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Effect of Zeolite on the Reduction of Cadmium Toxicity in Water and a Freshwater Fish, *Oreochromis mossambicus*

R. James, K. Sampath

Department of Zoology, V. O. Chidambaram College, Tuticorin 628 008, Tamil Nadu, India

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The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method and is the primary challenge of waste water treatment. The most widely used technique for the removal of toxic elements involves the process of neutralization and metalhydroxide precipitation (Himesh and Mahadevaswamy 1994). Chemicals can effectively remove certain toxic elements from industrial wastes or polluted media, but it is usually costly. However, there are some cheap chemicals which are also free from undesirable side effects. Zeolite is one such chemical and it acts as an ion-exchanging agent. Recently, it has been mainly used in detergency, aquaculture ponds and nuclear waste effluent treatment, but it also has potential for other applications in liquid waste treatment. Zeolite is a framework of sodium aluminosilicate ($Na_{12}[(AlO_2)_{12}(SiO_2)_{12}]\ 27H_2O$) and the valuable property of the substance is to exchange its sodium for cations like Ca^{2+}, Mg^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Zn^{2+} and other divalent cations in water. The complete removal or reduction of toxic elements in polluted environments is of the utmost importance (Boyd 1990; James *et al.* 1998). Reports (Piper 1984; Boyd 1990: Briggs and Smith 1996) document the use of zeolite in fish or shrimp ponds for the removal of ammonia. However, there is a paucity of information on the correlation between the introduction of zeolite in metal-polluted water and the reduction of metal toxicity. There is also not much information on the optimum dosage of zeolite that is required to reduce metal toxicity. The present work was designed to study the effect of the ion-exchanging agent zeolite on the reduction of cadmium toxicity in water and in the freshwater cichlid fish, *Oreochromis mossambicus*.

MATERIALS AND METHODS

Experimental animals, *Oreochromis mossambicus* (Tilapia) were collected from a local pond (latitude 8° 46; longitude 75° 5) and held for 30 d in laboratory conditions (temperature: 27.8 ± 1°C (± SD), pH: 7.8 ± 0.05; salinity 0.18 ppt; hardness 107 mg/ $CaCO_3$ and DO 4.65 mlO_2/l). During acclimatization, water was changed daily and fish were fed *ad libitum* with a pelletized diet containing 35% protein. Acclimated fish (12.52 ± 1.1g) were exposed to different concentrations of cadmium and mortality was observed for 96 hr. A static renewable bioassay method (Sprague 1973) was adopted for the determination of 96 hr median lethal concentration; probit analysis (Litchfield and Wilcoxon 1949) was followed for the calculation of 96 hr LC_{50}. A control group was maintained in metal-free freshwater. The 96 hr LC_{50} of cadmium for *O. mossambicus* was 12.0 ppm and its 95% confidence limits were 9.62 (lower) and 14.95 (upper). A stock solution of cadmium was prepared by dissolving 6.486 g of analar grade (Merck)

Correspondence to: R. James

cadmium sulphate ($CdSO_4 8 H_2O$) in 1 l of distilled water and then diluted with freshwater to obtain the desired concentration (6.0 ppm) for the experiments.

Active and healthy fish (12.5 ± 1.1g) (192 nos) were collected from the acclimation tank and starved for 24 hr prior to the commencement of the experiment. They were divided into six groups of 16 individuals each in duplicates and exposed for 45 d as follows:

Table 1. Experimental groups and their notation

S.No.	Groups	Total amount of zeolite added (g)	Notation
1.	Control (metal-free freshwater)	—	C
2.	Zeolite (4gZ*/l) alone	380	Z
3.	Cadmium (6.0 ppm) alone	—	Cd
4.	Cadmium (6.0 ppm) + 0.5gZ*/l	45	CdZ1
5.	Cadmium (6.0 ppm) + 2.0gZ*/l	180	CdZ2
6.	Cadmium (6.0 ppm) + 4.0gZ*/l	360	CdZ3

