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Research Article

Lead induced histological alterations in ovarian tissue of freshwater teleost *Mastacembelus pancalus* (Hamilton)

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Abstract

The Indian freshwater eel, *Mastacembelus pancalus* (Ham) was chronically exposed to lead nitrate for 30 days exposed at a dose of LC_{20} value 18.5 mg/l (in respect to 925ppm 96-h LC_{50} value). Results show reduced fecundity and GSI and deteriorated ootoxic conditions. Oolytic alteration includes lesion of oocytes, which resulted in dearth of any interfollicular spaces. Distorted appearance of oocytes was a major alteration observed. The effects of chronic exposure on growth and reproduction parameters were investigated in *M. pancalus*. The study demonstrated that lead can cause reproductive alteration in fishes. Present study insured that the gain in body weight and gonadal development along with egg laying capacity that is the fecundity and histological alteration of ovary of *Mastacembelus pancalus* is noticeably changed after 30 days exposure of lead. Lead exposure and growth rate, GSI were showed inverse relation. There were significant reduction on growth GSI, fecundity and histological attributes of the tissues of ovary due to chronic lead exposure. Exposure to lead can therefore, reduce fecundity of fish thereby can limit the numerical size of the succeeding generation.

Keywords: Lead nitrate, chronic toxicity, growth, GSI, fecundity.

1. Introduction

The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. The effects of different toxicants on the aquatic fauna, particularly fish have received attention of many investigators [2,4,11,17,15,23,28-30] as among animal species, fish are the inhabitants that cannot escape from the detrimental effects of these pollutants [8,22]. The toxicants like heavy metals after entering into aquatic environment accumulate in the tissues and organs of aquatic organisms. The amount of absorption and assembling depends on ecological, physical, chemical and biological condition and the kind of element and physiology of organisms [13].

Heavy metal accumulation in aquatic ecosystem shows that they are accumulated either in aquatic organisms [9,19] or in the sediment. Among the heavy metals lead is highly toxic to aquatic organisms, especially to fish [27]. The biological effects of sub-lethal concentrations of lead include delayed embryonic development, suppressed reproduction, and inhibition of growth, increased mucous formation, neurological problems, enzyme inhibition and kidney dysfunction [16,27]. Few other recent research reports also have indicated that lead can cause significant neurological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes in the animals [3,5,20,24,26].

The histological effects of heavy metals on the ovary have, however, received little attention [7,14,25]. *Mastacembelus pancalus* is an economically important as it is a very popular edible fish freshwater fish in India and very little information is available on the effects of lead, particularly through sub-lethal exposure on its ovarian maturation. The present work, therefore, investigated the chronic toxicological action of lead in the ovary of a freshwater omnivorous fish, *Mastacembelus pancalus* as any anomalies in histology of gonads in fish which reveals that can affect their progeny. In the present research, *Mastacembelus pancalus* was selected due to its adoptation in the particular niche of a polluted aquatic environment.

2. Materials and Methods

The study was carried out at laboratory condition. The live fish aliquots *Mastacembelus pancalus* were collected from local fishermen of uniform size in order to avoid the possible error due to size differences and brought to the laboratory in metallic pot containing the pond water.

The test fish M. *pancalus* of 19.33 ± 1.2 cm long and an average weight of 26.17 ±6 g. were acclimatized to ambient laboratory aquaria of 40 litres for 15 days. Prior to commencing any toxicological test the fish were disinfected regularly with dilute $KMnO_4$ (0.1%) and to keep the aquaria free from fungal growth and any other infection antibiotics were also used for a 1-1½ weeks within this period. The aliquots were fed with *Tubifex* sp. once daily. The aerator bubblers were also provided in all aquaria to avoid any oxygen depletion, and also combat with the possible alteration of carbon dioxide tension. The temperature of water of each aquarium was maintained at room temperature throughout the period of acclimatization. After 15 days the each fish were divided into two sets, containing 6 specimens each and were transferred to separate glass aquaria of 40 litre capacity, one for control and other one set for treatment. In the present experiment the fishes were exposed to lead nitrate for 30 days at a dose of LC_{20} value 18.5 mg/l (in respect to 925ppm 96-h LC_{50} value).

