

A STUDY ON HISTOPATHOLOGICAL CHANGES IN THE GONADS OF A FRESHWATER TELEOST FISH, *A. MOLA* EXPOSED TO THE HEAVY METALS

Shelke Abhay D.

P.G. and Research Dept. of Zoology,
B.P. Arts, SMA. Science and KKC Commerce College, Chalisgaon,
Dist. Jalgaon. State- Maharashtra, Pin 424101.

Abstract

The industrial effluents once released in to the water give way to a chain of happening and the resultant effect is felt on humans, aquatic life and on the riverine ecosystem. Polluted waters not only pose a threat to the aquatic species inhabiting in them but also to humans who often come in contact with the water either directly or indirectly by consuming fish. Every chemical released into ecosystem has the potential of getting in to the human diet food chain and for most people; diet is the primary means of exposure to environmental contaminants. In the present investigation *A. mola*. were exposed to chronic treatment up to 21 days (LC_{50/10} values of 96 hours) of heavy metals i.e. Mercuric chloride (0.04583 ppm.), Arsenic trioxide (0.2911 ppm.) and Cadmium chloride (0.6279 ppm.). The treated *A. mola* showed profound reduction in the size of the gonads and extensive destruction of the germinal elements in both the sexes. The mature stages were very largely destroyed while the earlier stages, particularly in the testis, underwent extensive atrophy at the chronic concentrations. An identical arrest of spermatogenesis was also observed, Severe damage in comparison in 21 days exposure indicate that damage in the gonads was indirectly related to dose and duration of treatment.

Key words: Heavy metals, histopathology, Gonads changes, *Amblypharyngodon mola*.

INTRODUCTION

Histopathology provides valuable information concerning changes in the cellular as well as sub-cellular structures of an organ or tissue much earlier than the external manifestations (Auffret, 1988; Livingstone and Pipe, 1992; Au, 2004). Heavy metals have high affinity to sulfhydryl groups and disulfide bonds which cause damage to secondary structure of proteins and alter the enzymatic activities (Siediecka and Krupa, 2002). The mechanism of action lies in their ability to form strong bonds with bases and phosphates of nucleic acids. They compete with other divalent cations and replace them in their physiological roles; further action of heavy metals is due to generation of reactive oxygen species and induction of oxidative stress (Tabaldi *et al.*, 2007).

The effects of organophosphorus and organochlorine insecticides on ovarian growth, steroidogenesis, ovulation and other aspects of reproduction in various species of teleosts have been investigated (Mani and Saxena, 1985, Haider and Inbaraj, 1988). Wester (1991) found 13-HCH-induced excessive vitellogenesis and yolk formation on premature oocytes in juvenile guppies. Wester *et.al.*, (1988) observed the effects of hormonal imbalances and stress on the ovaries a testies as a result of exposure to methyl bromide and sodium bromide.

Murugesan and Haniffa (1992) observed histopathological and histochemical changes in oocyte of the air breathing fish, *Heteropneustes fossilis* (Bloch.) after exposure to textile mill effluent. They observed complete Karyolysis, disappearance of the chromatin reticulum of the nucleoli and vacuolation of the cytoplasm. Sukumar and Karpagaganapathy (1992) found few mature oocyte, most of which had become atretic in the ovaries of fish, exposed to sublethal concentrations of carbofuran. Mukkapati, S. (1974), studied the effect of cadmium chloride on the testies of mangoose, *Herpestes auropunctatus* (Hodgson). Sangalag, G. *et.al* 1974), studied the effect of sublethal cadmium on maturation and testosterone and II - ketotestosterone production in vivo. in brook trout.

The fresh water teleost fish, *Amblypharyngodon mola*, is an edible fish, and is economically important as the protein food resources of the rural areas of North Maharashtra. Hence the present investigation

was undertaken to study the histopathological changes occurring in the gonads after exposure to some selected heavy metals, Mercuric chloride, Arsenic trioxide and Cadmium chloride.