* Sodium aluminosilicate

(For ease of presentation, hereafter control and experimental groups will be referred by their notation). Based on a separate pilot study, 3 concentrations of zeolite (0.5. 2.0 and 4.0 g/l) were chosen and added to the medium along with the cadmium (see Table I) on 0 d, to assess the effect of zeolite on metal distribution in water, sediment, fish and faeces and biochemical parameters and growth in *O. mossambicus*. The experiment was conducted in epoxy coated cement tanks (105 l capacity) containing 90 l of test medium. Pond sediment was added to all the experimental tanks up to 1 cm height and then 90 l of metal-free freshwater was tilled in it. The medium was mixed well after the addition of cadmium and zeolite and then 16 test individuals were introduced. A 35% protein feed was offered as diet to test animals in a feeding tray once in a day at 0800 hr and uneaten food was removed after 2 hr of feeding. Faecal matter was randomly collected by using feeding trays and was dried in a hot air oven at 60°C to estimate cadmium content. The medium was not changed during the experiment and the media were aerated for 14 hr/d. The hydrobiological parameters like D0, temperature, pH, salinity and hardness of different experimental exposures did not vary much and averaged to 4.13 mlO_2/l, 28.0 ± 1.0°C, 7.64, 0.13 ppt and 71 mg $CaCO_3$/l respectively.

At 15 d intervals, 4 fish were removed from each experimental group for estimation of chosen biochemical parameters (DNA, RNA and protein) and metal distribution in water, sediment and fish body. Gill, liver and muscle tissues were dissected out from the experimental fish for analysis of DNA, RNA and protein by the method of Searchy and MacInnis (1970 a and b) and Lowry *et al.* (1951). For DNA and RNA analysis, chosen tissues were homogenized in 0.5 N perchloric acid and heated in a water bath at 90°C for 20 minutes. After cooling, the tissue homogenates were centrifuged The supernatant was separated into two equal volumes and used for DNA and RNA analysis. One part of the homogenate was mixed with diphenylamine reagent and the OD was read after 20 hr at 595 nm for DNA. The other part of the homogenate was mixed with Dische-Orcinol and heated in water bath at 90°C for 15 minutes. After cooling at room temperature, OD was read at 655 nm for RNA. Standard DNA and RNA (Sigma chemicals) were dissolved in 0.5 N perchloric acid and used for the estimation of unknown samples.

Cadmium content in fish body (dry matter) and faeces was estimated following the method of FAO (1975). The cadmium concentration in water and sediment was also estimated by the method of APHA (1993) and Jan and Young (1978). All samples were analysed in Atomic Absorption Spectrophotometer (GBC 906 AA model). The instrument was calibrated using standards prepared from cadmium sulphate.

RESULTS AND DISCUSSION

The present study shows that addition of zeolite to cadmium contaminated media, significantly reduced ($P<0.05$) the metal level in the water and helped to eliminate metal from the fish body, which in turn improved the biochemical parameters as compared to fish exposed to cadmum alone. The concentration of DNA did not change, whereas levels of RNA and protein were found to increase in the tissues of control fish (Table 2 and 3) with increase of rearing time. The increase in RNA suggests that it is involved in protein synthesis without changing the amount of DNA in the control fish which supports the findings of previous workers (Brachet 1955; Love 1980). The amount of DNA in each cell nucleus is constant for a species (Love 1980) and it is considered as an index of cell numbers contributing to unit weight of tissue, while the concentration of RNA in a cell is related to protein synthesis (Brachet 1955) and metabolic activities of a tissue (Leslie 1955; Bulow 1970). Therefore, RNA : DNA ratio indicates the amount of protein synthesis and could be a more sensitive tool for measuring the growth rate of fish (Bulow 1971; Fauconneau 1985). In the present study, the RNA : DNA ratio has gradually increased with rearing time and it was 9-13 times more in control fish and 6-10 times in zeolite treated fish as compared to fish exposed to Cd alone at the end of the experiment. For instance, RNA : DNA ratio of Cd exposed fish was 0.23 and it was significantly ($P<0.01$) enhanced to 4.8, 4.6, 1.9, 2.2 and 3.6 in fish belonging to C, Z, CdZ1, CdZ2 and CdZ3 groups respectively in muscle on 45 d. A similar trend was obtained in liver and gill tissues also (Table 4). A significantly low RNA : DNA ratio and a concurrent reduction in growth rate was observed in fish exposed to Cd alone as compared to control and zeolite treated fish. The decline of RNA : DNA ratio in Cd exposed fish was due to the elevation of the DNA level and reduction in the levels of RNA and protein under metal stress. It indicates that Cd interferes with RNA and protein synthesis and simultaneously enhance the utilization of protein to mitigate stress during metal exposure and hence the poor growth rate. Chung and Robinson (1994) observed a reduction in RNA : DNA ratio reflecting on the growth rate in mussel. *Hiatella arctica* exposed to heating oil. The RNA : DNA ratio of zeolite alone treated fish did not show significant changes and it suggests that addition of zeolite to freshwater did not cause any side effect and it was evidently confirmed from the elevation of RNA and protein contents (Table 2 and 3) and no accumulation of cadmium in fish body (Table 5).

