

ZINC METAL CAUSED HEXOKINASE VARIATIONS IN DIFFERENT BRAIN REGIONS OF TELEOSTS AND INFLUENCE OF *SPIRULINA PLATENSIS* WITH THE SPECIAL REFERENCE OF DETOXIFICATION

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anichat@ymail.com**ABSTRACT**

The sub-lethal zinc metal concentrations in presence of *Spirulina platensis* caused significant variations in brain (cerebrum, diencephalons, cerebellum and medulla oblongata) enzyme *hexokinase* in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus*, in microbe presence to a lesser extent than metal exposure directly. Under detoxification studies the impact of *Spirulina platensis* on sub-lethal zinc toxicity on *hexokinase* in various brain regions, *Spirulina* may have potential as a precipitation agent. *Spirulina* rapidly adsorbed appreciable amount of zinc from the aqueous solutions. The autotroph *Spirulina platensis* has the detoxification ability and the change occurred in the enzyme levels of different brain regions of above fish species.

Key words: Zinc, *Spirulina plantesis*, Teleosts, *Hexokinase*.

INTRODUCTION

Heavy metals are highly toxic to both animal and human being. It's present in aquatic habitat due to its use in the various industries. Heavy metals are dangerous because they tend to bioaccumulation (Barron, 2003; Boyd, 2004). Few of the toxic effects of heavy metal exposure are severe visceral damage and testicular atrophy, renal dysfunction, hepatic damage, hyper-tension, gas exchange breakdown at lamellar regions, central nervous system injury, anemia, bio-chemical and physiological variations, less fecundity alterations in abiotic and biotic factors of the habitat and even in generic disorder are well established. Heavy metals further affect organisms directly by accumulating in their body or indirectly by transferring to the next trophic levels of the food chain. Heavy metal poisoning could result, for instance, from drinking-water contamination. High ambient air concentrations near emission sources or intake via the food chain (Barron *et al.*, 2003; Dosiet *et al.*, 2007; Das *et al.*, 2008). Hence the need of the man is to innovate some alternative technologies and devices to protect the nature gifted consumables and to boost the yield from natural water bodies. In the present investigation the author made an attempt to study the influence of on *Spirulina pletensis* sub-lethal concentration of zinc caused marked change in *hexokinase* in cerebrum, diencephalons, cerebellum and medulla

oblongata in *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus* (Bloch) under acute studies.

MATERIALS AND METHODS

Alive, healthy, mature, disease-free and active *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus* (Bloch.) 120-130 gm. of 18-20 cm. (standard length) were obtained from few selected local ponds to avoid ecological variation and acclimatized in the laboratory condition. Period of seven days were given for various exposures and investigations. Safety and sub-lethal concentrations of zinc was determined on *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* by the *probit analysis method*. Higher concentration of zinc was used and slowly reduced the amount of concentration to know the Lc 50/100 value for 96-hour exposure.

Acute studies:

The *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* (120-130 gm) of 18-20 cm (standard length) were taken separately and kept in twenty groups and each group consist of forty eight fish species. No food was given to the above fish species during this period (08, 16 and 24hrs). The first set of *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* were exposed to sub-lethal concentration of zinc and the detail were described (Shaffi and Kakaria, 2006).

the experiment, preparation of tissue extract and enzyme assays were described (Colowick and Kaplan, 1975; Shaffi and Habibulla, 1977). The experiments with acute studies were repeated at least seven times separately to subject the data for analysis of variance.

