HISTOPATHOLOGICAL ALTERATIONS IN VARIOUS TISSUES OF THE MUDSKIPPER, *BOLEOPHTHALMUS DUSSUMIERI* OF MAHI ESTUARY.

CHAPTER - V

HISTOPATHOLOGICAL ALTERATIONS IN VARIOUS TISSUES OF THE MUD-SKIPPER, BOLEOPHTHALMUS DUSSUMIERI OF MAHI ESTUARY

Previously it was reported that the effluents of the Nandesari Industrial belt from Baroda, conveyed through an effluent channel for discharge, contain alarmingly increasing levels of metal contamination and other unwarranted physico-chemical features (Chapter- I). The continuous discharge of the channel effluent into the Mahi estuary at the Gulf of Cambay has seriously affected the water quality and the faunistic characteristics of the estuary with ramification into the upstream areas (Chapters- I & IV). It was also recorded, that the mud skipper, *B.dussumieri*, more or less a common lone species at the Mahi estuary, has high levels of metal contents in its tissues (Chapter- IV). The high metal content of the estuarine water caused due to discharge of industrial effluent, could be a major factor for the very obvious shrinkage of the estuarine fauna. Though many of the metals are essential for plant and animal life, excess above the necessary optimum trace level, proves to be dangerous, often resulting in toxic manifestations. Amongst the aquatic fauna, fishes are one of the sensitive species towards aquatic pollutants. The toxic manifestations towards metals are greatly varied and, can affect a number of functions like respiration, osmoregulation, reproduction, development and neurotransmission and, damage to sensory membranes and other vital organs, like gills, liver, brain, gonads and kidney.

The degree and extent of toxic effects could also depend on the type and species of organism, the stage of life (larva, juvenile or adult), sex or, sexual stage of individuals, nutritional status and health, seasonal changes in physiological state and, degree of acclimation to natural environmental conditions or, to the toxic metals (Rand and Petrocelli, 1985). Even abiotic conditions can act as modifying factors which include temperature, pH, dissolved oxygen content, salinity or hardness and fluctuations of these conditions as well as suspended materials (organics and inorganics), dissolved salt or nutrients and their relative proportion, dissolved carbon dioxide and other gases, intensity of light and photoperiod, water movements and velocities, binding or chelating actions of substances in the water and, presence of other toxicants (Sprague, 1985). Most of the laboratory studies on metal toxicity, generate acute toxicity data using a few single species laboratory models and a single metal. Such studies overlook special interactions and thereby limit predictability when an attempt is made to directly apply results of single species test to the analysis of natural communities. There are also complications due to the difference in sensitivity of each species to metals. The results of acute toxicity test also usually do not provide substantive information about the sublethal or cumulative effects of a test material. Chronic toxicity studies are more preferable, as chronic effects occur usually as a consequence of repeated or long term exposures. This is more relevant as, there may be a relatively long latency period at low metal concentration. Chronic effects may be lethal or sub lethal; a typical lethal effect is, failure to produce a viable offspring and, the most common sublethal effects are behavioural, physiological, biochemical and histological ones. In the laboratory acute toxicity test, most of these sublethal effects may go unnoticed. Sublethal effects on fish tissue can be studied by various parameters, such as, changes of biological characteristics (like the growth rate and pattern, feeding, maturation, capability of fertilization and development of eggs, survival of fry etc.), pathophysiological alterations and, pathomorphological changes. Chronic effects are catagorised as immediate or delayed, reversible or irreversible and, local or systemic (Leland and Kuvabara, 1985). Above all these, another major drawback of laboratory toxicity studies, is focussing on a single metal, the results of which are often unsuitable for extrapolation for natural conditions as, the natural aquatic system harbours mixture of metals in different concentrations and, in such situations, there could be synergistic, additive or antagonistic effects (Rand and Petrocelli, 1985). Many such effect have been studied on aquatic plants and animals using different concentrations of metals and often resulting in varied effects (Kelly, 1988, Kabata Pendias and Pendias 1984, Wong and Beaver 1981, Leland and Kuvabara 1985). In this scenario, a pathological study of some tissues of the mud skipper, *B. dussumieri* in the Mahi estuary would be relevant, as, various metals in different concentrations are present and thereby provides the very best natural situation possible.

MATERIALS AND METHODS

Two sets of fishes, polluted and non-polluted were procured. The polluted group of fishes was captured from the estuarine area around the "J" point and, the non-polluted group from Hansot near Bharuch and also from Dumas near Surat. The fishes were brought alive into the laboratory and sacrificed. Gills, intestine, liver, muscle and ovary were excised and fixed in 10% formalin or Bouin's fluid. They were then processed routinely and embedded in paraffin wax. Sections of five micron thickness were cut on a microtome and stained with haematoxyline-eosin for histological observations.

