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Effect of Ion-Exchanging Agent, Zeolite on Removal of Copper in Water and Improvement of Growth in *Oreochromis mossambicus* (Peters)

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Abstract

The effect of different doses of zeolite (0, 0.5, 2, 4 and 8 g zeolite l^{-1}) on reduction of copper toxicity in water and on freshwater fish, *Oreochromis mossambicus* was studied for 180 days. The 96 h LC_{50} value of copper for *O. mossambicus* was 4.27 mg·l^{-1}. RNA:DNA ratio increased with exposure period in control fish that indicated the growth of fish during the experiment. However, in copper exposed fish the ratio did not show drastic variation when compared to control fish. In zeolite treated fish, RNA:DNA ratio increased like in the control fish. Among the treatments, 2 g zeolite l^{-1} added treatment showed better performance than the other treatments. When different levels of zeolite were added to copper media, the metal content was completely removed from the medium within a period of 150 days in 0.5 g zeolite l^{-1} added treatment, while in other treatments (2, 4 and 8 zeolite l^{-1}) it took 120 days. The copper level in Cu treatment was 0.830 mg·l^{-1} even at the time of the termination of the experiment. The removal of copper from the test media and transfer to sediment were more pronounced in 2 g zeolite l^{-1} added treatment than in other treatments. A similar trend was also observed in the elimination of accumulated copper from the body tissues. Metal removal from water and body tissues and subsequent improvement of RNA:DNA ratio and protein in fish were maximum in 2 g zeolite l^{-1} added treatment than in other treatments (0.5, 4 and 8 g zeolite l^{-1}), hence it is considered as an optimum dose.

Introduction

Heavy metal pollution of water bodies is a major concern following industrialization. Almost all heavy metals are toxic at high concentrations and some are highly toxic even at low concentrations. Metals and pesticides have a tendency to accumulate and undergo food chain magnification (Vinikour et al. 1980). They could also cause catastrophic diseases like Minamata and Itai-Itai. Some of the probably affected organisms such as fish are consumed by human beings. Hence reduction of toxic elements in aquatic environments is

one of the primary challenges in wastewater treatment. The most widely used technique for the removal of heavy metals involves the process of neutralization. Chemicals can effectively remove certain toxic substances from industrial wastes or polluted medium but it is presumed to be costly. However, there are some cheap chemicals which are also free from side effects. Zeolite is one of such chemicals and it acts as an ion-exchanging agent. Recently, it has been used in detergents, aquaculture ponds and nuclear treatment, but it also has large potentials for other applications in liquid waste treatment. Sodium aluminosilicate ($Na_{12}[(AlO_2)_{12}(SiO_2)_{12}]_{27}H_2O$) is one of the natural zeolites (faujasite). Previous authors have studied the role of zeolite in reducing toxic metabolite levels in aquatic environments, settlement of suspended solids, absorption of gases like CO_2, SO_2 and H_2S in aquaculture ponds (Chamberlain 1988; Chien 1992; Briggs and Smith 1996) and improvement of sediment quality, pH (Clifford 1992) and mineral nutrition in fish and shrimp (Battes et al. 1981; Akiyama et al. 1992). However, there is paucity of information on how zeolite reduces the toxic elements in a polluted medium. There is also not much information on the duration of the treatment and optimum dosage of zeolite needed to reduce metal toxicity. The present work was designed to study the effect of zeolite on the removal of copper in water and improvement of growth in the freshwater cichlid fish, *Oreochromis mossambicus*.

Materials and Methods

Experimental fish, *Oreochromis mossambicus* were collected from a local pond (latitude 8° 46; longitude 75° 5) and acclimated for 30 days in laboratory conditions. During acclimatization, water was changed daily and fish were fed *ad libitum* with pellet feed containing 35% protein. Two series of experiments were performed.

In the first series of experiments, 96 h LC_{50} value was determined following the static renewable bioassay method. (Sprague 1973). Fish were starved for 24 h prior to the experiment and throughout the bioassay study. Stock solution of copper was prepared by dissolving 3.93 g of analar grade copper sulphate ($CuSO_4 7H_2O$) in 1 l of distilled water and this was diluted with freshwater to obtain the desired concentrations of the medium. Well acclimated and active fish (12.4 $\pm$ 1.1 g) were exposed to different concentrations of copper and mortality was observed for 96 h. A control was run in freshwater. The LC_{50} was calculated through probit analysis.