Fish of each aquarium were thoroughly examined and maintained carefully.

2.1 Parameter Studied:

Total weight and standard as well as total length were recorded for each individual at the beginning of the experiment and the prior mentioned data were also documented for controlled and test specimens after end of experimentation of 30 days. Growth parameters as Growth rate (GR) and Condition factor (CF) were considered in parallel.

GR and CF of fishes also were calculated during the experimentation by the following formula:

GR = (Final weight - Initial weight)/Time (days)

CF= [Total weight of fish/(length of fish)^3]× 100

Fish body weight and weight of gonads give the gonado somatic Index (GSI) and fecundity is the number of eggs laid in a single in one season by the species. In order to assess the population stock of any species the accurate estimation of the fecundity is essential. GSI and fecundity were calculated during the study by the following formula:

GSI = (Weight of gonad/ Weight of fish) X 100 [6, 10].

Fecundity (F) = (Total wt. of ovary X No.of mature ovum in sub-sample)/ Wt. of sub-sample [6,10].

Relative fecundity (RF) = (absolute fecundity / body weight) [6, 10].

For histological observations paraffin sections were made for light microscopic study through Delafield's Haematoxylin and Eosin stain. For the histological study the desired tissues of the selected gonad of the experimental fish were collected and fixed and paraffin sections of 4-5 micra were made for ultramicroscopic observations.

2.2 Physico Chemical Analysis of water:

Physicochemical analysis of aquarium water was also included as there are very essential for any toxicity tests.

The statistical analysis was made by using the Microsoft Excel program.

3. Results and Discussion

Fig. 1 ***Mastacembelus pancalus***

The aquatic organisms as fishes are particularly very much susceptible to pollutants in almost all stages of their life cycle. Fish are often used as sentinel organisms for eco-toxicological studies because they play a number of roles in the trophic structure, accumulate toxic substances and even respond to low concentration of the toxicants. Therefore, the use of fish reproductive parameters as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems. Exposure to contaminants in the environment has been associated with a number of abnormalities, such as decreased fecundity, GSI. It is also reported that heavy metals cause reproductive disability [21].

3.1 Growth parameter:

Table 1: Fundamental growth parameters in controlled and treated condition:

Parameter	Controlled condition	Treated condition
Weight (gm)	29.00 ±2.76	18.50±1.70
Total length (cm)	21.85±1.56	21.85±1.55
Standard length (cm)	19.51±1.20	19.41±1.26

Table 2 (a) & (b). Significant growth parameters

(a)

Parameter	After 30 Days of Treatment Condition
Growth rate (GR)	- 0.35

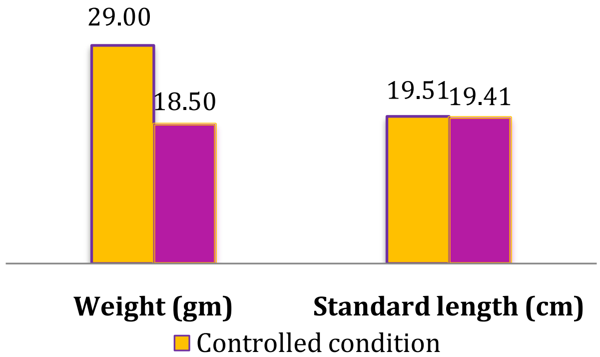

Chart. 1 Graphical representation of significant change in growth parameters after 30 days of treated condition (n=6 for each set)

(b)

Parameter	Before starting the experiment	Controlled Condition	Treated Condition
Condition Factor (CF)	0.28	0.39	0.25

