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Experimental Studies on the Accumulation of Copper and Zinc in the Green Mussel

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Toxicity of copper and zinc to the green mussel, *Mytilus viridis* was evaluated by short-term bioassays. Probit analysis was used for calculating 48 h median tolerance limits, 95% fiducial limits and slope functions (*S*). The rates of copper and zinc accumulation were determined by analysing the metal content in the soft parts of the mussels at fixed intervals of time by atomic absorption spectrometry. Bioaccumulation was determined as a function of metal concentration in the experimental medium. The correlations for the regression lines ($P < 0.1\%$) indicate that the uptake of metal is linear at least for the first five weeks. During a more extended experimental period, adjustment to a steady state probably occurs.

Zinc and copper are attracting the closest scrutiny at present because their concentrations are increasing significantly in the sea. Both the metals are essential to many organisms. However, the concept of 'essentiality' is under constant review and 'harmfulness' and 'toxicity' vary widely with the organism and concentration and their interpretations vary from country to country. All metals being cumulative poisons are potentially harmful

to most organisms at some level of exposure and absorption (Miettinen, 1974). A good review on the entry, distribution, fate and movement of metals through the food chains has been published by the working group reports of the proceedings of NATO Science Committee Conference (1974).

Mussels are ubiquitous in coastal waters in both northern and southern hemispheres. They are sessile and must, therefore, experience passively the varying conditions prevailing in the water mass (Bayne, 1976). For this very reason, Goldberg (1975) has argued for a global mussel watch on the concentrations of certain contaminants in the tissues of different species of *Mytilus* to provide an integrated index of environmental conditions.

The merits of using mussels in experiments on the effects and accumulation of metals are implicitly recognized in many studies (Brooks and Rumsby, 1965; Bryan, 1971; Brown & Newell, 1972; Abel, 1976). In the present study some aspects of the toxic effects of copper and zinc to *M. viridis* have been investigated, and the rate of accumulation of copper and zinc by *M. viridis*

TABLE 1
Average length, weight and natural metal content in *Mytilus viridis* and the water quality conditions during uptake experiments.

Metal	Length (mm)	Total wt (g)	Fresh wt (g)	Natural metal concentration ($\mu\text{g g}^{-1}$ wet wt)	Temp. ($^{\circ}\text{C}$)	Salinity (‰)	Oxygen (ml l^{-1})	Natural metal conc. in seawater (ppm)
Cu	21.45-22.21	1.038-1.161	0.1368-0.2543	2.268	29.0	33.60 \pm 1	4.8-5.1	0.0018
Zn	22.68-24.55	1.248-1.439	0.17779-0.2578	14.09	29.5	33.77 \pm 1	4.9-5.1	0.026

TABLE 2
Toxicity of trace metals to *Mytilus viridis* as indicated by acute toxicity tests.

Metal	No. of mussels exposed per concentration	Length (mm)	Probit regression equation	48 h LC ₅₀ ppm	95% fiducial limit. ppm upper lower	Slope function (S)
Cu	40	15.88-16.07	$y = 5.66172 + 6.1717(x - 1.2226)$	0.14	0.16 0.13	6.17
Zn	40	15.98-16.07	$y = 4.969 + 4.872(x - 2.359)$	2.31	2.64 2.03	4.87

and the potential of this mussel as an indicator of metal pollution has been discussed.

Material and Methods

Acclimation of test animals

Green mussels were collected from their natural beds at Velsao (Goa) during the period November 1976 to January 1977. Before use, all mussels were acclimatized to laboratory conditions for one week (temperature $29 \pm 1^{\circ}\text{C}$, salinity $33 \pm 1\text{‰}$, oxygen $4.8-5.2 \text{ ml l}^{-1}$). During this time, all mussels were fed on algal cultures.

