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ORIGINAL ARTICLE

PATTERN OF ZINC ACCUMULATION IN DIFFERENT TISSUES OF FRESHWATER FISH, *CHANNA PUNCTATUS* (BLOCH.) UNDER LONG-TERM EXPOSURE

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ABSTRACT

Bioaccumation pattern of Zinc in gill, liver and kidney of adult *Channa punctatus*, when exposed to sublethal concentration of Zinc-water containing 1/8th of 96 hrs LC_{50} level (4.42 mg L^{-1}) for long-term (60 days) experimentation was analyzed. The Zn showed a highest accumulation ($P < 0.05$) in the liver (46.45 μg g^{-1}) and followed by kidney (29.12 μg g^{-1}). The lowest deposition of Zn was found in the gill (19.42 μg g^{-1}) after long-term exposure. There was a linear and statistically significant rise in the accumulation of Zn in all the tested tissues of fish with increasing of exposure periods. The differences in Zn accumulation in various tissues may be due to the physiological difference among the tissues of fish.

Keywords: *Channa punctatus*, Zinc, bioaccumulation, long-term exposure.

1.INDRODUCTION

Bioaccumation 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. Fish being the top consumer in the aquatic food chain accumulates large amounts of heavy metals in their body (Chezhian *et al*., 2010). The chemicals once absorbed are transported by the blood to either a storage point (bones and liver) or further transported to other organs (kidney, gill, and muscles) (Dural, 2007).

The pattern of metal accumulation in fish tissue can be utilized as effective indicator of environmental contamination (Sultana and Rao,1998). Zinc, required as an indispensable micronutrient by the fish can be obtained from water and diet (Wood, 2001). However, higher intake of Zinc and its over accumulation could cause harmful effects on fish health as well as to those who consume them directly or indirectly through food chain (Hayat *et al*., 2007).

As seen in earlier reports, there were many fish species tested for zinc accumulation with the exposure of Zinc metal. The comparison among these species regarding Zinc accumulation is not practical since different exposure conditions, animal stages and treatment methods for Zinc residue were used in the studies. However, some information could be extracted from these reports. Laboratory experiments conducted to determine the bioaccumulation pattern of Zinc in four fish species (*Labeo rohita, Cirrhina mrigala, Catla catla* and *Ctenopharyngodon idella*) separatedly in accordance with the acute toxicity. Among the exposed fish species *Cirrhina mrigala* exhibited significantly higher ability to amass Zinc (243.0 ± 190.55 μg g^{-1}) followed by *C. idella*, *L. rohita* and *catla catla*. Liver showed higher tendency to accumulate Zinc followed by gill, and kidney with significant difference (Kousar and Javed, 2014). *Channa punctatus* exposed to two different sublethal concentration of Zn after chronic exposure reported significant higher accumulation in liver, kidney intestine and gill. Further, a linear relationship of Zinc accumulation with exposure concentrations was noted in all tissues of test fish (Murugan *et al*., 2008).

It is evident from the bioaccumulation of Zinc in tissues of *Oreochromis mossambicus* the pattern of accumulation found to be increased in direct proportional with the ambient concentration of Zinc and exposure duration (Celik *et al*., 2013). Many of the previous studies documented that accumulation of Zinc mostly depend on their concentration in the medium and their exposure period (Moraes *et al*., 2003) and gain the entry from water into the body through gill respiration and absorption of contaminated food (Firat and Kargin, 2010).

The tendency of accumulation of Zinc in different concentration mostly depends on the analysed species and tissues, attributed to the function of their membrane

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permanently, which is highly species specific (Kousar and Javed, 2014).

2.MATERIALS AND METHODS

Healthy *C. punctatus* (19–21 g in weight; 10–12 cm in length) were collected from local freshwater bodies in and around Annamalai University, Annamalai-nagar. Fishes were separately maintained at 27 ± 1 °C in 1000 L tank with continuously aerated and dechlorinated tap water (pH 7.2–7.4; hardness 185–200 mg L^{-1} as $CaCO_3$; alkalinity 165–175 mg L^{-1} as $CaCO_3$) at least one month prior to the experiments. The laboratory photoperiod was 12D:12L. Fish were fed with boiled chicken eggs and small pieces of earthworm on alternate days and then 60% water was renewed every day. Feeding was suspended 24 hr before conducting the mortality test for the fish. However, during the accumulation experiments, the fishes were fed with earthworm, once a day for 30 min before the renewal of test water and after 30 min, the left over feed was removed.

The heavy metal Zn in the form of zinc sulfate ($ZnSO_4.7H_2O$-Analar grade, Merck) was used in the present study. The 96 hr LC_{50} concentration of zinc was 35.37 mg L^{-1} for *C. punctatus* as calculated adopting Probit analysis method (Finney, 1971). Zinc accumulation was investigated in fish exposed to $1/8^{th}$ of 96 hr LC_{50} concentration of Zn over 60 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). At each interval of 20, 40 and 60 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 and kidney were taken, washed in double distilled water and preserved in 10% formalin. Before analysis, formalin was removed using filter paper from each tissue and they were weighed and acid digested with a mixture of perchloric acid and concentrated nitric acid in the ratio of 1:2 (v/v) (FAO, 1975). The final acid digested extract was analyzed for Zn concentration using an atomic absorption spectrophotometer-3100 (Perkin Elmer). The Zn concentration in tissue was recorded □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 to compare treatment groups against control.

