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ASSESSMENT OF ZINC BIOACCUMULATION IN FISH CHANNA PUNCTATUS EXPOSED CHRONICALLY

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ABSTRACT

Zn is an essential element for every organism, but in excess amount it is toxic for that organism and causes physiological disorders. It gets accumulated in different parts/organs of fish and through the food chain ultimately it reaches to the body of human being. The present study was designed to evaluate bioaccumulation of Zn in fresh water fish *Channa punctatus*. Fish were distributed into control and two exposed groups which were decided according to permissible limit (P.L.) and above permissible limit *i.e.* two times of P.L. (as per WHO, USEPA & BIS) of Zinc Sulphate for chronic exposure. The bioaccumulation of Zn was measured in liver, kidney, gill, muscle, testis and ovary after the different intervals of exposure periods *viz.*, 7 days, 14 days, 21 days and 28 days. The element Zn was assayed using Shimadzu AA-7000F Atomic Absorption Spectrophotometer. All the values were measured in $\mu g g^{-1}$ of dry tissue weight. It is evident from the findings that treated fish have elevated level of Zn in comparison to control fish and bioaccumulation was comparatively higher in liver and kidney than other organs. Our findings of this experimental study will be helpful for accumulated quantification of Zn metal in several tissues of fish, *Channa punctatus* in different regions having metal contamination.

KEY WORDS: Bioaccumulation, liver, kidney, gill, muscle, testis, ovary.

INTRODUCTION

Zinc (Zn) gains access to aquatic habitats by several ways. The largest natural emission of zinc to water bodies results from erosion. Natural inputs of zinc to air are mainly due to igneous emissions and forest fires. Anthropogenic sources of zinc include mining, zinc production facilities, iron and steel production, corrosion of galvanized structures, coal and fuel combustion, waste disposal, incineration, and the use of zinc-containing fertilizers and pesticides (Centre water commission CWC 2014). Zinc is widely used in semiconductors, solar panel devices (Zimmermann et al., 2012), paints, personal care products (sunscreens) and even in waste water treatment. Although, zinc is an essential trace element for both plants and animals but it induces toxicity at elevated concentrations particularly under conditions of low pH, low alkalinity, low dissolved oxygen and elevated temperatures (Eisler 1993). Partially but relatively quickly, zinc dissolves in water, and releases free zinc ions which become primary source of aquatic toxicity (Blinova et al., 2010; Buerki-Thurnherr et al., 2013; Franklin et al., 2007). Moreover, in combined state (ZnO) higher toxicity of zinc has been reported (Bai et al., 2010; Hu et al., 2009; Fernández et al., 2013), than dissolved Zn (II) alone. Zinc is the only metal to be involved in all six classes of enzymes viz; oxido-reductases. transferases. hvdrolases. lvases. isomerases and ligases (Barak and Helmke, 1993). In higher animals and humans it is estimated that approximately 3,000 proteins contain Zn prosthetic groups (Tapeiro and Tew 2003). Zn ions also act as potent

neurotransmitters, and cells in the salivary glands, prostate, immune system and intestine use Zn signalling (Herschfinkel et al., 2007). Zn plays a key role in physical growth and development, functioning of immune system, reproductive health, sensory function and neurobehavioral development (Hotz and Brown, 2004). Similarly, in aquatic habitats it is also required in traces for normal growth and functioning of the fish. However, chronically higher intake of zinc would lead towards bioaccumulation in different body organs of fish (Nussey et al., 2000) that becomes toxic to the fish. Thus, it's over accumulation is hazardous to exposed organisms as well as to those who consume them directly or indirectly through food chain. In human beings long-term intake of excess Zn induces sideroblastic anemia, leukopenia and hypochromic microcytic anaemia (Simon-Hettich et al., 2001). In plants, zinc is one of the 17 essential elements necessary for the normal growth and development. It is among eight micronutrients essential for plant growth. Zinc plays a key role in plants with enzymes and proteins involved in carbohydrate metabolism, protein synthesis, gene expression, auxin metabolism, pollen formation, maintenance of biological membranes, protection against photo-oxidative damage and heat stress, and resistance to infection by certain pathogens (Alloway, 2008). High concentration of zinc in plants causes chlorotic and necrotic leaf tips, interveinal chlorosis in new leaves, retarded growth of entire plant and injured roots resembling barbed wire (Kabata-Pendias and Pendias, 1992). Application of zinc based fertilizers and irrigation

