A PRELIMINARY STUDY FOR ESTABLISHING *PERNA VIRIDIS* (MOLLUSCA: BIVALVIA) AS A BIOLOGICAL MONITOR FOR POLLUTION IN KARACHI COASTAL WATER

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ABSTRACT: Adverse effects of toxic substances on the environmental quality have become a subject of concern in recent years. Toxicity of heavy metals has never been in dispute and therefore their presence in our natural environment is undesirable. This study was undertaken to establish the capability of *Perna viridis* as a monitor for pollution in the Manora channel. Accumulation of Zinc, Copper, Iron and Manganese by marine mussels, sampled from Manora channel, was determined. Metal load varied markedly between individuals from the same populatin. This variability was partly accounted for systematic relationship between metal load and body weight and age. The distribution of metal between the major organs was considered, but the analysis of separate organs showed no advantage for their use as a biological monitor. comparison between Iron, Manganese, Copper and Zinc concentration in ambient sea water and in the mussel showed no clear correspondence. The results suggest that the mussel is capable of acting as a biological monitor, although may not be a good short term monitor of Iron, Manganese, Zinc and Copper. It may have potential as a long term and site comparison monitor for metals, once inherent variability is taken into account.

KEY WORDS: *Perna viridis* - Biological monitor - Pollution - Karachi coastal water.

INTRODUCTION

There is a considerable interest in the use of organisms as biological monitor for heavy metals. Water, sediments and living organisms have been widely targeted for investigating pollution of environment. The use of a living organism as a biological monitor ideally obviates the necessity of multiple sampling because of its ability to accumulate metal contents present in the surrounding environment. Contrary to that metal concentrations in sediments and water are prone to variation in depth, locality or the time of sampling. The metal concentration in organisms may be 10^3 to 10^6 times higher than the surrounding environment and thus it is possible to proceed with direct analysis without preconcentration. Biological monitoring also provides a direct picture of pollutant's bioavailability. Thus an ideal monitor could be used firstly to identify area of pollution and secondly to monitor the progress of counter pollution measures. A wide variety of marine organisms have been shown to reflect, through investigations on their metal accumulation, the extent of contamination of coastal waters inhabited by them. Some of them are worth mentioning; Ahmed, M. 1977; Ahmed, M. and S.H.N. Rizvi, 1981; Ayling, 1974; Boyden, 1973, 74; Bryan, 1973; Bryan *et al.*, 1977; Chan, 1988; Cheung, *et al.*, 1992; Luis, *et al.*, 1989; Chow, *et al.*, 1976; Mackay *et al.*, 1975; Martincic, *et al.*, 1984; Mathews, R. *et al.*, 1984; Palmer, S.J. *et al.*, 1993; Philips, D.J.H. *et al.*, 1988; Philips, 1978; Philips and D.A. Seagar, 1986 and Saegar *et al.*, 1971.

The investigation reported here is based upon the estimation of heavy metals Cu, Zn, Fe and Mn in the mollusc *Perna viridis* inhabiting Manora Channel, Karachi.

The prime objects of the study have been:
1. To assess the practical potential of *Perna viridis* as a monitor for iron, copper, zinc and manganese.
2. To investigate the variability of metal concentration among individual mussels of different ages and body weight within a population.
3. To examine the variability caused by gut material.
4. Discover the advantages of separate organ analysis for monitoring.
5. Determine the response of the mussel to the changing ambient concentration of metals.

The reason for the selection of *Perna viridis* as a biological model for monitoring the pollution are: a) its abundance and easy accessibility b) long life span c) sedentary way of life which prevents them from migrating away from the source of pollution and d) its inhabitance in marine environment which is susceptible to pollution.

MATERIAL AND METHODS

1. Samples

About 150 individuals of *Perna viridis* were collected from Manora Channel, between the period September 1988 to February 1989. Manora Channel was targeted for this sampling because of its being ultimate dump outlet for Karachi's domestic and industrial waste. Samples collected in September were investigated for determining variation in metal content caused by the gut material. The specimens were immediately enclosed in polythene bags following their collection, washed with sea water and subsequently frozen on their arrival in the laboratory.

The samples collected in January and February were analysed for their metal content after they were made to eject all of their undigested food. This was done by keeping them for 48 hours, in aquarium containing filtered sea water, following their collection. Later they were stored in a deep freezer.