In the second series of experiments, animals were exposed to different treatments for 180 days. Acclimated fish were divided into six groups of 60 individuals each and starved for 24 h before the experiment. Table 1 presents the groups and their notations.

For convenient presentation, control and experiment groups hereafter would be referred to according to their notations. The chosen levels of zeolite and a constant level of copper (2.14 $mg \cdot l^{-1}$; half of LC_{50}) were added to the medium on day 1. The experiment was conducted in triplicates in epoxy-coated cement tanks containing 1.3 tons of test medium. Pond sediment was

added to all the experimental tanks up to five cm thick to simulate field condition and then 1.3 tons of freshwater were pumped in it. After adding copper and zeolite, the medium was mixed well and then test animals were introduced. Zeolite treated fish did not show any deleterious effects (James and Sampath 1999 a and b), hence individual effect of zeolite was not studied here. A feed with 35% protein was given to test animals in a feeding tray once a day at 0800 h; uneaten food was removed two hours after feeding. Water was not changed during the experiment and aerated for 14 h to avoid depletion of oxygen. Water quality during periods without aeration were: DO: 4.16 $\pm$ 0.23 ml·l^{-1}, temperature: 29.2 $\pm$ 1°C, pH: 7.80 $\pm$ 0.08, salinity: 0.15 $\pm$ 0.02 ppt and hardness: 58.0 $\pm$ 4.0 mg $CaCO_3$ l^{-1}.

On the first week of every month from April to September 1997, eight fish were removed from each group for estimation of DNA, RNA, protein and metal accumulation in the gills, liver and muscle. The nucleic acids and protein were estimated following the methods of Searchy and MacInnis (1970 a and b) and Lowry et al. (1951) respectively. RNA:DNA ratio was calculated by dividing the value of RNA by DNA. Copper content in fish body and feces (dry matter) was estimated following the method of FAO (1975). The zeolite deposited over the sediment was carefully removed prior to sampling of sediment and dried at 60°C in a hot air oven for two days. Copper concentration in water and sediment was estimated using the method of APHA (1993) and Smith and Windom (1972). All samples were analyzed in an Atomic Absorption Spectrophotometer (GBC 906 AA model). The instrument was calibrated using standards prepared from copper sulphate. Students 't' test was used to determine the significance of mean values between control and experiment groups at different days. Regression analysis was carried out based on the least square method (Zar 1974).

Results and Discussion

Toxicity of copper

The 96 h LC_{50} value of copper for *O. mossambicus* was 4.27 mg·l^{-1}. The 95% confidence limits were 3.7 to 4.0 mg·l^{-1}. The slope value was 1.2 mg·l^{-1}.

Table 1. Experimental groups and their notations.

S. No.	Groups	Total amount of zeolite added (kg)	Notation
1	Control (Freshwater)	—	C
2	Copper (2.14 mg·l^{-1}) alone	—	Cu
3	Copper (2.14 mg·l^{-1}) + 0.5 g zeolite l^{-1}	0.650	CuZ1
4	Copper (2.14 mg·l^{-1}) + 2.0 g zeolite l^{-1}	2.600	CuZ2
5	Copper (2.14 mg·l^{-1}) + 4.0 g zeolite l^{-1}	5.200	CuZ3
6	Copper (2.14 mg·l^{-1}) + 8.0 g zeolite l^{-1}	10.400	CuZ4
	Zeolite: Sodium aluminosilicate		

Half of the LC_{50} value of copper (2.14 $mg \cdot l^{-1}$) was used as the test dose along with the chosen levels of zeolite.

