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# Removal of Copper Toxicity by Zeolite in Java Tilapia *Oreochromis mossambicus* (Peters)

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Reduction of toxic elements in aquatic environments is one of the primary challenges in waste water treatment. The most widely used technique for 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 such chemical and it acts as an ion-exchanging agent. Recently, it has been used in detergency, aquaculture ponds and nuclear treatment, but it also has large potential for other application 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 metabolites, settlement of suspended solids and absorption of gases like $CO_2$, $SO_2$ and $H_2S$ in aquaculture ponds (Chaberlain 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). However, there is paucity of information on how zeolite reduces the toxic elements in polluted medium. There is also not much information available on the duration of the treatment and optimum dosage of zeolite needed to reduce the metal toxicity. The present work was designed to study the effect of zeolite on the removal of copper at two different levels (sublethal and median lethal) from water and tissues of Java tilapia, *Oreochromis mossambicus*.

## MATERIALS AND METHODS

*Oreochromis mossambicus* (Peters) were collected from a pond and acclimated for 30 d in laboratory conditions. During acclimatization, water was changed daily and fish were fed *ad libitum* with pellet feed containing 35% protein. Three 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 then diluted with

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freshwater to obtain the desired concentrations of the medium. Well acclimated and active 10 fish (12.4 ± 1.1 g) were exposed to different concentrations of copper (0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 mg $l^{-1}$) and mortality was observed for 96 h. A control was run in freshwater. The $LC_{50}$ was calculated adopting probit analysis. The 96 h $LC_{50}$ value of copper for *Oreochromis mossambicus* was 4.27 mg $l^{-1}$ and its 95% confidence limits were 3.72 (lower) and 4.90 (upper) mg $l^{-1}$.

In the second and third series of experiments, animals were exposed for 180 d to estimate metal accumulation in different tissues of fish and also metal content was determined in the medium and sediment. Acclimated animals (12.4 ± 1.1 g) recruited from stock were divided into six groups of 60 individuals each. They were exposed to the sublethal level of copper (2.14 mg $l^{-1}$) alone and with different levels of zeolite (series 2). Similarly another set of six groups of 60 individuals each was prepared and exposed to median lethal level of copper (4.27 mg $l^{-1}$) alone and alongwith different levels of zeolite (series 3). The animals were starved for 24 h before commencement of the experiment.

**Table 1.** Experimental groups and their notations.

| S.No. | Groups | Total amount of zeolite added (kg) | Notation |
|---|---|---|---|
| | ***Sublethal treatments*** **(SL)** | | |
| 1 | Control (Freshwater) | — | C |
| 2 | Copper (2.14 mg $l^{-1}$) alone | — | $^{SL}Cu$ |
| 3 | Copper (2.14 mg $l^{-1}$) + 0.5gZ*$l^{-1}$ | 0.650 | $^{SL}CuZ1$ |
| 4 | Copper (2.14 mg $l^{-1}$) + 2.0gZ*$l^{-1}$ | 2.600 | $^{SL}CuZ2$ |
| 5 | Copper (2.14 mg $l^{-1}$) + 4.0gZ*$l^{-1}$ | 5.200 | $^{SL}CuZ3$ |
| 6 | Copper (2.14 mg $l^{-1}$) + 8.0gZ*$l^{-1}$ | 10.400 | $^{SL}CuZ4$ |
| | ***Median lethal treatments*** **(ML)** | | |
| 1 | Control (Freshwater) | — | C |
| 2 | Copper (4.27 mg $l^{-1}$) alone | — | $^{ML}Cu$ |
| 3 | Copper (4.27 mg $l^{-1}$) + 0.5gZ*$l^{-1}$ | 0.650 | $^{ML}CuZ1$ |
| 4 | Copper (4.27 mg $l^{-1}$) + 2.0gZ*$l^{-1}$ | 2.600 | $^{ML}CuZ2$ |
| 5 | Copper (4.27 mg $l^{-1}$) + 4.0gZ*$l^{-1}$ | 5.200 | $^{ML}CuZ3$ |
| 6 | Copper (4.27 mg $l^{-1}$) + 8.0gZ*$l^{-1}$ | 10.400 | $^{ML}CuZ4$ |
| | * Sodium aluminosilicate | | |