2. Age Determination

Length frequency plot method was employed to ascertain the age of *Perna viridis* collected for this study. (Bayne, B.L. 1976).

3. Equipment and the Reagent

Parkin Elmer model 2380 Atomic Absorption spectrophotometer was employed for analysing all the metals reported herein. All the reagents used for digesting samples were of BDS A R grade quality. Doubly distilled water was used whenever needed.

4. Homogenates

Frozen animal was first defrosted and then weighed. Subsequently it was removed from its shell and dried keeping it for 48 hours at 75°C. The weight of the dried animal was then recorded. This was followed by crushing of the dried body by employing a grinder and then the resulting powder was stored in air tight containers. Some of the samples collected in January and February were analysed after dissecting them to obtain various anatomical organs. Organs were the gills, labial palps, mantle, foot, kidney and the visceral mass comprising of the remaining organs. For organs having smaller size, six specimens of same shell length were pooled together to provide sufficient material for analysis.

5. Digestion

Metal analysis was carried out on samples prepared by digesting 0.8 to 1 gm of dry powder of *Perna viridis* in a warm (7:1) mixture of concentrated nitric acid and perchloric acid. The resulting solution was dried at 150°C and crystals thus recovered were dissolved in 10ml 1:1 mixture of 1 molar nitric and hydrochloric acid. This solution was later diluted to 1/5 of its concentration by using doubly distilled water. This diluted sample was then directly aspirated into A.A. Spectrophotometer for the required analysis. (Annon, 1960).

RESULTS AND DISCUSSIONS

Correlation of metal concentration with wet body weight of the animal has always been questionable in this study. Metal concentrations of the animals have been interpreted vis-a-vis their dry weight, as dry weight measurements are easy to manage and also the procurement of dry animal is very convenient.

Variation of metal concentration in the same population

It was observed that the individual animals of the same population had varying amount of metal concentration. Variability of the metal concentration among individuals was estimated by calculating percentage coefficient of variations tabulated in Table 1.

Table 1. Variability of metal concentration of *Perna viridis* for two collections.

Sample	N		Concentration (mg/g dry wt.)			
			Iron	Maganese	Copper	Zinc
January	16	X	232.40	15.67	12.17	208.10
		S.D	190.00	02.59	1.699	45.18
		C.V	81.76	16.56	13.96	21.70
February	24	X	152.56	11.95	13.13	155.80
		S.D	84.19	03.34	02.96	26.69
		C.V	55.18	27.97	22.61	17.13

N=number of mussels in a sample; X=sample average; S.D=standard deviation; C.V=coefficient of variation (%).

This type of variability in metal content has also been demonstrated through a number of other studies (Bryan, 1973; Bryan *et al.*, 1977; Ayling, 1974; Phillips, 1976a,b). Variations of metal content of individuals of the same population of *Perna viridis* is a discouraging factor for its establishment as a bioindicator (Chow *et al.* 1976). However, such variations may be attributed to a systematic variation in factors such as body weight, age and sex (Latouchie and Max, 1982) and these are being taken into consideration in the discussion that follows.

Metal concentration vis body weight

In order to describe metal concentration dependence on the body weight, the same was plotted against body weight on both linear and double logarithmic scales. The corre-

Table 2. Correlation coefficients for plot of *Perna viridis* dry body weight against metal concentration on both linear and double logarithmic scales.

METAL	N	METAL CONCENTRATION linear	BODY WEIGHT Log-Log
IRON:			
January	16	-0.15	-0.20
February	24	-0.41*	-0.43*
MANGANESE:			
January		-0.20	-0.12
February		-0.33*	-0.22
ZINC:			
January		-0.05	0.076
February		-0.54****	-0.58
COPPER:			
January		-0.18	-0.40
February		0.584****	0.41*

*P=0.05; **P=0.02 ***P=0.001 (P represents the probability of falling with in a range).

Table 3. Correlation coefficients for plot of *Perna viridis* age (years) against metal concentration on both linear and double logarithmic scales.