The increase in RNA : DNA ratio was observed in fish exposed to Cd + Zeolitc and it was evidently due to the elevation of RNA production for protein synthesis without changing the DNA level significantly ($P<0.05$) as in the case of control fish. Zeolite reduced the Cd toxicity in water and fish which inturn enhanced the RNA : DNA ratio and consequently improved growth rate; this was more pronounced in CdZ3 group than in others. The protein is an important component to stimulate growth in fish and the linear increase of protein content with rearing time (except early exposure period) in fish exposed Cd + Zeolite confirmed the increase of growth rate over those exposed to Cd alone. The result showed that all the tested biochemical parameters were improved in

control, zeolite alone and zeolite added groups and it was more pronounced in CdZ3 group than CuZ1 and CuZ2; hence, CuZ3 treatment (4 gZ/l) is considered as optimum dose.

The present study also reveals that the addition of zeolite to the cadmium media significantly ($P<0.05$) reduced the cadmium level in water. However, the Cd level in sediment was found to increase in all exposures during the experiment (Table 5). Zeolite can bind with Cd^{2+} ions forming ion-exchanged zeolite complex which could be deposited in the sediment. Further, the ion-exchanged zeolite complex could readily enter into the fish body; however it appears that the complex was not accumulated in the body but eliminated through faeces, thus reducing the metal burden. This was evident from the estimation of cadmium in faecal matter; cadmium elimination through faeces in fish exposed to Cd alone was 0.15 mg/g dry matter as against 5.08 mg/g dry matter in groups CdZ3. Addition of zeolite to cadmium contaminated media significantly ($P<0.05$) reduced the Cd level in water and fish body (through faeces) which reduced the Cd toxicity and improved the biochemical and growth parameters. This was more pronounced in the group CdZ3 (4 g zeolite/l) than in other groups (CdZ1 and CdZ2) and hence 4 g Z/l is considered as optimum amount for treatment.

The Cd and zeolite could interact in the experimental medium as follows: zeolite has extra framework ion (Na^+) and framework ions (Al^{3+} and Si^{4+}) which 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°; the ionic radius of Cd^{2+} is 0.97A° (Sanderson 1960; Huheey 1983) which is suitably, matched to the ionic radius of Na^+ (0.95A°) in zeolite, and hence both ions could be easily exchanged with each other than Al^{3+} and Si^{4+} ions. Perhaps, Cd^{2+} ions can bind with easily exchangeable extra framework Na^+ ion in zeolite. Briggs and Smith (1996) found that zeolite has the capacity to remove ammonia and other metabolites from freshwater by ion-exchange and adsorption. James *et al.* (1998) reported that addition of EDTA in Cu contaminated medium caused the formation of stable ion (Cu^{2+}) exchanged EDTA complex and elimination of more amount of copper in faeces, which significantly reduced the metal burden in tissues and improved the haematological parameters in *Oreochromis mossambicus*. Muramota (1980) found that metal-chelating compounds like NTA (Nitrilotriacetic acid) and EDTA reduce the metal toxicity in fish by preventing the accumulation of metal in tissues. He also suggested that cadmium complexed to EDTA is indeed taken up, but the complex is quickly excreted through urine and not accumulated (Babiker and Rankin 1975).