MATERIAL AND METHODS

Live specimens of fish *A. mola* were collected from Gadad and Girna river dams near Chalisgaon city Dist. Jalgaon, stored in 50 liter glass aquarium containing dechlorinated water with aeration system, during experiment the fishes fed with leafy green cabbage. The physiological properties of water as follows. PH 7.1 ± 0.25 , Air temp. $32 \pm 2.0^\circ\text{C}$, water temp. 26.5 ± 2.0 , Dissolved oxygen 4.36 ± 0.94 , Bicarbonates 68 ± 10 , Total alkalinity $74 \pm 86 \pm 10.50$, Acidity 4.36 ± 2.57 (expect PH and temp.) all parameters are expressed in mg/l. Animals were acclimatized to laboratory condition for 10 days. During the experiment healthy fishes of uniform size and weight were selected for the treatment. Fishes were divided in to two groups; one group was maintained as control while the remaining one group was separately exposed to the chronic treatment.

Each groups contain 10 fishes, were exposed to Mercuric chloride (HgCl_2), Arsenic trioxide (As_2O_3) and cadmium chloride (CdCl_2) to different concentration separately for studying LC_{50} , in aquaria containing 60 litter of water. During this period the medium was changed 24 hourly. The LC_{50} values for different intervals 24, 48, 72 and 96h were calculated by Probit analysis method (Finney, D. J.M. 1971).

In the chronic treatments the experimental groups of fishes were exposed to 0.04583 ppm. Mercuric chloride, 0.2911 ppm. Arsenic trioxide and (0.2911 ppm.) Cadmium chloride ($\text{LC}_{50/10}$ values of 96 hours) up to 21 days. At the end of 7, 14 and 21 days of treatment, the control and experimental fishes were scarified by decapitation method, gonads were dissected out from the fish and preserved overnight in Bouins and 10% neutral buffered formalin, following paraffin embedding, section were cut out at 6μ and were stained with haematoxylin and counter stained with eosin.

OBSERVATIONS AND RESULTS

Control Ovary (photo plate - 1)

The organ appeared more or less circular in outline, in transverse section. Most of the section was packed by several large mature oocytes each containing an irregular germinal vesicle surrounded by a lot of carminophilic yolky matter and outside of which a distinct band of aniline blue positive reticulate cytoplasm could be seen. Each mature oocyte was bound by a thick zona radiata. Between them oocytes aggregates of the younger stages and a few follicles undergoing atresia were observed. Most of the younger elements showed a homogenous basophilic cytoplasm (Fig.-1).

Treatment of mercuric chloride (photo plate - 1)

As compared to control ovary of freshwater teleost fish, *A. mola* showed the most striking effect in the ovary of the treated animals was a total disappearance of the mature and maturing yolky oocytes, because of which the ovarian diameter was reduced substantially in treated animals observed after 14 days exposure (Fig.-2).

Total disappearance of the mature and maturing yolky oocyte. The yolk material reduced in the mature oocyte, reduction in follicular cells was observed after 21 days exposure (Fig.-3).

Treatment of Arsenic trioxide (photo plate -1)

As compared to control ovary of freshwater teleost fish, *A. mola* showed inhibition of growth of oocyte, especially the atretic follicles was obviously observed after 21 days exposure (Fig.-4).

Treatment of Cadmium Chloride (photo plate - 2)

As compared to control ovary of freshwater teleost fish *A. mola* showed large amount of yolky matter disappeared, follicular cells disappeared, destruction in nucleus, nucleolus and follicular epithelium observed after 7 days exposure (Fig.-6)

Yolky matter becomes disappeared, in some mature the oocytes. Overall destruction in the structure, follicular cells disappeared, follicular epithelium destructed, observed after 14 days exposure (Fig.-8).