with zinc enriched water paves the way for greater accumulation of zinc in plants as well. Fish being aquatic organism quickly register even minute changes in physicochemical regime of water. Thus, the pattern of zinc metal accumulation in fish tissues can be effectively utilized as a sensitive indicator of environmental contamination (Sultana and Rao, 1998); accumulation was more in water having high concentration of metals. In present investigation zinc uptakes in different fish tissues were studied, to understand the extent of bioaccumulation by different tissues as a critical research need in developing an understanding to establish a correlation between zinc availability in contaminated water and exposure based accumulation pattern in vital fish organs, *viz*; liver, kidney, gill, muscle and gonads.

MATERIALS & METHODS

Live and apparently healthy fish specimens (30-40g, 12-15 cm) of *Channa punctatus*, available throughout the year in greater part of India, were procured from local fishermen of Lucknow, Uttar Pradesh. The fish were subjected to repeated washing with tap water and 0.1% KMnO₄ solution for 30 minutes, to remove external infections. Fishes were acclimatized to laboratory condition for ten days in aerated glass aquaria (100X 40X40 cm³) in 10 days aged tap water. They were fed with special aquarium food pallets manufactured by Perfect companion group Company limited, Thailand and minced goat liver. Emplura grade test chemical, Zinc sulphate of Merck specialties private limited, Worli, Mumbai was used in the present study. Experiments were designed for different

intervals of chronic exposure periods viz. 7 days, 14 days, 21 days, and 28 days. The concentration of test chemical Zinc sulphate (ZnSO₄) was used as per WHO. BIS and USEPA guidelines. Experiment was designed using three concentrations of the test chemical 0 mg/l (control), 5.0 mg/l (permissible limit) and 10 mg/l (2 times of permissible limit) for different intervals of chronic exposure period in triplicate. During the experiment proper supply of air was maintained by stone diffusers and fishes were fed at regular intervals. Fish loading rate in aquarium was maintained at 4 g/l according to Burress (1975). After expiry of exposure periods fishes from each experiment group was taken out and anesthetized by using 2-phenoxy ethanol (0.1%). Liver, kidney, muscle, gill, testis and ovary of fishes were excised and washed with phosphate buffered saline (PBS). 500 mg of tissue from each organ was dried in hot air oven at 105°C and then transferred in glass bottles (20 ml) containing nitric acid and perchloric acid (10:1 v/v) for autolysis and digestion. The tissue digestion bottles were heated at 100°C on hot plate and digested samples were diluted with double distilled water. Zinc concentration was assayed in every sample by using Shimadzu AA-7000F Atomic Absorption Spectrophotometer and all the results were measured in ug per g of dry tissue weight. The operating parameters for the zinc element were set as recommended by manufacturer (Table 1). The data obtained from experiment were statistically analyzed using SPSS (Version 15) and the results so obtained were expressed as mean ±S.D, statistical significance for data of various experimental sets was evaluated using one way ANOVA.

TABLE 1: Analytical conditions of Shimadzu AA- 7000F for Zn metal assay

Element	Acetylene (L min ⁻¹)	Air (L min ⁻¹)	Wavelength (nm)	Slit width (nm)
Zn	2.0	17.0	213.9	0.7

RESULTS & DISCUSSION

Zn (II) concentration in different tissues of fish, *Channa punctatus* was determined on dry weight basis. Differential bioaccumulation of Zn in fish, based on exposure to test chemical and time interval, reflects that the liver has highest Zn (II) concentration, followed by kidney, muscle, gill, ovary and testis (Table 2). In liver, the maximum Zn (II) accumulation, over and above control, was recorded after 14 days of exposure period when fishes are subjected to 10mg/l concentration of ZnSO₄. However, analysis of percentage variation of zinc accumulation, in comparison to control, reveals that maximum increase of 151.12% was for 28 days exposure period in 10mg/l concentration of test chemical and minimum increase of 35.48% was for 7 days exposure period in 5 mg/l concentration of test chemical.