From the results obtained in the present study, it is recommended that, application of 4 g Z/l to metal polluted water could reduce the cadmium toxicity on fish and other commercially important organisms. Gworek and Borowiak (1990) reported that application of synthetic zeolite results in the immobilization of heavy metals and recommended for clean up method. In aquaculture practices, the application of the low cost ion-exchanging agent zeolite in ponds before stocking fry or during the pond preparation is suggested (Briggs and Smith 1996). In aquaculture systems, when the concentration of ammonium ions exceeds the permissible limit it becomes toxic to fish life (Sampath *et. al.* 1991; James *et al.* 1993) and it is advisable to reduce the concentration below the permissible limit by application of zeolite. Other chelating agents such as EDTA, NTA and citrates are in use in aquaculture systems or industrial wastes to remove unwanted toxic substances (Muramota 1980: Simon 1981; James *et al.* 1998). However, over doses of chelating agent like EDTA could cause deleterious effects on survival, development and growth in crustacean larvae (Davis 1976) and survival and

Table 2. Effect of sublethal concentration of cadmium and the role of zeolite on nucleic acids, RNA and DNA (mg/g wet tissue) as a function of time in *Oreochromis mossambicus*. Each value is the mean ($\overline{X}$ ± SD) of three estimations.

Exposures	Exposure period (days)			
	0	15	30	45
Ribonucleic acid - Liver				
C	8.98 ± 0.71	11.25 ± 2.76	15.23 ± 0.79	16.50 ± 0.18
Z	8.98 ± 0.71	12.78 ± 0.08	16.45 ± 0.96	17.54 ± 0.71
Cd	8.98 ± 0.71	3.07 ± 1.65	3.50 ± 1.08	1.31 ± 0.51
CdZ1	8.98 ± 0.71	3.63 ± 0.24	4.73 ± 0.16	8.59 ± 0.96
CdZ2	8.98 ± 0.71	6.60 ± 0.30	9.12 ± 0.45	12.62 ± 1.44
CdZ3	8.98 ± 0.71	12.25 ± 2.84	12.83 ± 0.59	13.12 ± 0.70
Gill				
C	3.03 ± 0.48	6.65 ± 1.20	12.70 ± 1.37	13.29 ± 2.55
Z	3.03 ± 0.48	7.05 ± 0.74	13.49 ± 0.07	14.73 ± 0.08
Cd	3.03 ± 0.48	3.06 ± 1.04	2.10 ± 0.07	1.50 ± 0.35
CdZ1	3.03 ± 0.48	4.09 ± 0.32	6.77 ± 2.09	8.99 ± 0.75
CdZ2	3.03 ± 0.48	7.75 ± 0.51	8.29 ± 0.62	10.81 ± 1.52
CdZ3	3.03 ± 0.48	10.30 ± 2.22	13.65 ± 2.47	13.80 ± 2.06
Muscle				
C	11.04 ± 0.89	12.45 ± 2.47	15.14 ± 2.97	24.82 ± 2.01
Z	11.04 ± 0.88	12.75 ± 0.48	15.78 ± 0.21	27.49 ± 0.30
Cd	11.04 ± 0.89	1.15 ± 0.21	2.33 ± 0.60	2.40 ± 0.28
CdZ1	11.04 ± 0.89	11.92 ± 1.17	12.54 ± 0.69	13.28 ± 2.82
CdZ2	11.04 ± 0.89	8.10 ± 1.03	11.11 ± 0.60	15.34 ± 2.55
CdZ3	11.04 ± 0.89	10.83 ± 1.56	13.82 ± 2.79	21.67 ± 2.14
Deoxyribonucleic acid - Liver				
C	6.48 ± 0.62	6.78 ± 0.89	7.05 ± 0.20	6.94 ± 0.36
Z	6.48 ± 0.62	6.94 ± 0.18	7.45 ± 0.63	7.76 ± 0.07
Cd	6.48 ± 0.62	8.84 ± 0.57	10.60 ± 1.06	12.79 ± 1.62
CdZ1	6.48 ± 0.62	7.79 ± 0.77	8.50 ± 0.18	7.84 ± 0.98
CdZ2	6.48 ± 0.62	7.36 ± 0.56	7.68 ± 2.47	7.29 ± 0.98
CdZ3	6.48 ± 0.62	7.05 ± 0.22	7.25 ± 0.69	7.06 ± 0.63
Gill				
C	3.22 ± 0.08	3.05 ± 0.61	3.18 ± 0.33	3.48 ± 0.63
Z	3.22 ± 0.08	3.15 ± 0.07	3.49 ± 0.14	3.78 ± 0.62
Cd	3.22 ± 0.08	4.26 ± 0.40	6.67 ± 0.54	8.50 ± 0.35
CdZ1	3.22 ± 0.08	3.25 ± 0.35	4.70 ± 1.48	5.30 ± 0.97
CdZ2	3.22 ± 0.08	3.50 ± 0.08	4.39 ± 2.99	3.95 ± 0.75
CdZ3	3.22 ± 0.08	3.74 ± 0.66	4.74 ± 0.49	3.89 ± 1.29
Muscle				
C	4.90 ± 0.14	5.24 ± 0.88	5.07 ± 0.02	5.20 ± 0.73
Z	4.90 ± 0.14	5.46 ± 0.62	5.49 ± 0.14	6.48 ± 0.40
Cd	4.90 ± 0.14	6.88 ± 0.66	7.85 ± 0.35	10.65 ± 0.42
CdEDTA1	4.90 ± 0.14	5.69 ± 1.98	6.49 ± 1.34	7.15 ± 0.48
CdEDTA2	4.90 ± 0.14	5.40 ± 1.27	6.64 ± 0.24	6.90 ± 0.72
CdEDTA3	4.90 ± 0.14	5.25 ± 1.24	6.06 ± 0.81	5.95 ± 1.90