TESTIS

Control testis (photo plate - 3)

Testis of control fish were composed of lobules showing active spermatogenesis. Sperm nests were found in majority of the lobules. Spermatids were the predominant, followed by dividing spermatocytes and secondary spermatogonia. Majority of the spermatogenic nests were in active state

of division. The peripheral part of lobules had a normal healthy look. There were no intercellular fissures. Sertoli cells were normal with granular cytoplasm containing a large nucleus with a peripheral nucleolus. In a number of places the inter-lobular septa were marked by the presence of small and large aggregations of polygonal Leydig cells. These possessed large carminophilic nuclei containing granular chromatin and two or more nucleoli. Their cytoplasm was faintly aniline blue positive and often contained small vacuoles. Usually small capillaries were associated with these cell groups. (Fig.- 9).

Treatment of mercuric chloride (photo plate 3)

As compared to control testis of freshwater teleost fish, *A. mola* showed a complete arrest of spermatogenetic activity at the secondary spermatocyte level. This stage constituted the predominant form in all lobules.

There was complete lack of division in this stage and there were no post-spermatocyte stage. Some earlier stages like secondary spermatogonia were noticed but these also were mostly inactive, observed after 7 days exposure (Fig.- 10).

Treatment of Arsenic trioxide (photo plate -3)

As compared to control testis of freshwater teleost fish *A. mola*, observed that the premeiotic germ cells were significantly more numerous, while the post meiotic stages were destroyed. Although the pre-meiotic stages are numerically more, most of these cells exhibited a high degree of nuclear degeneration, cytoplasmic clumping and vacuolation. The secondary spermatocytes were shrivelled and showed small pycnotic nuclei. Most of the degenerating cells showed aniline blue positive contents as against the distinctly carminophilic nuclei and practically chromophobic cytoplasm of the corresponding control cells. Further, the areas occupied by the spermatids and sperms were largely filled with amorphous masses containing some darkly stained nuclei and a few large, irregular cells, probably phagocytes, observed after 7 days exposure (Fig.-11).

The nuclei of the secondary spermatocytes were pycnotic which pointed to their gradual degeneration. The cytoplasm of Sertoli cells was vacuolated and fissures had appeared between the lobule wall and the peripheral layer of lobular cells. Intercellular fissures were also common between the cells at the periphery of the lobules. The nuclei of Sertoli cells were pycnotic, many of whom were in various stages of degeneration observed after 14 days exposure (Fig.-12).

Treatment of Cadmium chloride (Photo plate 4)

As compared to control testis of freshwater teleost fish, *A. mola* showed the lobular structure of the organ was completely lost. Similarly, the interstitial component appeared to consist of small cells containing scanty cytoplasm and crumpled pycnotic nuclei. A picture of extensive necrosis induced by cadmium thus emerges very clearly, observed 14 days exposure (Fig.-14).

Intercellular fissures were also common between the cells of the periphery of the lobules. The nuclei of Sertoli cells were pycnotic, many of whom were in various stages of degeneration; strongly basophilic granules were clustered around the nucleus of some Sertoli cells. This probably represented an early stage in degeneration. The lobular structure of the organ was completely lost observed after 21 days exposure (Fig.-16).

In the present investigation after chronic exposure of the fish to various heavy metals, Mercuric chloride, Arsenic trioxide and Cadmium chloride, severely affected the testis and ovary. The organs appeared straight, thin and nearly white in colour. In the present investigation found that damage in testis and ovary indirectly related to dose and duration of the treatment.

