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COMPARATIVE QUALITATIVE AND QUANTITATIVE CHANGES IN THE OVARY OF SEXUALLY MATURE Channa punctatus Bloch CHALLENGED BY SUB-LETHAL DOSES OF LEAD NITRATE AND ZINC SULPHATE FOR VARYING DURATIONS.

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KEYWORDS

Sublethal, ovary, histopathology, vitellogenesis, Channa punctatus, oocyte, mature oocytes, growth cell division

ABSTRACT

Heavy metal ions e.g. lead, zinc etc are extracted and used for variety of purposes. Effluxes from smelters which use large quantities of water for industrial cooling are recycled into water bodies. Such a water not only contains metal ions and their salts but has elevated temperature. Although the literature on lead (Pb) and zine (Zn) as toxicants has grown in recent years, much of it either pertains to mammals or to select species of teleostei and elasmobranchi. Further, no comprehensive and integrated study has been undertaken to focus on the structural changes (qualitative and quantitative) in the ovary of teleost exposed to sub-lethal doses of Pb-, and Zn-salts for varying durations.

In the present study, using sexually mature breeding females of Channa punctatus as a 'model' an effort has been made to generate reproducible and reliable data on the inductive changes (qualitative & quantitative) in the ovary due to sub-lethal doses of $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$ after 7,21 and 35 days of treatment vis-a-vis control. The results of these findings are summarised as follows:-

Nuclear pycnosis, chromatolysis, breakdown of nuclear membrane, cell lysis., atresia, cessation of vitellogenesis and breakdown of yolk granules were used as parameters for monitoring the cytopathologies in the growing, dividing, maturing, pre-vitellogenic and vitellogenic ovarian cell types induced by Pb-, and Zn-salts after exposure for 7,21, and 35 days vis-a-vis control.

Pb appeared to be the most deleterious cytotoxic agent as compared to Zn. Pre-vitellogenic and vitellogenic oocytes were the targeted cells for this cation. It also caused significant changes in the number of these cells as well as of mature oocytes. The cytoarchitectural aberrations induced by Zn were much more sharply defined after prolonged exposure of 21 and 35 days. These observations indicate that the oogenetic stages of this fish are differentially responsive to the deleterious effects of Pb and Zn, with the former being more profound in its effects. Further, it is clear that a loss of nearly 40-50% oocytes will have a diverse effect on the fecundity and thus the population dynamics of this fish in nature.

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INTRODUCTION

Aquatic biota of ponds, lakes and rivers is constantly exposed to diverse pollutants that are discharged as industrial effluents. In addition they may reach water bodies via rain water, erosion and leaching, precipitation, dust fallout too. Heavy metal ions e.g., lead., cadmium, mercury, zinc etc.. form an important class of toxicant which induce a variety of histopathologies in somatic and gonadal tissues. Further, they also cause severe aberrations in metabolic pathways., DNA- RNA protein synthesis; often leading to cell death (cf:- Train, 1979.,Pickering and Henderson, 1966).

Salts of lead are known to have mutagenic. carcinogenic., co-carcinogenic and teratogenic effects. No organ system is exempt from the deleterious effects of this heavy metal, be it the nervous system renal tissues., blood vascular system or reproductive organs. Gametogenic activity is severly disturbed due to exposure to lethal., and sub-lethal doses for an extended period of time. Further, enzyme and substrate relationships (qualitative quantitative) also undergo significant aberrative alteration affecting cellular functions e.g.,growth., division., differentiation and maturation, including oogenesis (Eisler, 1988., Hodson et al., 1984). Compared to lead salts, salts of zinc have been shown to express their effects only when they are deficient or in excess. Thus, while Zn-deficiency has a marked effect on fecundity., and cellular metabolism; high doses of this metal induce significant histo-; and cyto-pathologies. Over 70 metalloenzymes are known to require Zn for their normal function. In addition, Zn has been shown to have a protective function by competing with such deleterious metals as lead by dislodging them from binding sites. However, there is no evidence of Zn-salts being carcinogenic, mutagenic , Co-carcinogenic or teratogenic... (Brungs., 1969., Vallee., 1957., Goel and Gupta, 1985., Taneja and Kaur, 1988, Eisler and Gardner, 1973., Eisler, 1971., Goyer et al, 1980).

