ZINC INDUCED ARCHITECTURAL ALTERATIONS AND ACCUMULATION IN THE OVARY OF FRESH WATER TELEOST, CHANNA PUNCTATA (BLOCH)

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Abstract: Fresh water murrel, Channa punctata were kept in aqueous solutions of zinc (Sub-lethal concentrations of 8 mg/l, 10 mg/l and 15 mg/l) for 15 days. Results show reduced GSI and ootoxic conditions. Oolytic changes include shrinkage of oocytes, which resulted in large interfollicular spaces. Distorted appearance of oocytes was a major alteration observed. Atretic follicles also increased in number while immature oocytes decreased. Simultaneously, zinc accumulation was also estimated in the ovary. Zinc accumulation recorded continuous elevation till the end of the experiment. Results are linearly proportional to dose and duration of the experiment. Exposure to zinc can therefore, reduce fecundity of fish thereby limiting the numerical size of the succeeding generation.

Key words: Zinc, Ootoxicity, Fish.

Introduction
Zinc is an essential micronutrient involved in a wide range of biological processes including enzyme catalysis, protein structure, protein-protein interaction and protein oligonucleotide reactions (Liyaquat et al. 2003; Jat and Kothari 2006; Srivastava 2007). The effects of different toxicants on the aquatic fauna, particularly fish have received attention of many investigators (Loganathan et al. 2006; Gupta and Srivastava 2006; Athikesavan et al. 2006; Srivastava et al. 2006; Ayas et al. 2007; Shukla et al. 2007; Srivastava 2007; Tilak et al. 2007; Joshi et al. 2007). The historical and biochemical effects of heavy metals on the ovary have, however, received little attention (James et al. 2003; Deshmukh and Kulkarni 2005; Popek et al. 2006). Any anomaly in the normal metabolism or histology of gonads in fishes can affect their progeny. The present work, therefore, describes the accumulation and toxicological action of zinc in the ovary of a fresh-water fish, Channa punctata (Bloch).

Materials and Methods
Adult specimens of Channa punctata (average length 18-20 cm. and average weight 65 to 70 gm) were procured from nearby water bodies. They were acclimatized for 15 days in 500L glass aquaria containing water from a tube well (Temperature- 14 to 22°C, Dissolved oxygen- 6.62 to 6.76mg/l, Alkalinity- 62 to 68mg/l, CO₂ Nil). Control fish (Group- I) were kept in normal tap water. Zinc sulphate (ZnSO₄·7H₂O, mol.wt 287.55, E.Merck, India (Ltd.)) containing 65.38 Zn, was used for the experiment. A stock solution was prepared in 500 ml distilled water, which was further diluted to give working dilutions of 10mg/l (group II), 15mg/l (group III) and 25mg/l (group IV). On the basis of Lc50 (56.52mg/l) it works out to be approximately 20, 25 and 50% of Lc50.
Five fish from each group were sacrificed on day 8, 10 and 15. The ovary of control and test fish were dehydrated through graded series of ethanol for routine histological examination. Gonadosomatic Index (GSI) was calculated using the following formula:

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GSI = \frac{\text{Weight of ovary} \times 100}{\text{Weight of fish}}
\]

Ovarian tissue was weighed for each group and samples were digested in a dia-cidic solution (HNO₃ : HClO₄ = 5:1) for noting zinc accumulation. Digested samples were analyzed by atomic absorption spectrophotometer in air acetylene flame at 213 µ for estimation of zinc content. Data obtained was subjected to statistical analysis.

Results

Ovaries of group I (control) show a histological picture similar to that of a normal fish with a number of oocytes showing various stages of maturation. Immature oocytes are small in size with large nuclei and several nucleoli scattered in the nucleus (figure 1). In some oocytes nucleoli are ranged on the periphery of the nucleus. Fatty yolk globule formation starts from the periphery of the oocytes (figure 1).

In groups II, III and IV however, pathological conditions are progressively observed. In group IV, the histopathological changes are most pronounced, progressive and prominent (Figures 2-4). In this group majority of oocytes are immature; they tend to lose shape and show drastic reduction in size. Atretic oocytes increase in number.

Gonadosomatic-index (GSI) shows a dose and duration dependent decrease (figure 5). Accumulation of zinc by the ovary increases significantly dose and duration of the experiment (figure 6).

Discussion

Similar to the present study, Kumar and Pant (1984) earlier reported a significant atresia in the ovary with major damage to younger oocytes in Puntius conchonius, after exposure to zinc; they suggested a direct action of zinc on gonads. Baruah and Das (2002) noted partial lysis, swelling, atresia and changes in nucleus and cytoplasmic organization after exposure of Heteropneustes fossilis to paper mill effluent for 20 days. They suggested that alterations were due to the influence on the pituitary-gonadal axis. Similarly, Olfat and El-Greisy (2007) observed extensive necrosis of oolema, hypertrophy and hyperplasia of the follicular cells of oocytes, atresia in the large vacuolated mature follicles of the ovary in Siganus rivulatus after exposure to different waste sources (containing Zn) for 42 days.

Ovarian changes noted in the present study can be attributed to either direct action of zinc on the ovary (Kumar and Pant 1984) and/or inhibitory action on the pituitary- gonadal axis (Baruah and Das 2002; Agrawal and Srivastava 2003a).

Similar to present study, Masud et al. (2003) observed decreased GSI, retardation of oocyte development, denaturation of yolk material in Cyprinus carpio exposed to mercurial compounds for 45 and 60 days. Kaur and Kaur (2006) also noted a decline in GSI of the fish Channa punctata (Bloch) under stress of nickel chrome electroplating effluent. Exposure of Siganus rivulatus to different waste sources (containing Zn) for 42 days (Olfat and El-Greisy 2007) also resulted in a decline in GSI.

In this study, the decrease in GSI of treated groups can be attributed to low occurrence of yolky oocytes as well as to greater occurrence of atretic oocytes. Atresia of vitellogenic follicles may be a result of insufficient amounts of endogenous gonadotrophin (s) (Agrawal and Srivastava 2003a).

Zinc accumulation in the ovary of Channa punctata, in the present study, is significantly raised in all treated groups at
**Figure 1.** showing ovary of group I (control) with different developing stages of oocytes.

**Figure 2.** showing ovary of group II at day 15.
Figure 3. showing ovary of group III at day 15.

Figure 4. showing ovary of group IV at day 15.
all intervals, as zinc has binding sites in
the ovary. Maiti and Banerjee (1999) ob-
served highest accumulation of zinc in the
ovary of Oreochromis nilotica in compari-
sion to other organs. High zinc concen-
tration in the ovary of the treated fish is a re-
sult of greater metallothionein production
for binding, as suggested by Shukla et al.
(2007). Duration dependent accumulation
of zinc is similar to observations by James
et al. (2003) on Cu accumulation in X. hel-
leri.

It has earlier been suggested that if the
concentration of zinc is high in tissues it
may cause severe structural damage and
impair physiological functions (Agrawal
and Srivastava 2003 b; Gupta and Srivastava 2006). In the present study also
histological damage can be well correlated
with high concentrations of zinc in the
ovary of all treated groups.
Conclusion
High zinc accumulation by the ovary of Channa punctata can be directly correlated to histopathological damage seen in the organ. A short exposure of 15 days is sufficient to cause drastic degenerative changes. If this persists for longer duration it may be transferred to developing eggs and cause deleterious effects on embryonic development. Exposure to zinc, therefore, can reduce the fecundity of fish and lower survival potential of the young ones, thereby limiting the numerical size of the succeeding generation.

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References
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