Cadmium Toxicity of Catfish *Hetropneustes fossilis* by short Term Exposure to Chelating Agent, Zeolite

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Abstract

The aim of present study, heavy metal (Cd) taxicity in Catfish *Heteropneustes fossilis* was analyzed by short time exposure of 96 hrs. Heavy metal of Cadmium content in muscle, liver, gills, kidney and gonad tissues of *H. fossilis*. This study infers that the order of heavy metals accumulation is muscle > gills > gonads > liver > kidney and also heavy metal entry to the organisms through sediments due to the deposition of effluents intake of food materials, absorption of body tissue, drinking water, respiration via and metabolism.

Key words: *Heteropneustes fossilis*, Cadmium, Toxicity, Authoor, Tuticorin

Introduction

Human activity has continuously disturbed the natural environment particularly the aquatic ecosystems. Industrial development has resulted in heavy metal contamination of river water. The environmental pollution affects the general quality of our surroundings and poses risk to our health and well-being (Karthikeyani and Sivakumar, 2010). Heavy metals such as mercury, lead, cadmium, arsenic, chromium, zinc, copper and iron are mainly aquatic releases from industrial operations, domestic sewage discharges, atmospheric releases from fossil fuel burning and land run-off. These heavy metals due to their properties like long half life period, bioaccumulation, biomagnification in the food chain and non-biodegradability are hazardous to the aquatic organisms and their consumers which on being exposed to these heavy metals can suffer from immense health problems and risk of life (Onkar Singh and Kaur Manjeet, 2015).

The toxic effects of various heavy metals may hinder the physiological and metabolic functions, rate of growth, reproductive efficacy and ultimately causes mortality in fishes (Woodward *et al*., 1994). Lead is well known to cause neurological, haematological, immunological, gastrointestinal, reproductive, circulatory, histopathological and histochemical changes in vertebrate and invertebrate animals (Reglero *et al*., 2009; Abdallah *et al*.,2013; Rout and Niak,2013). The bioaccumulation of the highly toxic heavy metal has been observed in various tissues of the fishes like scales, bones, gills, kidneys and liver (Dallas and Day,1993). Toxicity tests have been performed on fishes to evaluate the effect of toxicants on various aquatic organisms under laboratory conditions. Therefore, the present study has been undertaken to study the effect of chelating agent, zeolite on the reduction of cadmium toxicity in cat fish, *H. fossils.*

Materials and Methods

Collection and acclimation of fish

The experimental animals of *Heteropneustes fossilis* were collected from local ponds of Authoor, Tuticorin District, Tamilnadu. They were brought to the laboratory and acclimated for three weeks. During acclimation period, the fishes were fed as *ad libitum* with fresh pieces of beef liver. The aquarium water was changed daily. The experimental setup, triplicate in three series of experiments were conducted to study the effect of zeolite on the reduction of cadmium toxicity in *H. fossilis*. A stock solution of cadmium (ppt) was prepared by dissolving 6.846g of cadmium sulphate ($CdSO_4$ $8H_2O$) crystals 1ltr of distilled water. From this stock solution, the desired concentration of test solution was prepared. Zeolites are recent and fascinating selective absorbents used in aquaculture industry. Sodium aluminosilicate (Na_{12} [AIO_2)12]27 H_2O) is one of the synthetic zeolites, having large open structured anions and balancing cations. Because of the open structure, zeolites can take up water molecules, gases like CO_2, NH_3, alcohol and mercury, copper and cadmium reversibly into the interstices of their structures.

Determination of LC_{50} Value

96 hours LC_{50} value can be defined as the lethal concentration at which 50% of the mortality in 96hours. To find out LC_{50} of cadmium, a series of concentrations were prepared separately. Well acclimated six fishes were exposed to each concentration and mortality was recorded every 1hr intervals up to 24 hrs, 3 hrs intervals upto 48 hours and 6 hours intervals upto 96 hours. Fresh concentrations were prepared by daily to maintain constant toxic concentration (Sprague, 1971). The static renewable bioassay method was adopted to estimate the LC_{50} (Sprague, 1973) method. The 96 hours LC_{50} value, its 95% confidence limit and slope function were determined following the method of Litchfield and Wilcoxon

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(1949) method. One third of LC_{50} was taken as the maximum sublethal concentration (Konar, 1969) for the present study.