In cyclically breeding oviparous teleosts, the process of oogenesis entails proliferation of oogonia; their growth, cell division (meiotic); differentiation; maturation; vitellogenesis and ovulation which culminates in spawning. The response of various ovarian cell types during the aforesaid phases is highly variable and complex. Very little is known about the chronic exposure of sexually mature breeding females to sub-lethal concentration of lead nitrate ($Pb(NO_3)_2$) and zinc sulphate ($ZnSO_4.7H_2O$) for varying durations of time.

The present report deals with the effect of the aforesaid metal ions on the extent of changes (qualitative and quantitative) in the ovary of sexually mature breeding adults of the Channa punctatus (Bloch).

MATERIAL AND METHODS

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1. TEST ANIMAL-

Channa punctatus is a cosmopolitan fresh water teleost, which is found in abundance in the Lake waters of Udaipur. This species is commonly knowm as murrels". It is carnivorous, mud dweller and an air breathing fish. It feeds on small fishes and insects. Sexually mature females of C. punctatus were collected from the non-polluted aquatic reservoirs in the vicinity of Udaipur during their breeding season (May-September). Fishes weighing 60.0 ± 5.0 gm were acclimated for two weeks in cement tanks (100x50x40cm) containing water with stable physico-chemical properties as described elsewhere (vide infra). The animals were used to evaluate the short and long term effect of sub-lethal concentration of Pb $(NO_3)_2$ and $ZnSO_4.7H_2O$.

ii) Preparation of test concentration of Pb $(NO_3)_2$ and $ZnSO_4.7H_2O$ for determining LC_{50} value.

a) Pb $(NO_3)_2$: Reagent grade (E.Merck., India) salt of Pb was used to prepare series of test concentrations ranging from 5mg/1 to 20. The test concentration of 10mg/l was determined to be sub-lethal as no mortality resulted upto 35 days which was the maximum duration of exposure of fishes to this cation.

b) $ZnSO_4.7H_2O$: Reagent grade (E.Merck India) of this salt was used to make series of test concentration to determine LC_{50} value. The test concentration of 20mg/1 was computed to be sub-lethal to this fish.

iii) Maintenance of Fishes: Static bioassay test of water was done according to the "Standard Methods for the examination of water and waste

water (APHA et al., 1975). The test water displaying the following physico-chemical characteristics was replaced after every 24h.

a) Temperature 29°C - 31.5°C, b) pH : 7.96, c) Dissolved Oxygen : 7.6 ppm., d) Alkalinity : 102 ppm, e) Salinity : 99.30 ppm, f) Photoperiodic conditions were 12 h. light: 12 h dark. The fishes were fed with minced liver, or egg white every alternate day.

iv) Experimental protocol

Fishes (3-6) were used to set up control and experimental groups., Group I : Control, Group II : Experimental: exposed to 10mg/1 Pb $(NO_3)_2$ for 7 days., Group III : experimental: exposed for 21 days to the above test concentration., Group IV : experimental exposed for 35 days to sub lethal concentration of Pb $(NO_3)_2$

Similar groups (Group V -VIII) were set up for testing the effects of 20mg/1 of $ZnSO_4.7H_20$ on the ovarian histoarchitecture.

v) Surgical procedures:

Fishes from control and experimental groups were sacrified by cephalic stunning after 7, 21, and 35 days of exposure. The paired ovaries were dissected out surgically under semisterile conditions. They were freed off excess fascia, blood clots., and washed in chilled physiological saline (at 4°C).

vi) Treatment of tissues for histopathological evaluation.

Pieces of ovaries were fixed in aqueous Bouin's for 16-18h. Consequent to this, the tissues were washed in running water to remove excess fixative. They were dehydrated in graded ETOH series (30%-100%), cleared in xylene., infilterated with and embeded in paraffin wax (m:p 60°c).