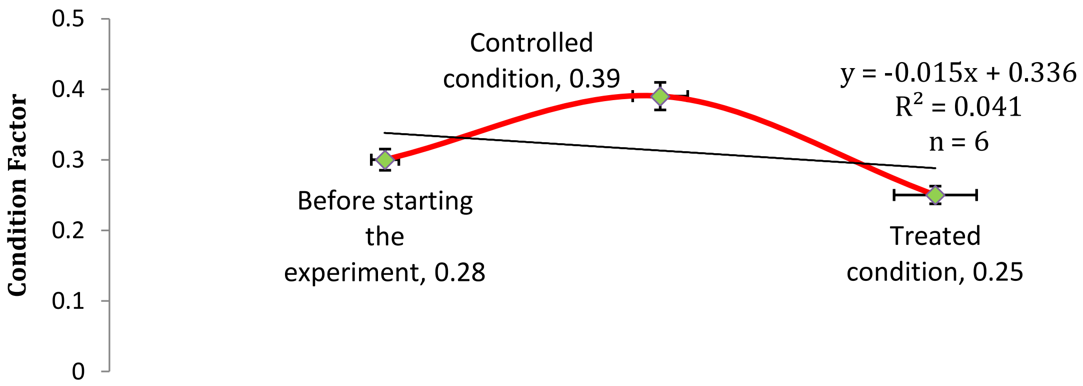

Chart. 2 Graphical representation of Condition Factor (K) of experimental aliquots prior to the experimentation and 30 days after controlled and treatment condition of experimentation

Length-weight relationship is an essential biological parameter needed to appreciate the suitability of the environment for any fish species. That is why many fishery biological studies give an importance to it. As fish grow in length, they increase in weight. The linear equations of morphometric relationship between weight and length not always give ideal statistics. The present study indicates that under long term, heavy metal stress the study samples have loosened their weights.

In Table 2(b) Fulton's condition factors (K) were recorded just prior to the experimentation and 30 days after controlled and treatment condition of experimentation.

Lead exposure induced a gradual decline in the condition factor of the exposed group relative to the controls, although, all fish continued to gain weight during the experiment and the changes can be expressed as $y = -0.015x + 0.336$, $R^2 = 0.041$. Here it is important to emphasize that the 'K' values of selected specimens that were recorded just prior to the experimentation were also not satisfactorily good and that was 0.28. Possible reasons for that poor condition factor may be the lack of food/prey, increased competition, habitat unsuitability or an infection.

The 'K' values growth rate both were declined after 30 days methodical intoxication by $PbNO_3$. The negative growth rate value (-0.35) indicates obvious retardation in development [Table 2 (a)].

The CF and GR of exposed fish were significantly different from control, and there was a significant inverse relationship between weight gains and in general fish that are heavier than the standard weight for their length are considered healthier, having more energy reserves for normal activities, growth and reproduction.

3.2 Reproduction:

Table 3: Analysis and observations of relations between length, weight and other gonadal parameters of female *Mastacembelus pancalus* in controlled condition after 30 days of experiment

No of samples	Std. L (cm)	Wt .of fish (g)	Length of ovary (cm)	Wt. of ovary (g)	No of ova	No of ova/ gm ovary wt.
6	19.51	29.00	1.98	1.84	352	191.30
	No of ova /gm wt. of body	**No of ova/ fish length (cm)**	**No of ova/ ovary length (cm)**	**Absolute Fecundity**	**Relative Fecundity**	**GSI (%)**
	12.13	18.04	177.77	350.72	12.093	6.34

Table 4: Analysis and observations of relations between length, weight and other gonadal parameters of female *Mastacembelus pancalus* in treated condition after 30 days of experiment

No of samples	Std. L (cm)	Wt .of fish (g)	Length of ovary (cm)	Wt. of ovary (g)	No of ova	No of ova/ gm ovary wt.
6	19.41	18.50	0.651	0.605	90	141.76
	No of ova /gm wt. of body	**No of ova/ fish length (cm)**	**No of ova/ ovary length (cm)**	**Absolute Fecundity**	**Relative Fecundity**	**GSI (%)**
	4.8	4.63	138.24	89.72	4.85	3.27