Feeding Experiments

Healthy fish of *H. fossilis* (18 ±1.5g) were selected from the acclimation tank and they starved for 24hrs to ensure the evacuation of gut content prior to commencement of the experiment. The fishes were divided into 8 groups of single individuals each and they were exposed as follows for 21 days. Hereafter control and experimental fishes were expressed as A, B, C, D, E, C1, D1 and E1.

Group	I part of the experiment	Notation of exposures
1	Control (Metal free water)	A
2	Cadmium (5ppm) alone	B
3	Cadmium (5ppm) + 0.5g z*/l	C
4	Cadmium (5ppm) + 1.0 g z*/l	D
5	Cadmium (5ppm) + 2.0 g z*/l	E
	II Part of the experiment	
6	Cadmium (5ppm) + 0.25 & 0.25g z*/l	C1
7	Cadmium (5ppm) + 0.5 & 0.5g z*/l	D1
8	Cadmium (5ppm) + 1.0 & 1.0g z*/l	E1

*Zeolite (sodium aluminosilicate)

The chosen levels of zeolite was added on day 0 to cadmium exposures group C to E to study the role of synthetic zeolite on the reduction of cadmium toxicity in food transformation of *H. fossilis*. The medium was thoroughly mixed after the chosen levels of zeolite was added to respective cadmium exposures and then test animals were introduced. Like first part of experiment in second series experiment, the chosen levels of zeolite was added to 6, 7 and 8th exposures, but the same level of zeolite added two times by equal parts on 10 days intervals (i.e day 0 and 10 respectively. Hereafter, the exposures of 6, 7 and 8th were referred to as C1, D1 and E1 respectively. The experiment was conducted in circular plastic trough containing 10 litres of test medium in triplicates. Animals were fed with *ad libitum* and food utilization was studied for 21 days. The medium was not changed throughout the experimental period.

Feeding and collection of faeces

Test individuals of *H. fossilis* fed on weighed quantities of minced beef liver as libitum once in a day at 09.00 hours for a period of one hour. The consumed food remaining in the aquarium was collected after the feeding time with a pipette causing least disturbance to the fish. Water content of the food sample was estimated by drying a known weight at 80^{o} C

To estimate the growth of the fish at the commencement and termination of the experiment "Sacrifice method" (Maynard and Loosli, 1962) was followed. At the end of the experiment, the test animals were starved for 24 hrs to ensure complete evacuation of the gut and they were killed and dried at 80^{o}C to estimate the growth of the animals. All weighing were made in an electrical monopan balance to an accuracy of 0.1mg.

Rates of feeding, absorption, conversion were collected by dividing the respective quantities by the product of initial wet weight of the fish and duration (day) of the experiment. The rates were expressed as mg/g live fish/day. Absorption efficiency was calculated by relating food absorbed to food consumed and expressed as percentage. Gross and net production efficiencies were calculated by relating production to consumption and absorption respectively and expressed as percentage.

Accumulation of Cadmium in Tissues

Like second series of experiment, another set of exposures were maintained separately to study the effect of chelating agent, zeolite on cadmium accumulation in different tissues of test animals *H. fossilis*. Metal concentration was analysed in chosen tissues, liver, kidney, muscle, gill and gonad on day 0 and 22 of the experiment. The animals were anesthetized and dissected out the chosen tissues and they were subjected to acid digestion following the method (FAO,1975). Total cadmium in tissue was estimated by taking 0.5 - 1gm of wet tissue and digesting with the mixture of concentrated nitric acid and perchloric acid in the ration of 1:3 until the formation of the white residue. The cooled residue was dissolved completely by adding 10 ml of IN HCL and made upto 25 ml with distilled water. The content was filtered by cotton wool and the filtrate was subjected to metal analysis in Atomic Absorption Spectrophotometer (Perkin and Elmer model 2380). The instrument was calibrated using cadmium sulphate as standard.

Results and Conclusions

The 96 hr LC value of cadmium for *Heteropneustes fossilis* was 15 ppm (Fig.1). No mortality was observed below the concentration of 9 ppm. However, the concentration of 10ppm and above were observed to be toxic. The 95% confidence limits were 11.67 (lower) and 19.25 ppm (upper). The value of the slope S was 1.33 ppm (Table - 1).