Serial sections (5-7µm) were stained with Weigert's -iron-haematoxylin and counterstained with alcoholic eosin. Dehydrated and cleared sections were mounted in DPX. Every alternate section of the ovary was microscopically evaluated to determine the extent and type of structural changes in the ovarian cell types of fishes challenged by the aforesaid metal ions for different durations. Appropriate areas in the section exhibiting significant changes were microphotographed at various magnifications. In some cases the prints of such photographs were magnified to highlight details of microscropic alterations.

The parameters of monitoring qualitative changes in the ovary were:-
(a) Nuclear pycnosis., (b) Chromatolysis (c) breakdown of nuclear membrane, (d) cell lysis., (e) atresia., (f) cessation of vitellogenesis (g) breakdown of yolk granules. These changes were visually appraised in growing, dividing, maturing., pre-vitellogenic and vitellogenic ovarian cell types.
Quantitative changes were determined by making counts of various cell types in the ovary. These data were subjected to statistical analyses.

RESULTS AND DISCUSSION

The present comparative studies highlight the effect of sublethal -doses of Pb $(NO_3)_2$ and $ZnSO_4.7H_2O$ on the ovarian histoarchitecture (qualitative and quantitative alterations) of sexually mature breeding females of Channa punctatus. The duration of exposure was used as a variable. Both the aforesaid metal ions induced visible and measureable albeit variable changes in the ovarian cell types undergoing processes of growth, cell division., vitellogenesis. and ovulation. For the sake of clarity, meaningful interpretation and tangible discussion with other known information, these changes with respect to each cation species are discussed separately as under:-

I. Effect of $Pb(NO_3)_2$.

A. Qualitative and quantitative alterations in the ovarian histoarchitecture.

Effect of sub-lethal dose of Pb $(NO_3)_2$ (10mg / 1) on the ovarian cell types manifested variations that appear to be linked with the duration of exposure (Plate 1 and 2, Tables 1 and 3). Pre-vitellogenic and vitellogenic oocytes after 7 days of challenge showed significant cytopathologies which were characterised by nuclear pycnosis., chromatolysis.. vacuolisation of cytoplasm and eventual cytolysis. Marked reduction in the percentage of vitellogenic and mature oocytes were noticed vis-a-vis control. Further, aggravation of cytopathological changes were observed in the ovarian cell types after 21 and 35 days of chronic exposure to $Pb(NO_3)_2$. Reduction in the number

of nucleoli in the vitellogenic and mature oocytes was followed by karyolysis. The percentage of pre-vitellogenic oocytes was not affected and they actually manifested sustained augmentation (63.48%) on day 35 vis-a-vis-control (51.04%). However, nearly 50% reduction was noticed in the percentage of vitellogenic and mature oocytes. Interestingly enough the percentage of atretic oocytes was more than doubled. Significant changes also occured in the diameter of the oocyte types over a period of time. These qualitative and quantitative changes in the ovary of C. punctatus in response to $Pb(NO_3)_2$ clearly show that vitellogenic and mature oocytes are most susceptible to the detrimental influence of this cation. The pre-vitellogenic oocytes appear to be relatively resistant as they remain unaffected by this challenge and actually show an increase of their number. The duration of exposure does not appear to interfere with their developmental progress. It is also significant to record here the fact that there is a 2-fold loss of oocytes (in various stages of development) due to atresia. This indicates that the out put of fertile/viable eggs is substantially reduced due to Pb-toxicity. It is quite logical to suggest that these decline in fecundity would be detrimental to the population dynamics and therefore, survival of the species in nature.