For convenient presentation, control and experimental groups hereafter would be referred by their notation. The chosen levels of zeolite and a constant level of copper [2.14 mg $l^{-1}$ (50% of $LC_{50}$) in the case of sublethal exposure and 4.27 mg $l^{-1}$ in the case of median lethal exposure] were added to the medium separately on 0 d. The experiment was conducted in epoxy coated cement tank containing 1300 l of test medium. Pond sediment was added to

all the experimental tanks upto 5 cm height to simulate field condition and then 1300 l of freshwater was pumped in it. The medium was mixed well after the addition of copper and zeolite and then test animals were introduced. Zeolite treated fish did not show any deleterious effects but it improved the growth and heamatological parameters during the experimental period of 45 days in fishes (James and Sampath 1999 a and b, James 2000), hence, individual effect of zeolite was not studied in the present study. 35% protein feed was offered as diet to test animals in a feeding tray once in a day at 0800 h and uneaten food was removed 2 h after feeding. The medium was not changed during the experiment and the medium was aerated for 14 h a day to avoid depletion of oxygen. The hydrobiological parameters like dissolved oxygen, temperature, pH, salinity and hardness of media were estimated during non-aeration and these parameters averaged to 4.16 $\pm$ 0.2 or 4.07 $\pm$ 0.4 ml $l^{-1}$, 29.2 $\pm$ 1 or 29.0 $\pm$ 1°C, 7.80 $\pm$ 0.08 or 7.82 $\pm$ 0.08, 0.15 $\pm$ 0.02 or 0.14 $\pm$ 0.02 $g^{-1}$ and 58.0 $\pm$ 4.0 or 60.0 $\pm$ 3.0 mg $CaCO_3 l^{-1}$ in sublethal or median lethal exposures respectively. First week of every month, eight fish were removed from each group for estimation of metal accumulation in tissues gill, liver and muscle following the method of FAO (1975). There was no mortality in all experimental groups except $^{ML}$Cu which recorded 16.7% mortality. The zeolite deposited over the sediment was carefully removed prior to sampling of sediment and dried at 60°C in hot air oven for 2 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 experimental groups at different days.

## RESULTS AND DISCUSSION

Trace level of Cu was found in control water (0.005 mg $l^{-1}$) and sediment (0.024 mg $l^{-1}$) in the present study. The initial level of Cu in all exposures was 2.14 or 4.27 mg $l^{-1}$ in sublethal or median lethal exposures and it gradually declined with extension of exposure period from 0 to 180 days (Fig.1 and 2). When zeolite was added to sublethal level of Cu media, the metal content was completely removed within 120 days in $^{SL}$CuZ2 - $^{SL}$CuZ4 groups and 150 days in $^{SL}$CuZ1 group. However, in median lethal exposures, Cu was completely removed on 180 days in $^{ML}$CuZ2 - $^{ML}$CuZ4 groups (except $^{ML}$CuZ1). This indicates that zeolite took 60 more days for the complete removal of Cu in median lethal exposures as compared to sublethal exposures. The Cu level in $^{SL}$Cu and $^{ML}$Cu groups was 0.850 and 1.39 mg $l^{-1}$ even at the time of termination of the experiment. The Cu level in sediment and medium was inversely proportional (Fig.1 and 2).

Trace level of copper was also found in tissues of control fish and the amount of Cu had linearly increased with extension of exposure period in