Table 3. Impact of sublethal level of cadmium and the role of ion-exchanging agent zeolite on protein content (mg/g wet tissue) indifferent tissues of *Oreochromis mossambicus.* Each value is the mean ($\overline{X}$ ± SD) of three estimations.

Exposures	Exposure period (days)			
	0	15	30	45
Liver				
C	117.1 ± 10.14	122.8 ± 10.08	122.4 ± 14.30	156.4 ± 7.07
Z	117.1 ± 10.14	125.7 ± 6.91	127.9 ± 6.83	137.8 ± 6.92
Cd	117.1 ± 10.14	66.5 ± 6.81	32.4 ± 2.69	35.3 ± 6.06
CdZ1	117.1 ± 10.14	58.5 ± 4.26	78.2 ± 4.72	101.0 ± 10.03
CdZ2	117.1 ± 10.14	77.8 ± 2.64	112.4 ± 10.75	124.5 ± 8.47
CdZ3	117.1 ± 10.14	83.0 ± 6.44	125.2 ± 10.13	131.0 ± 10.52
Gill				
C	79.00 ± 6.28	83.1 ± 4.36	92.2 ± 6.80	104.7 ± 11.45
Z	79.00 ± 6.28	82.9 ± 5.79	93.9 ± 5.37	106.7 ± 7.19
Cd	79.00 ± 6.28	25.8 ± 2.66	33.9 ± 2.32	34.6 ± 6.24
CdZ1	79.00 ± 6.28	56.9 ± 6.91	78.6 ± 6.52	63.4 ± 3.37
CdZ2	79.00 ± 6.28	66.6 ± 12.01	109.7 ± 16.33	108.4 ± 10.25
CdZ3	79.00 ± 6.28	78.6 ± 6.52	96.7 ± 4.46	112.2 ± 10.80
Muscle				
C	163.4 ± 10.33	179.8 ± 7.06	187.9 ± 10.78	218.2 ± 11.01
Z	163.4 ± 10.33	181.7 ± 3.48	184.3 ± 4.51	210.3 ± 4.51
Cd	163.4 ± 10.33	45.8 ± 4.63	28.9 ± 2.69	26.5 ± 2.77
CdZ1	163.4 ± 10.33	57.3 ± 6.83	64.8 ± 6.47	93.6 ± 10.21
CdZ2	163.4 ± 10.33	68.0 ± 8.74	92.9 ± 6.49	130.5 ± 6.92
CdZ3	163.4 ± 10.33	79.8 ± 6.47	107.6 ± 8.35	155.4 ± 10.07

Table 4. Effect of sublethal level of cadmium and the role of zeolite on RNA : DNA ratio in different tissues of *Oreochromis mossambicus.* Each value is the mean ($\overline{X}$+SD) of three observations.