A comparison of the present findings with other studies on Pb-toxicity to ovarian cell types shows many interesting parallels as well as differences. Thus, Banerji (1992) found that 5mg/1 of $Pb(NO_3)_2$ in Channa punctatus induced decremental changes in the gonadosomatic index (GSI) over a period of time. A variety of structural and developmental abnormalities were noticed over a period ranging from 15 to 90 days. These inductive effects were clastogenic in nature. Ovarian cells were observed to be arrested in the early maturational stages during initial exposures.. folliculogenesis and early vitellogenesis were in a state of suspended animation. Cytotoxic alterations were observed in ovarian cells particularly relating to folliculogenesis, vitellogenesis and steroidogenesis. The results of the present studies are at variance with this, since at 10mg/1 of $Pb(NO_3)_2$ the cytopathological changes occur. at a much earlier stage. Further, by day 35 the population of vitellogenic and mature oocytes are drastically reduced. The literature on lead-induced structural aberrations in the ovary of teleost fish is rather fragmented and scant. Kumar and Pant (1984) have reported a variety of cytological and developmental abnormalities in the ovary of Puntius conchonius challenged by 127 ppb of lead. This dose is very much lower as compared to the one used in the present studies. Results of other studies on lake trout (Demayo et al., 1982)., brook trout (Wong et al.. 1978)., Ictalurus punctatus (EPA, 1980), three-spine stickleback (Hawkaley, 1967) and guppies (Crandall and Goodnight, 1962) indicate that various test concentrations of lead cause differential reduction in the kinetics of egg production thus altering fecundity. It seems that species of fishes have not only variable but also characteristic threshold of sensitivity towards Pb which neverthless is toxic.

Sub-lethal concentration of $ZnSO_4.7H_2O$ (20mg/1) caused a variety of cytological aberrations in the growing, dividing, maturing, and vitellogenic oocytes. These syndromes manifested time dependent relationships (Plates 3 and 4, Tables 2 and 4). Early effects (after 7 days) of this cation were targeted at mature and vitellogenic oocytes which showed intense and irregular vacuolization. These oocytes also showed a decrease in their percentage vis-a-vis a control. Pre-vitellogenic oocytes, however, showed a slight elevation in their percentage. As the duration of chronic exposure to this cation increased, a further aggravation in cytopathologies were noticed. Thus, after 21 days of treatment the number of yolk globules showed a significant decline. Concomitant

accumulation of eosinophilic material in the follicular layer was discernible. Atretic oocyte number increased by 50% and the percentage of mature and vitellogenic oocytes was substantially reduced (40-50%). Significant clastogenic effects were noticed in the mature oocytes after 35 days of treatment. While the vitellogenic and mature oocyte, percentage was drastically reduced., while the previtellogenic oocyte percentage actually increased. This clearly shows their ability to circumvent the deleterious effects of $ZnSO_4$. That this assumption is true is also indicated by little change in the diameter of these oocyte type, and that too after 35 days of challenge. However, the diameter of vitellogenic and mature oocytes was significantly altered. These observation indicates that the oogenetic stages of Channa punctatus are differentially responsive to the deleterious effects of zinc. It also suggests that while the survival of the fish is not at stake, its reproductive performance in terms of number of viable eggs produced seems to be greatly altered. That even such eggs are hatchable and can produce normal fry remains to be studied. Entry of zinc into the body of fish via mouth, gills, intestinal absorption may over a period of time lead to its bioaccumulation in tissues. Long exposures of fish to this cation probably ensure bio accumulated zinc in the ovary reaches a threshold level where it initiates degenerative changes in susceptible oocyte types e.g., vitellogenic and mature oocytes. That this is so is further substantiated by the observed increase in the percentage of atretic oocytes and the fact that even the surviving populations of oocytes display substantially reduced diameter. The literature on zinc-induced aberrations on the ovary of teleost fishes is nebulous., although comparable information on mammalian ovary is fairly extensive. Thus, Brungs (1969) studied the chronic toxicity of zinc to fat head minnow and found that the number of eggs produced per female was only 17% as compared to control. This correlates well with the present studies on Channa punctatus which manifested a relatively lesser ovum loss due to degenerative changes affected by zinc. It is of interest to record here the observations of Benoit and Holcombe (1978) who observed a significant reduction in the hatchability of eggs and larval survival of fat-head minnow challenged by 295mg of zinc/l. They also found developmental defects in the juveniles hatched from eggs. Similar reduction in reproductive performance due to zinc toxicity have been reported in the flag fish Jordanella floridae (Spehar, 1976)., guppy Poecilia reticulata (Uviovo and Beatty, 1979)., fat-head minnow Pimephales promelas (Eaton, 1973), Cyprinus carpio (Kapur and Yadav., 1982)., and Clupea harengus (Soma Sundaram et al., 1984).

