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Effect of Natural Ion Exchanger Chabazite for Remediation of Lead Toxicity : An Experimental Study in Teleost Fish *Heteropneustes fossilis*

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Abstract : Zeolites are synthetic and naturally occurring sodium/calcium aluminosilicate minerals having unique adsorption, cation-exchange, dehydration-rehydration, and catalytic properties. They are used for the removal of ammonia, phosphates and heavy metals, and also as dietary supplement in animal diets in pet litters and as ammonia filters in kidney-dialysis unit. In the present investigation, toxicity of lead nitrate with reference to protein contents in fish tissue and the role of natural zeolite chabazite over it, has been studied. The experimental teleost fish *Heteropneustes fossilis* divided in to four equal groups. Group I worked as control while group II, III and IV exposed to sublethal concentration of lead nitrate, lead nitrate + chabazite and only chabazite respectively. Fish were dissected after 60, 90, 120, 150 and 180 days of exposure, their tissue brain, liver, kidney and gills removed and processed for the estimation of protein contents. The data thus obtained was statistically evaluated by applying student'tʼ test. It has been observed that lead nitrate cause deleterious effects in all the tissue with reference to protein contents. When chabazite added with lead nitrate, protein contents improved towards normal. In fish of group IV, exposed to chabazite only, the protein contents further improved in comparison to control. The data thus obtained is statistically significant. The observations of the present study indicate the specificity of chabazite for lead adsorption and an inexpensive mean for its removal from exposure water. Also, chabazite may be useful as feed additive to improve quality of fish by increasing protein contents.

Key words : Lead, ion exchanger, chabazite, protein, fish tissues.

Introduction

Metal accumulation in the environment continuously increases owing to the anthropogenic activities and they tend to concentrate in all the aquatic matrices. Heavy metals including lead are found in various tissues of fish and shrimps (Vazquez *et al.*, 2001). High level of trace metals is found in liver, kidney, and muscles of Antarctic penguin *Pygoscelis adeliac* (Patricia *et al.*, 2006). Lead poisoning is associated with carcinogenicity, reduced fertility, miscarriages, spermatoxicity and gonadotoxicity (Kazantsis, 1981; Thomas & Brogan, 1983). Lead is among the most toxic heavy element in the atmosphere. Fergusson (1990) reported that aerosol lead enters the human blood stream by way of the respiratory tract and indirectly by surface deposition in the elementary tract followed by absorption. Accumulation of heavy metals, including lead in goat meat has been recorded 0.080mg/kg in liver (Abou-Arab, 2001). Slawomir *et al.* (2005) investigated that exposure to even small dose of lead may cause raised arterial blood pressure and this may be connected with peroxidative action of ä-aminolivulinic acid. Exposure of fish to lead via gills effects adversely the body weight and digestive enzymes (WHO, 1989; Shafi and

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Choundhary, 1979; Sastry and Gupta, 1978 and Jain *et al*., 1996). Acute toxicity of heavy metals, including lead has been studied in rainbow trout *Oncorhyncos myleies* (Svecevicius, 2001). Lead increases with age in liver and brain (Zaccaroni *et al*., 2003). Remediation of lead toxicity in teleost fish *Heteropneustes fossilis* has also been studied by Jain *et al*. (1997), Jain and Shrivastava (2000) and Shrivastava *et al*. (2001). Kalay *et al*. (1999) studied that the gills of teleost fish play an important role on ion regulation, gas exchange, acid-base balance and nitrogenous waste excretion, which means it has a key role at the interface of fish with its environment. Immunotoxic studies in *Clarias batrachus* due to subacute lead toxicity has been done by Rout and Naik (1998).