Zn (II) accumulation in liver of fish gradually increased up to 28 days but during 7 days and 14 days exposure periods zinc amount was accumulated abruptly. After the completion of 28 days exposure period of test chemical, mean value of Zn (II) concentration with standard deviation was found $21.78\pm0.58 \ \mu g.g^{-1}$ and $24.04\pm0.37 \ \mu g.g^{-1}$ for 5mg.L⁻¹ and 10 mg.L⁻¹ zinc sulphate exposed groups respectively.(Figure -1) Zn (II) contents in kidney of fish exposed to two different concentrations of zinc

sulphate (5 mg.L⁻¹ or 10 mg.L⁻¹), were recorded significantly higher (p<0.05) than the control groups for 14, 21, 28 days exposure periods except values of Zn(II) content for 7 days. At the end of the exposure period of 28 days, zinc accumulation was found 15.32 \pm 0.49 µg.g⁻¹ and 15.83 \pm 0.85 µg.g⁻¹ for 5 mg. L⁻¹ and 10 mg. L⁻¹ of test chemical respectively.

However, percentage change of zinc accumulation in kidney, in comparison to control, reveals that maximum increase of 60.17% was for 28 days exposure period in 10 mg/l concentration of test chemical and minimum increase of 7.81% was for 7 days exposure period in 5 mg/l concentration of test chemical.

However, accumulated zinc in muscle tissue of fish was lower than that in liver and kidney. Zn (II) level in muscle of fish was found significantly higher (p<0.05) in both concentrations of test chemical for all the exposure periods than control. The accumulation in 5 mg L⁻¹ exposed group for 7 and 14 days exposure periods was non significant (Table No -2). While the percentage change over control was maximum for 28 days exposure period in 10 mg/l concentration of test chemical and minimum increase of 2.14% was for 7 days exposure period in 5 mg/l concentration of test chemical.

Name of fish tissues	Exposure period (in days)	Zn accumulation in $\mu g g^{-1}$ dry fish tissue			
		Control (without ZnSo ₄) 0 mg/l ZnSo ₄	Experimental (exposed with ZnSo ₄)		
			5 mg/l ZnSo4	10 mg/l ZnSo4	
Liver	7	9.60±0.53	13.01±0.17*(35.48%)	18.77±0.26*(95.43%)	
	14	9.78±0.22	18.02±0.11*(84.22%)	22.52±0.52*(130.23%)	
	21	9.68±0.26	20.97±0.16*(116.65%)	22.52±0.93*(132.73%)	
	28	9.57 ± 0.40	21.78±0.58*(127.61%)	24.04±0.37*(151.12%)	
Kidney	7	9.75±0.32	10.51±0.47 (7.81%)	11.06±0.70 (13.39%)	
	14	9.70 ± 0.28	11.71±0.94* (20.62%)	12.90±0.34* (32.94%)	
	21	9.81±0.14	13.90±0.57* (41.69%)	14.51±0.63* (47.93%)	
	28	9.89 ± 0.84	15.32±0.49* (54.93%)	15.83±0.85* (60.17%)	
Muscle	7	9.08±0.27	9.28±0.40 (2.14%)	10.46±0.54* (15.10%)	
	14	9.50 ± 0.95	10.27±0.15 (8.08%)	11.47±0.66* (20.66%)	
	21	9.44±0.20	11.17±0.38* (18.25%)	11.84±0.58* (25.44%)	
	28	9.75±0.39	12.02±0.89* (23.21%)	13.60±1.06* (39.46%)	
Gill	7	8.15±0.27	8.61±0.81 (5.66%)	8.99±0.77 (10.29%)	
	14	8.24±0.23	8.94±1.11 (8.48%)	9.37±0.98 (13.69%)	
	21	8.61±1.41	10.90±2.04 (26.59%)	11.64±2.11 (35.25%)	
	28	8.72±0.92	11.68±1.96 (33.99%)	12.83±1.21* (47.19%)	
Ovary	7	7.85 ± 0.35	8.28±0.26 (5.45%)	8.79±0.23* (11.96%)	
	14	8.07±0.13	8.64±0.20 (7.01%)	9.36±0.35* (15.92%)	
	21	8.14±0.09	8.87±0.18* (8.88%)	9.81±0.37* (20.49%)	
	28	8.39±1.14	9.38±0.36 (11.89%)	10.47±0.45* (24.82%)	
Testis	7	6.78±0.36	7.28±0.26 (7.29%)	7.66±0.27* (12.85%)	
	14	6.81±0.33	7.64±0.20* (12.23%)	8.03±0.38* (18.04%)	
	21	6.81±0.50	8.00±0.22* (17.47%)	8.34±0.23* (22.54%)	
	28	7.05 ± 0.75	8.72±0.39* (23.59%)	9.47±0.57* (34.24%)	