Very little is known about the exact mechanism(s) involved in the induction of toxicity by metal ions. However, many correlative data point towards their inhibitory role on ovarian processes. Bioaccumulation of heavy metals follows two distinct phases. During the first phase, there is a rapid intake of metals at constant rate till it reaches a reversible threshold level, followed by declination of intake in second and third phase in which accumulation is irreversible. The result of present studies do show this trend, for upto 15 days of exposure the extent of changes are not so magnified as they are after 35 days of challenge. It is pertinent here to record the observations that heavy metals can be stored in (a) non-toxic form as inorganic precipitates (b) membrane vesicles, and (c) lysosomes. They can also be trapped by cytosolic cystein rich proteins (metallothioneins) (Roesijadi, 1981). Viarengo (1989) suggested that metal free to interact with cell structure and/or enzymes in their way, affecting metabolic pathways. This needs to be further supported by experimental evidence.

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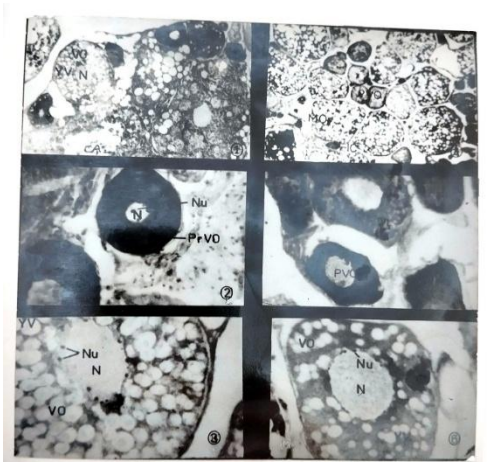
A comparison of the relative toxicity of $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$ clearly shows that the former has a cytotoxic effects on the ovarian cell types at a concentration of 10mg/1. $ZnSO_4$ on the other hand. mimics the syndromes induced by Pb at twice the concentration. It also seems likely that zinc gains entry into the body of fish and perhaps bioaccumulates in ovary as well. Its effects become pronounced as the duration of challenge increases. It seems logical to suggest that chronic exposure upto 35 days induces the development of an aberrant milieu interior in the ovary of Channa punctatus which is not conducive to normal development of ovum in qualitative and quantitative terms. However, the synergistic/competitive effects of Pb and zinc salts on the ovary remains an enigma.

Plate – 1
Histopathological changes in the ovary of C. punctatus induced by Pb $(NO_3)_2$. (10mg/l)
Figs. 1 – 3
Ovary of control fishes showing active oogenesis. Well-developed mature and vitellogenic oocytes can be seen. (100X and 400X)
Figs. 4 – 6
Ovarian histopathologies after 7th day of treatment. Oocytes showing nuclear pycnosis, chromatolysis and loss of shape. (40X and 400X)



VO- Vitellogenic oocytes, Nu- Nucleolus, YV- Yolk vesicle, N-Nucleus, IC- Interstitial cell; MO – Mature oocytes; PVO – Previtellogenic oocyte.

