

## Bioaccumulation Pattern of Zinc in Freshwater Fish *Channa punctatus* (Bloch.) After Chronic Exposure

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Received 03 September 2007  
Accepted 07 January 2008

### Abstract

The pattern of zinc (Zn) residue in various tissues of *Channa punctatus* exposed to two treatments of zinc (6.62 and 13.24 mg L<sup>-1</sup>) for a period of 45 days was analysed. The concentrations of Zn was found to follow the order of liver > kidney > intestine > gill > muscle. Zn accumulated significantly (P<0.05) in all the tissues was studied except muscle tissue. Present findings revealed that liver and kidney are the prime sites of Zn accumulation and zinc load in the muscle was for low as compared to other organs. In the muscles of *C. punctatus*, 4.95 and 5.29 µg Zn g<sup>-1</sup> was accumulated in fish exposed to lower and higher sublethal concentration of Zn at 45<sup>th</sup> day of exposure, respectively. These values were low as compared to 49.72 and 67.31 µg Zn g<sup>-1</sup> of accumulated level in prime site of liver tissue of test fish exposed to lower and higher sublethal concentration of zinc, respectively. Further, a linear relationship of Zn accumulation with exposure concentrations was noted in all tissues of test fish. These data will constitute a reference for future studies on the evaluation of Zn accumulation tendency in relation to the ecotoxicological testing scheme for hazard assessment.

*Key words:* zinc, *Channa punctatus*, residues, organs, long-term exposure.

### Introduction

Among the various toxic pollutants, heavy metals are particularly severe in their action due to tendency of bio-magnification in the food chain. The global heavy metal pollution of water is a major environmental problem. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated (Khare and Singh, 2002). Industrial discharges containing toxic and hazardous substances, including heavy metals (Gbem *et al.*, 2001; Woodling *et al.*, 2001) contribute tremendously to the pollution of aquatic ecosystem. Zinc is essential element acting as structural component and having specific properties indispensable for life (Bengari and Patil, 1986). The danger of zinc is aggravated by its almost indefinite persistence in the environment because it cannot be destroyed biologically but are only transformed from oxidation state or organic complex to another. Zn is a potential toxicant to fish (Everall *et al.*, 1989), which causes disturbances of acid-base and ionoregulation, disruption of gill tissue and hypoxia (Hogstrand *et al.*, 1994).

Bioaccumulation of metals reflects the amount ingested by the organism, the way in which the metals are distributed among the different tissues and the extent to which the metal is retained in each tissue type. Accumulation of zinc has attained a serious dimension causing a pathogenic stage like Alzheimer's disease. Zn in certain concentration is desirable for the growth of freshwater animals but its over accumulation is hazardous to exposed organisms as well as to those who consume them directly or

indirectly through food chain. The pattern of metal accumulation in fish tissue can be utilized as effective indicator of environmental contamination (Sultana and Rao, 1998). Fish exposed to high concentrations of trace metals in water may take up substantial quantities of these metals. Hogstrand *et al.* (1994) suggested an adaptation to water borne Zn by a change of *K<sub>m</sub>* of a mutual Ca<sup>2+</sup>/Zn<sup>2+</sup> carrier which may have reduced Zn influx. When exposure to high Zn level occurs and the liver's capacity may be removed by the exceeded level of Zn and the more toxic type of Zn (Zn<sup>2+</sup>) may be transported through blood stream to other organs. Zinc can be accumulated via the gills and/or the digestive track, however the role of water as source of Zn uptake is not fully elucidated (Spry *et al.*, 1988). Hence the present study was aimed to investigate the bioaccumulation of Zn in gill, liver, kidney, intestine and muscle tissues of freshwater fish *Channa punctatus* at laboratory condition.

### Materials and Methods

Healthy *C. punctatus* (18–20 g in weight; 9–11 cm in length) were collected from local freshwater bodies in and around Annamalai University, Annamalai Nagar. Fish were separately maintained at 27±1°C in 1,000 L tank with continuously aerated and dechlorinated tapwater (pH 7.2–7.4; hardness 185–200 mg L<sup>-1</sup> as CaCO<sub>3</sub>; alkalinity 170–175 mg L<sup>-1</sup> as CaCO<sub>3</sub>) at least one month prior to the experiments. The laboratory photoperiod was 12 h D: 12 h L. Fish were fed with boiled chicken eggs and small pieces of earthworm on alternate days and then 60% water was

renewed everyday. Feeding was suspended 24 h earlier during the mortality test for the fish. Whereas during the accumulation experiments, the fish were fed with earthworm pieces, once a day for 30 min before the renewal of test water, after 30 min, the remaining food was removed.