Photoplate No. 1

Histopathological Studies

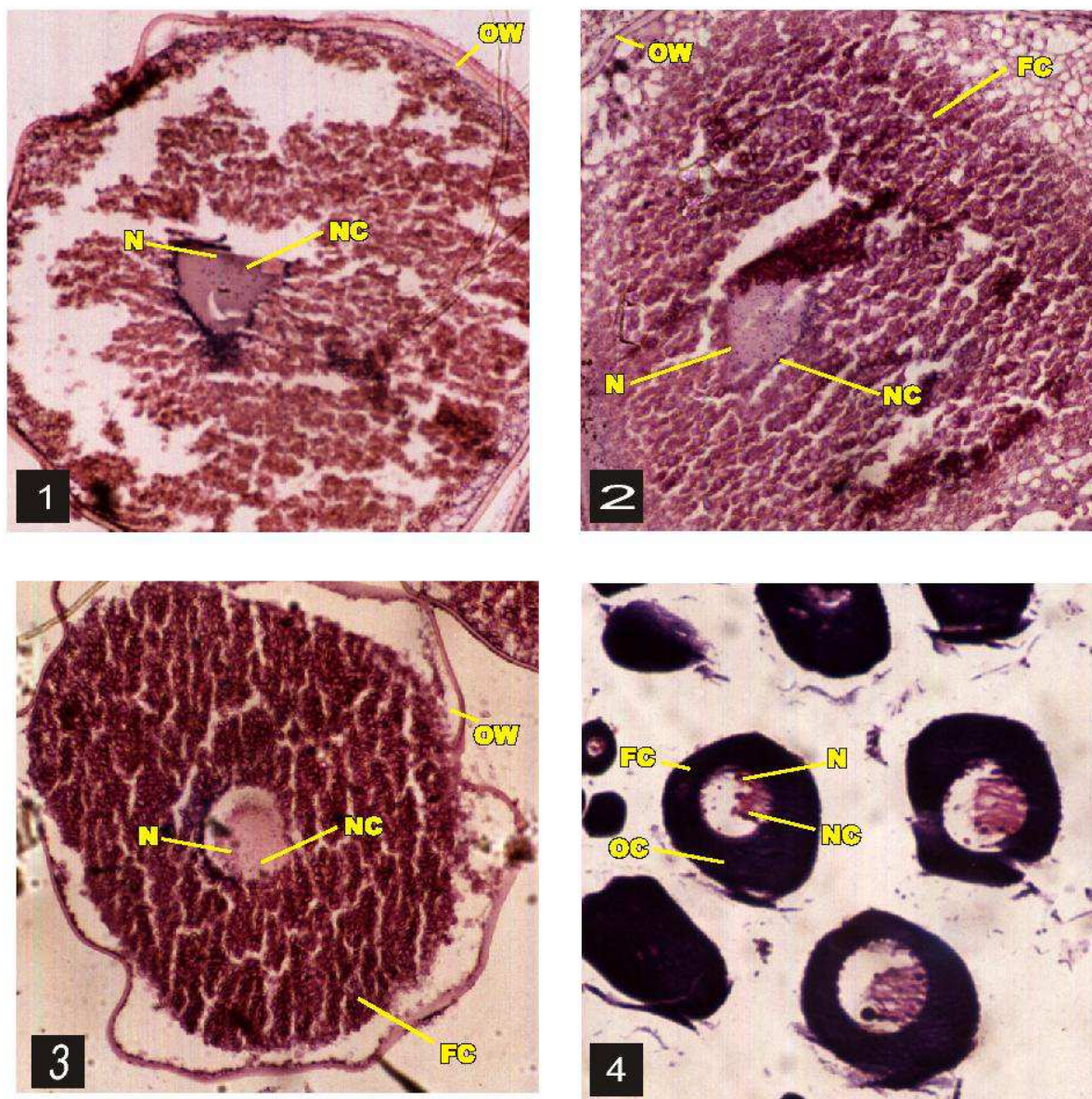


Fig. 1. :- T.S. of Ovary of A. mola from Control fish - 10 x 40

Fig. 2. :- T.S. of Ovary of A. mola exposed to Mercury Chloride for 14 days. - 10 x 40

Fig. 3. :- T.S. of Ovary of A. mola exposed to Mercury Chloride for 21 days. - 10 x 40

Fig. 4. :- T.S. of Ovary of A. mola exposed to Arsenic for 21 days. - 10 x 10

N - Nucleus; NC - Nucleolus; OC - Oocyte

FC - Follicular cell of oocyte; FE - Follicular epithelium.