Plate – 2
Histopathological changes in the ovary of C. punctatus exposed to Pb $(NO_3)_2$.
Figs. 1 – 3
After 21 days of treatment
Irregular vacuolization in the mature and vitellogenic oocytes can be visualised. (40X, 100X, 400X)
Figs. 4 – 6
On 35th day of treatment
Mature and vitellogenic oocytes showing atretic changes. Irregular vacuolization in the previtellogenic oocytes can be seen. (40X, 100X, 400X)

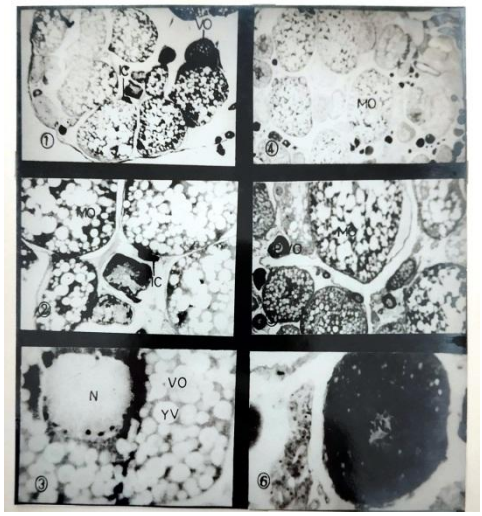


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Plate – 3

Histopathological aberrations in the ovary of sexually mature C. punctatus challenged by Zn (SO_4).7H_2O (20mg/lit.).

Figs. 1 – 3

Ovary of untreated fishes, showing several pre-vitellogenic, vitellogenic and mature oocytes (100X, 400X).

Figs. 4 – 6

Ovarian histopathology after 7 days of treatment

Increase in the size of previtellogenic oocytes, irregular vacuolization of mature oocytes can be seen. (40X, 100X, 400X)

CA- Cortical alveoli, N- Nucleus, VO- Vitellogenic oocytes, IC-Interstitial cell, Nu- Nucleuolus.

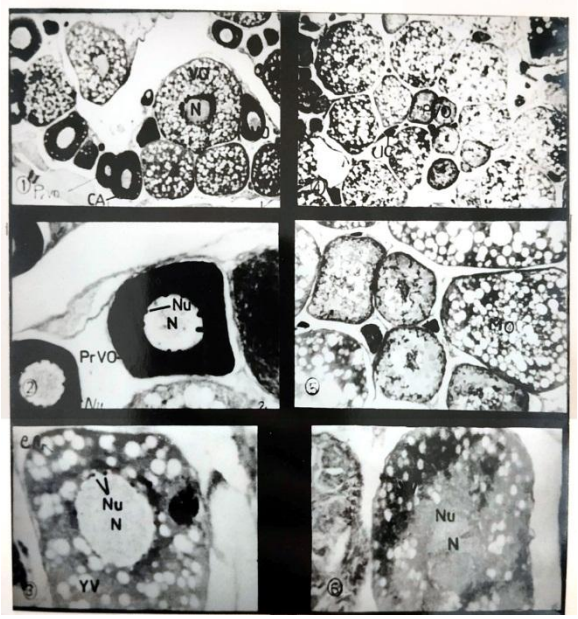

Plate – 4

Histopathology of ovary of sexually mature C. punctatus treated with Zn (SO_4).7H_2O.

Figs. 1 – 3

After 21st day of treatment

Accumulation of eosinophilic substances (arrows) in the vitellogenic oocytes can be seen. (40X, 100X, 400X)

Figs. 4 – 6

After35th day of treatment

Mature oocytes displaying atretic changes. Intense vacuolization can be observed. (40X, 100X, 400X)

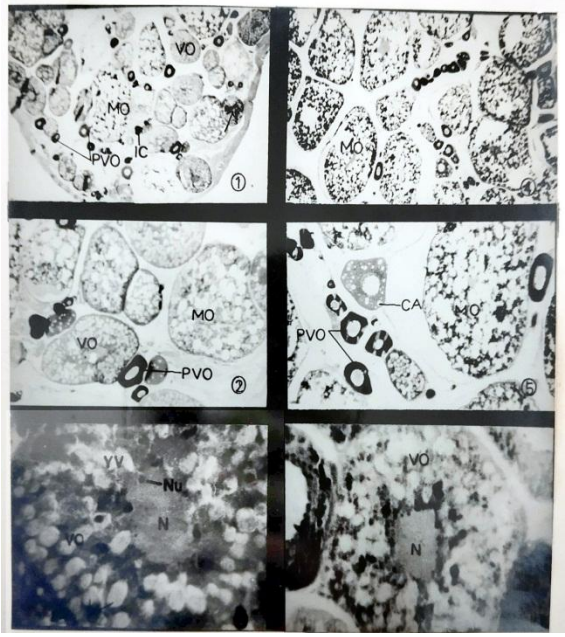

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TABLE-1

Percentage of oocytes after 10mg/l. Pb $(NO_3)_2$ treatement (duration being the variable)