The heavy metal zinc in the form of zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ -Analar grade, Merck) was used in the present study. The 96 h  $\text{LC}_{50}$  concentration of zinc was  $48.68 \text{ mg L}^{-1}$  for *C. punctatus* as calculated by using probit analysis method (Finney, 1971). Zinc accumulation was investigated in fish exposed to  $1/3^{\text{rd}}$  ( $13.24 \text{ mg L}^{-1}$ ) and  $1/6^{\text{th}}$  ( $6.62 \text{ mg L}^{-1}$ ) of 96 hr  $\text{LC}_{50}$  concentration of zinc over 45 days of exposure. The experiments were carried out in glass aquarium (100 L water capacity) with six replications (30 fish in each zinc concentration and control). No zinc was put into the aquarium containing the control fish. The water in the control and Zn containing aquarium was renewed everyday in order to minimize decrease in the Zn concentrations. At each interval of 15, 30 and 45 days of long-term exposure, six fish were sampled from each group for determination of zinc in different organs.

The fish were dissected and different organs of gill, liver, kidney, intestine and muscle were taken from the experimental fish as well as control fish separately and the tissues were washed in double distilled water and preserved in 10% formalin. Before analysis, formalin was removed using filter paper from each tissue. Five hundred mg from each tissue was placed in separate digestion flask and perchloric-nitric acid mixture in the ratio of 1:2 (v/v) was added (FAO, 1975). The digestion flasks were gradually brought and kept at  $130^\circ\text{C}$  on hot plate until all materials dissolved and the digests were diluted with deionized water. The final acid digested extract was analysed for Zn concentration using Perkin Elmer Atomic Absorption Spectrophotometer-3100. The Zn concentration in tissue was recorded  $\mu\text{g g}^{-1}$  wet tissue.

Data analyses were carried out using SPSS (version number-10) statistical package. Analysis of variance (ANOVA) was used to determine differences between various data sets. While the Dunnett test was used to compare experimental treatment groups against control.

## Results and Discussion

Large variations occurred in the pattern of Zn accumulation in different tissues of *Channa punctatus* after the exposure to 6.62 and  $13.24 \text{ mg Zn L}^{-1}$  for a long term (45 days) (Table 1 and 2). Organ-wise distribution of residual zinc revealed that the liver is the prime site of accumulation with highest persistence, which followed by kidney, gills and intestine in the test fish throughout the exposure period. The muscle tissue was always contained a significantly lower ( $P < 0.05$ ) levels of Zn than any other tissues sampled during the experimentation. Zn enters

the body mainly via ingestion and absorption through the gills and skin (Romanenko *et al.*, 1986). The present results are in equal to the effect of Zn on bioaccumulation in different tissue of several fish species exposed to Zn in contaminated system (Murphy *et al.*, 1978; Hofer *et al.*, 1989; Seymore *et al.*, 1994). Furthermore, the present results have clearly proved the increasing accumulation of Zn in tissue over a long-term exposure (45<sup>th</sup> day) of examined fish, which can be regarded as an indicator of cumulative contamination (Madhusudan *et al.*, 2003).

In the present investigation, the liver tissue always contained a significantly higher level of Zn residue compared to control fish. These results clearly indicate that the liver appears to be one of the most important sites for Zn accumulation as it was also evident from some of the earlier findings of Heath (1987) and Seymore *et al.* (1994). The high levels of Zn in liver can be ascribed to the bindings of Zn to metallothionein (MT) which was at highest concentration in liver (Kendrick *et al.*, 1992).

The differences in the level of accumulation in the different organs of the test fish can primarily be attributed to the differences in the physiological role of each organ (Karuppasamy, 2004). Regulatory ability and functions are also other factors that could influence the accumulation differences in the different tissues. The Zn concentration in the liver (not in direct contact with Zn in water) which play a major role in detoxification as well as storage, would therefore differ from the concentration detected in the gill (in direct contact with the Zn in the water) which play a role in the uptake and excretion of the Zn (Romanenko *et al.*, 1986).