Table 2 and 3 presents the data on sublethal effect of cadmium and the role of synthetic zeolite on food transformation in catfish *H.fossilis*. Animals exposed to

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chosen sublethal level of cadmium showed the significant ($P<0.05$) reduction in the rates of feeding, absorption and conversion than control and other exposures. The feeding rate of control fish (A) was 23.4 mg/g live fish/day and it decreased to 7.3 mg/g live fish/day in animals exposed to group B (Fig. 2 & 3). However, animals exposed to sublethal level of cadmium with different levels of zeolite showed an improvement of same parameters as compared to exposure B. For instance animals exposed to C, D and E groups showed an individual improvement of feeding rate was 22, 53 and 12% (Table 2) and it was 79, 61 and 24% (Table 3) in C1, D1 and E1 exposures respectively as compared to animals exposed to group B. It suggests that, animals exposed to sublethal level of cadmium with different levels of zeolite enhanced the feeding rate than animals exposed to cadmium alone. The feeding rate was greatly enhanced in exposure D (53%) of the I part of the experiment and C1 (79%) and D2 (61%) exposures in the II part of the experiment.

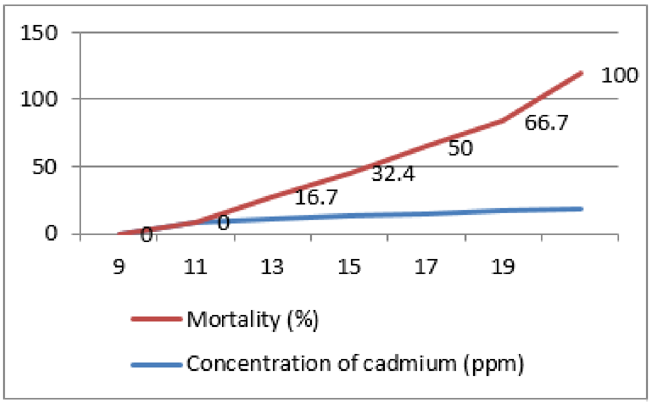

Fig. 1 Effect of different concentrations of cadmium on percent mortality in *Heteropneustes fossilis* exposed for 96 hr.

Table- 1: Effect of cadmium concentration on relative percent mortality of *Heteropneustes fossilis* exposed for 96 hours

Concentration of cadmium (ppm)	Mortality (%)	Lethal concentration at (ppm) 16%	50%	84%	Slope function S (ppm)	95% confidence limit Lower (ppm)	Upper(ppm)
9	0	10.9	14.99	19.29	1.33	11.67	19.25
11	16.7						
13	32.4						
15	50						
17	66.7						
19	100						

Table- 2: Effect of sublethal level of cadmium and 0 day application of synthetic zeolite to cadmium exposures on food transformation parameters in *Heteropneustes fossils*.

Parameters	Exposures A	B	C	D	E
Feeding rate	23.40 ±1.72	7.30 ±1.23	8.90 ±1.62	11.19±1.06	8.17 ±1.14
Absorption rate	22.78 ±1.86	6.38 ±1.44	8.07±1.90	10.36±1.24	7.71 ±1.08
Conversion rate	4.35 ±0.68	0.68±0.05	0.94±0.05	1.36 ±0.08	0.93 ±0.06
Metabolic rate	16.61 ±2.16	5.14 ±1.30	6.43±0.94	8.11 ±0.14	6.11 ±0.86
Absorption efficiency	97.36 ±3.68	87.48 ±4.28	90.71 ±3.44	92.54±2.48	89.75 ±4.86
Gross conversion efficiency(K1)	18.60 ±1.03	09.27 ±1.08	10.59±1.16	12.16 ±0.56	11.41 ±0.23
Net conversion efficiency (K2)	19.10 ±1.41	10.60 ±1.48	11.68 ±1.08	13.14 ±1.31	23.40 ±1.72

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Table -3: Food utilization parameters of *Heteropneustes fossilis* exposed to sublethal level of cadmium and 0 and 10th day application of zeolite to cadmium exposures.

Parameters	Exposures				
	A	B	C1	D1	E1
Feeding rate	23.40 ±1.72	7.30 ±1.23	13.08±1.11	11.72±0.94	9.03 ±0.75
Absorption rate	22.78 ±1.86	6.38 ±1.44	12.19±1.34	10.96±0.74	8.23 ±0.64
Conversion rate	4.35 ±0.68	0.68±0.05	1.79±0.18	1.39 ±0.06	1.06 ±0.03
Metabolic rate	16.61 ±2.16	5.14 ±1.30	9.37±1.70	8.63 ±1.03	6.46±0.21
Absorption efficiency	97.36 ±3.68	87.48 ±4.28	93.21 ±3.45	93.05±2.73	91.14 ±4.27
Gross conversion efficiency(K1)	18.60 ±1.03	09.27 ±1.08	13.74±0.86	12.78 ±0.44	11.73 ±0.53
Net conversion efficiency (K2)	19.10 ±1.41	10.60 ±1.48	14.74 ±0.75	13.67 ±1.01	12.87 ±1.12