RNA:DNA ratio

RNA:DNA ratio is considered as a sensitive measure of the growth rate of fish (Love 1980). Figure 1 presents the changes in the levels of RNA: DNA ratio in different tissues of *O. mossambicus* at different exposures. RNA :DNA ratio showed a steady increase in control fish and zeolite treated groups, whereas there was no significant variation in the Cu exposed group. Among the zeolite treatments, the CuZ2 group elicited a higher ratio than the control fish at the termination of the experiment. The observed reduction in the RNA:DNA ratio in the Cu exposed fish was due to the low production of RNA for protein synthesis (Table 2) and metal burden in tissues (Table 3). The increase in RNA:DNA ratio in the control and zeolite treated groups was due to

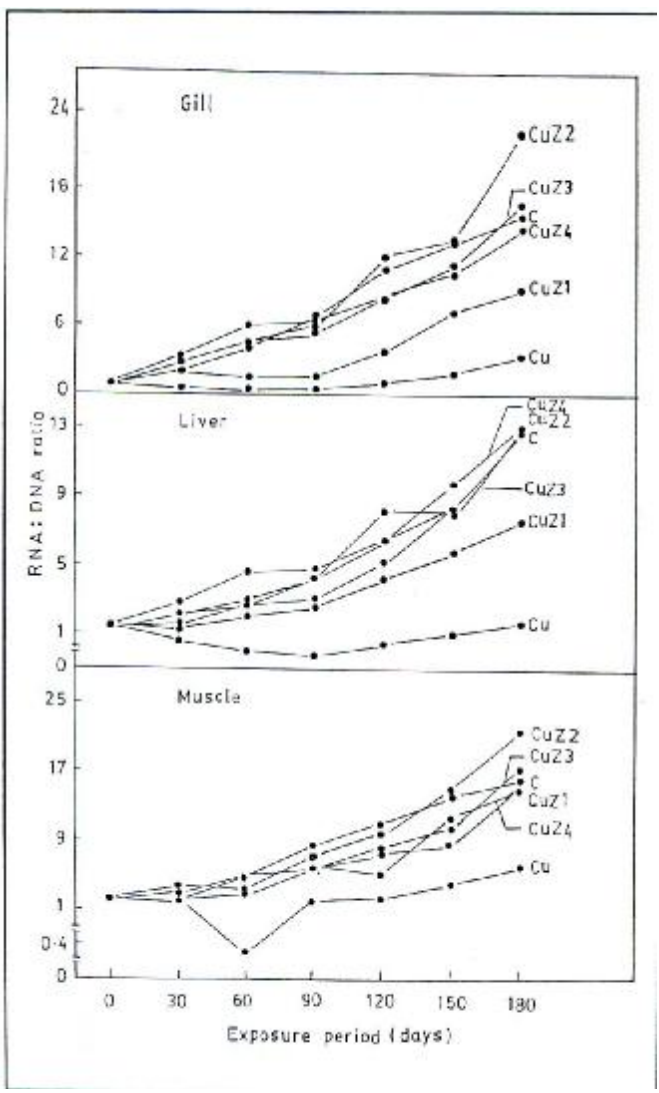

Fig. 1. RNA : DNA ratio of *O. mossambicus* exposed to sublethal concentrations of copper and different doses of zeolite.

the elevation of RNA production for protein synthesis (Table 2) without changing the DNA level. Regression lines obtained for protein and RNA:DNA ratio in the control and zeolite treated groups are clearly segregated and placed above the lines obtained in the Cu exposed group. The regression lines obtained for the CuZ2 group is on top (Fig. 2). A similar pattern of regression lines was obtained for the RNA and RNA:DNA ratio that indicates the close relationship

Table 2. Effect of sublethal concentration of copper and addition of zeolite on protein content ($\mu g \cdot g^{-1}$ wet tissue) in tissues of *O. mossambicus*. Each value is the mean (X±SD) of three fish.