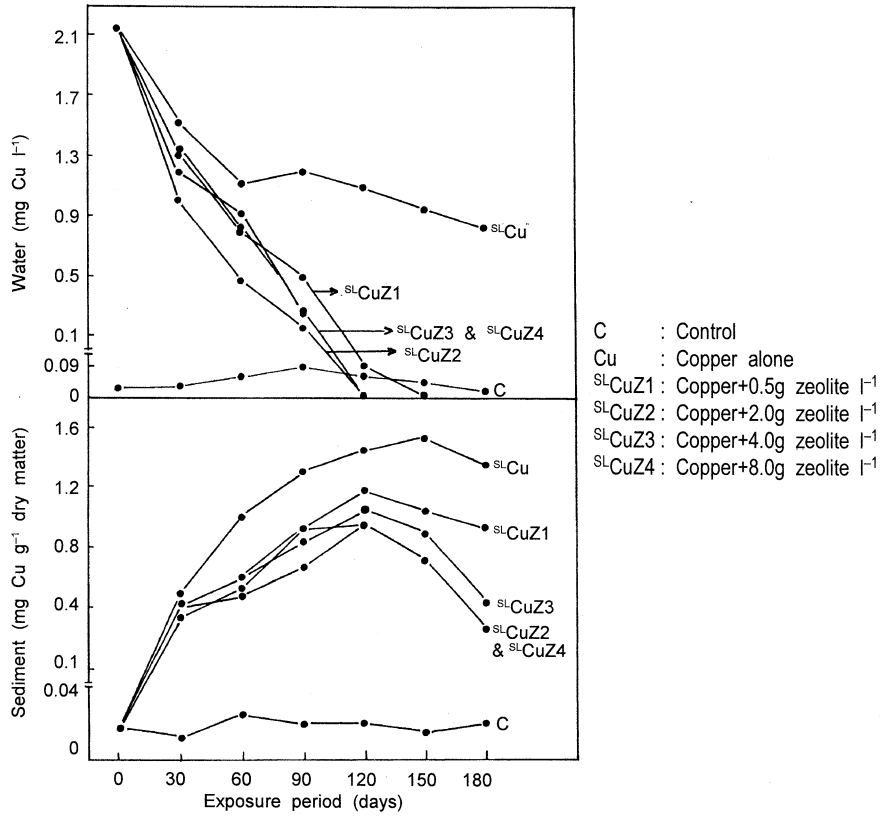


**Figure 1.** Effect of sublethal concentration of copper (2.14 mg $l^{-1}$) and addition of different doses of zeolite on copper distribution in water and sediment.

fish exposed to sublethal and median lethal levels of Cu alone. However, in zeolite treatments, there was initial increase in Cu content and the accumulation attained maximum on days 30 and 60 (except $^{SL}$CuZ1 and $^{ML}$CuZ1) and afterwards it drastically declined and completely eliminated from tested tissues on days 120 and 180 in sublethal and median lethal exposures respectively (Table 1 and 2). Fish exposed to median lethal exposures took 60 days more for the complete removal of Cu from tissues than those exposed to sublethal level.

Briggs and Smith (1996) found that zeolite have the capacity to remove ammonia and other metabolites from freshwater by ion-exchange and adsorption. James *et al.* (1998) reported that addition of another chelating agent EDTA to Cu contaminated medium cause 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 NTA (Nitrilotriacetic acid) and EDTA reduced the metal toxicity in fish by preventing the accumulation of metal in tissues. He also

**Table 2.** Effect of sublethal concentration of copper (2.14 mg $l^{-1}$) and addition of zeolite on the copper accumulation (μg.$g^{-1}$ wet tissue) in tissues of *Oreochromis mossambicus*.

| Exposures | Exposure period (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| **Gill** | | | | | | | |
| C | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 |
| $^{SL}$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 |
| $^{SL}$CuZ1 | 0.01 ± 0 | 0.88 ± 0.01 | 0.33 ± 0.02 | 0.09 ± 0.16 | 0.07 ± 0 | CuNd | CuNd |
| $^{SL}$CuZ2 | 0.01 ± 0 | 0.27 ± 0.01 | 0.20 ± 0.01 | 0.02 ± 0 | CuNd | CuNd | CuNd |
| $^{SL}$CuZ3 | 0.01 ± 0 | 0.31 ± 0.02 | 0.11 ± 0.01 | 0.02 ± 0 | CuNd | CuNd | CuNd |
| $^{SL}$CuZ4 | 0.01 ± 0 | 0.40 ± 0.03 | 0.13 ± 0.02 | 0.02 ± 0 | CuNd | CuNd | CuNd |
| **Liver** | | | | | | | |
| C | 0.05 ± 0.01 | 0.05 ± 0 | 0.04 ± 0 | 0.06 ± 0 | 0.04 ± 0 | 0.05 ± 0 | 0.05 ± 0 |
| $^{SL}$Cu | 0.05 ± 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 |
| $^{SL}$CuZ1 | 0.05 ± 0.01 | 0.92 ± 0.11 | 0.98 ± 0.06 | 0.13 ± 0.02 | 0.07 ± 0 | CuNd | CuNd |
| $^{SL}$CuZ2 | 0.05 ± 0.01 | 0.98 ± 0.06 | 0.62 ± 0.06 | 0.12 ± 0.03 | 0.01 ± 0 | CuNd | CuNd |
| $^{SL}$CuZ3 | 0.05 ± 0.01 | 0.99 ± 0.11 | 0.73 ± 0.03 | 0.15 ± 0.04 | 0.03 ± 0 | CuNd | CuNd |
| $^{SL}$CuZ4 | 0.05 ± 0.01 | 1.06 ± 0.13 | 0.85 ± 0.05 | 0.17 ± 0.01 | 0.02 ± 0 | CuNd | CuNd |
| **Muscle** | | | | | | | |
| C | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 |
| $^{SL}$Cu | 0.01 ± 0 | 0.72 ± 0.06 | 1.85 ± 0.02 | 2.97 ± 0.04 | 4.06 ± 0.06 | 5.17 ± 0.14 | 4.23 ± 0.23 |
| $^{SL}$CuZ1 | 0.01 ± 0 | 0.15 ± 0.01 | 0.11 ± 0 | 0.01 ± 0 | CuNd | CuNd | CuNd |
| $^{SL}$CuZ2 | 0.01 ± 0 | 0.06 ± 0 | 0.03 ± 0 | CuNd | CuNd | CuNd | CuNd |
| $^{SL}$CuZ3 | 0.01 ± 0 | 0.10 ± 0 | 0.10 ± 0 | CuNd | CuNd | CuNd | CuNd |
| $^{SL}$CuZ4 | 0.01 ± 0 | 0.09 ± 0 | 0.25 ± 0.01 | 0.03 ± 0 | CuNd | CuNd | CuNd |

Each value is the mean ($\bar{X}$±SD) of three fish; CuNd: Copper not detected.

