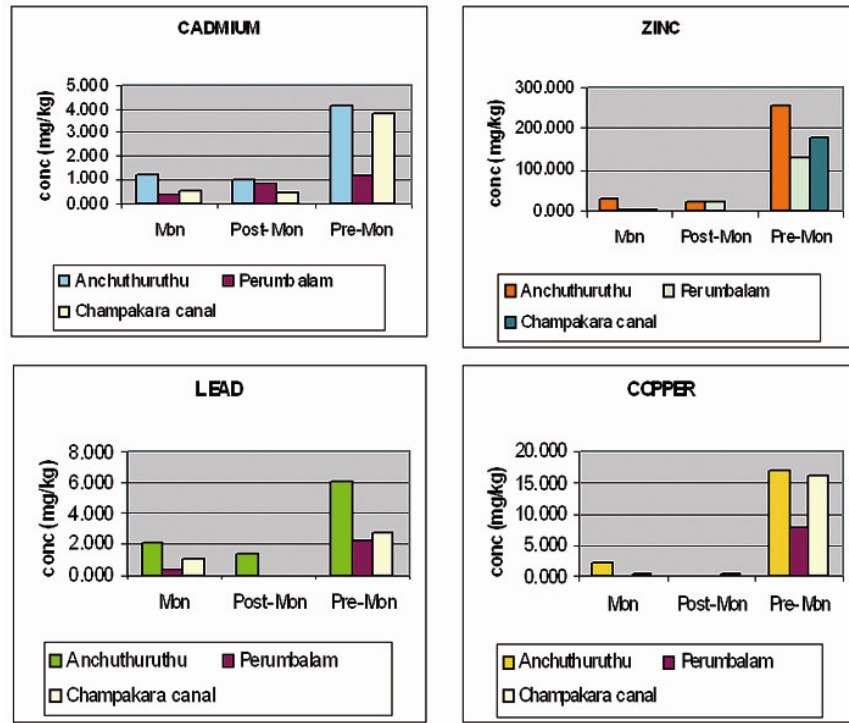


Fig. 3—Graphical representation of metal concentration

basis of temperature regime. Cu is more readily bioaccumulated when present as the free cupric ion.

The concentrations of Zn in bivalves were fairly high compared to other metals. All the stations show maximum concentration during premonsoon 255.163, 126.699 and 179.900mg/kg in station 1, 2 and 3 respectively and minimum 18.34, 25.030, 1.916mg/kg during post monsoon in stations 1, 2 and 3 respectively and the monsoonal lies between these two seasons (31.963, 3.316, 3.340mg/kg). Zn is essential trace element in living systems for the normal cell differentiation and growth. It forms an essential part of a number of metalloenzymes and a co-factor for regulating the activity of zinc specific enzymes²². It also acts as a structural component in many enzymes taking part in the energy metabolism. Earlier studies by Kiekens²³ shows deficiency of Zn can result in severe growth depression, skin scratch and sexual immaturity. But prolonged exposure to sub lethal concentration of Zn could cause extensive edema and necrosis of liver tissues²². Several authors do investigations on the accumulation of metals by marine organisms, which support our results^{24,25,26,27}.

Mollusk have the ability to accumulate Cd to the great extend. All the station shows maximum values in premonsoon 4.133, 1.184 and 3.863mg/kg in station 1, 2 and 3 respectively. Minimum value in post

monsoon 1.025, 0.813, 0.438mg/kg and the values noticed in monsoon lies between other two seasons 1.226, 0.325, 0.525mg/kg in stations 1, 2 and 3 respectively. Cd is a highly toxic non-essential metal. Its chemical resemblance to Zn enables it to mimic the essential element Zn in its metabolic functions. Several studies have been carried out on the accretion of Cd by organisms^{25,26,27,28,29,30}. Peak value of Cr noticed during premonsoon 15.233, 6.735 and 5.863mg/kg in station 1, 2 and 3 respectively and minimum value (1.651mg/kg and not detected in station 2 and 3) during monsoon. Cr compounds frequently encountered as environmental pollutants and have been known to produce toxic, mutagenic and carcinogenic effects in biological system although Cr is an essential metal in glucose metabolism, playing a cofactor role in insulin action³¹. Hexavalent Cr is also one of the substances whose use is restricted by the European Restriction of Hazardous Substances directive³².