As it is seen from Table 1 and 2, the Zn concentration in the liver of fish gradually increased upto 45 days, where the Zn concentration in the gill of fish again decreased by the end of exposure period (45<sup>th</sup> day) compared to the levels found at 30 days exposed fish in both sublethal concentrations, which indicate the induction of regulatory process. Zn seemed to accumulate upto a certain level and then remains constant in tissues due to several biochemical mechanisms (Evans *et al.*, 1993). Zn is an essential element which can be regulated by fish over wide range concentrations (Spry *et al.*, 1988).

The Zn level in gills of fish exposed to two different sublethal concentrations of Zn, was significantly higher ( $P < 0.05$ ) than the level found the control groups at all exposure periods. These high Zn levels in gill tissue can possibly due to the fact that they are the main sites for Zn uptake, particularly in freshwater fish and due to the large surface that is in contact with environmental water and the very thin barrier separating the external and internal media of the animal. The large surface area of *C. punctatus* (Karuppasamy, 2000) may be favour for metal uptake from water. However accumulated Zn in the gill tissue of this species was lower than that in the liver and kidney. Lower amounts of Zn in gills suggest that

**Table 1.** Zinc concentration in tissues of *C. punctatus* exposed to lower-sublethal concentration (6.62 mg L<sup>-1</sup>) of Zn under long-term condition

Tissue	Control Group	Treated groups			Significant level between exposure groups (F-value)
		Exposure periods (days)			
		15	30	45	
Gill	17.1±1.17 <sup>a</sup>	25.02±1.36 <sup>b</sup>	41.41±1.41 <sup>c</sup>	32.97±1.16 <sup>c</sup>	183.66*
% COC		-31.65	58.70	48.13	
Liver	9.7±0.86 <sup>e</sup>	16.81±0.98 <sup>a</sup>	23.66±1.25 <sup>b</sup>	49.72±1.29 <sup>g</sup>	122.35*
% COC		42.30	59.00	80.49	
Intestine	6.13±1.36 <sup>f</sup>	11.76±1.54 <sup>e</sup>	26.74±1.33 <sup>b</sup>	38.66±1.15 <sup>e</sup>	189.62*
% COC		47.87	77.07	84.14	
Kidney	12.55±0.91 <sup>e</sup>	22.35±1.10 <sup>b</sup>	39.21±0.98 <sup>c</sup>	45.91±0.82 <sup>g</sup>	165.43*
% COC		43.84	67.99	72.66	
Muscle	4.62±0.23 <sup>f</sup>	5.21±0.28 <sup>f</sup>	5.11±0.33 <sup>f</sup>	4.95±0.43 <sup>f</sup>	4.11 <sup>NS</sup>
% COC		11.32	09.58	06.66	

Mean ± SD of six individual observed

Values are expressed µg g<sup>-1</sup> wet tissue

\*Indicates significant at 5% level (by one way analysis of variance)

Different letter designations % denotes significance at 5% level (Dunnet test)

NS – not significant

% COC - % change over control.

**Table 2.** Zinc concentration (µg g<sup>-1</sup> wet wt.) in tissues of *C. punctatus* exposed to higher sublethal concentration (13.24 mg L<sup>-1</sup>) of Zn under long-term conditions

Tissue	Control group	Treated Groups			Significant level between exposure groups (F-value)
		Exposure periods (days)			
		15	30	45	
Gill	16.33±1.21 <sup>a</sup>	32.31±1.77 <sup>b</sup>	58.36±0.89 <sup>c</sup>	50.51±1.11 <sup>c</sup>	122.55*
% COC		49.45	72.01	67.67	
Liver	10.11±1.08 <sup>a</sup>	26.54±1.06 <sup>b</sup>	49.98±1.66 <sup>c</sup>	67.31±1.29 <sup>e</sup>	146.92*
% COC		61.90	79.77	84.97	
Intestine	5.28±0.96 <sup>d</sup>	19.81±0.87 <sup>a</sup>	28.56±1.12 <sup>b</sup>	42.16±1.00 <sup>c</sup>	137.92*
% COC		73.34	81.51	87.47	
Kidney	13.21±1.41 <sup>a</sup>	37.51±1.57 <sup>b</sup>	52.51±1.49 <sup>c</sup>	62.29±1.62 <sup>e</sup>	110.54*
% COC		64.78	74.84	78.79	
Muscle	4.14±0.32 <sup>d</sup>	5.83±0.66 <sup>d</sup>	5.63±0.33 <sup>d</sup>	5.29±0.11 <sup>d</sup>	2.16 <sup>NS</sup>
% COC		28.98	16.53	19.14	

Mean ± SD of six individual observed

Values are expressed µg g<sup>-1</sup> wet tissue

\*Indicates significant at 5% level (by one way analysis of variance)

Different letter designations % denotes significance at 5% level (Dunnet test)

NS – not significant

% COC - % change over control.