Bioassay procedure

The toxicity tests were carried out as described by Portman & Connor (1968) and Ahsanullah (1976). The mussels were neither fed during the bioassays nor for two days before the start of the experiment. Briefly, 10 test animals, approximately of the same size (15.88 to 16.07 mm shell length) were exposed to serial dilutions in seawater of the metal under test. The test solutions (2 l.) were kept static but constantly aerated and were held at 29°C during the period of the test. The usual test period was 48 h. *M. viridis* was considered dead when it did not close even when the shell valves were pressed together. In practice, when the valves tended to close partially, after they were pressed, the animal was transferred to pure seawater to establish whether it was capable of recovery. Only the animals that failed to recover were considered dead.

Bioaccumulation

Mussels were exposed to copper and zinc in tanks containing ten litres of medium, which was changed every alternate day and the tanks were carefully washed without disturbing the animals unduly. Individuals similar in size were selected. The average length, weight, natural metal concentration in the soft parts and water quality conditions are given in Table 1. The animals were fed $4 \times 10^6 \text{ cells}^{-1} \text{ ml}^{-1}$ individual of unicellular algae *Synechosystis* sp. daily. The experimental mussels along with the controls were sampled weekly for a period of five weeks. A composite sample of soft parts from ten mussels without pallial fluid was prepared for the metal analysis. During the preparation of sample,

special precautions were taken to minimize contamination of the sample. For this purpose, the procedures given in the Manual of Methods in Aquatic Environmental Research (1976) were used. After digestion, the samples were analysed for zinc and copper by atomic absorption spectrophotometry, using a Hilger & Watts Atomspek H1 550.

Results

The results of the toxicity tests are expressed as LC₅₀ i.e. the concentration of a substance killing 50% of the test organisms during the period of exposure. The LC₅₀ values were estimated by Probit analysis (Fisher & Yates, 1949; Finney, 1971). This method permitted the utilization of all experimental data above the minimum threshold concentration of metal required to provide a lethal response. It also permitted the calculation of slope function and the 95% fiducial limits (Table 2). For each metal, the 48 h LC₅₀ was determined by plotting mortalities at 48 h, transformed to probits, against the log concentrations of the metal. From Figs 1 and 2, it is evident that the sensitivity will be represented by the slope of the line. The greater the slope, the narrower is the range in dosage for a given range in the percentage kill (Goulden, 1952). The 48 h LC₅₀ for copper and zinc was 0.14 and 2.31 ppm respectively. The two metals follow the expected general pattern of toxic behaviour, copper being more toxic than zinc. Martin *et al.* (1975) found metal toxicity in *M. edulis* to decrease in the same sequence.

In the experiments reported here, copper concentrations of 0.25 ppm or greater than this, produced a mortality of 100% within 48 h and concentrations of 4 ppm zinc and above produced 100% mortality within 48 h. In the experimental tanks, *M. viridis* usually gaped, and when they were transferred to copper solutions of concentrations less than 0.05 ppm, they continued to do so. At higher concentrations, they kept their shells closed. In contrast, *M. viridis* usually kept their shells opened when transferred to zinc solutions. Treatment of mussels with zinc does not seem especially detrimental, at least as judged by short-term exposures. However, the enrichment of zinc within the organism, and hence its transfer through the food chain may constitute a danger to man.

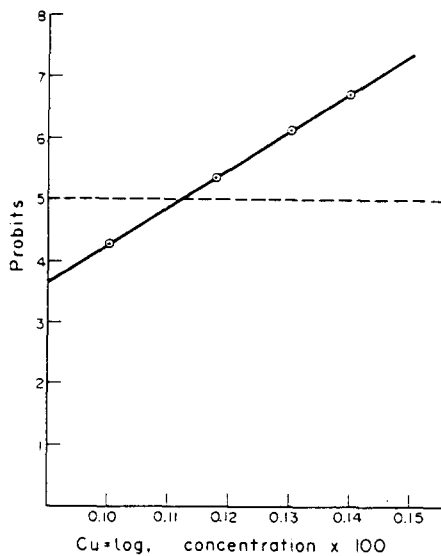


Fig. 1 Dose-mortality response of copper to *M. viridis*.