TABLE No. 2 Differential bioaccumulation of Zn (II) in various tissues of Fish, *Channa punctatus*. Mean \pm SD of three observed values of each group. Percentage change over control in paranthese, *represents the values are significant at 5% level or p<0.05 (by one way analysis of variance)

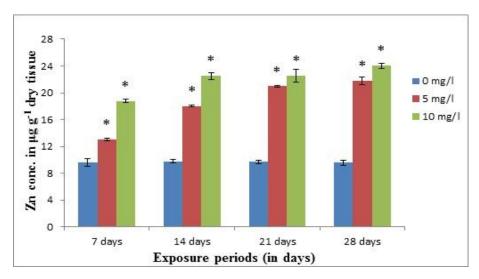


FIGURE1. Variations in bioaccumulation of Zn (Mean \pm Standard deviation) in liver of fish, *Channa punctatus* exposed to different concentrations of ZnSO₄ (5 and 10 mg L⁻¹) and control (0 mg L⁻¹) after 7 days, 14 days, 21 days and 28 days intervals of exposure period. (* represents the values significant at p<0.05 level)

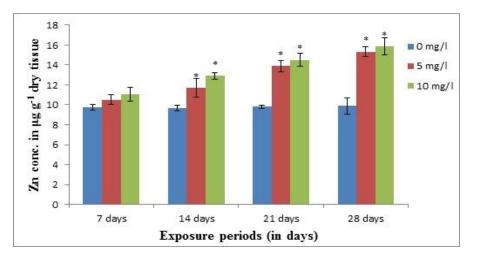


FIGURE 2. Variations in bioaccumulation of Zn (Mean \pm Standard deviation) in kidney of fish, *Channa punctatus* exposed to different concentrations of ZnSO₄ (5 and 10 mg L⁻¹) and control (0 mg L⁻¹) after 7 days, 14 days, 21 days and 28 days intervals of exposure period. (* represents the values significant at p<0.05 level)

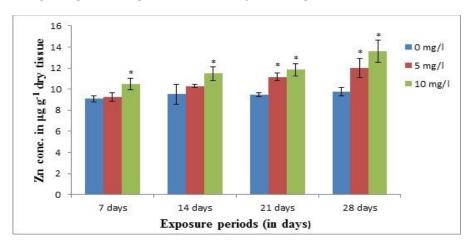


FIGURE 3. Variations in bioaccumulation of Zn (Mean \pm Standard deviation) in muscle of fish, *Channa punctatus* exposed to different concentrations of ZnSO₄ (5 and 10 mg L⁻¹) and control (0 mg L⁻¹) after 7 days, 14 days, 21 days and 28 days intervals of exposure period. (* represents the values significant at p<0.05 level)