Zn is excreted more rapidly and reduce the body burden of Zn and suggest that Zn are not accumulated in prolonged period in gill tissue.

The Zn content in the intestine of fish exposed to various sublethal concentrations was lower than in the other organs tested, except muscle in the present study. However the level of Zn content in the intestine of treated groups are significantly (P<0.05) higher when compared to the control groups. Alimentary canal can be considered as the interface of the organisms and its ambience. It is the system which receives the metal like Zn directly from ambient source (Matheissen and Brafield, 1975). The accumulated level of Zn in the intestine of test fish, *C.*

*punctatus*, highly resembles to levels found in the other fish of *Cyprinus carpio* (Yamamoto *et al.*, 1977) and *Oreochromis mossombicus* (Pelgrom *et al.*, 1995) exposed to the essential metals. Since fresh water fish drink very little water, this could not explain the accumulation of heavy metal in the intestine. A hypothesis could be considered and needs to be investigated, i.e. the route for metal uptake through intestine contamination from Zn excreted with bile into gastrointestinal track, posterior to the stomach and a possible alternative route for metal excretion, as suggested by Pelgrom *et al.* (1995), in the same way it was reported for rats (Stonard and Webb, 1976). In the present investigation the Zn may be absorbed through

the gills and transported to the intestine via circulatory system.

Zn concentration in the kidney of the fish exposed 13.24 and 6.62 mg L<sup>-1</sup> of Zn was progressively increased with a significant level (P<0.05) towards the end of experiments. Our result indicates kidney as target organ to Zn storage in *C. punctatus*. This corresponds to the field study where the Zn content of the kidney was found to be positively correlated to the Zn concentration of the river (Hofer *et al.*, 1989), which points out kidney to be a suitable indicator of Zn contamination in *C. punctatus*.

Zn concentration in the muscle tissue of *C. punctatus* exposed to both higher (13.24 mg L<sup>-1</sup>) and lower (6.62 mg L<sup>-1</sup>) sublethal concentration was found to be 5.29 and 4.95 µg Zn g<sup>-1</sup> wet mass, respectively at the end of experimentation (45 days). These values are very low (10 to 13 times less) compared to liver accumulation of 49.72 and 67.31 µg Zn g<sup>-1</sup> wet mass, respectively in fish exposed to lower and higher concentrations of Zn, at the same period (45 days) of sampling. Similarly, black marlin accumulated 5.5 times of Zn concentration in liver compared to muscle tissue (Mackay *et al.*, 1975). The present results correspond to the field study, where the muscle Zn content was 16-82 mg kg<sup>-1</sup> wet weight in omnivorous and 3-9 mg kg<sup>-1</sup> wet weight in carnivorous fish collected from industrial and agricultural areas of lower great lakes (Brown and Chow, 1977). Similarly, yellow perch, blue gill and black crable inhabiting industrial zone rivers in the USA had average muscle burden of 108, 100 and 101 mg kg<sup>-1</sup> dry weight respectively (Adams *et al.*, 1980; Vinikour *et al.*, 1980).

According to Madhusudan *et al.* (2003), the excessive Zn in muscle tissue was transferred to other organs in the fish exposed to Zn contaminated system. It is evident that the test fish of *C. punctatus* had a tendency to push zinc burden to other tissue like kidney from muscle during metallic stress, perhaps may be upto some limit of exposure concentration and time. But this Zn metabolism in fish definitely does not allow for excessive ambient metal in muscle tissue to pose a threat to fish. This ability of deloading of fish is advantageous to consumer who are using fish muscle as their food.

To summarize, these results indicate that the fish *C. punctatus*, as a representative fish species of South India, can be a useful vertebrate bio-indicator organisms of Zn contamination water. This species is also a highly sensitive type to zinc pollution in the environment.

### Acknowledgement

Our thanks are due to the Professor and Wing Head, Department of Zoology, Annamalai University for providing necessary facilities.

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