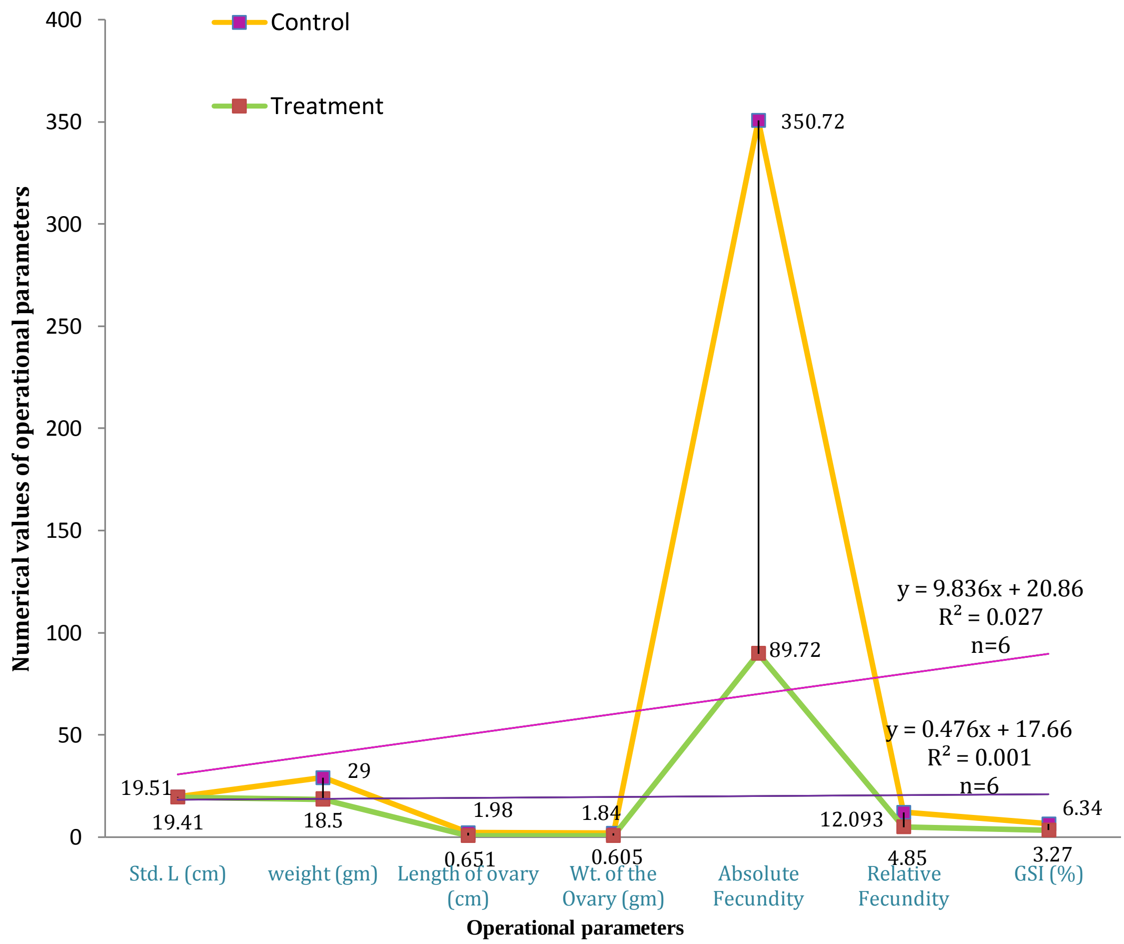

Chart. 3 Graphical representation of significant relation between fish and ovary length to Absolute fecundity after 30 days of controlled and treated condition (Ref. Table.3 & 4)

Fecundity is one of the important parameter of fish biology and key for the assessment of wild stock population of fish. Similarly, it is an important tool to determine the egg production, spawning potential and for the successive management of cultivation. In the present study, it was observed that the absolute, relative fecundity and GSI was reduced by 25.58%, 40.02% and 51.57%. The statistical co-relationship of fecundity with variables as standard length, body weight, length of ovary, weight of ovary of fish, GSI was also estimated both in controlled and treated condition. The relationship between fecundity and standard length, body weight, length of ovary, weight of ovary of fish, GSI were found linear in both controlled and treated condition and expressed as y = 9.836 (x) (R^2 = 0.027) with no intoxication and, y = 0.476 (x) + 17.66 (R^2 = 0.001) with lead intoxication for 30 days. The fecundity was found highly correlated with the observed parameters. Therefore, fish exposed to lead nitrate showed changes in reproductive behaviour, but these effects were not less prominent. Worth mentioning variations in the absolute and relative fecundity were registered.