Table - 4: Sublethal effect of cadmium and 0 day application of zeolite to cadmium exposures on tissue concentration of cadmium (μg/ g^{-1} wet tissue) in *Heteropneustes fossilis*. Each value is the mean of two observations

Exposures					
Tissues	A	B	C	D	E
Liver	0.48	5.97	3.97	2.42	4.16
Kidney	0.5	3.93	2.81	1.3	3.15
Gill	0.39	1.89	0.85	0.71	1.07
Muscle	0.23	0.47	0.32	0.26	0.34
Gonad	0.29	0.51	0.42	0.23	0.23

Table - 5: Effect of sublethal concentration of cadmium and application of zeolite by two intervals (day 0 & 10) to cadmium exposures on tissue concentration of cadmium (μ g g^{-1} wet tissue) in *Heteropneustes fossilis.*

Tissues	Exposures				
	A	B	C1	D1	E1
Liver	0.48	5.97	2.59	2.29	3.75
Kidney	0.5	3.93	2.44	2.1	2.78
Gill	0.39	1.89	1.1	0.75	0.84
Muscle	0.23	0.47	0.34	0.3	0.37
Gonad	0.29	0.51	0.32	0.28	0.45

The results obtained for absorption rate was similar to those of feeding rate. The absorption rate was observed to be enhanced in synthetic zeolite added cadmium exposure than cadmium exposure alone. For instance, the increased absorption rate of C, D and E exposure was to 26, 62 and 21 % (Table 2) and it was 91, 68 and 29 % in C1, D1 and E1 exposures (Table 3) respectively as compared to exposure B. The absorption efficiency of *H. fossilis* exposed to group B was 87.48% and it increased to 90.71, 92.54 and 89.75 % in animals exposed to C, D and E groups (Table 2), however, it was 93.21, 93.05 and 91.14% in C1, D1 and E1 exposures respectively.

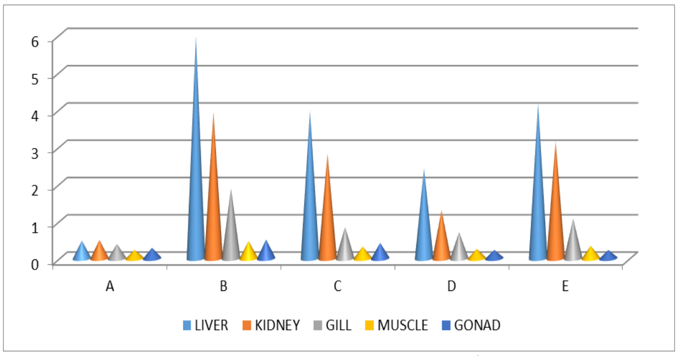

Fig. 2. Uptake of cadmium (μ g g^{-1} wet tissue) in *Heteropneustes fossilis* exposed to sublethal level of cadmium with different levels of zeolite (day 0).

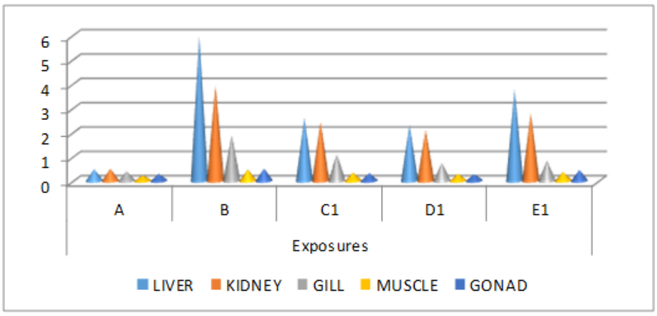

Fig. 3. Uptake of cadmium (μg/g^{-1} wet tissue) in *Heteropneustes fossilis* exposed to sublethal level of cadmium and cadmium with different levels of zeolite (day 0 & 10).