Photoplate No. 2

Histopathological Studies

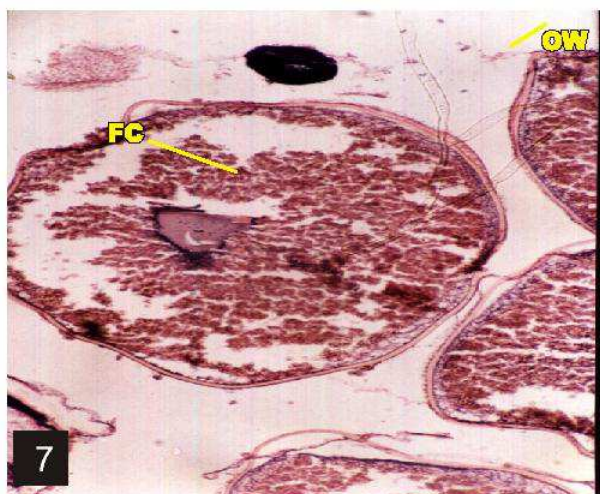
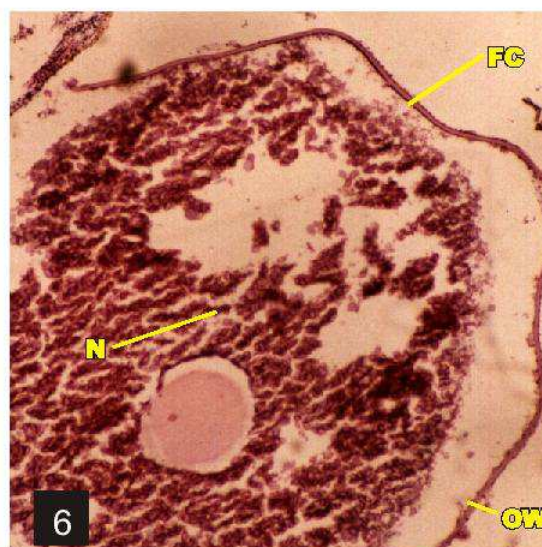
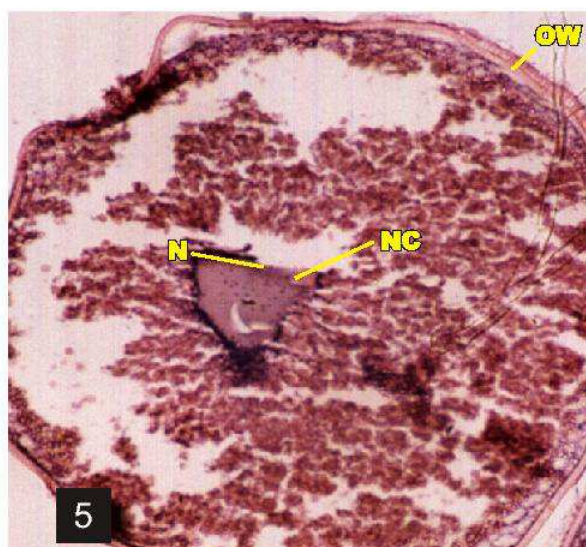


Fig. 5 . :- T.S. of Ovary of A. mola from Control fish - 10 x 40

Fig. 6 . :- T.S. of Ovary of A. mola exposed to Cadmium Chloride for 7 days. - 10 x 40

Fig. 7 . :- T.S. of Ovary of A. Mola exposed to from Control fish - 10 x 40

Fig. 8 . :- T.S. of Ovary of A. mola exposed to Cadmium Chloride for 14 days. - 10 x 40

N - nucleus; NC - nucleolus; OC - Oocyte

FC - follicular cell of oocyte; FE - follicular epithelium.