Exposures	Exposure period (days)						
	0	30	60	90	120	150	180
				Gill			
C	97.9 ± 3.3	98.1 ± 6.6	110.3 ± 4.9	123.4 ± 2.9	127.3 ± 3.2	135.4 ± 3.8	133.8 ± 4.7
Cu	97.9 ± 3.3	76.1 ± 2.0	68.5 ± 4.8	65.7 ± 3.4	74.8 ± 1.2	82.5 ± 4.1	90.9 ± 3.9
CuZ1	97.9 ± 3.3	88.1 ± 2.1	94.0 ± 5.4	95.7 ± 8.8	97.9 ± 8.1	99.7 ± 4.6	103.8 ± 9.3
CuZ2	97.9 ± 3.3	89.7 ± 6.4	103.1 ± 3.4	119.7 ± 3.6	123.2 ± 3.5	130.8 ± 6.9	145.4 ± 14.7
CuZ3	97.9 ± 3.3	85.2 ± 2.3	98.3 ± 6.2	103.7 ± 2.8	114.6 ± 4.9	120.5 ± 8.8	128.8 ± 10.7
CuZ4	97.9 ± 3.3	89.5 ± 3.2	99.7 ± 8.0	116.1 ± 8.9	121.7 ± 11.4	136.6 ± 11.9	147.6 ± 13.2
				Liver			
C	126.3 ± 4.3	126.2 ± 6.2	135.7 ± 7.9	138.5 ± 13.1	146.2 ± 13.9	158.9 ± 11.1	163.1 ± 14.7
Cu	126.3 ± 4.3	95.1 ± 8.7	84.8 ± 6.0	83.5 ± 2.7	89.5 ± 3.8	92.5 ± 2.7	99.8 ± 9.5
CuZ1	126.3 ± 4.3	104.4 ± 3.3	111.0 ± 5.1	114.8 ± 11.4	119.7 ± 12.0	125.9 ± 10.9	132.1 ± 13.5
CuZ2	126.3 ± 4.3	109.5 ± 5.1	120.2 ± 6.7	132.8 ± 8.5	146.5 ± 14.3	161.4 ± 13.2	188.1 ± 12.5
CuZ3	126.3 ± 4.3	111.3 ± 6.9	119.5 ± 4.8	124.4 ± 11.2	134.1 ± 10.3	136.9 ± 13.2	140.7 ± 8.7
CuZ4	126.3 ± 4.3	115.2 ± 4.3	126.4 ± 9.3	127.7 ± 3.6	132.1 ± 10.0	137.0 ± 12.7	142.0 ± 9.0
				Muscle			
C	111.7 ± 4.6	130.2 ± 3.8	141.5 ± 6.1	157.1 ± 14.2	181.7 ± 14.9	206.7 ± 3.1	213.5 ± 12.9
Cu	111.7 ± 4.6	88.5 ± 4.1	72.2 ± 1.8	71.5 ± 6.1	89.4 ± 3.1	110.7 ± 8.7	121.6 ± 10.2
CuZ1	111.7 ± 4.6	115.5 ± 2.4	122.1 ± 3.9	124.7 ± 13.3	139.8 ± 11.9	164.1 ± 14.4	189.1 ± 12.2
CuZ2	111.7 ± 4.6	124.8 ± 6.4	133.1 ± 9.0	159.8 ± 12.1	188.6 ± 12.7	204.4 ± 15.8	222.6 ± 13.7
CuZ3	111.7 ± 4.6	126.4 ± 5.8	137.0 ± 8.3	141.3 ± 11.9	157.4 ± 13.9	166.0 ± 12.0	204.7 ± 14.2
CuZ4	111.7 ± 4.6	127.9 ± 4.4	140.7 ± 6.7	147.5 ± 12.7	153.8 ± 11.0	169.1 ± 14.7	188.5 ± 14.4

Table 3. Effect of sublethal concentration of copper and addition of zeolite on the copper accumulation ($\mu g \cdot g^{-1}$ wet tissue) in tissues of *O. mossambicus*. Each value is the mean (X±SD) of three fish.