Exposures	Exposure period (days)			
	0	15	30	45
Liver				
C	1.39 ± 0.12	1.66 ± 0.10	2.16 ± 0.17	2.38 ± 0.21
Z	1.39 ± 0.12	1.84 ± 0.13	2.21 ± 0.12	2.26 ± 0.24
Cd	1.39 ± 0.12	0.35 ± 0.01	0.33 ± 0.04	0.10 ± 0.01
CdZ1	1.39 ± 0.12	0.47 ± 0.03	0.56 ± 0.06	1.10 ± 0.07
CdZ2	1.39 ± 0.12	0.90 ± 0.11	1.19 ± 0.13	1.73 ± 0.14
CdZ3	1.39 ± 0.12	1.74 ± 0.09	1.77 ± 0.18	1.86 ± 0.12
Gill				
C	0.94 ± 0.05	2.18 ± 0.15	3.99 ± 0.23	3.82 ± 0.28
Z	0.94 ± 0.05	2.24 ± 0.18	3.87 ± 0.29	3.90 ± 0.31
Cd	0.94 ± 0.05	0.72 ± 0.06	0.31 ± 0.01	0.18 ± 0.01
CdZ1	0.94 ± 0.05	1.26 ± 0.10	1.44 ± 0.13	1.70 ± 0.11
CdZ2	0.94 ± 0.05	2.21 ± 0.17	1.89 ± 0.17	2.47 ± 0.24
CdZ3	0.94 ± 0.05	2.75 ± 0.21	2.88 ± 0.21	3.55 ± 0.17
Muscle				
C	2.25 ± 0.15	2.38 ± 0.17	2.99 ± 0.16	4.77 ± 0.29
Z	2.25 ± 0.15	2.34 ± 0.12	2.87 ± 0.10	4.61 ± 0.33
Cd	2.25 ± 0.15	0.17 ± 0.02	0.30 ± 0.04	0.23 ± 0.03
CdZ1	2.25 ± 0.15	2.09 ± 0.13	1.93 ± 0.16	1.86 ± 0.14
CdZ2	2.25 ± 0.15	1.50 ± 0.16	1.67 ± 0.08	2.22 ± 0.20
CdZ3	2.25 ± 0.15	2.06 ± 0.19	2.28 ± 0.20	3.64 ± 0.24

Table 5. Effect of sublethal concentration of cadmium and the role of ion-exchanging agent, zeolite on cadmium distribution in water, sediment, fish body and faeces at the termination of the experiment. Each value is the mean $(\overline{X} \pm SD)$ of three estimations.

Exposures	Water (mg Cd/l)	Sediment (mg Cd/g dry matter)	Fish body (mg Cd/g dry matter)	Faeces (mg Cd/g dry matter)
C	0.004 ± 0	0.008 ± 0	0.003 ± 0	0.006 ± 0
Z	Nil	Nil	Nil	Nil
Cd	5.20 ± 0.48	1.14 ± 0.00	1.78 ± 0.03	0.15 ± 0.01
CdZ1	3.79 ± 0.22	2.90 ± 0.01	1.36 ± 0.07	0.94 ± 0.03
CdZ2	2.73 ± 0.10	3.47 ± 0.10	1.17 ± 0.14	2.34 ± 0.02
CdZ3	1.50 ± 0.07	4.19 ± 0.14	0.90 ± 0.16	5.08 ± 0.10

haematology in fish (James *et al.* 1998). *James and Sampath* (1998) reported that application of even the higher dose of zeolite (8 gZ/l) did not cause any adverse effects in *O. mossambicus*.

Comparatively, zeolite is cheaper, causes no side effects, and is more suitable than EDTA and NTA and hence it may be considered as best chemical agent to remove toxic elements from polluted environments. In conclusion, zeolite reduces the Cd level in water and eliminates the same from fish body and improve the biochemical and growth parameters. However, the treatment period given i.e. 45 d was not sufficient for complete removal of metal from the medium and the fish body and it requires still longer duration.

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