Photoplate No. 3

Histopathological Studies

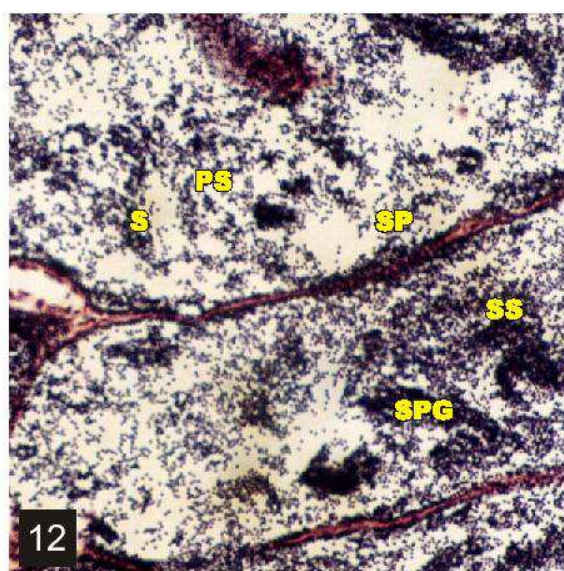
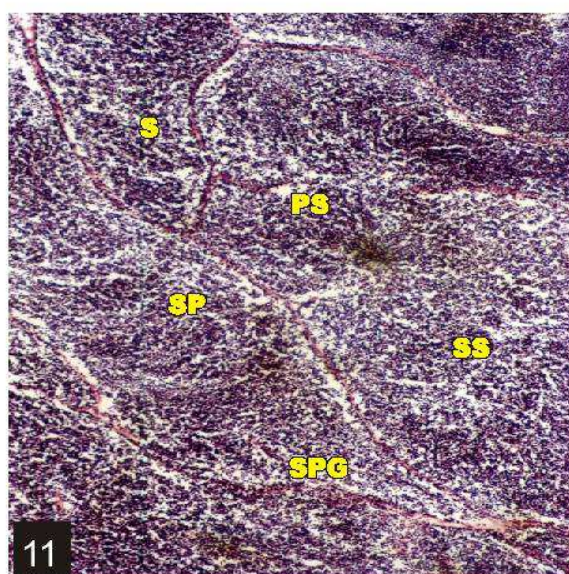
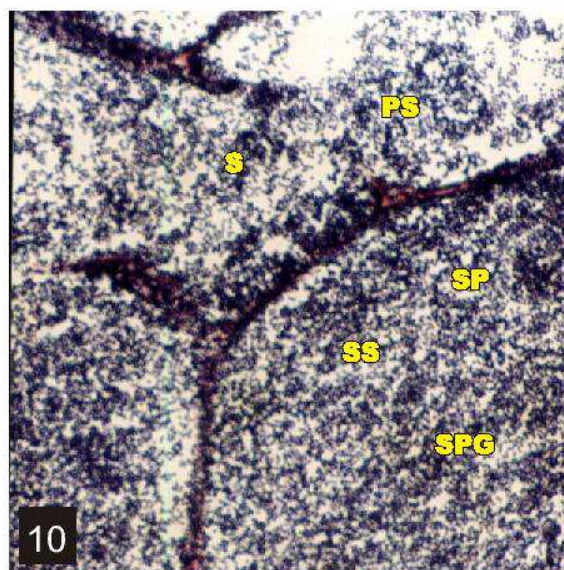
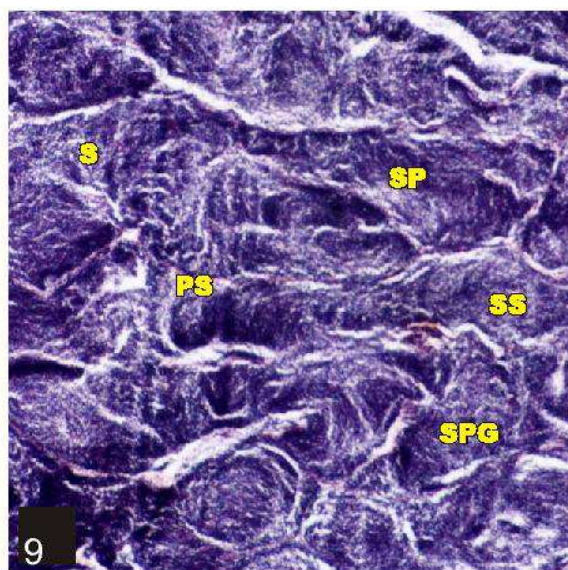