3.RESULTS

Bioaccumulation of Zinc

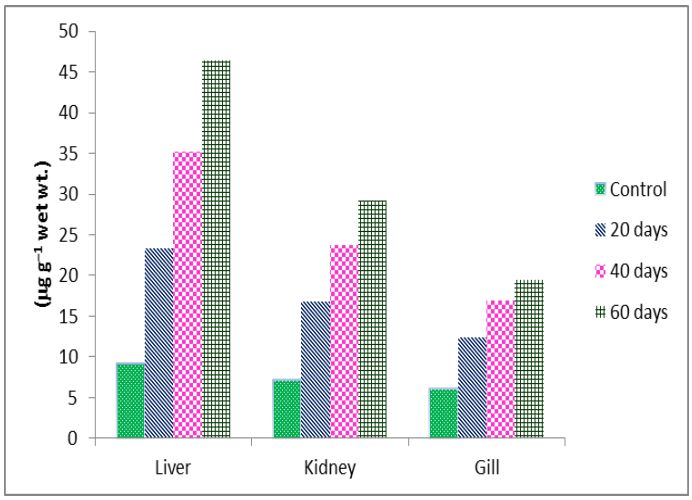
The results of bioaccumulation level of Zinc in the liver, kidney and gill of *Channa punctatus* are given in table - 1. The concentration of Zinc in the liver, kidney and gill after the long term exposure of 60 days was found to be 46.45 ± 1.19 µg/g^{-1}, 29.12 ± 0.22 µg/g^{-1} and 19.42 µg/g^{-1} respectively.

The results showed statistically significant increase ($P < 0.05$) in all the intervals (20, 40 and 60 days) of treated groups in comparison to control groups. Zinc concentration in treated fish was found to be increased with the increase in the days of exposure. The highest Zinc accumulation was observed in liver followed by kidney and gill. The order of Zinc accumulation pattern in the test fish was liver > kidney > gill.

Table 1Accumulation trend of Zinc (µg/g^{-1} wet wt.) in organs of *Channa punctatus* exposed to 4.42 mg L^{-1} concentration of Zinc during long-term exposure

Organ	Control group	Treated groups: Exposure duration (in days)			Significant level between exposure days
		20	40	60	F-value
Liver	9.12 ± 0.74	23.25 ± 1.21	35.14 ± 0.98	46.45 ± 1.19	98.66*
% CoC		60.77	74.04	80.36	
Kidney	7.2 ± 1.04	16.75 ± 1.06	23.76 ± 0.96	29.12 ± 0.87	118.71*
% CoC		55.63	69.65	75.24	
Gill	6.13 ± 0.96	12.36 ± 1.02	16.86 ± 0.95	19.42 ± 1.12	121.34
% CoC		50.40	63.64	68.43	

Fig. 1 Bioaccumulation level of Zinc (µg/g^{-1} wet wt.) in tissues of *Channa punctatus* exposed to sublethal concentration of Zn (4.42 mg L^{-1})



4.DISCUSSION

A large variation occurred in the pattern of Zinc accumulation in different tissues of *Channa punctatus* after exposed to Zinc 4.42 mg L^{-1} for long term (60^{th} day) exposure (Table - 1). Organ-wise distribution of residual zinc revealed that the liver is the prime site of accumulation with highest persistence, which followed by kidney and gill in the test fish throughout the exposure period. The similar pattern of results was reported in a study conducted with four species of fish (*L. rohita, C. catla, C. mrigala and C. idella*) exposed to sublethal concentration of Zinc recorded highest Zinc accumulation in liver followed by that of kidney and gill (Kousar and Javed, 2014). The pattern of Zinc accumulation in the *Channa punctatus* was Liver > Kidney > Intestine > gill > muscles (Murugan *et al.*, 2008) for long time exposure (45 days) of Zinc. Whereas the pattern of bioaccumulation of zinc in *Capoeta fusca* was kidney > liver > gill > muscle (Pourkhabbaz *et al.*, 2014). The Zinc enters the body mainly via ingestion and absorption through the gill and skin (Romanenko *et al.*, 1986). The present results are in equal to the effect of Zinc bioaccumulation in different tissue of

several fish species exposed to Zinc in contaminated system (Murphy *et al*., 1978; Holfer *et al*., 1989; Seymore, 1994). Further, the present results indicated that the accumulation of Zinc in tissues were increased with (60^{th} day) increasing time of examined fish that can be regarded as an indicator of cumulative contamination (Madhusudan *et al*., 2003). Similar trend of Zinc accumulation was noted in test fish *Channa punctatus* (Wagh 2010) and in *O. mossambicus* (Celik *et al*., 2013).