Among the various cation exchangers, zeolites especially natural zeolite viz chabazite, phillipsite and clinoptilolite are preffered due to their high selectivity for heavy metal cations and low cost compared with synthetic cation exchange materials (Sherman, 1978; Semmens & Seyfarth, 1978). Natural zeolites help in protein digestion in cattle, sheep and goat (Petkova *et al*., 1984), out of which clinoptilolite is an ion exchanger widely used for drinking water treatment (Gaspard, 1983), and removal of ammonia from waste water (Aral *et al*., 1999). In European seabass, the influence of zeolite observed on protein digestibility and growth etc. (Jorge *et al*., 1998). Role of natural and synthetic zeolite for the remediation of lead toxicity from fish exposure water (Jain *et al*., 1997; Jain & Shrivastava, 2000; Shrivastava *et al*., 2001 and Jain 1999, 2001) and cadmium toxicity in a freshwater fish observed (James and Sampath, 1999). Looking in to the various applications of natural zeolites, the present investigation has been planned to study whether natural zeolite chabazite play role for the remediation of lead toxicity in brain, liver, kidney and gills of teleost fish *Heteropneustes fossilis* and also for the improvement of fish quality with reference to protein contents as an indicator.

Material and Methods

Living specimen of teleost fish *Heteropneustes fossilis* were collected from local fishery and acclimatized in aquaria (50L) containing tap water for two weeks prior to experimentation. 96h LC_{50} value for lead nitrate determined by interpolation method (105 mgl^{-1}). Fish were divided into four equal groups. Group I served as control and received only fish feed. Group II, III and IV exposed to sublethal concentration of lead nitrate, lead nitrate+chabazite, and only chabazite respectively. For experimental study, doses of lead nitrate and chabazite, each 10 mgl^{-1} day^{-1} (10% of 96h LC50) have been applied by sprinkling in to the exposure water. All experiments were run in triplicate. Fish were weighed and sacrificed after 60, 90, 120, 150 and 180 days of exposure, their tissues brain, liver, kidney and gills were removed and processed for quantitative estimation of protein by Lowry method (Plummer, 2002). Student't' test was applied for the statistical evaluation of data.

Result and Discussion

According to table the protein contents in brain, liver, kidney and gills of control fish are almost similar to that of initial control fish. Sublethal concentration of lead nitrate (group II) cause significant decrease in protein content in the experimental tissue. But when fish of group III exposed to lead nitrate along with chabazite, reversal of deleterious effect in all the tissue has been observed. In fish exposed to chabazite only (group IV) as feed additive, protein content in all the tissues increase in comparison to initial control fish.

Enzyme inhibition due to lead nitrate has been reported by Jain *et al*. (1997), Jain & Shrivastava (2000), Shrivastava *et al*. (2001),

Table 1 : Chronic lead toxicity in the brain, liver, kidney and gills of fish *Heteropneustes fossilis* with reference to protein contents (mg/g) and protective action of natural zeolite chabazite over it (values are mean±SE, and P value)

Tissues	Days of Exposure	Control	$Pb(NO_3)_2$	$Pb(NO_3)_2$ + Chabazite	Only Chabazite
Brain	60	70.25±1.53	61.48 ± 1.43 P<0.001	65.18 ± 1.47 P<0.001	71.22 ± 1.54 P<0.001
Liver		83.94 ± 1.67	71.52 ± 1.54 P<0.001	75.49 ± 1.58 P<0.001	87.18± 1.70 P<0.001
Kidney		71.80 ± 1.54	62.42 ± 1.44 P<0.001	67.36 ± 1.49 P<0.001	74.62± 1.57 P<0.001
Gills		65.90 ± 1.48	56.96 ± 1.37 P<0.001	60.15 ± 1.41 P<0.001	68.10± 1.50 P<0.001
Brain	90	70.27 ± 1.53	61.45 ± 1.43 P<0.001	65.22 ± 1.47 P<0.001	71.29±1.54 P<0.01
Liver		83.98 ± 1.67	71.48 ± 1.54 P<0.001	75.58± 1.58 P<0.001	87.28± 1.70 P<0.001
Kidney		71.82 ± 1.54	62.38 ± 1.44 P<0.001	67.40± 1.49 P<0.001	74.66± 1.57 P<0.001
Gills		65.92 ± 1.48	56.88 ± 1.37 P<0.001	60.21± 1.41 P<0.001	68.15± 1.50 P<0.001
Brain	120	70.28 ± 1.53	61.40 ± 1.43 P<0.001	65.30 ± 1.47 P<0.001	71.34± 1.54 P<0.001
Liver		84.10±1.67	71.42 ± 1.54 P<0.001	75.65± 1.58 P<0.001	87.45± 1.70 P<0.001
Kidney		71.83 ± 1.54	62.32 ± 1.44 P<0.001	67.48± 1.49 P<0.001	74.72± 1.57 P<0.001
Gills		65.94 ± 1.48	56.72 ± 1.37 P<0.001	60.25± 1.41 P<0.001	68.21± 1.50 P<0.001
Brain	150	70.30 ± 1.53	61.34 ± 1.42 P<0.001	65.36±1.47 P<0.001	71.40± 1.54 P<0.001
Liver		84.13 ± 1.67	71.38± 1.54 P<0.001	75.72±1.58 P<0.001	87.52±1.70 P<0.001
Kidney		71.84 ± 1.54	62.28± 1.44 P<0.001	67.56±1.50 P<0.001	74.76±1.57 P<0.001
Gills		65.95 ± 1.48	56.65± 1.37 P<0.001	60.28±1.41 P<0.001	68.28±1.50 P<0.001
Brain	180	70.32 ± 1.53	61.31± 1.42 P<0.001	65.41± 1.47 P<0.001	71.46±1.54 P<0.001
Liver		84.15 ± 1.67	71.35± 1.54 P<0.001	75.78±1.58 P<0.001	87.58±1.70 P<0.001
Kidney		71.86 ± 1.54	62.22± 1.44 P<0.001	67.65± 1.50 P<0.001	74.81±1.57 P<0.001
Gills		65.96 ± 1.48	56.58± 1.37 P<0.001	60.32± 1.41 P<0.001	68.33±1.50 P<0.001