**Table 3.** Effect of median lethal concentration of copper (4.27 mg $l^{-1}$) and addition of zeolite on metal accumulation (μg/g wet tissue) in tissues of *Oreochromis mossambicus*.

| Exposures | Exposure period (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| **Gill** | | | | | | | |
| C | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 |
| $^{ML}$Cu | 0.01 ± 0 | 4.87 ± 0.31 | 9.62 ± 0.71 | 19.26 ± 1.16 | 23.33 ± 1.65 | 28.11 ± 2.16 | 29.77 ± 2.74 |
| $^{ML}$CuZ1 | 0.01 ± 0 | 2.45 ± 0.11 | 4.84 ± 0.26 | 6.80 ± 0.61 | 8.34 ± 0.48 | 7.74 ± 0.44 | 5.83 ± 0.36 |
| $^{ML}$CuZ2 | 0.01 ± 0 | 1.36 ± 0.03 | 2.43 ± 0.08 | 1.10 ± 0.06 | 0.70 ± 0.06 | 0.09 ± 0 | CuNd |
| $^{ML}$CuZ3 | 0.01 ± 0 | 1.40 ± 0.01 | 2.87 ± 0.12 | 1.42 ± 0.10 | 0.92 ± 0.03 | 0.06 ± 0 | CuNd |
| $^{ML}$CuZ4 | 0.01 ± 0 | 1.68 ± 0.13 | 2.27 ± 0.16 | 1.51 ± 0.09 | 1.13 ± 0.07 | 0.06 ± 0 | CuNd |
| **Liver** | | | | | | | |
| C | 0.05 ± 0 | 0.05 ± 0 | 0.05 ± 0 | 0.06 ± 0 | 0.06 ± 0 | 0.06 ± 0 | 0.05 ± 0 |
| $^{ML}$Cu | 0.05 ± 0 | 7.74 ± 0.15 | 18.97 ± 1.36 | 29.44 ± 2.26 | 36.17 ± 3.51 | 43.43 ± 3.08 | 45.86 ± 4.11 |
| $^{ML}$CuZ1 | 0.05 ± 0 | 2.14 ± 0.11 | 6.90 ± 0.14 | 7.09 ± 0.19 | 9.24 ± 1.10 | 10.75 ± 0.93 | 8.26 ± 0.76 |
| $^{ML}$CuZ2 | 0.05 ± 0 | 2.81 ± 0.06 | 5.26 ± 0.28 | 4.16 ± 0.21 | 2.21 ± 0.01 | 0.60 ± 0 | CuNd |
| $^{ML}$CuZ3 | 0.05 ± 0 | 3.76 ± 0.17 | 6.91 ± 0.16 | 5.83 ± 0.28 | 2.96 ± 0.14 | 0.84 ± 0.01 | CuNd |
| $^{ML}$CuZ4 | 0.05 ± 0 | 1.89 ± 0.04 | 7.44 ± 0.31 | 7.84 ± 0.31 | 3.21 ± 0.30 | 0.87 ± 0.01 | CuNd |
| **Muscle** | | | | | | | |
| C | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 |
| $^{ML}$Cu | 0.01 ± 0 | 1.31 ± 0.02 | 3.03 ± 0.24 | 4.10 ± 0.33 | 6.37 ± 0.48 | 9.39 ± 0.75 | 11.18 ± 0.97 |
| $^{ML}$CuZ1 | 0.01 ± 0 | 0.63 ± 0.01 | 1.28 ± 0.02 | 1.93 ± 0.01 | 2.60 ± 0.06 | 2.05 ± 0.03 | 0.063 ± 0 |
| $^{ML}$CuZ2 | 0.01 ± 0 | 0.51 ± 0.03 | 0.73 ± 0 | 0.60 ± 0 | 0.27 ± 0 | 0.11 ± 0 | CuNd |
| $^{ML}$CuZ3 | 0.01 ± 0 | 0.62 ± 0.07 | 0.97 ± 0.01 | 0.82 ± 0 | 0.46 ± 0 | 0.04 ± 0 | CuNd |
| $^{ML}$CuZ4 | 0.01 ± 0 | 0.74 ± 0.05 | 1.23 ± 0.03 | 0.93 ± 0.01 | 0.53 ± 0 | 0.08 ± 0 | CuNd |

Each value is the mean ($\bar{X}$±SD) of three fish; CuNd: Copper not detected.

