

## ACCUMULATION AND TRANSFER OF ZINC AND COPPER IN A FOOD CHAIN FROM SEA WATER TO A TERRESTRIAL ANIMAL

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### ABSTRACT

Bioaccumulation and transfer of copper and zinc from the primary producers to the higher trophic levels have been studied using molluscs exposed to short term contamination. The bivalves *Meretrix meretrix*, were fed phytoplankton enriched with zinc and copper. Next these bivalves were eaten by carnivorous gastropods *Chicoreus virgineus*. At the tertiary consumer level, four mice were fed with contaminated *C. virgineus* for 21 days. The activity of the animals was monitored. The transfer of copper and zinc showed decreasing trend towards higher trophic levels. The retention time was 4 times higher with metals absorbed from food than from water. Mice fed *C. virgineus* for 21 days did not accumulate zinc, but copper was found in kidney, brain, liver, heart, muscles and skin. The liver seemed to have absorbed copper more than other organs. Loss of weight and activity were observed in mice.

### INTRODUCTION

Potentially hazardous metals find their way in ever increasing quantities into the marine environment from mining operation, metal processing facilities, chemical industries and numerous other sources. The intake of heavy metals by organisms and their passage through the food chain have been recognized as a major mechanism for their transfer to the different regions of the sea. Contaminants are often visualized as moving along a food chain from prey to predator with concentrations in the tissues of the organisms, either decreasing or increasing at each successive trophic level. Copper and zinc in trace quantities are essential for marine organisms within the biosphere. As they pass through higher trophic levels, the quantity of these metals is reduced. Reduction of their uptake occurs at two stages: at every point from one trophic level to the next, and at the point of their transfer from the chemical to the biological state. Many studies have been made on distribution of heavy metals in tissue (George and Coombs, 1975) and their seasonal variation in molluscs, (Bryan, 1973). But knowledge of the mechanism of the intake of the metals and their subsequent transfer to higher trophic levels is scanty.

Few studies have been made on the actual or potential pathways of pollutant flow from one population of bivalves to or through the populations of the predatory gastropods. The present study is aimed at investigating the accumulation and transfer of zinc and copper from plankton to *M. meretrix* (bivalves) to *C. virgineus* (muricid gastropod) to mice.

### MATERIALS AND METHODS

A series of rectangular fiber glass tanks of 35 l capacity were used in the study. They were non toxic with smooth interior surfaces. The animals selected for the study were groups of similar size. The bivalve *Meretrix meretrix* (1.5-2 cm size) and the muricid gastropod *C. virgineus* (4-5 cm size) were used in the study. The molluscs were stocked in large aquarium tanks and acclimatized for 10 days in 34 ppt sea water, pH 8.0-8.2, and dissolved oxygen content of 5.4 mg/l. Controls were maintained for all the sets of experiments. Copper was given as cupric sulphate and zinc as zinc sulphate. The test concentration of copper and zinc was selected based on tolerance limits. Sublethal doses of copper and zinc were added for 7 days.

### Experiment - I

The marine diatom *Pleurosigma elongatum* were collected from the Vellar estuary by towing a plankton net and culturing them in the laboratory. The culture medium was the Guillard f/2 medium. Stocks of *P. elongatum* were cultured in 500 ml medium with a known amount of added pollutants. Seven cultures were stocked on subsequent days in order to feed *M. meretrix* with plankton culture exposed to the metals for exactly seven days.

### Experiment - II

The second experiment was conducted to investigate the absorption of zinc and copper by the clam *M. meretrix* from water and phytoplankton respectively. Animals in seven tanks, 20 animals in each tank, were fed with 200 ml of plankton stock solution ( $10^5$  cells/ml). Care was taken to feed the animals with exactly 7 days plankton culture. Another set of animals was exposed to 0.02 ppm copper and 3 ppm zinc concentration respectively in water in addition to zinc and copper enriched phytoplankton. After 7 days, five animals were sacrificed and the whole soft bodies of the animals oven-dried at 80°C. Another set of 10 animals was exposed to zinc and copper contaminated medium for 7 days.

### Experiment - III

About 20 *C. virginicus*/tank, in 2 tanks, were fed with *M. meretrix* enriched with zinc and copper respectively at the rate of one bivalve per day. Care was taken to feed the snails with *M. meretrix* exposed to the pollutants for 7 days. Another set of 10 animals was exposed to medium containing 0.02 ppm copper and 5 ppm zinc in addition to food enriched with copper and zinc. After 7 days, 5 animals were sacrificed and various body parts like gill, mantle, gut, digestive gland and foot were dissected for metal analysis. About 5 animals were kept in natural sea water to study the retention time of these metals in *C. virginicus*. Another set of about 10 *C. virginicus* were exposed to sea water with 0.02 ppm copper and 3 ppm zinc for 7 days.

### Experiment - IV

Four mice were fed with zinc and copper contami-

nated *C. virginicus*. The contamination period lasted for 21 days.

### Analyses of the samples

The tissues were acid digested following Topping (1973). Each specimen was dried, weighed and digested in a mixture of nitric acid and perchloric acid in the ratio 3:1. The digested samples were made up to 25ml with double distilled water. Each digested sample was analyzed for copper at 324 nm, and for zinc at 211 nm in a Verion Techtron AA 120 Atomic absorption Spectrophotometer. The metal concentration was expressed in  $\mu\text{g/g}$  of dried soft tissue.