METAL	N	METAL CONCENTRATION linear	BODY WEIGHT Log-Log
IRON:			
January	16	-0.31	-0.384
February	24	-0.27	-0.290
MANGANESE:			
January		-0.43	-0.334
February		-0.23	-0.203
ZINC:			
January		-0.03	0.0925
February		-0.50	-0.4900
COPPER:			
January		-0.24	-0.134
February		0.47	0.468

*P=0.05; **P=0.02 ***P=0.001 (P represents the probability of falling with in a range).

lation coefficient thus determined are shown in Table 2 and 3. January collection have provided non significant values whereas February samples show significant values of the correlation coefficients. January's non significant values may be a result of very small sized samples having only 0.4 to 1.58 gram dry weight. Boyden (1977) found that a range of less than 10 fold usually gave non significant relationship due to the inherent biological variability. Thus the size range involved in January sample is not sufficient for reliable interpretation of the size effect. The metal concentration of the mussel may also change with its age and the partial correlation coefficient estimated for February samples demonstrate that the age factor does influence the weight and metal concentration relationship. Therefore multiple linear regression of metal concentration against age and body weight were calculated. The value of multiple R^2 multiplied by 100 estimates the percentage of variation determined by the multiple regression. The B weighting allows comparison of the relative effect of each independent variable on the dependent variable. These calculations help to understand the variation of metal concentration with body weight, between mussels of different ages (Table 4). Our findings suggest that concentrations of the metals Zinc, Iron and manganese drop systematically with the increasing body weight, in accordance with the previous reports (Boyden, 1974). However copper shows a different pattern and its concentration increases. According to Ayling (1974) this may be due to the selective absorption of particular heavy metal by mollusc.

Table 4. Summary of multiple regression of *Perna viridis* body weight and age against metal concentration for February 1989 collection.

Metal	Multiplier (multiple R)	Parameter	Contribution to Mult. R^2	B weighting	Overall F
Fe	0.1345	W	0.1765	-0.490	1.632
	(0.37)	A	-0.0420	0.146	
Mn	0.1240	W	0.1990	0.600	1.486
	(0.35)	A	-0.0750	0.310	
Zn	0.2950	W	0.2484	-0.460	4.394**
	(0.54)	A	0.0467	-0.093	
Cu	0.2800	W	0.2850	0.540	3.900**
	(0.53)	A	-0.0040	-0.008	

W=dry weight(g); A=age (year's); *P=0.05; **P=0.01; ***P=0.001; number of mussels=24

Variation of metal concentration with age

In order to establish systematic variation of metal concentration with age, these variables were plotted on linear and double logarithmic scales. Results reflect that linear plots provide better demonstration of the systematic variation between these two variables. Though the age and metal concentration relationship is not very plausible, the partial correlation coefficients for the samples collected in February establish that these are influenced by the relationship between body weight and the metal concentration.

These conclusions are amply supported by the multiple, regression analysis of the February collections and it has been shown that iron and manganese concentrations are behaving totally different with age as compared to weight changes, as the age has shown to help the animal to increase its metal load. However copper and Zinc concentrations decrease with age. Previous studies have focussed attention on age factor as compared to weight. Ayling (1974) studied Pacific Oyster *Crassostrea gigas* for its Cadmium, Zinc and Copper concentration and found that these metals enhance their concentration with age. On the other hand Remoril (1974) and McKay *et al.* (1975) claim that concentration of Zinc, Copper and Cadmium drop with age. The drop in metal load with increasing age may be attributed to the fact that growth rate of the mollusc is far beyond the metal accumulation rate of the mollusc.

Role of major organs in metal accumulation

Bivalve molluscs have been shown to contain remarkably higher concentration of trace metals in some organs. for example high levels of both manganese and zinc are found in the kidneys (Bryan, 1973), and lead is concentrated in the gills (Chow *et al.*, 1976). Therefore, it was advantageous to analyse various body organs for their metal load as they may also have additional advantage of less deviations in variability.

Major organs like, labial palp, gills, mantle, foot, kidney and visceral mass consisting of remaining organs, were recovered from January and February, 1989 collections and subsequently analysed for their metal content. High concentration of the different trace metals was observed in individual organ (Table 5). As with the metal load of the whole body there has been a considerable variation between individuals. This variability was measured in terms of percentage coefficient of variation (Tables 6-9). To account for the variability in metal concentration of organs, mussel concentration in individual organs have been plotted against whole body weight and age to establish systematic variation. Variation in metal concentration in the kidney, palp and gill was the most systematic. However, partial correlation coefficient values have not been as high as for whole body values. Hence from point of view of reducing random variability there is no justification for separate organ analysis.

