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PROTECTIVE ROLE OF ZEOLITE ON SHORT- AND LONG-TERM LEAD TOXICITY IN THE TELEOST FISH *Heteropneustes fossilis*.

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ABSTRACT

The high ion-exchange capacity of zeolite (sodium aluminium silicate) enhances the removal of lead from water, thus decreasing its availability to fish. Zeolites are very important in the field of environmental preservation due to the low cost and ecological compatibility. Zeolites can adsorb metallic ions by cation exchange reactions. Continuous exposure of the teleost fish *Heteropneustes fossilis* to sublethal concentrations of lead nitrate in water solution for short (35 days) and long (120 days) periods decreased both the soluble protein, RNA and glycogen contents in the liver and the body weight, but increased the cholesterol content. The presence of zeolite in the exposure solution decreased all of the adverse effects. In fish exposed to zeolite as feed additive, all the parameters improved in comparison to control fish, indicating that zeolites can be used safely in biological systems.

INTRODUCTION

Lead is an important contaminant with different toxic effects to various species [1]. Lead concentrations in natural waters have been summarized in some reviews [2,3]. Further, exposure of fish to lead via gills [4] has been shown to adversely affect body weight [5,6], digestive enzymes and lipase [7]. Natural and synthetic zeolites (sodium aluminium silicates) are known to easily adsorb metal ions by exchange reactions [6]. In particular, removal of lead from waste water using zeolite has been reported [8].However, zeolites have not yet been used extensively to remove heavy metals from water, despite their documented affinity towards many toxic cations and the evident advantage of ion exchange with respect to methods based on precipitation [8]. In this work, biochemical changes in the fish liver due to short- and long-term exposure to lead and the usefulness of zeolite to adsorb lead in water and to decrease its adverse effects to fish, have been investigated.

MATERIALS AND METHODS

Samples of *Heteropneustes fossilis* (cat fish) were obtained from the nursery of the city of Sagar in Central India, and acclimatized to laboratory conditions for two weeks in aquaria containing tap water (pH, 7.3; temperature, 20 ± 3°C; dissolved O_2, 3.6 mgL^{-1}) prior to start experiments. All fish were fed regularly with fish meal available locally. Fish were then divided into two sets of four groups each, and placed into eight tanks. Four different treatments were experimented: Pb $(NO_3)_2$, zeolite, Pb $(NO_3)_2$ + zeolite, and

TABLE 1.

Doses (mg L^{-1} d^{-1}) of $Pb(NO_3)_2$ and Zeolite used in the experiments

Treatment	Short term experiments	Long term experiments
A $Pb(NO_3)_2$	60	20
B $Pb(NO_3)_2$ + Zeolite	60 50	20 20
C Only Zeolite	50	20

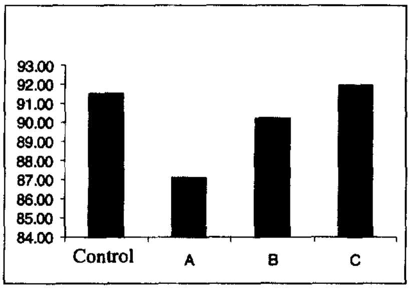

Fig.1 Protein (mg/g)

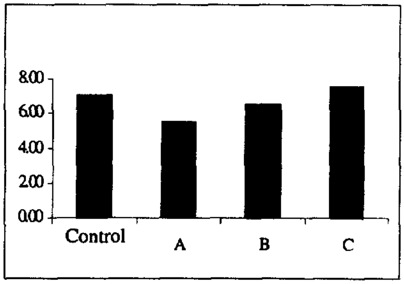

Fig.2 RNA (mg/g)

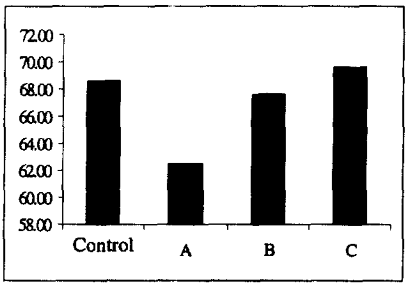

Fig.3 Glycogen (mg/g)

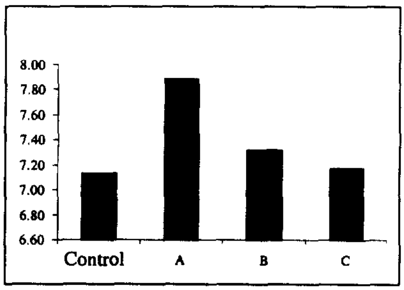

Fig.4 Cholesterol (mg/g)

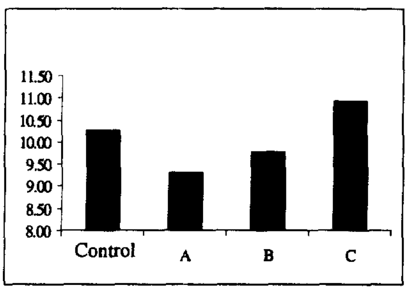

Fig.5 Body Weight (g)

A – $Pb(NO_3)_2$
B - $Pb(NO_3)_2$ + Zeolite
C – Zeolite only

Figures 1-5. Contents of Protein, RNA, Glycogen and Cholesterol in the fish liver and Body Weight of fish under three different conditions (A, B, C) in the short-term study control (Table 1). Control fish were only fed. Aquaria were cleaned every week. The 96h-LC50 value determined for Pb $(NO_3)_2$ was 105 mg L^{-1}.