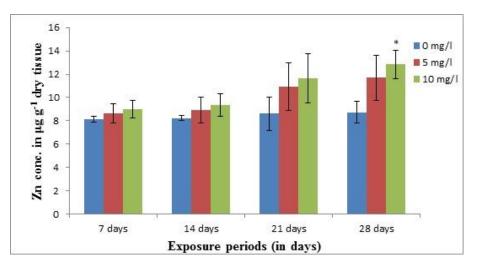


FIGURE 4. Variations in bioaccumulation of Zn (Mean \pm Standard deviation) in gill of fish, *Channa punctatus* exposed to different concentrations of ZnSO₄ (5 and 10 mg L⁻¹) and control (0 mg L⁻¹) after 7 days, 14 days, 21 days and 28 days intervals of exposure period. (* represents the values significant at p<0.05 level)

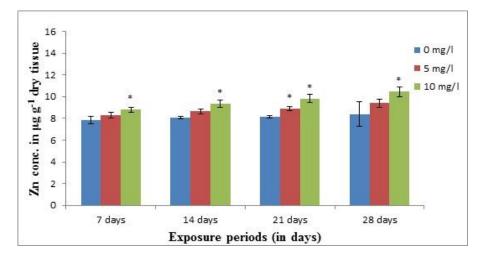


FIGURE 5. Variations in bioaccumulation of Zn (Mean \pm Standard deviation) in ovary of fish, *Channa punctatus* exposed to different concentrations of ZnSO₄ (5 and 10 mg L⁻¹) and control (0 mg L⁻¹) after 7 days, 14 days, 21 days and 28 days intervals of exposure period. (* represents the values significant at p<0.05 level)

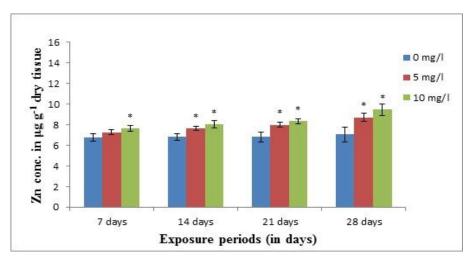


FIGURE 6. Changes in bioaccumulation of Zn (Mean \pm Standard deviation) in testis of fish, *Channa punctatus* exposed to different concentrations of ZnSO₄ (5 and 10 mg L⁻¹) and control (0 mg L⁻¹) after 7 days, 14 days, 21 days and 28 days intervals of exposure period. (* represents the values significant at p<0.05 level)

Zn (II) concentration in different tissues of fish, *Channa punctatus* was determined on dry weight basis. Differential bioaccumulation of Zn in fish, based on exposure to test chemical and time interval, reflects that the liver has highest Zn (II) concentration, followed by kidney, muscle, gill, ovary and testis (Table 2). In liver, the maximum Zn (II) accumulation, over and above control, was recorded after 14 days of exposure period when fishes are subjected to 10mg/l concentration of ZnSO₄. However, analysis of percentage variation of zinc accumulation, in comparison to control, reveals that maximum increase of 151.12% was for 28 days exposure period in 10mg/l concentration of test chemical and minimum increase of 35.48% was for 7 days exposure period in 5 mg/l concentration of test chemical.

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However, accumulated zinc in muscle tissue of fish was lower than that in liver and kidney. Zn (II) level in muscle of fish was found significantly higher (p<0.05) in both

concentrations of test chemical for all the exposure periods than control. The accumulation in 5 mg L⁻¹ exposed group for 7 and 14 days exposure periods was non significant (Table No -2). While the percentage change over control was maximum for 28 days exposure period in 10 mg/l concentration of test chemical and minimum increase of 2.14% was for 7 days exposure period in 5 mg/l concentration of test chemical.