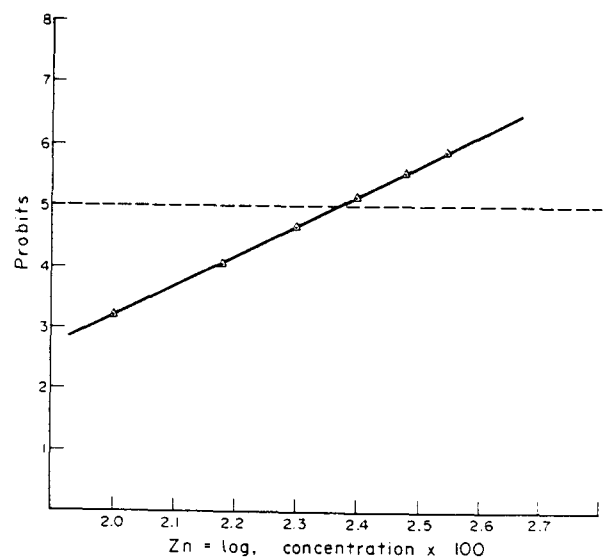


Fig. 2 Dose-mortality response of zinc to *M. viridis*.

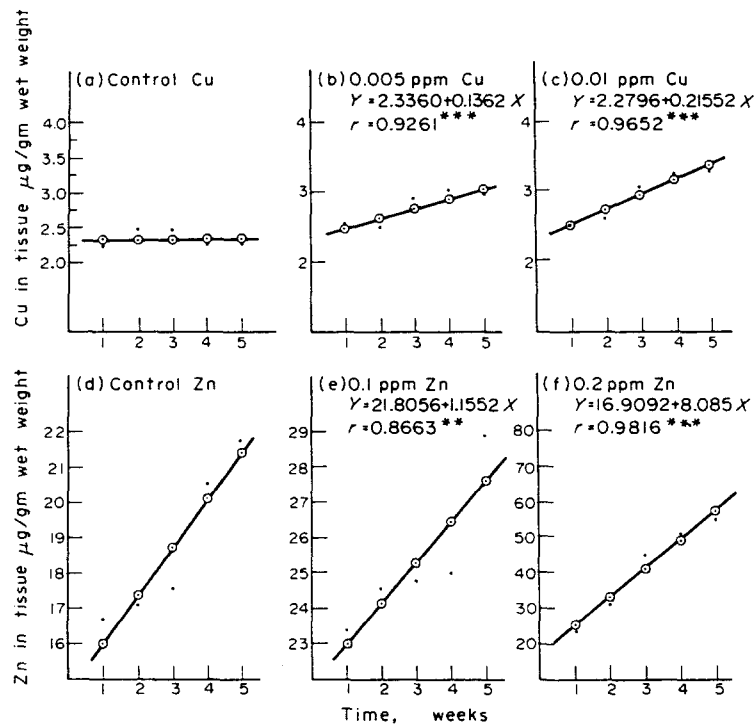


Fig. 3 (a-f) Copper and zinc uptake by *M. viridis* at different concentrations in the medium. Significance of correlation r (valid for all figures).
 ***: $P < 0.1\%$; **: $P < 0.2\%$; *: $P < 0.5\%$.

During the experiment on metal uptake from the medium, the mussels were kept for five weeks in seawater with the following metal concentrations: 0.005, 0.01 ppm Cu and 0.1, 0.2 ppm Zn. The pattern of metal uptake was similar at all concentrations used. Copper and zinc were continuously taken up into the soft parts of *M. viridis* throughout the experiment. The correlations for the regression lines ($P < 0.1\%$) indicates that uptake was linear at least for the first five weeks. This relationship is given by $Yt = a + b.t$ where Yt = metal concentration in the animal at time t , $b.t$ = factor of proportionality which is equal to the rate of uptake; a = natural metal concentration in the mussel. The copper concentration of the control mussels remained almost constant whereas

the zinc concentrations of control mussels increased slightly. This increase may be due to the selective absorption of zinc which is related to high intake of calcium in filter feeding organisms (Coombs, 1972).