Fig. 9. :- T.S. of Testis of A. mola from Control fish - 10 x 40

Fig. 10. :- T.S. Of Testis of A. mola exposed to Mercury Chloride for 7 days. - 10 x 40

Fig. 11. :- T.S. Of Testis of A. mola exposed to Arsenic Trioxide for 7 days. - 10 x 40

Fig. 12. :- T.S. Of Testis of A. mola exposed to Arsenic Trioxide for 14 days. - 10 x 40

PS - Primary Spermatocytes ; S - Spermatozoa; SP - Spermatids

SPG - Spermatogonia; SS - Secondary Spermatocytes.

Photoplate No. 4

Histopathological Studies

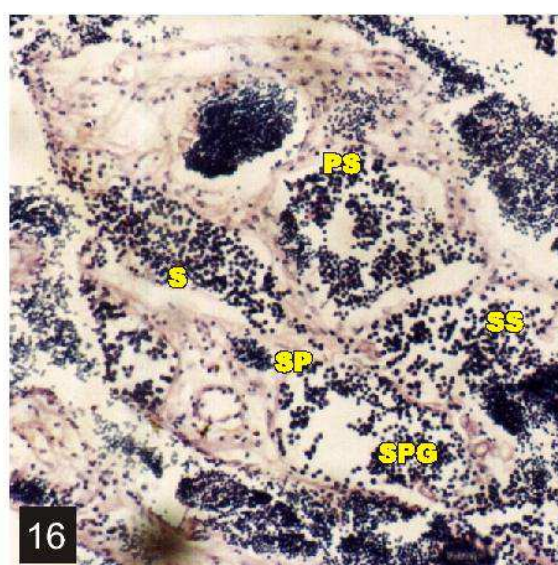
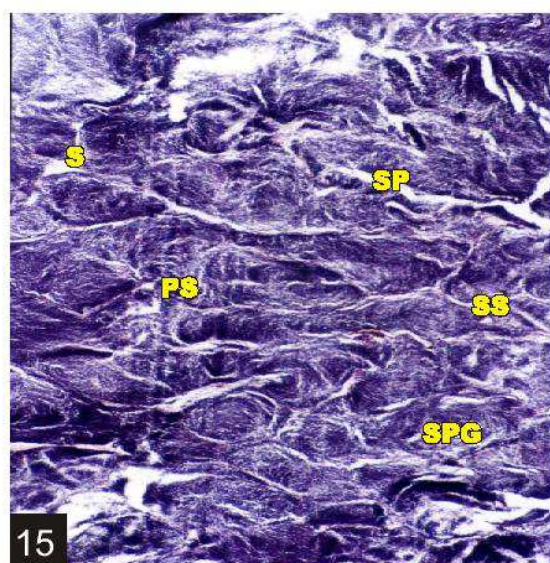
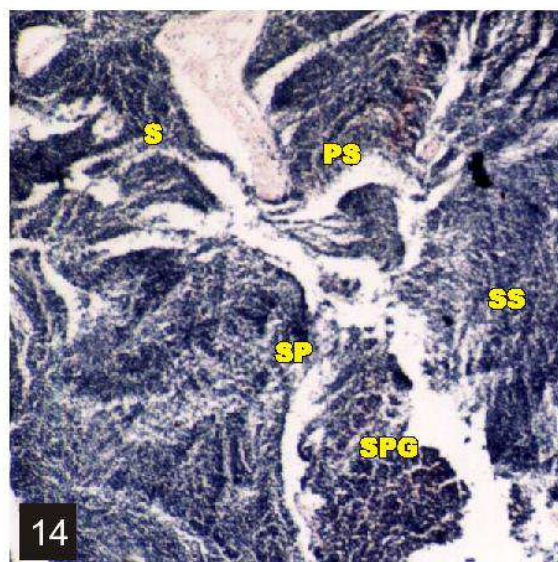
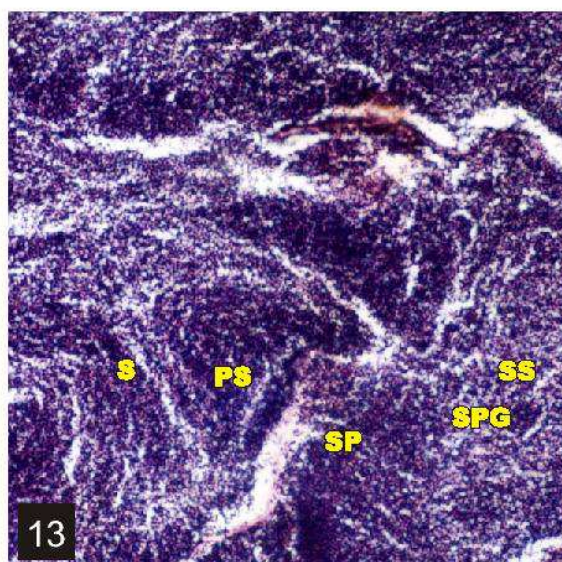