S.No	Duration of treatment	Previtellogenic oocytes	Vitellogenic oocytes	Mature oocytes	Atretic oocytes
1.	Control	51.01±2.63	23.13±1.87	18.84±2.33	6.99±1.26
2.	7 day	*56.77±4.86 (+11.29)	*22.19±2.48 (-4.06)	12.51±2.56 (-33.59)	*8.53±1.51 (+22.03)
3.	21 day	*61.80±4.92 (+21.15)	*18.01±1.14 (-22.13)	10.09±2.23 (-46.44)	10.09±3.36 (+44.34)
4.	35 day	63.48±5.37 (+24.45)	13.20±5.45 (-42.93)	9.22±4.84 (-51.06)	14.10±0.80 (+101.71)

( ) Values in parenthesis are difference in increase or decrease vi-a-vis control

* Non significant, ± S.E.

P < 0.001

TABLE-II

Percentage of oocytes after 20mg/l. $Zn(SO_4).7H_2O$ treatement (duration being the variable)

S.No	Duration of treatment	Previtellogenic oocytes	Vitellogenic oocytes	Mature oocytes	Atretic oocytes
1.	Control	51.01±2.63	23.13±1.87	18.84±2.33	6.99±1.26
2.	7 day	*54.90±3.50 (+7.62)	*18.46±2.09 (-20.19)	*15.97±2.07 (-15.23)	10.69±2.31 (+52.93)
3.	21 day	*58.79±2.70 (+15.25)	16.23±3.27 (-29.83)	12.97±4.21 (-31.15)	12.24±3.31 (+75.10)
4.	35 day	62.85±3.35 (+23.21)	12.5±1.78 (-45.95)	10.79±2.34 (-42.72)	13.08±2.88 (+87.12)

( ) Values in parenthesis are difference in increase or decrease vi-a-vis control

* Non significant, ± S.E.

P < 0.001

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TABLE-III

Diameter of oocytes after 10mg/l. Pb $(NO_3)_2$ treatement (duration being the variable)

S.No	Duration of treatment	Previtellogenic oocytes	Vitellogenic oocytes	Mature oocytes
1.	Control	0.123	0.382	0.665
2.	7 day	0.093	0.205	0.529
3.	21 day	0.098	0.228	0.399
4.	35 day	0.123	0.249	0.337

TABLE-IV

Diameter of oocytes after 20mg/l. $Zn(SO_4).7H_2O$ treatement (duration being the variable)

S.No	Duration of treatment	Previtellogenic oocytes	Vitellogenic oocytes	Mature oocytes
1.	Control	0.123	0.382	0.665
2.	7 day	0.134	0.312	0.658
3.	21 day	0.129	0.261	0.501
4.	35 day	0.104	0.246	0.410

REFERENCES

Banerji, M. and Lall, S.B. (1989): Enzymological perturbations in the ovary of Channa punctatus (teleostei) challenged by cadmium chloride., Env. Start and Bioscience, 215, Ed.R. Prakash, J.B.A., New Delhi.

Benoit, D.A. and Holcombe, G.W. (1978): Toxic effects of zinc of fat -head minnow Pimephales promelas, in soft water. J. Fish. Biol. 13, 701-708.

Brungs, W.A. (1969): Chronic toxicity of zinc to the fat head minnow, Pimephales promelas Rafinesque. Trans. Am. Fish. Soc. 98: 272.