Assuming a linear time course during the five weeks of the experiment, the rate of uptake was calculated to be 0.14 and 0.22 $\mu\text{g g}^{-1} \text{ week}^{-1}$ in 0.005 and 0.01 ppm copper respectively. The rate of uptake in 0.1 ppm and 0.2 ppm zinc was estimated as 1.16 and 8.01 $\mu\text{g g}^{-1} \text{ week}^{-1}$ respectively. The accumulated copper and zinc per unit wet weights are shown in Fig. 3 (a-f). It is evident that in no experiment was an equilibrium of metal exchange between the organism and the surrounding water achieved within five weeks. The estimated metal concentration in

the organism was increasing with an increase in the metal concentration of water at a given time after the start of the experiment.

Discussion

The present work clearly shows that short-term exposures to metals has a detrimental effect on *M. viridis*. The severity of the effect is primarily dependent upon the form of metal, the presence of other metals, the physiological conditions of the organism and the environmental conditions (Bryan, 1971). The physiological conditions, which effect the toxicity of heavy metals, include the size and age of the organism, stage in the life history and activity and acclimatization of metals. Brown and Ahsanullah (1971) showed that 48 h old larvae of *Artemia* were more susceptible to poisoning to copper and zinc than were the adults. Saliba & Ahsanullah (1973) showed that *Artemia* which has been acclimated in low concentrations of copper showed greater tolerance in LC₅₀ tests.

During the present studies, several differences between the toxic effects of copper and zinc have become apparent, which suggests that copper may act in a different manner. The range of concentrations from the no-effect level to that which is lethal is very narrow for copper as compared to zinc. Zinc differs in that it seems to act more slowly than copper and there is no significant response in the first 24 h after the exposure to concentrations that later prove lethal. Zinc, though toxic to marine organisms at elevated concentrations, is much less harmful than copper at equivalent concentrations. However, copper in organic form does not appear to be excessively toxic. Inorganic ions of copper, on the other hand, have been found to be toxic to a wide variety of vertebrates and invertebrates and its toxicity varies significantly between various species of organisms and with different physical and chemical properties of the water (Stemann-Nielsen & Wium-Anderson, 1970).

In environmental toxicology, the problem is whether very low concentrations have an effect on biological processes exposed continuously to those levels. It has long been known that there is a relationship between the effect of toxic levels of substances and the period over which they are absorbed by the organisms. This implies that a large amount taken in over a short period will have a similar effect to small amounts over a long period. This was first shown as Haber's Rule for equitoxic doses (Haber, 1924).

Sublethal effects of metals on *Mytilus* have been studied by several authors. Martin *et al.* (1975) found that 0.3 mg l⁻¹ copper and 1.8 mg l⁻¹ zinc reduced byssal thread production by 50%. During our experiments, it was observed that 0.01 mg l⁻¹ copper and 0.2 mg l⁻¹ zinc increased mucus secretion and reduced byssal thread production. Other important effects of sublethal exposure of organisms to heavy metals include changes in growth, development, morphology and behaviour. As a result of the exposure to very low concentrations of copper, (5 µg Cu l⁻¹) *M. viridis* lost weight probably due to inhibition of feeding. Brown & Ahsanullah (1971) observed growth inhibition when they exposed the polychaete *Ophryotrocha labronica* to copper concentrations of ≥ 50 µg Cu l⁻¹

The uptake of metals by *M. viridis* measured at different copper and zinc concentrations indicates an important linear relation which is valid at least for limited experimental times. There is a dependence of the rate of uptake on the concentration in the medium. Pringle *et al.* (1968) suggested that metal uptake by mussels is directly proportional to the external concentration. The present data confirm this. Over the experimental period, the relationship between toxicity and concentration was linear. The median lethal concentration (LC₅₀) of each metal for *M. viridis*, tested over a 48 h period, exceeded the concentration found in natural water by a factor 100. For longer test periods, the LC₅₀ would then be considerably reduced. Hence, it can be concluded that the continued addition of these metals to confined waters which do not provide adequate dilution must give a cause for concern.

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