Exposures	Exposure period (days)						
	0	30	60	90	120	150	180
				Gill			
C	0.01 ± 0	0.01 ± 0	0.009 ± 0	0.01 ± 0	0.011 ± 0	0.005 ± 0	0.004 ± 0
Cu	0.01 ± 0	1.51 ± 0.02	2.63 ± 0.14	4.65 ± 0.31	5.90 ± 0.12	8.17 ± 0.41	10.07 ± 0.26
CuZ1	0.01 ± 0	0.879 ± 0.01	0.326 ± 0.02	0.086 ± 0.16	0.07 ± 0	Nil	Nil
CuZ2	0.01 ± 0	0.270 ± 0.01	0.196 ± 0.01	0.023 ± 0	Nil	Nil	Nil
CuZ3	0.01 ± 0	0.314 ± 0.02	0.113 ± 0.01	0.018 ± 0	Nil	Nil	Nil
CuZ4	0.01 ± 0	0.397 ± 0.03	0.131 ± 0.02	0.020 ± 0	Nil	Nil	Nil
				Liver			
C	0.047 ± 0.01	0.048 ± 0	0.044 ± 0	0.061 ± 0	0.040 ± 0	0.045 ± 0	0.049 ± 0
Cu	0.047 ± 0.01	1.98 ± 0.02	3.25 ± 0.04	8.63 ± 0.26	12.74 ± 1.31	18.11 ± 1.26	21.35 ±2.08
CuZ1	0.047 ± 0.01	0.920 ± 0.11	0.976 ± 0.06	0.126 ± 0.02	0.074 ± 0	Nil	Nil
CuZ2	0.047 ± 0.01	0.980 ± 0.06	0.617 ± 0.06	0.117 ± 0.03	0.010 ± 0	Nil	Nil
CuZ3	0.047 ± 0.01	0.994 ± 0.11	0.728 ± 0.03	0.145 ± 0.04	0.028 ± 0	Nil	Nil
CuZ4	0.047 ± 0.01	1.058 ± 0.13	0.850 ± 0.05	0.165 ± 0.01	0.021 ± 0	Nil	Nil
				Muscle			
C	0.007 ± 0	0.005 ± 0	0.004 ± 0	0.004 ± 0	0.006 ± 0	0.005 ± 0	0.004 ± 0
Cu	0.007 ± 0	0.720 ± 0.06	1.85 ± 0.02	2.97 ± 0.04	4.06 ± 0.06	5.17 ± 0.14	4.23 ± 0.23
CuZ1	0.007 ± 0	0.151 ± 0.01	0.106 ± 0	0.008 ± 0	Nil	Nil	Nil
CuZ2	0.007 ± 0	0.060 ± 0	0.032 ± 0	Nil	Nil	Nil	Nil
CuZ3	0.007 ± 0	0.104 ± 0	0.098 ± 0	Nil	Nil	Nil	Nil
CuZ4	0.007 ± 0	0.085 ± 0	0.254 ± 0.01	0.033 ± 0	Nil	Nil	Nil

between them. An increase in the RNA:DNA ratio in zeolite treated groups likewise suggests an improvement in the growth of test animals. The RNA:DNA ratio was higher in zeolite treated fish than those exposed to Cu alone. Evidently it was due to the reduction of copper toxicity in fish by zeolite (Table 3).

Cu in water, sediment and fish tissues

Trace level of Cu was found in control water (0.003 $mg \cdot l^{-1}$) and sediment (0.02 $mg \cdot g^{-1}$ dry matter). In all exposures (except control), the initial level (day 1) of Cu in water was 2.14 $mg \cdot l^{-1}$ and it gradually declined with the extension of exposure period from 0 to 180 days (Fig. 3). When zeolite was added to the Cu media, the metal content declined in the test media very quickly and it was completely removed within 120 days in the CuZ2 to CuZ4 groups and 150 days in the CuZ1 group. The copper level in the Cu group was 0.850 $mg \cdot l^{-1}$ even at the time of termination of the experiment. The results obtained for Cu level in sediment was opposite to that of the Cu level in water.

Trace level (0.007 to 0.047 $\mu g \cdot g^{-1}$ wet tissue) of copper was found in tissues of control fish while the Cu linearly increased with the exposure period in fish exposed to Cu alone. However in zeolite treatments, Cu accumulation was at its maximum on the 30th day (except CuZ1 on 60 days) and afterwards it drastically declined and was completely eliminated from the tested tissues (Table 3). Among the zeolite treatments, the CuZ2 group elicited a fast removal of Cu from polluted medium and fish tissues compared to the other groups.