Crandall, C.A. and Goodnight, C.J. (1962) : Effects of sublethal concentration of several toxicants on growth of the common guppy Labistes reticulatus. Limnol. Oceanogr. 1, 233,

Demayo, A., Taylor, M.C. Taylor., K.W. Hudson, P.V. (1982): Toxic effects of lead compounds on human health, aquatic life, wild life, plants and live stock. CRC Crit., Rev Environ. Cont. 12. 257.

Eaton, J.G. (1973): "Chronic toxicity of copper cadmium, and zinc mixture to the fat-head minnow, Pimephales promelas Rafinesque"., Water Res., 7, 1723-1736.

Eisler, R. and Gardner, G.R. (1973): Anti-toxicity of dissolved metallic salts to Polycelis nigra (Moller) and Gammarus pulex (L). Br. J. Exp. Biol. 14, 351-363.

Eisler, R. (1971): Cadmium poisoning in Fundulus heteroclitus (Pisces: Cyprinodontidae) and other marine organisms. J. Fish. Res. Bd. Can. 28, 1225-1234.

Eisler, R. (1988): Lead hazards to fish and wildlife invertebrates. A synoptic review. US. Fish and Wild life. Ser. Biol. Rep. 85 (114-134).

EPA (1980): Ambient water quality criteria for Lead. USEPA. Rep. 440/5-80-057, 151.

Goel, K.A. and Gupta, K. (1985): Haematobiochemical characteristics of Heteropneustes fossilis under the stress of zinc. Ind. Jour. Fish 32,255-260.

Hawksley, R.A. (1967): Advanced water pollution analysis by a water laboratory. Analyser, 8, 13.

Hodson, P.V., Whittle, D.M. Wong., P.T.S., Borgman, U., Thomas, R.L., Chan, Y.K., Nriagu, J.O. and Hallet, D.J. (1984): Lead contamination of the great lakes and its potential effects on aquatic biota. Ed. J.O. Nriagu and M.S. Simons. John Wiley, New York.

Kapur, K., Yadav, N.A. (1982): Effects of certain heavy metal salts on the development of eggs in common carp, Cyprinus carpio var communis. Acta Hydrochem. Hydrobiol. 10, 5, 517.

Kumar, S. and Pant, S.C. (1984): Comparative effects of the sublethal poisoning of zinc, copper and lead on gonads of the teleost Puntius conchonius (H). Toxicol. Lett. 23, 189.

Pickering , Q.H., and C. Henderson (1966): The acute toxicity of some heavy metals to different species of warm water fishes. Air/Water Poll. 10, 453.

Roesijadi, G. (1981): The significance of low molecular weight, metallothionein – like proteins in marine invertebrates. Current status. Mar. Environ. Res. 4: 167.

Somasundaram . B., P.E. King., and S.E. Shackley (1984): Some morphological effects of zinc up on the yolk sac larvae of Clupea harengus L. J. Fish. Biol. 25, 333-343.

Spehar, R.L. (1976): "Cadmium and zinc toxicity to flag fish (Jordanella floridae)"; J. Fish. Res. Bd. Can. 33, 1939-1945.

Taneja, S.K. and R. Kaur, (1988): Effect of dietary Zn-deficiency on the distribution of lipids in the ovary of mature mouse. Ind. J. exp. Biol. 26, 271-273.

Train, R.E, (1979) : Quality criteria for water, U.S. Environmental Protection Agency Washington.

Uviovo. E.J. and Beaty. (1979): Bull. Environ. Contam. Toxicol. 23, 650.

Vallee. B.L. (1957): Zinc and its biological significance. Arch. Indust. Health, 16, 147.

Viarengo, A. (1989): Heavy metals in some marine invertebrates. Mechanism of regulation and toxicity at cellular level. Rev. Aquat. Sci. 12, 295.

Wong, P.T.S., Silverberg, B.A., Chan, Y.K. and Hodson, P.V. (1978): Lead and aquatic biota. In Biogeochemistry of lead in The Environment Part B. "Biological effects". . Ed. Nriagu., J.O. Elsevier/North Holland Biomedical Press Amsterdam.