The study outweighs the following findings:

- Specimens were obtained from station 3 is considered to be relatively population free zone when compared to other two stations.
- Maximum values were detected during premonsoon.

- When compared to other stations, station 1 showed maximum value.
- The relative abundance of metal studied in animal tissue, in the order,
- Zn > Cu > Cr > Pb > Cd.
- The animal is most sensitive to Zn and least sensitive to Cd.

Conclusion

Toxicity is not only influenced by the intrinsic toxicity of the element, but also by its availability as determined by occurrence, complexation of other chemical reactions and absorption potential. The findings show that the mollusk can be used as more responsive bioindicator of pollution. Besides these Chemo toxicity can be preferred as a model for assessing metal pollution of the CES to a great extent.

References

- 1 Rasalam, E.J and Sebastian, M.J (1976). The lime shellfisheries of Vembanad Lake, Kerala. J. Marine Biological Association of India, 18 (2), pp. 323-355.
- 2 Appukuttan, K.K and Lakxmilatha, K.K (2002). A review of Black Clam (*Villorita Cyprinoides*) fishery of Vembanad Lake. Indian J. Fisheries, 49 (1) pp. 85-91.
- 3 Bryan, G.W and Hammerstone, L.G (1978). Heavy metals in the burrowing bivalve *Scrobicularia plana* from contaminated and non-contaminated estuaries. *J.Mar.Biol.Ass. U.K.*, 58:401-419.
- 4 David J.H.Philips (1980). Quantitative aquatic biological Indicators, Applied Science Publishers Ltd, London.
- 5 Kurup, B.M., J.Sebastian, T.M Sankaran and P.Rabindranath, 1990c. Exploited fishery resources of the Vembanad Lake. Part III, Clam fisheries. *Mahasagar*, 23:127-137.
- 6 Arun, A.U. (2002). Biology, Experimental culture and toxicity studies of *Villorita cyprinoids* in Cochin Estuary. PhD Thesis, Cochin University of Science and Technology.
- 7 Babukutty, Y (1991). Studies on intercompartmental exchange of trace metals in an estuarine system. PhD Thesis. Cochin University, Cochin.
- 8 Sujatha, C.H., S.M. Nair, and J. Chacko (1995). Tissues lipid levels of the clam, *Villorita Cyprinoides* Var *Cochinensis* following exposure to endosulphan, malathion and methyl parathion. *Envir.Toxicol. Wat.Qual.* 10:231-235.
- 9 Karthikeyan, P. (1995). Competitive interactions of Aluminium ions in modifying trace metal availability to an estuarine clam. PhD Thesis, Department of Chemical Oceanography, Cochin University, Cochin.
- 10 Sujatha, C.H., S.M. Nair, and J. Chacko (1995). Pesticide induced physico logical change in an estuarine clam. *Oebalia*. XXI: 181-186.
- 11 Sujatha, C.H., S.M. Nair, and J. Chacko (1995). TBTO induced physiological and biochemical changes in a tropical estuarine clam. *Bull.Environ.Contam.Toxicol.* 56: 303-310.
- 12 Vinu Chandran R. (2002) Intracellular Osmoregulation in the estuarine mollusk, *Villorita Cyprinoides* Var *Cochinensis*. PhD Thesis, Cochin University, Cochin.
- 13 Dr.Ravindran K, Dr.Appukuttan K.K, Dr.Sivasankarapillai V.N and Dr.Boopendranath M.R. (2006). Report of the committee of experts on ecological and environmental impacts of dredging at vaduthala kayal and vaikom kayal, Kerala. Govt. of Kerala.
- 14 Lakshmanan, P.T and Nambisan, P.N.K (1983). Seasonal variations in trace metal content in bivalve mollusk, *Villorita Cyprinoides* Var *Cochinensis* (Hanley), *Meretrix casta* (Chemnitz) and *Perna Virdis* (Linnaeus). *Ind.J.Mar.Sci.* 12:2,100-103.