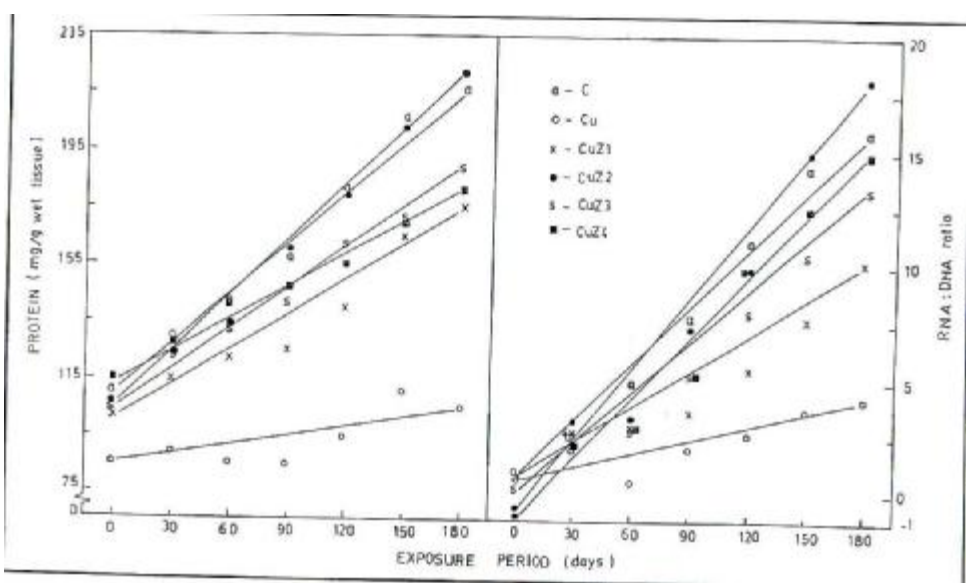

Fig. 2. Regression lines representing the relationship between muscle protein and RNA:DNA ratio in *O. mossambicus* exposed to sublethal levels of copper and different doses of zeolite.

Interactions of zeolite and copper

Copper and zeolite could interact in the experimental medium as follows: zeolite has extra framework ion (Na^+) and framework ions (Al^{3+} and Si^{4+}) that are easily exchangeable and non-exchangeable respectively. The ionic radii of Na^+, Al^{3+} and Si^{4+} are 0.95, 0.50 and 0.54A° respectively. However, the ionic radius of Cu^{2+} is 0.96A° (Sanderson 1960; Huheey 1983) which is suitably matched to the ionic radius of Na^+ (0.95A°) in zeolite, hence both ions could be easily exchanged with each other than Al^{3+} and Si^{4+} ions. Perhaps, Cu^{2+} ions could bind with easily exchangeable extra framework Na^+ ion in zeolite and it would leave a lesser number of free Cu^{2+} ions reducing the chance for metal uptake by fish which in turn evidently improved the RNA:DNA ratio and protein content (Fig. 1; Table 2) in *O. mossambicus*.