Our investigations on the various body organs of *Perna viridis* have produced similar analytical results as reported previously (Bryan, 1973; Hobden, 1967 and D. Martincic *et al.*, 1984) for other marine mussels, and these are described below.

Kidney

Kidneys are excretory organs and contain higher metal concentration as compared to other body organs. Our data in this respect agrees with the similar studies reported by Martincic *et al.* (1984). Similarly concentration of Copper in kidneys was found totally similar to the values reported by Bryan (1973).

Foot

Metal concentration in the foot is significantly higher, particularly that of iron. As explained by Hobden (1967), the metal concentration is higher in case of recently fed bivalves. In these animals the foot can have iron attached to its external surface, even though the animal was allowed to starve for two to three days prior to its analysis. Martincic *et al.*, (1984) have as well reported that foot is an effective cadmium zinc

accumulator. High metal content in foot of the animal also shows that granules are not the only site of metal accumulation, as suggested by Bryan *et al.* (1977).

Visceral mass

Against the results of Martincic *et al.* (1984) our results show lesser metal concentration in visceral mass.

Table 5. Mean concentration of the metals in the body and major organs of *Perna viridis* for two collections.

Sample		Metal whole body Concentration soft parts (μg/g)	ORGANS ANALYSED				
			Gill+palp	Foot	Mantle	Kidney	visceral mass
January	Fe	232.200	2071.7	4687.3	969.97	4096.4	930.660
1989	Zn	208.100	206.4	182.48	274.01	300.25	137.265
N=16	Cu	12.1700	29.520	24.170	24.590	36.320	11.9600
	Mn	15.6700	102.23	146.52	43.70	208.73	55.5990
February	Fe	1076.27	1076.3	1103.8	608.29	1126.0	367.940
1989	Zn	148.090	148.09	81.960	1955.4	194.27	114.110
N=24	Cu	12.5100	12.510	16.880	14.760	24.995	11.2100
	Mn	37.350	37.350	0.100	117.31	54.034	31.5250

CONCLUSION

Presence of significant concentrations of metals in *Perna viridis* demonstrates the aptitude of this bivalve to accumulate environmental accesses. However, the variability in the metal concentration of the bivalves of the same population limits its usage as an indicator for pollution. Variability in the metal content is of the same magnitude as reported by Chow *et al.* (1976) for *Mytilus* sp. and Phillips (1967a,b) for *M. edulis*. However *Perna viridis* could be used as a long time monitor if sampling size is large or a large number of samples are pooled and homogenate sub samples are analysed.

Table 6. Correlation coefficient for relationship of metal concentration in organs with whole body dry weight.

The correlation between whole body concentration and these variables are shown for comparison February (1989) sample.

N=24

| Meta / organ | WHOLE BODY DRY WEIGHT | |
	Metal conc. in organ	Metal conc. in whole body
IRON		
gill+palp	-0.34*	
mantle	-0.63****	
foot	-0.26	-0.36*
kidney	-0.46**	
visceral mass		
MANGANESE		
gill+palp	-0.58****	
mantle	-0.64****	
foot	-0.25	-0.33*
kidney	-0.454**	
visceral mass	-0.708	

*P=0.05 **P=0.02; ***P=0.01; ****P=0.001

Table 7. Correlation coefficient for relationship of metal concentration in organs with age.

The correlation between whole body concentration and these variables are shown for comparison February (1989) sample.

February 1989 sample.
N=24

| Meta / organ | AGE | |
	Metal conc. in organ	Metal conc. in whole body
IRON		
gill+palp	-0.26	
mantle	-0.59****	
foot	-0.37*	-0.27
kidney	-0.1104	
visceral mass	0.028	
MANGANESE		
gill+palp	-0.49**	
mantle	-0.895****	
foot	-0.36*	-0.23
kidney	-0.090	
visceral mass	-0.58****	

*P=0.05 **P=0.02; ***P=0.01; ****P=0.001

Table 8. Correlation coefficient for relationship of metal concentration in organs with whole body dry weight.

The correlation between whole body concentration and these variables are shown for comparison February (1989) sample.

N=24

Meta / organ	WHOLE BODY DRY WEIGHT	
	Metal conc. in organ	Metal conc. in whole body
IRON		
gill+palp	-0.16	
mantle	-0.43**	
foot	-0.654****	-0.086***
kidney	-0.67****	
visceral mass	-0.086	
MANGANESE		
gill+palp	-0.62	
mantle	0.0468	
foot	-0.275	-0.53***
kidney	-0.3196*	
visceral mass	-0.63****	

*P=0.05 **P=0.02; ***P=0.01; ****P=0.001

Table 9. Correlation coefficient for relationship of metal concentration in organs with age.