RESULTS AND DISCUSSION

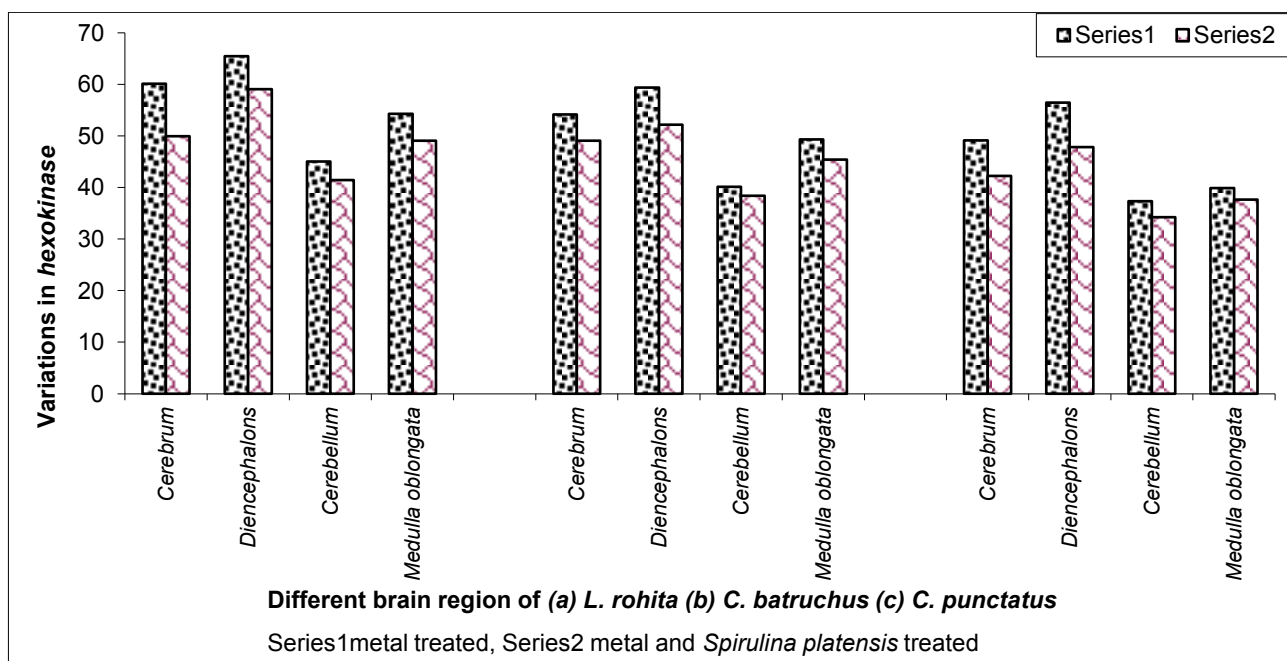
Exposure to sub-lethal concentration of zinc caused marked changes in *hexokinase* in cerebrum, diencephalons, cerebellum and medulla oblongata of *Labeo rohita* (sub-lethal concentration of Zn-0.72 mg/ltr.), *Clarias batrachus* (sub-lethal concentration of Zn- 2.75mg/ltr.) and *Channa punctatus* (sub-lethal concentration of Zn-2.90mg/ltr.) under acute studies. Safety level concentrations of zinc metal was determined for *Labeo rohita*(Zn-0.10 mg/ltr.), *Clarias batrachus* (Zn-0.14 mg/ltr.) and *Channa punctatus* (Zn- 0.18 mg/ltr). The influence of *Spirulina platensis* on sub-lethal concentration of zinc was investigated in table No. 1 and fig. No. 1.

The exposure to sub-lethal concentrations of zinc in presence of *Spirulina platensis* led to highest fall in diencephalons, *hexokinase* in comparison to cerebrum, medulla oblongata and cerebellum in *Labeo rohita*. The maximum fall in *hexokinase* was in diencephalons followed by cerebrum, medulla oblongata and cerebellum at 08

hrs. exposure than at 16 & 24 hrs. exposure in *Clarias batrachus*. The fall in *hexokinase* in *Channa punctatus* was optimum at 16 hrs. in diencephalon, in comparison to, medulla oblongata, and cerebellum than at 08 hrs. and at 24 hrs.in exposure to sub-lethal concentrations. The *hexokinase* fall was highest in diencephalon exposed to sub-lethal concentrations of zinc in microbe presence at 08 hrs. than at 16 & 24 hrs. in comparison to cerebrum, medulla oblongata & cerebellum in *Labeo rohita* than in *Clarias batrachus* and *Channa punctatus* (Table-1 & chart digram No.1.).

The sub-lethal zinc concentrations in presence of *Spirulina platensis* caused significant variations in brain (cerebrum, diencephalons, cerebellum & medulla oblongata) enzymes *hexokinase* in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* in microbe presence to a lesser extent than metal exposure directly. Under detoxification studies the impacts of *Spirulina platensis* on sub-lethal zinc toxicity on *hexokinase* in various brain regions of brain i.e. cerebrum, diencephalon, cerebellum and medulla oblongata in three important inland teleost viz. *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* under short term exposure studies.

Fig. 1: Influence of *Spirulina platensis* on zinc (sub-lethal) caused hexokinase variations in different brain regions of three fresh water fish species.



In the present investigation to a new strategy was adapted to detoxify the metal caused toxicity on brain enzyme compartmentation in three fish species. The sub-lethal zinc concentration in presence of *Spirulina platensis* caused significant variations in brain (cerebrum,

diencephalons, cerebellum & medulla oblongata) enzyme (*hexokinase*) in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* in microbe presence to a lesser extent than metal exposure directly.