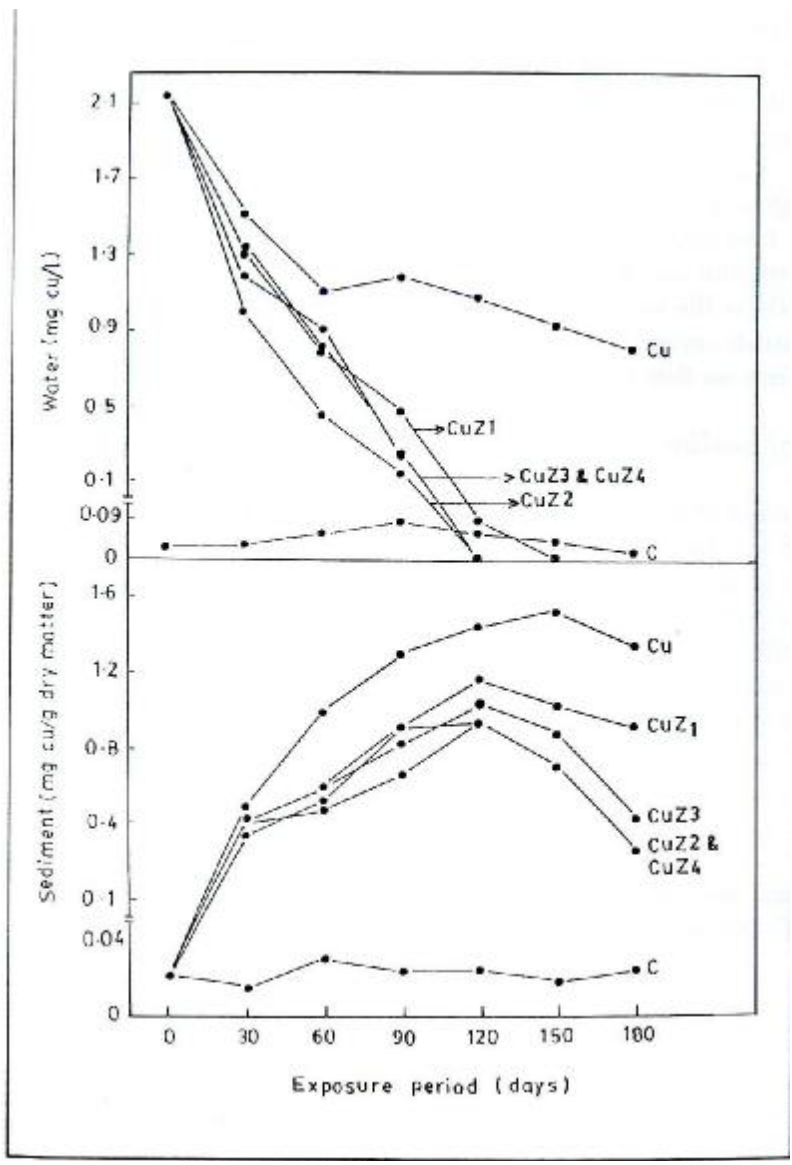

Fig. 3. Effect of different doses of zeolite on copper distribution in water and sediment.

Optimum dosage of zeolite

The present study reveals that protein content and RNA:DNA ratio are higher in the zeolite treated groups than in the Cu exposed group. It manifests that zeolite caused the complete elimination of Cu in tissues and neutralized the metal burden in fish (Table 3) which in turn enhanced the RNA:DNA ratio close to the level of control fish. Among the zeolite treated groups, CuZ2 elicited a better performance (0.5, 4 and 8 g zeolite l^{-1}); hence an addition of 2 g zeolite l^{-1} (CuZ2) is considered as an optimum dose. It is recommended that application of 2 g zeolite l^{-1} to metal polluted environment could remove the heavy metal copper and reduce its toxicity on fish and other commercially important organisms. James and Sampath (1999b) reported that addition of 1 g zeolite l^{-1} to cadmium polluted environment, produced least accumulation of metal (Cd) and maximum improvement in food intake and growth in the cadmium exposed *Heteropneustes fossilis*. In aquaculture practices, application of zeolite is suggested in ponds before stocking fry or during pond preparation (Briggs and Smith 1996). When the concentration of ammonium ions exceeds the permissible limit in aquaculture ponds, it becomes toxic to fish life (Sampath et al. 1991; James et al. 1993) hence it is advisable to reduce the concentration below the permissible limit by application of zeolite. Overdoses of chelating agent like ethylenediamine tetraacetic acid (EDTA) could cause deleterious effects on the survival, development and growth of crustacean larvae (Davis 1976) and on the survival and haematology in fish (James et al. 1998). In the present study, even the maximum dose of 8 g zeolite l^{-1} did not produce any adverse effect on fish (Table 2; Fig.1).

Applicability of zeolite

The application of 2, 4 and 8 g zeolite l^{-1} to Cu contaminated media, completely removed Cu from water (Fig. 3) and eliminated Cu from fish tissues (Table 2) within a period of 120 days; 2 g zeolite l^{-1} was a more economical optimum dose than other higher dosages. Comparatively, zeolite is the cheapest, causes no side effects and is more suitable than EDTA and nitrilo triacetic acid (NTA) hence it may be considered as the best chemical agent to remove toxic elements from polluted environments.

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