In the present investigation, Zn accumulation in liver tissue was increased significantly with exposure period. The concentration of Zn in liver was elevated from 9.12 to 46.45 $\mu g\ g^{-1}$ and exceeded five times than that of control at the end of exposure. The highest level of Zn accumulation was recorded in the liver tissue among the organ studied (Table - 1 & fig. 1). These results are in equal trend to the reports of highest Zn accumulation in *C. mrigala* (Kousar and Javed, 2014); *C. punctatus* (Wagh, 2010). These results are clearly indicates that the liver appears to be one of the most important site for Zinc accumulation. According to Sultana and Rao (1998) the liver is the principle site involved in the storage of metals. It was also evident from some of the earlier studies in fish reported a higher accumulation of Zn in liver tissue than other organs (Heath, 1987; Seymore, 1994; Muguran *et al*., 2008 and Wagh, 2010).

Liver showed significant higher tendency to accumulate heavy metals than other tissue (Javed, 2012) due to alterations in biochemical parameters of fish (Narayanan and Vinodhini, 2008). Higher amassing of Zn in selected tissues of fish may probably due to increased metal concentration in ambient water (or) due to slow rate of elimination from body (Yousafzai and Shakoori, 2008a). It is also evident that the metal concentration in the liver reflects its varied role in detoxification, storage processes and redistribution; hence, it is an active site of pathological effects induced by metal toxicants (Palaniyappan *et al*., 2009 and Al-Yousuf *et al*., 2000). Liver plays a vital role in bioaccumulation and detoxification of heavy metals (Yousafzai, 2008). The elevated levels of heavy metals induced the synthesis of metallothionine, a metal binding protein which helps in the detoxification and accumulation of metal ions in the liver (Hogstrand and Hauk (1991); Kendrick *et al*., 1992). In the present investigation, the highest level of accumulation of Zinc in the liver of *Channa punctatus* can be ascribed to the bindings of Zinc to metallothionine (MT) which was at highest concentration in liver and serve as a detoxification mechanism.

In the present investigation, Zn concentration in the kidney of the fish exposed to sublethal concentration was progressively increased from 7.21 to 29.12 $\mu g\ g^{-1}$ with significant level ($p < 0.05$) towards the end of experiments (Table – 1 & fig. 1). Zn accumulation in the kidney of *Channa punctatus* was four fold higher than that in the control group and indicates that the kidney as a target organ to Zn storage. According to Thomas *et al*., (1985) kidney has shown a good potential organ for accumulation of metals. The proportionate increase in the accumulation of Zn with duration of exposure is consistent with the result of (Murugan, *et al*., 2008 and Pallavi Gupta, 2006). High accumulation of Zn in the kidney of treated fish is probably a result of the kidney being one of the major organs for detoxification and elimination of metals (Dallinger *et al*., 1987; Gupta and Srivastava, 2006 and Pourkhabbaz *et al*., 2014). Murugan *et al*., (2008) pointed out that the excessive Zn in muscle was transferred to other organs in the fish. It is also evident that the test fish (*C. fusca*) has a tendency to push Zn burden from muscle to other tissue like kidney (Pourkhabbaz *et al*., 2014). Dalinger *et al*., (1987) reported a high accumulation of several heavy metals in liver and kidney and suggested that these organs as target organ for final deposition of metals. Hence, the increase of Zn concentration in the kidney of *C. punctatus* indicates the kidney as a target organ for final deposition.

The Zinc level in gill of *Channa punctatus* exposed to sublethal concentration was significantly higher ($p < 0.05$) than control groups at all exposure periods (Table – 1 & fig. 1). The concentration of Zn in gill was increased from 6.13 to 19.42 $\mu g\ g^{-1}$ and was three fold higher than the control group. Among the organs studied, Zn accumulation in gill was found to be lower than the liver and kidney. The accumulation level of Zn in gill tissue can possibly due to the fact that are the main site for Zinc uptake, particularly in freshwater fish, due to the large surface area 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, 2000c) may be favour for metal uptake from water. However, accumulated Zinc in the gill tissue of *C. punctatus* was lower than in the liver and kidney. Lower amounts of Zinc in gill suggested that Zn are excreted more rapidly and reduces the burden of Zinc and that Zinc is not accumulated in prolonged period in gill tissue (Murugan *et al*., 2008).

Further, the Zn accumulation through fish gill is a combination of absorption by the gill surface and subsequent transfer of metal across the gill to the blood stream (Karuppasamy, 2004). This suggested that the Zn is taken up through the gill and is possibly transported to the storage organs like liver and kidney. It is evident from the result of this investigation that the lower concentration of Zn detected in gill, which play a role in the uptake, transfer and excretion of Zn.

The result of this study indicates that Zn has a highest level of accumulation in the liver of *Channa punctatus* followed by kidney and gill, the difference in the accumulation level of Zn in different tissues may be attributed to the metabolic activities. Several factors including time, temperature, physical and chemical properties of water, age and metabolism of fish influence the bioaccumulation in fish tissues.

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

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