Fig.13. :- T.S. of Testis of A. mola from Control fish - 10 x 10

Fig.14. :- T.S. Of Testis of A. mola exposed to Cadmium Chloride for 14 days. - 10 x 10

Fig. 15. :- T.S. of Testis of A. mola from Control fish - 10 x 40

Fig.16 . :- T.S. Of Testis of A. mola exposed to Cadmium Chloride for 21 days. - 10 x 40

PS - Primary Spermatocytes ; S - Spermatozoa; SP - Spermatids
SPG - Spermatogonia; SS - Secondary Spermatocytes.

DISCUSSION

The environmental biological findings and in particular of the data of Hughes *et al.* (1979) are quite obvious. It is only rarely that in nature aquatic organisms such as fishes may transiently be exposed to high levels of heavy metal pollutants. On an overall basis, concentrations of toxicants producing chronic effects would normally be of limited value from the aspects of reproductive ability and environmental management. It's harmful changes more subtle than extremely premature death or gross trauma that are of real concern.

Administration of sublethal dose of cadmium salts leads to varying degrees of gonadal dysfunction in a number of vertebrates (Ramaswami, 1981). Most of these studies show that cadmium impaired the testicular blood supply and caused widespread destruction of the spermatogenic cells and affected development of ova. Bird testes however appear to be largely unaffected by cadmium (Lofts and Murton, 1967).

The endocrine component of the testies revealed that its constituent leading cells were markedly injured as evident from the pycnotic nuclei, cytoplasmic vacuolation and cell shrinkage. Pycnosis of leydig cell nuclei was also noted in *C. batrachus* by Ahsan and Ahsan (1974) and these cells did not regenerate in the damaged testies of *S. fontinalis* (Sangalang and O' Halloran, 1972). Moreover, the sertoli cells were also degenerated in *C. batrachus* (Ahsan and Ahsan, 1974). All these findings strongly suggest that the androgenic activity was suppressed in the heavy metal intoxicated testies.

An identical arrest of spermatogenesis was also reported by Ahsan and Ahsan (1974) in cadmium injected *C. batrachus*. However, no sign of haemorrhagic necrosis of the testis was observed by Sangalang and O. Halloran (1972) in the brook trout was evident in the in *C. batrachus*.

In the present investigation on *A. mola* revealed a profound reduction in the size of the gonads and extensive destruction of the germinal elements in both the sexes following chronic exposure to different concentrations of heavy metals. The mature stages were very largely destroyed while the earlier stages, particularly in the testis, underwent extensive atrophy at the chronic concentrations. In the female *A. mola*, the marked increase in the number of primary oocytes suggested that heavy metal blocked the transformation of these cells in to maturational stages. The numerical increase of the atretic follicles further indicated degeneration of the maturing follicles.

In the present investigation, histopathologically Mercuric chloride, Arsenic trioxide and cadmium chloride treated fish; *A. mola* gonads shown severe damage up to 21 days exposure indicate that damage in the gonads is indirectly related to dose and duration of treatment.

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