Exposure to the sublethal level of cadmium significantly affected the rate and efficiency of food conversion and it improved in zeolite added media. Test animals exposed to B was converted the food into flesh at the rate of 0.68 mg/g live fish/day and it significantly increased to 0.94 (t=5.20; P<0.01), 1.36 (t = 10.19; P<0.01) and 0.93 (t = 4.53; P < 0.02) mg/g live fish/day in C, D and E exposures of the I part of experiment and 1.79 (t = 8.54; P < 0.01), 1.39 (t = 12.86, P<0.01) and 1.06 (t= 9.23; P<0.01) mg/g live fish/day in C1, D1 and E1 exposures respectively. There was 38, 100 and 37% an increase of conversion rate in C, D and E exposures and it was 163, 104 and 59 % in C1, D1 and E1 exposures as compared to exposure B. The efficiency with which the food was converted into body tissues also followed the rates of feeding, absorption and conversion (Fig. 2 & 3).

The present study reveals that the uptake of cadmium was greatest in liver of *H.fossilis* followed by kidney, gill, muscle and gonad tissues. For instance, animals exposed to B, attained the concentration of 5.97, 3.93, 1.89, 0.47 and 0.51 μg g^{-1} cd wet tissue. Similar result was obtained in other exposures also.
Food transformation parameters reveals that application of different levels of zeolite by two intervals on day 0 to 10 to chosen cadmium exposure greatly enhanced than application of zeolite by one time on day 0. However, metal accumulation study did not show any significant variation between the application of zeolite to cadmium exposure by one or two intervals during the experimental period.

In the present study, toxic effect of sublethal level of cadmium and chelating agent, zeolite on food consumption, utilization and accumulation of cadmium in chosen tissues of the catfish *Heteropneustes fossilis* were exposed to sublethal level of cadmium with different levels of synthetic zeolite showed an improvement of food transformation parameters and reduced the accumulation of cadmium in tested tissues of *H.fossilis*. Accumulation of cadmium was more amount in liver of *H.fossils*, followed by kidney, gill, muscle and gonad. Application of chelating agent, zeolite on day 0 and 10 to chosen cadmium exposures showed significant variations in food transformation parameters; however metal accumulation in tested tissues of *H.fossilis* did not show significant variation. Optimum level of zeolite for the effective reduction or replacement of cadmium contaminated medium which was evidently proved by food transformation parameters and accumulation of cadmium in tissues of *H.fossilis*.

References

Karthikeyani, R. and Sivakumar, A. A. 2010. A preliminary studies on the water quality of the River Varaganathi at Periyakulam (Theni Dt.,TamilNadu). *Proc. Zool. Soc. India.* Budhgaya, 9 (1):9-14.

Onkar Singh and Kaur Manjeet, 2015. Determination of LC_{50} of lead nitrate for a fish, *Labeo rohita* (Hamilton Buchanan) Brraich). *Int. Res. J. Biological Sci.*, 4(8): 23-26.

Woodward, D.F., Brumbaug, W.G., Delonay, A.J. and Smith C.1994. Effects on rainbow trout of metals contaminants diet of benthic invertebrates from the Clark Fork river, Moutana, *Trans. American fish*. Soc., 23, 51-62.

Reglero, M.M., Taggart, M.A., Monsalve-Gonzalez, I. and Mateo, R. 2009. Heavy metal exposure in large game from a lead mining area: effects on oxidative stress and fatty acid composition in liver. *Environ. Pollut*., 157:1388-1.

Abdallah, G.M., El-Shayed, S.M. and Abo-Salem, O.M.2010. Effect of lead toxicity on coenzyme Q levels in rat tissues. *Food. Chem. Toxicol*., 48: 1753-1756.

Rout, P.C. and Niak, B.N.2013. Quantitative precipitation tests for anti avidin during experimental plumbism in *Clarias batrachus*, Linn. *Asian resonance*, 2(3):

Dallas, H.F. and Day, J.A.1993. The effect of water quality variables on riverine ecosystem. A review: *Water Research Commission Report* No., 351-360.

Sprague, J.B 1971. Measurement of pollutant toxicity to fish in sublethal effects. *Water. Res*.,5: 245-266.

Sprague, J.B.1973. ABC's of pollutant bioassay using fish. In: Biological methods for assessment of water quality. ASTM STP 528. American society for testing materials,p.6-30.

Litchfield, J. T. and Wilcoxon, F.1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Therapeut*., 96, 99-113.

Konar, S.K. 1969. Two organophosphorus insecticides DDVP and phosphomidon as selective toxicants. *Trans.Amer.Fish.Sci*., 98: 430 -437.

Maynard, A.L. and Loosli, K.C. 1962. In: Animal Nutrition. McGraw-Hill, NewYork, pp.533.

FAO, 1975. Manual of methods in aquatic environment research. Part I. Publ.Div., FAO, Rome, Italy, pp.223.