Histology:

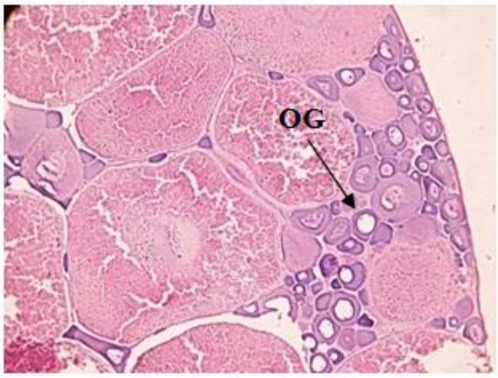

Fig. 2 The histological view of ovarian structure of M. *pancalus* under controlled condition (10X)

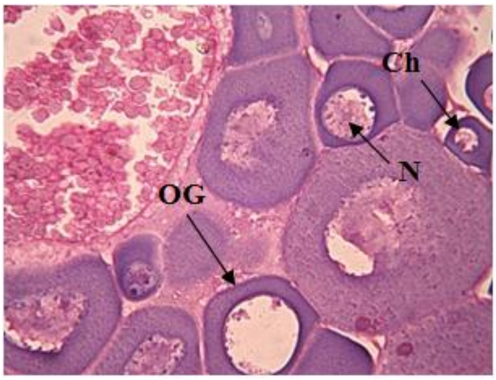

Fig. 3 The histological view of ovarian structure of M. *pancalus* under controlled condition (100X)

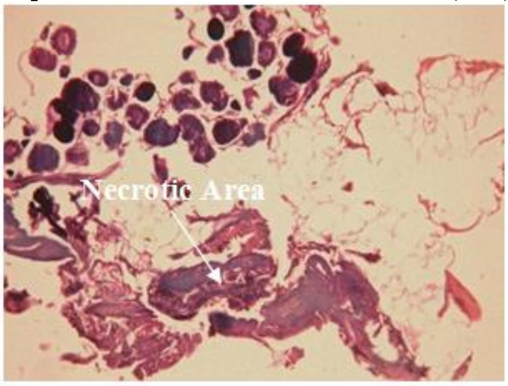

Fig. 4 The histological view of ovarian structure of M. *pancalus* under treated condition (10X)

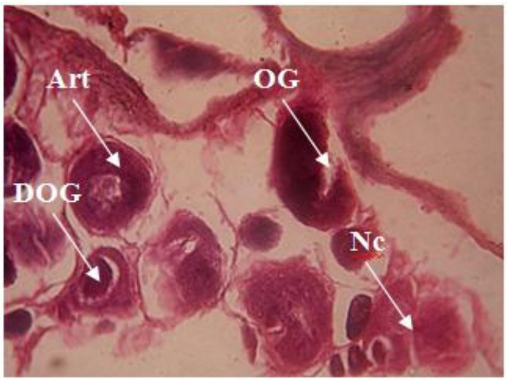

Fig. 5 The histological view of ovarian structure of M. *pancalus* under treated condition (100X)

The normal ovary of *Mastacembelus pancalus* was mainly composed of clusters of oogonia (OG) thin indistinct peripheral zone along with these histological facet showed chromatin nucleolar oocytes (Ch). From the investigations on GSI and histological analysis of lead (Pb) on the ovary of freshwater fish *Mastacembelus pancalus* revealed that lead induced a serious effect on fertility and productivity of the experimental freshwater fish. Considerable degrees of alterations in the histology of ovary were observed such as oocytic abrasion (OA), artesia (Art), denaturation of yolk globules (DYG) *etc*. Increased artretic and necrotic structures of oocytes (Nc) were indicative a degenerative effect of toxicity of lead. Distorted appearance of oocytes is one of the most noteworthy features for concluding loss of reproductive potential and reproductive defect in fishes that can limit the numerical size of the succeeding generation.