In the short-term study, n° 30 fish samples were collected from each treatment and sacrificed, after 35 days. In the long-term study fish were sacrificed after 60, 90 and 120 days of exposure (10 fish samples were sacrificed each time). Liver was removed, homogenized in fish saline suspension (0.59%) and processed for the analysis of contents of soluble proteins (Lowry method), RNA (Orcinol method), glycogen (Anthron method) and cholesterol (Lieberman-Burchard method), as described by Plummer [9]. All experiments were repeated three times. Student 't' test was employed for the statistical evaluation of data.

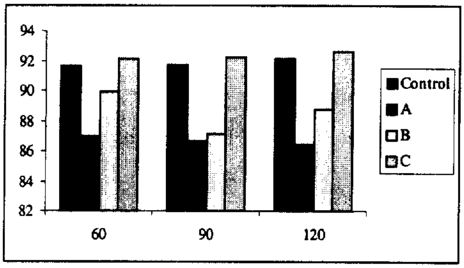

Fig.6 Protein (mg/g)

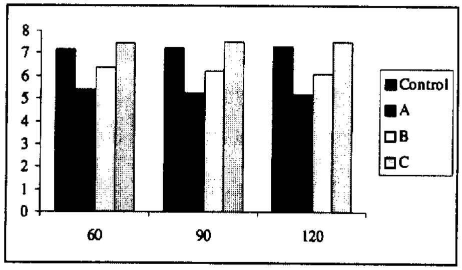

Fig.7 RNA (mg/g)

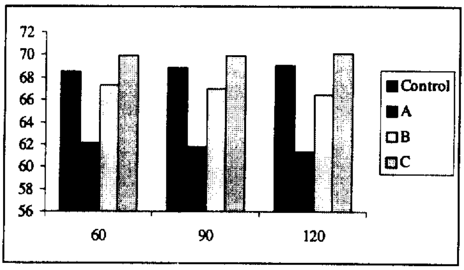

Fig.8 Glycogen (mg/g)

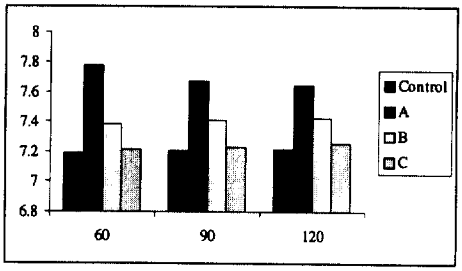

Fig.9 Cholesterol (mg/g)

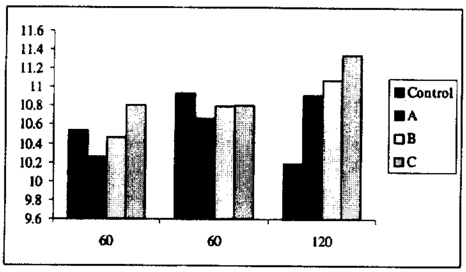

Fig.10 Body Weight (g)

A – $Pb(NO_3)_2$
B - $Pb(NO_3)_2$ + Zeolite
C – Zeolite only

Figures 6-10. Contents of Protein, RNA, Glycogen and Cholesterol in the fish liver and Body Weight of fish under three different conditions (A, B, C) in the long-term study

RESULTS AND DISCUSSION

In both short-term and long-term experiments, lead nitrate was observed to cause a significant decrease of soluble protein, RNA and glycogen contents of liver (Figs. 1-10). These results suggested the inhibitory role played by lead in cellular metabolism, possibly through enzyme inhibition. Lead nitrate also caused a decrease of body weight, possibly due to an excessive loss of body constituents [6]. The increase of cholesterol content and the decrease in soluble protein and glycogen contents suggested the possible involvement of carbon skeleton of amino acids and acetyl CoA into cholesterol biosynthesis [10], which otherwise would have provided body weight gain and normal carboydrate metabolism.

Results obtained showed that zeolite was able to protect fish against lead toxicity by decreasing the adverse effects of lead nitrate. Further, zeolite alone did not cause any adverse effect when added to exposure water. Because of the high cation exchange capacity of zeolite, ionic lead may replace other cations on zeolite, thus becoming less available to fish. Some authors [9,11] have found a high affinity and a rapid exchange kinetics of lead onto zeolites. Further, zeolites added to food have been shown to increase the body weight and haemoglobin contents in cattles [6]. Similarly, results of this study showed that when fish were exposed to zeolite only, a little but statistically significant increase in all the parameters was measured at all intervals of time. These results suggest that zeolites are not only harmless, but beneficial for fish exposure.

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