The level of zinc accumulation in the gill of fish was lower than the organs like liver, kidney, and muscle except gonads (testis and ovary). Accumulation of zinc in both exposed groups were found mostly non-significant (p>0.05) than the accumulated value of Zn (II) in control group for each exposure period. Only for 28 days exposure period in 10 mg L⁻¹ ZnSO₄ treated group the accumulation value was significant (p<0.05). At the end of 28 days exposure period accumulation values of Zn (II) were found 11.68±1.96 µg g⁻¹ and 12.83±1.21 µg g⁻¹ for 5 mg L¹ and 10 mg L⁻¹ of zinc sulphate respectively. In comparison to the value $(7.05\pm0.75 \ \mu g \ g^{-1})$ of control group, quantity of Zn (II) accumulation in testis were calculated 8.72±0.39 $\mu g g^{-1}$ and 9.47±0.57 $\mu g g^{-1}$ for 5 mg L⁻¹ and 10 mg L⁻¹ of Zn exposed groups respectively after the completion of 28 days. Zn (II) accumulation in testis was found significantly (p<0.05) high in each exposed group for different exposure periods except for 5 mg L⁻¹ treated group at 7 days exposure period where accumulation value was non-Comparatively (p>0.05). Zn significant (II)bioaccumulation in ovary was more than the testis. Accumulation values of Zn for 10 mg.L⁻¹ exposed group in ovary were significantly higher (p<0.05) than the control group. But in the case of 5 mg L^{-1} exposed group, only one value of Zn accumulation i.e. 8.87 ± 0.18 µg.g⁻¹ was significant (p<0.05) at 21 days exposure period, the Zn concentrations were calculated 9.38±0.36 µg.g-1 and 10.47±0.45 μ g.g⁻¹ for two exposed groups (5 mg L⁻¹ and 10 mg L^{-1} of ZnSO₄ respectively) with respect to the 8.39 ± 1.14 µg.g⁻¹ value of control group. The percentage change over control was maximum for 28 days exposure period in 10 mg/l concentration of test chemical and minimum increase of 5.45% was for 7 days exposure period in 5 mg/l concentration of test chemical.

Cu, Ni, Zn and Fe are essential elements but they can also produce toxic effects when intake is excessively elevated (Pe' rez-Cid et al., 2001). Although it is detected that certain degree of bioaccumulation occurs for both essential and non-essential elements, the maximum concentrations reported corresponded to the essential elements Fe, Zn and Cu. (Sa'nchez-Chardi et al., 2007; Abdullah et al., 2007). This observation may be related to the involvement of these elements in the regulation of key enzymatic detoxification processes (Agtas et al., 2007) or to disrupted metal metabolism (Sa'nchez-Chardi et al., 2007). Positive correlations between elements in fish tissues may also denote the participation of essential elements (Cu, Ni, Zn, Fe) in the detoxification of nonessential elements (Cd, Hg, Pb) as part of the enzymes of the anti-oxidant systems, such as superoxide dismutase and, in metallothioneins (Sa'nchez-Chardi et al., 2007; Yılmaz et al., 2007). These relationships may also explain the same bioaccumulation pattern together with the same source of pollution, especially in case of non-essential elements (Yılmaz et al., 2007). However, prolonged

exposure to them may result in an impaired detoxification response to essential elements (e.g. Cu and Zn), thus leading to liver bioaccumulation (Fernandes, 2007). It is reported that active metabolite organs such as gill, liver and kidney accumulate more amount of heavy metal than other tissues like muscle (Dural et al., 2007). Muscle is generally considered to have a weak accumulating potential (O. Erdo rul and F. Erbilir 2007; Uysal et al., 2009; Bervoets and Blust 2003). High accumulating ability of the liver is a result of the activity of metallothioneins, the proteins that can be binded to some metals, such as Cu, Cd and Zn, thus reducing their toxicity and allowing the liver to accumulate them at high concentrations (Ploetz et al., 2007; Uysal et al., 2009; Wu et al., 2006). Due to the above discussed reasons, liver has been recommended by many authors as the best environmental indicator of both the water pollution and chronic exposure to heavy metals (Dural et al., 2006; Agah et al., 2009; Messaoudi et al., 2009). Our findings are in conformity with those of above cited researchers (Table 2). Among the six organs of fish assayed for bioaccumulation of zinc, the maximum accumulation was recorded in liver in comparison to other organs for all the four sets of exposure periods, viz., 7, 14, 21 and 28 days, in our experiments. We have recorded a maximum of 151.12% elevation in zinc accumulation in liver of fishes exposed to 10mg/l concentration of ZnSO₄ for 28 days, over controls. Even when exposure concentration was just 5mg/l for 7 days period, liver accumulated 35.48% higher zinc over and above controls. However, in other organs zinc accumulation was recorded 2.14% to 35.48% (p<0.05) higher than controls for 5mg/l after an exposure period of 7 days while for 28 days exposure period it was significantly (p<0.05) higher than controls in all the organs. Lenhardt et al. (2012) also observed higher levels of zinc in carp liver, muscle and gill samples which are in line with our observations. Our results also exhibit that maximum accumulation is in liver and similar results of high Zn, Cu and Cd in liver were observed in many studies (Mustafa and Guluzar 2003; Hamed and El-Moselhy 2000; Hanna and Muir, 1990). Among the fish organs, the liver and kidney appear to have a significantly higher tendency for the accumulation of most of the metals whereas gill and muscle had minimum concentrations of these metals (Javed, 2005; Agarwal et al., 2007; Rauf et al., 2009).