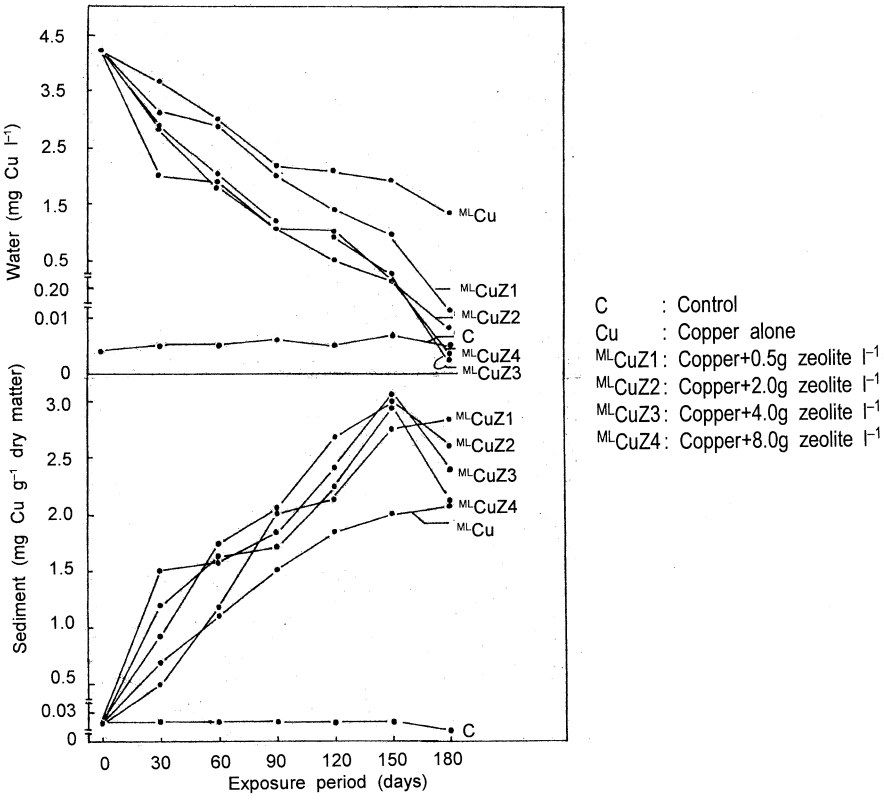


**Figure 2.** Effect of median lethal concentration of copper (4.27 mg $l^{-1}$) and addition of different doses of zeolite on copper distribution in water and sediment.

suggested that cadmium complexed to EDTA is indeed taken up, but the complex is quickly excreted through urine (Babiker and Rankin 1975).

The present study revealed that metal uptake by test animals was lower in zeolite treated groups than in the Cu exposed group. It was found that zeolite caused the elimination of Cu in polluted water (Fig.1 and 2) which in turn reduced the metal burden in fish (see Table 1 and 2). Among the zeolite treated groups, $^{SL}$CuZ2 in sublethal exposures and $^{ML}$CuZ2 in median lethal exposures (2 g Z $l^{-1}$) have elicited the better performances than other groups (0.5, 4 and 8 g Z $l^{-1}$) and hence an addition of 2 g zeolite $l^{-1}$ is considered as optimum dose. Based on the present study, it is recommended that, application of 2 g zeolite $l^{-1}$ to metal polluted environment could remove the heavy metal, copper at any level of concentration 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, resulted in least accumulation of metal (Cd) and maximum improvement in food intake and growth in the cadmium exposed

*Heteropneustes fossilis*. Gworek and Borowiak (1990) reported that application of synthetic zeolite cause the immobilization of heavy metals and recommended for clean up method. In aquaculture practices, application of zeolite is suggested in ponds before stocking fry or during the pond preparation (Briggs amd Smith 1996). When the concentration of ammonium ion 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. However, doses of chelating agent like EDTA could cause deleterious effects on survival, development and growth in crustacean larvae (Simon 1981) and on survival and haematology of fish (James *et al.* 1998). Comparatively, zeolite is the cheapest, causes no side effects and more suitable than EDTA and NTA and hence it may be considered as one of the best chemical agents to remove toxic elements from polluted environments.

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