Jain (1999, 2001) and James and Sampath (1999). Liver is the most important target organ for lead and cadmium toxicity (Holcombe *et al*., 1976 and Dallinger *et al*., 1987). The over all decrease in protein content is probably due to enzyme inhibition which plays an important role in protein synthesis (Sastry and Gupta, 1978). The kidney is a major site of antagonistic interaction between essential trace metals and cadmium (Friberg *et al.*, 1985 and Bremner, 1987). Mercury is known to inhibit protein synthesis in the brain of rats (Carmichael, 1976). Mumcilovic and Kostial (1974) and Levesey *et al*. (1986) observed high uptake of lead in the brain in immature rodents. Tulasi *et al*. (1992), Allen (1994) and Roesijadi and Robinson (1994) observed that accumulation of lead in gills is higher than in the liver. In the aquatic animals gills are the most important organ of respiration. Deleterious effects in gills due to heavy metals and pesticides have been observed by number of workers (Couch, 1979; Khangarot, 1982; Pawar and Katdare, 1983; Nilkant and Sawant, 1993).

In particular, removal of lead from waste water using zeolites has been reported by Pansini & Cololla (1989). Pond & Yen (1983) observed the effectiveness of both natural and synthetic zeolite in protecting piglets from cadmium induced anemia. Zeolites are also used as feed additive in cattles and chelation therapy for remediation of metal ion toxicity (Sharma, 1995). The use of clinoptilolite and its sodium form for removal of heavy metal from municipal waste water has been reported by Gaspard (1983) and Faghihian *et al*. (1999). For the remediation of metal toxicity, zeolites have been applied in Jawa tilapia (James & Sampath, 2003). The observations of the present investigation are also in accordance with the study performed on mercuric toxicity in fish with reference to natural zeolites stilbite (Chaurasia *et al*., 2007).

Zeolite added to food has been shown to increase the body weight and haemoglobin contents in cattles (Jain *et al*., 1996). The result of the study suggests the inhibitory role played by lead in cellular metabolism through enzyme inhibition as reported by Jain (1999). According to the table it is clear that chabazite is able to protect fish against lead toxicity by decreasing the adverse effects cause due to lead nitrate. It is also very interesting to note that zeolites alone did not cause any adverse effect when added to exposure water. Due to high ion exchange capacity of the chabazite, ionic lead is replaced by the cation Ca^{++} present on zeolites and therefore the lead becomes less available to fish. Looking at above, it is suggested that the natural zeolites may be considered as mineral commodity, the use of which promise to expand even more in the future in biological system.

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