- 15 Sankaranarayanan V.S., and S.V.Panampunnayil, (1979). Studies on Organic Carbon, Nitrogen and Phosphorus in Sediments of Cochin Backwaters. *Indian Journal of Marine Sciences* 8: 27-30.
- 16 P.S.Harikumar., U.P.Nazir., and M.P.Mujeebu Rahman. (2009). Distribution of heavy metals in the core sediments of a tropical wetland system. *Int. J. Environ. Sci. Tech.*, 6 (2), 225-232, Spring 2009.
- 17 Denton, G.R.W and Burdon-Jones, C. (1981). Influence of temperature and salinity on the uptake, distribution of mercury, cadmium and lead by the black lip Oyster *Saccostrea Echinata*. *Mar.Biol.* 64:317-326.
- 18 Lakshmanan, P.T (1982). Investigations on the chemical constituents and trace metal interactions in some bivalve mollusks of the Cochin backwaters. Phd Thesis. Cochin University, Cochin.
- 19 Ajmal, M.Raziuddin and Khan, A.U (1987). Heavy metals in water, sediments, fish and plants of river Hindon. U.P., India. *Hydrobiologia*, 148:151-157.
- 20 Malm,O., Pfeiffer, W.C., Fiszman, M and Azcme, J.M(1988). Transport and availability of heavy metals in the Paraiba Dosul-Guandu river system, Rio de Janeiro state, Brazil. *Sci.Tot.Environ.*, 75:210-209.
- 21 Lakshmanan, P.T and Nambisan, P.N.K (1989). Bioaccumulation and depuration of some trace metals in the mussel, *Perna Virdis* (Linnaeus). *Bull.Environ.Toxicol.*43: 131-138.
- 22 Leland, H.V and Kuwabara, J.D (1985). Trace metals. In: Rand, G.M and Petrocelli, S.R (eds). *Fundamentals of Aquatic Toxicology*. Hemisphere Publishing Corporation, Washington.
- 23 Kiekens, L. (1990). Zinc. In: Alloway, J.B (ed). *Heavy metals in soils. Blackie and Sons Ltd*, London. 261-279.
- 24 Styron, C.E., Hagon, T.M., Campbell, D.R., Harvin, J., Whitten Burg, N.K., Baughman, G.A., Bransford, M.E., Saunders, W.H., Williams, D.C., Woodle, C., Dixon, N.K and Mc Neil, C.R (1976). Effects of temperature and salinity on growth and uptake of ⁶⁵Zn and ¹³⁷Cs for six marine algae. *J. Mar.Biol.Ass. U.K.*, 56:13-20.
- 25 Ahsanullah, M., Negilski, D.S and Mobley, M.C (1981). Toxicity of Zinc, Cadmium and Copper to the shrimp *Callinassa australiensis*. III, accumulation of metals. *Mar.Biol.* 64:311-316.
- 26 Amiard-triquet, C., Berthet, B and Metayer, C(1986). Contribution to the ecotoxicological study of Cd, Cu and Zn in the mussel *Mytilus Edulis*.II. Experimental Study. *Mar.Biol.* 92:7-13.
- 27 Chan, H. (1988). Accumulation and tolerance to Cadmium, Copper, Lead and Zinc by the green mussel *Perna Virdis*. *Mar.Ecol.Prog.Ser.* 48:295-303.
- 28 Amiard, J.C., Amiard-triquet, C., Berthet, B and Metayer, C., (1987). Comparative study of the patterns of bioaccumulation of essential (Cu, Zn) and non essential (Cd, Pb) trace metals in various estuarine and coastal organisms. *J.Exp.Mar.Biol.Ecoll.* 106:73-89.

- 29 Giles, M.A (1988). Accumulation of cadmium by rainbow trout *salmo gairdneri* during extended exposure. *Can. J.Fish.Aquat.Sci.* 45:1045-1053.
- 30 Marigomez, J.A and Ireland, M.P (1989). Accumulation, distribution and loss of cadmium in the marine prosobranch *littorina littorea*. *Sci.Tot.Environ.*78: 1-12.
- 31 Debetto P, Arslan P, and Luciani S (1988a) Uptake of chromate by ray thymocytes and role of glutathione in its cytoplasmic reduction. *Xenobiotics* 18: 657-664.
- 32 Parlak, S. Katalay, and B. Büyükisik. Accumulation and Loss of Chromium by Mussels (*M. galloprovincialis*) H. *Bull. Environ. Contam. Toxicol.* (1999) 62:286-292.