## RESULTS

The zinc and copper inputs into *M. meretrix* and *C. virginicus* from sea water with known amounts of metal concentrations have been calculated from the accumulated concentration of zinc and copper in the body parts. The metal input which *M. meretrix* and *C. virginicus* obtained from food have been calculated by dividing the value of the "Concentration of metals in tissue at that time" by the "mean value of metals in food".

The results are presented in Appendix 1 and 2. At the end of the feeding experiment, the amount of zinc and copper exchanged from phytoplankton to *M. meretrix* was 365  $\mu\text{g/g}$  dry wt and 77  $\mu\text{g/g}$  dry wt respectively. The rate of intake from *M. meretrix* to *C. virginicus* was 116  $\mu\text{g/g}$  dry wt for zinc and 16  $\mu\text{g/g}$  dry weight for copper. In *M. meretrix* the input of zinc from food was 52.1  $\mu\text{g/g}$  dry wt per day. In *Chicoreus virginicus* input of zinc from food was 16.5  $\mu\text{g/g}$  dry wt per day. The input of copper in *M. meretrix* from food was 12.1  $\mu\text{g/g}$  dry wt per day. In *C. virginicus* the input of copper was calculated at 2.3  $\mu\text{g/g}$  dry wt/day.

## DISCUSSION

The gastropod molluscs accumulated more metals from food than from water. Young (1975) showed that *Littorina obtusata* accumulated most of its iron and zinc from the brown seaweed *Fucus serratus*. The present study indicated that food chain transfer

of metal within the tissues of *M. meretrix* and *C. virgineus* is more prominent than direct intake from water.

Different parts of *C. virgineus* exhibited different concentrations, the digestive gland showed by far the highest rate of absorption regardless of the source. High concentration of zinc in the digestive gland may be due to the presence of zinc containing proteases in the digestive gland as reported by Ireland and Wootton (1977) for carnivorous species. The gut seems to have accumulated more zinc, when the animals were fed zinc enriched *M. meretrix*. Walker *et al.* (1975) reported the presence of granules containing zinc phosphate in parenchymal cells surrounding the mid gut of *B. balanoides* and this probably constitutes a site of high storage. The low rate of absorption from both water and food in foot and mantle indicated their insignificant role.

The absorption of zinc and copper from the food into all organs is greater than that from the medium. Romeril (1971) proposed different mechanisms involved in the accumulation of heavy metals in marine animals. According to him, the metal may be absorbed by active or passive diffusion from the sea water across semipermeable membranes into the body. Another mechanism is the process of adsorption between water membrane interfaces. The third mechanism involves ingestion of ions with food or in combination with particulate matter or mucus and

absorption through the gut wall. High concentrations of zinc and copper were recorded in digestive gland and the stomach of the animals fed with zinc and copper enriched *M. meretrix* and it is probable that the third mechanisms proposed by Romeril (1971) might have played a role. Gills were also found to have more accumulation. The difference in metal content at each trophic level may be due to excretion across the body, surface or gills, excretion via the gut and via the urine.

In the tertiary consumer level zinc accumulation was observed only in the skin. All the body parts showed copper accumulation within the short period of study. Liver has accumulated more copper than the other organs and loss of activity and weight in mice was noticed at the end of the period of study.

Transfer of zinc from the primary producer level to the primary consumer level was 37.9%, and 31.4% from primary consumer level to the secondary consumer level. The intake of copper from the primary producer level to the primary consumer level was 23.2%, and 22.3% from the primary consumer level to the secondary consumer level. At the tertiary consumer level the rate of intake of copper was 7.5%, whereas for zinc it was 1.0%. Compared with the primary and secondary consumer levels copper was found to accumulate more in the tertiary consumer level than zinc.

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### Appendix 1.

Zinc 3 ppm

Sample	Control value	From water	Net uptake from water	From food and water	Net uptake from food & water	From food alone	Net uptake from food
<b>Plankton</b>	11	966	---	---	---	---	---
<i>M. meretrix</i>	125	460	335	980	855	490	365
<i>C. virgineus</i>							
1) Mantle	100	180	80	300	200	206	106
2) Gill	160	260	100	415	255	310	150
3) Dig. gland	1080	1360	280	2165	1085	1859	779
4) Gut	220	240	200	420	200	395	175
5) Foot	80	140	60	230	150	160	80
<b>Mice</b>							
1) Kidney	80	---	---	---	---	80	---
2) Brain	100	---	---	---	---	100	---
3) Liver	120	---	---	---	---	130	10
4) Heart	40	---	---	---	---	40	---
5) Skin	120	---	---	---	---	140	20

### Appendix 2.

Copper 0.02 ppm

Sample	Control value	From water	Net uptake from water	From food and water	Net uptake from food & water	From food alone	Net uptake from food
<b>Plankton</b>	5	365	---	---	---	---	---
<i>M. meretrix</i>	64	91	27	176	112	141	77
<i>C. virgineus</i>							
1) Mantle	90	98	8	112	22	102	12
2) Gill	96	108	12	130	34	113	19
3) Dig. gland	86	104	18	136	48	112	26
4) Gut	82	91	9	117	35	103	21
5) Foot	66	74	8	87	21	77	11
<b>Mice</b>							
1) Kidney	42	---	---	---	---	54	12
2) Brain	50	---	---	---	---	60	10
3) Liver	58	---	---	---	---	72	14
4) Heart	50	---	---	---	---	50	---
5) Skin	42	---	---	---	---	56	14