TABLE No. 1: Influence of *Spirulina platensis* on zinc (sub-lethal) caused *hexokinase* variations in different brain regions of three fresh water fish species.

Regions of the Brain	Control	Duration of sub-lethal Concentration exposure			% of fall/Rise	Duration of sub-lethal concentration exposure with <i>Spirulina platensis</i>			% of fall/rise
		08 Hrs.	16 Hrs.	24 Hrs.		08 Hrs.	16 Hrs.	24 Hrs.	
(A) <i>Labeo rohita</i> (HAM)									
Cerebrum	0.386 ±.098	0.198 a ±.029	0.172 ±.032	0.154 a ±.032	60.10	0.284 c ±.064	0.242 ±.042	0.196 a ±.034	49.92
Diencephalon	0.298 ±.064	0.178 c ±.032	0.142 ±.024	0.103 ±.019	65.43	0.208 c ±.026	0.181 ±.028	0.122 b ±.022	59.06
Cerebellum	0.222 ±.048	0.159 ±.022	0.138 c ±.019	0.122 c ±.022	45.04	0.188 ±.021	0.159 ±.019	0.130 c ±.018	41.44
Medulla Oblongata	0.326 ±.054	0.199 c ±.032	0.162 ±.021	0.149 c ±.032	54.29	0.232 c ±.038	0.192 ±.022	0.166 c ±.024	49.07
(B) <i>Clarias batrachus</i> (LINN.)									
Cerebrum	0.371 ±.030	0.341 ±.066	0.236 c ±.024	0.170 ±.028	54.17	0.226 c ±.024	0.199 b ±.026	0.189 ±.032	49.05
Diencephalon	0.251 ±.041	0.219 ±.022	0.148 c ±.020	0.102 ±.019	59.36	0.184 ±.019	0.162 c ±.032	0.120 b ±.019	52.19
Cerebellum	0.172 ±.029	0.158 ±.019	0.124 ±.019	0.103 c ±.021	40.11	0.143 ±.021	0.128 ±.018	0.106 c ±.021	38.37
Medulla Oblongata	0.282 ±.039	0.266 ±.028	0.184 ±.014	0.143 c ±.036	49.29	0.162 c ±.023	0.136 ±.020	0.154 ±.022	45.38
(C) <i>Channa punctatus</i> (BLOCH)									
Cerebrum	0.291 ±.041	0.266 ±.042	0.184 c ±.026	0.148 ±.024	49.14	0.204 ±.032	0.196 c ±.019	0.168 ±.026	42.26
Diencephalon	0.209 ±.028	0.178 ±.026	0.126 ±.018	0.091 a ±.018	56.45	0.162 ±.028	0.139 ±.012	0.109 c ±.016	47.84
Cerebellum	0.190 ±.036	0.162 ±.022	0.138 ±.014	0.119 a ±.021	37.36	0.156 ±.018	0.142 ±.026	0.125 c ±.014	34.21
Medulla Oblongata	0.247 ±.042	0.229 ±.019	0.168 c ±.022	0.148 ±.026	39.91	0.196 ±.019	0.172 ±.018	0.154 c ±.026	37.65

Values are mean ± SDM of seven replicates. The data was subjected to test of ANOVA. The super scripts (a, b & c) indicates that P > 0.01, P > 0.02, & P > 0.05 respectively

The *Spirulina platensis* influenced the sub-lethal effect of zinc caused variations in brain compartmentation (cerebrum, diencephalons, cerebellum and medulla oblongata) of *hexokinase* in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* under acute or short term exposure. The sub-lethal levels of zinc inhibited the *hexokinase* to a highest extent in diencephalon than in cerebrum, medulla oblongata and cerebellum in *Labeo rohita* in comparison to *Clarias batrachus* and *Channa punctatus* but lesser than the fall of the enzymes in the above said fish species directly exposed to sub-lethal levels of zinc directly without any microbe compelled us to develop an insight to understand the positive impact on important bio-chemical parameters like enzymes that are important to promote a variety of anabolic and catabolic processes in an organism effectively reflects that microbes act as antidote effect fall heavy metal toxicity and the less fall of the four enzymes under investigation may be that microbes has a soothing impact and hence the microbes are able to decrease the sub-lethal toxicity of sub-lethal level zinc.