Ebrahimi and Taherianfard (2011) studied the effect of heavy metal on the reproductive system of cyprinid fish, and confirmed that exposure of heavy metals decrease the estrogen and androgen hormone secretion in fish i.e., it disrupt the quality and quantity of sperm and ova production [18]. The toxic effects usually associated with heavy metals exposure are carcinogenicity, immune-suppression and impaired reproduction [1,21,22] and the present experimental data, histological observations are providing evidences that the growth rate along with fecundity and GSI of

Mastacembelus pancalus were noticeably declined after 30 days exposure of lead nitrate.

Hence, lead exposure and growth rate, GSI and were showed inverse relation. In chronic exposure to lead is critically perilous and leads to a complete loss of normal configuration of ovary, necrosis, elongated ovarian follicles, and fragmented ovum with malformation. No fish mortality was recorded in all treatments throughout the experimental period.

Table 6: Water chemistry of treatment aquarium during the experimentation period

Parameter	Instrument/ Method Used	Unit	Avarage	±SD	CV
Temperature	Thermometer	°C	24.6	1.6	0.065773
pH	PCSTestr-35 Multi-parameter	-	6.8	1.4	0.033322
TDS	PCSTestr-35 Multi-parameter	ppm	387.3	22.1	0.029220
Salinity	PCSTestr-35 Multi-parameter	ppm	209.9	10.5	0.020953
Conductivity	PCSTestr-35 Multi-parameter	S	449.3	27.1	0.023454
DO	Iodometric method	(mg/L)	4.9	0.5	0.10204
Hardness	EDTA method	mg $CaCO_3$/l	210.0	3.21	0.015285

According to Walker *et al.* (1996), the values obtained by toxicity testing are dependent on the conditions under which tests were performed, so, the interpretation of physicochemical water parameters were very crucial aspect of the total investigation [31].

Fundamental physicochemical parameters as temperature, pH, TDS, salinity, conductivity were measured and these were within the limits acceptable for fish culture. Considerable importance was given in recording of hardness of water as high water hardness reduces lead toxicity to fish due to a significant inorganic complexation process that controls lead availability to fish [12].

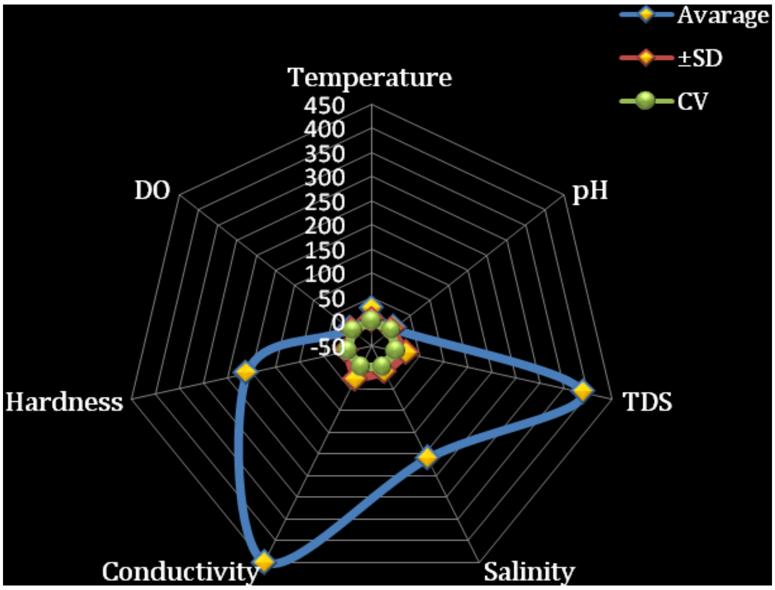

Chart No. 1 Water parameters of treatment aquarium during the experimentation period

4. Conclusion

In conclusion, this study showed that heavy metal contamination of aquatic system not only directly affects the fish health, but it can also decrease the fish maturation, spawning and finally production. This study proves that *Mastacembelus pancalus* can be used as aquatic model and appeared to have the greatest potential for future applications in developmental toxicity. It is also clear that heavy metals like lead can cause adverse effects on the reproductive system of *Mastacembelus pancalus*.

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