February

N=24

Meta / organ	AGE	
	Metal conc. in organ	Metal conc. in whole body
IRON		
gill+palp	-0.12	
mantle	-0.61****	
foot	-0.41*	-0.47**
kidney	0.61****	
visceral mass	-0.07	
MANGANESE		
gill+palp	-0.53****	
mantle	0.61****	
foot	-0.065	0.5***
kidney	0.29	
visceral mass	-0.59****	

*P=0.05 **P=0.02; ***P=0.01; ****P=0.001

REFERENCES

Annon, Analytical methods committee (1960). Methods for the destruction of organic matter. Analyst 85, 643.

Ayling, G. 1974. Uptake of Cadmium Zinc, Copper, Lead and Chromium in the Pacific oyster "*Crassostrea gigas*" grown in the Tamar River, Tasmania. Water Res. 8: 729-738.

Bayne, B.L. 1976. Marine Mussels, their ecology and physiology. Cambridge University Press.

Boyden, C.R. 1974. Trace element content and body size of molluscs. Nature (London) 251: 311-314.

Boyden, C.R. 1977. Effect of zie upon metal content of shellfish. Journal Marine Biological Association, U.K. 57: 675-714.

Bryan, G.W. 1973. The occurrence and seasonal variation of trace metals in scallops, *Pecten maximums* (L) and *Chlamys opercularis* (L). Journal Marine Biology, U.K. 53: 145-166.

Bryan, G.W., Potts, G.W. and Forster, G.R. 1977. Heavy metals in the gastropod mollusc *Halliotis tuberculata* (L).

Chow, T.J., Snyder, H.G. and Syder, C.B. 1976. Mussel (*Mytilus* sp.) an indicator of lead pollution. Sci. Total. Environ. 6: 53-63.

Davies, I.M. and J.M. Pirie, 1980. Evaluation of "Mussel watch" project for heavy metals in scottish coastal water. Marine Biology 57: 87-93.

Hobden, D.J. 1967. Iron metabolism in *Mytilus edulis*. L. Variation in total content and distribution. Journal Marine Biology Association, U.K. 47: 597-606.

Latouche, Y.D. and Michael, C. Max. 1982. The effect of Depuration, size and sex on trace metal level in Bay Mussels. marine Pollution Bulletin 13(1): 27-29.

Mackay, M.J., Williams, R.G., Kacprzac, J.J., Kazacos, N.N. Collins, A.J. and Auty, E. 1975. Heavy metals in cultivated oysters (*Crassostrea Commercials Saceostrea cucullata*) from the estuaries of New South Wales. Australian Marine Freshwater Research 26: 31-46.

Martincic, D., H. Nurenberg, M. Sloeppler an dM. Branica, 1984. Bio-accumulation of heavy metals by bivalves from lun Fjord (Northan Adriatic sea). Marine Biology 81: 177-188.

Phillips, D.J.L. 1976a. The common mussel *MYtilus edulis* as an indicator of pollution by Zinc, Lead, Cadmium and Copper. 1. Effect of environmental variables on uptake of metals. Marine Biology 38: 71-81.

Phillips, D.J.L. 1978. The common mussel *Mytilus edulis* as an indicator of trace metals in scandinavian waters. II Lead, Iron and Manganese. Marine Biology 46: 147-156.

Phillips, D.J.L. and Douglas, A.S. 1986. Use the Bio-Indicators in monitoring conservative contaminants. Marine Pollution Bulletin 20(1): 10-17.

Remeril, M.G. 1974. Trace metal in sediment and bivalve mollusca in Southhampton water and the solent. Revue internationale d' oceanographie medicale 33: 31-47.

Sericano, J.L. 1993. National status and trend mussel watch program: Chlordane-related compounds in Gulf of Mexico oysters, 1986-90. Environmental Pollution 82(1): 23-32.

Theede, H. 1993. Heavy metals as biomonitors in the Baltic Biological monitoring of the Baltic sea in the Institute of Marine Sciences, Kiel 1985-1992. Ber. Inst. Meeres KD. Christianmeeres albrechts. Univ. Kiel. No.240: 199-207.