The following finding may help to understand the microbe-metal interaction and sub sequent detoxification of the metal to a less

extent in a better way. The sub-cellular regions of Cyanobacteria and *Anabaena cylindrica* could trap the lead through its phosphate and precipitates in the form of lead phosphate on the cell wall inside the cell (Jayprakash *et al.*, 2005, Bert *et al.*, 2009). *Spirulina platensis* has the detoxication ability and the present change of enzyme levels in different brain regions of three fish species (Kushwaha *et al.*, 2004, Cristina *et al.*, 2005; Gelagutashvili, 2006; Shaffi *et al.*, 2007 and Page *et al.*, 2009). Similar kind of mechanism might have taken place in the present findings i.e. less fall of enzymes in which the cellular components of *Spirulina platensis* might have precipitated the metal into compound with the help of its cellular components and the present findings i.e. less fall of enzymes in presence of a autotroph than the enzyme fall when directly exposed to zinc sub-lethal should understand on similar lines.

In the present investigation the zinc metal might have accumulated in *Spirulina platensis* and the less impact of zinc noticed in the fall of *hexokinase* in various brain regions of *Clarias batrachus* and *Channa punctatus* in comparison to directly expose to zinc sub-lethal.

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LITERATURE CITED

- Barron MG, 2003.** Bioaccumulation and bioconcentration in aquatic organisms. *Handbook of ecotoxicology* 2nd Edi. Lewis Publishers- New York. 877-892.
- Bert V, Seuntjens P, Dejonghe W, Lacherez S, Thi HTT and Vandecasteele B, 2009.** Phyto-remediation as management option for contaminated sediments in tidal marshes, flood control areas and dredged sediment land fill sites. *Environ. Sci. Poll., Res.*, **16(7)**:117-125.
- Boyd RS, 2004.** Ecology of metal hyperaccumulation. *New Phytol.* **162**: 563-567.
- Cristina SF, Armando AHV and Otaciro RN, 2005.** The metal binding capacity of anabaena siroides extracellular polysaccharide: an EPR study. *Cell Host and Microbes Process. Biochem.* **40** (6):2215- 2224.
- Colowick SP and Kaplan NO, 1975.** Methods in Enzymology. Vol. *XLI (B) Aca. Press. New York*
- Das N, Karthika P, Vimala R and Vinodhini V, 2008.** Use of natural protects as biosorbent of heavy metals- An overview. *Nat. Prod. Radiance*, **7(2)** :133-139.
- Dosi H, Ray A and Kothari IL, 2007.** Bio-remediation potential of live and dead *Spirulina*: spectroscopic, kinetics and SEM studies. *Biotechnol. and Bioeng.* **96**: 1051-1063.
- Finney DT, 1971.** *Probit Analysis Method.* 2nd Edi. Camb. Uni. Press.8
- Gelagutashvili E, 2006.** Interaction of Cu(II) and Cd(II) ions with DNA from *Spirulina platensis*. *J. Therm. Ana. and Calo.* **1572-8943** (85): 491-494.
- Holden JF and Adams MWW, 2003.** Microbe-metal interactions in marine hydrothermal environments. *Curr. Opi Chem. & Biol.* **7**: 160- 165.
- Jayprakash K and Chinnaswamy P, 2005.** Effect of *Spirulina* & Liv-52 on cadmium induced toxicity in albino rats. *Curr. Sci.*, **85** (2): 925-929.

Kushwaha PK, Pachiappan A, Abraham SK, Santhiya ST and Ramesh A, 2004. Protective effect of *Spirulina fusiformis* on chemical induced genotoxicity in mice. *Fitotera*, **75**: 24-26.

Mehta SK and Gaur JP, 2001. Removal of nickel and copper from single and binary metal solutions by free and immobilized *Chlorella vulgaris*. *Euro. J. Protistol.* **37**: 261-271.

Page V and Schwitzguébel JP, 2009. The role of cytochromes P-450 and peroxidases in the detoxification of sulphonated anthraquinones by rhubarb and common sorrel plants cultivated under hydroponic conditions. *Environ. Sci. Pollut. Res.*, **16**(7):243-248.

Shaffi SA and Habibulla M, 1977. Differential distribution of glycogen, lactate & pyruvate indifferent brain regions of rat. *Ind.j.exp.Biol.*, **14**:307-308.

Shaffi SA and Kakaria VK, 2006. Comparison of the sub-lethal effect of metal mixture on gluconeogenic enzymes compartmentation and recovery in brain of three fresh water teleosts: *J. and Cell Tissue Res.* **6**:3.

Shaffi SA, Prakash BVBS, Avinash Nichat and Mangala R, 2007. Microbes as antidote to detoxify the metal caused toxicity in some fresh water teleost : *National Sem. on New Horizont in Toxicol&Sustance of life* : 19-20.

Thomas DJ, Styblo M and Lins, 2001. The cellular metabolism and systemic toxicity of arsenic. *Toxicol. Appl. Pharmacol.* **176**:127-144.