Zinc is needed for a variety of physiological function of living tissues and regulates many biological processes. It plays an important role in protein synthesis. However, it also causes severe toxicological effects on aquatic ecosystem, when discharged into natural water at elevated concentrations and greater discharges through sewage, industrial waste and from mining operation. Zinc shows fairly low concentration in surface water due to its restricted mobility from the place of rock weathering or from natural sources. Thus greater chances are there for more zinc accumulation in column or bottom feeding fishes. Fernandes et al. (2007) have also emphasised that the feeding habits and life style of species are strongly related to metal accumulation level. Through water bodies it reaches into the body of fishes and gets accumulated in various tissues. Zinc and other heavy metals also accumulate in various parts of the plants/crops through soil irrigated with contaminated water. They ultimately

reach to the body of animals at higher trophic levels including humans through food chain. Elevated concentrations of these heavy metals cause various physiological anomalies in animals as well as in plants. (Vinodhini, R. & Narayanan, M. 2007, Pandey, G. and Madhuri, S. 2014, Fallah et al., 2011). Though Zinc is involved in nucleic acid synthesis and participates in a variety of metabolic processes involving carbohydrates, lipids, proteins and nucleic acid (Mc. Dowell, 1992), it can be toxic when present in excess amount. Adverse changes in blood parameters and tissue structures have already been reported in animals on exposure to zinc (Gupta and Chakraborty, 1995; Banerjee, 1998). As discussed above, zinc accumulates in greater quantities in liver in comparison to other organs. This higher accumulation in liver may alter the levels of various biochemical parameters. This may also cause severe liver damage (Fergeuson, 1989; Mayers and Hendricks, 1984). The accumulation of essential metal like zinc in liver is linked to its role in metabolism (Zhao et al., 2012). High levels of zinc in hepatic tissues are usually related to a natural binding protein such as metallothioneins (Rahman et al., 2012) which act as an essential metal store to fulfil enzymatic and other metabolic demands (Meche et al. 2010; Elnabris et al., 2013). In fact, these pollutants have capability to accumulate in the food chain and could impose adverse effects in aquatic organisms (Gupta and Singh, 2011). Human who occupy top level of the food chain and consume metal-contaminated food may face greater risk of health problem. Long-term intake of Zn (150 - 2000 mg/day) induces sideroblastic anemia, leukopenia and hypochromic microcytic anaemia (Simon-Hettich et al., 2001).

CONCLUSION

It is evident from the result that the zinc metal contamination definitely affects the aquatic life of the freshwater fish. In chronically exposed fish internal environment is disturbed gradually at cellular level. This poses the physiological stress inside the various fish tissues due to the progressively Zn metal accumulation in the course of time. Hence a scientific method is required for detoxification so as to improve the health of these economically important fishes. And further research studies must need to be done to evaluate the toxic effects of heavy metals on fish health.

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