RESULTS

LIVER

The liver of fishes from the non-polluted areas showed well formed hepatic parenchymal cells, with about 6-8 hepatic cells arranged in rosette to form a cord like structure with channels of bile canaliculi etc. Each hepatic cell appeared more or less hexagonal with a prominent centrally located nucleus. There were blood sinusoids between the hepatic cells and a clear division into lobules as characteristic of mammalian liver was not evident. (Fig- 1a)

The liver of fishes from the polluted area showed a complete fatty liver condition. Normal looking hepatic cells were not visible in any part of the liver. The blood channels and sinusoids appeared to be engorged with RBC. Even amongst the heavily fatty infiltrated hepatic cells, lumps of RBC in a nodular form were visible. (Figs- 1b-d)

GILLS

The gills of control fishes showed normal appearing primary lamellae and secondary lamellae. The epithelia of the secondary lamellae were of normal size (Figs- 2a,c). The gills of experimental fishes showed swollen secondary lamellae with hypertrophied lamellar cells. The vascular

PLATE I : Photomicrographs of sections of liver.

Figure 1a: Section of liver from control fish, showing closely packed well formed hepatocytes with prominent nuclei (X 320)

Figure 1b: Section of the liver from fish from Mahi estuary showing blood channels engroged with RBCS, erythropoitic nodules and a fatty liver state (X 160)

Figure 1c: Englarged version of 1b (X 690), note RBC filled blood channel and fat laden hepatocytes.

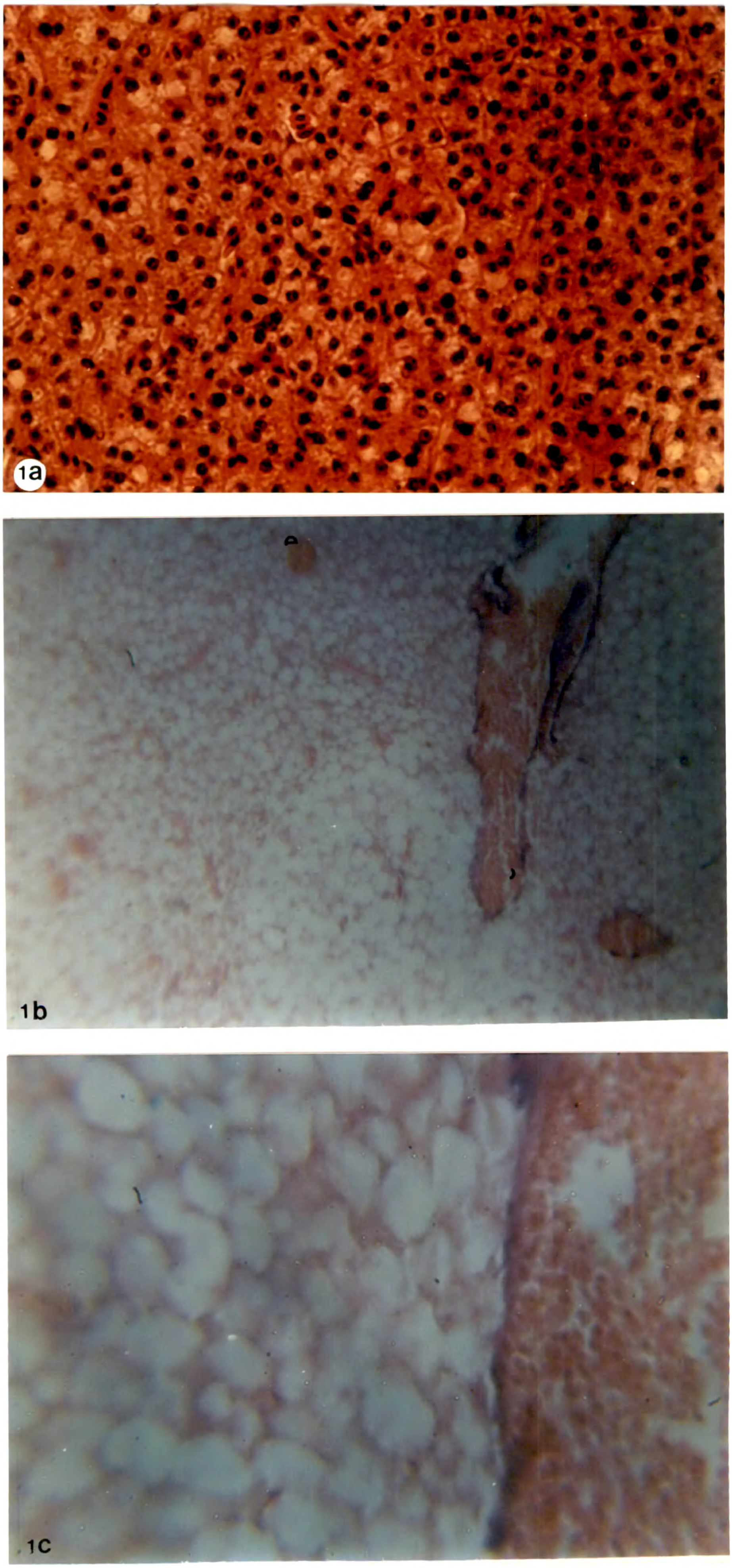
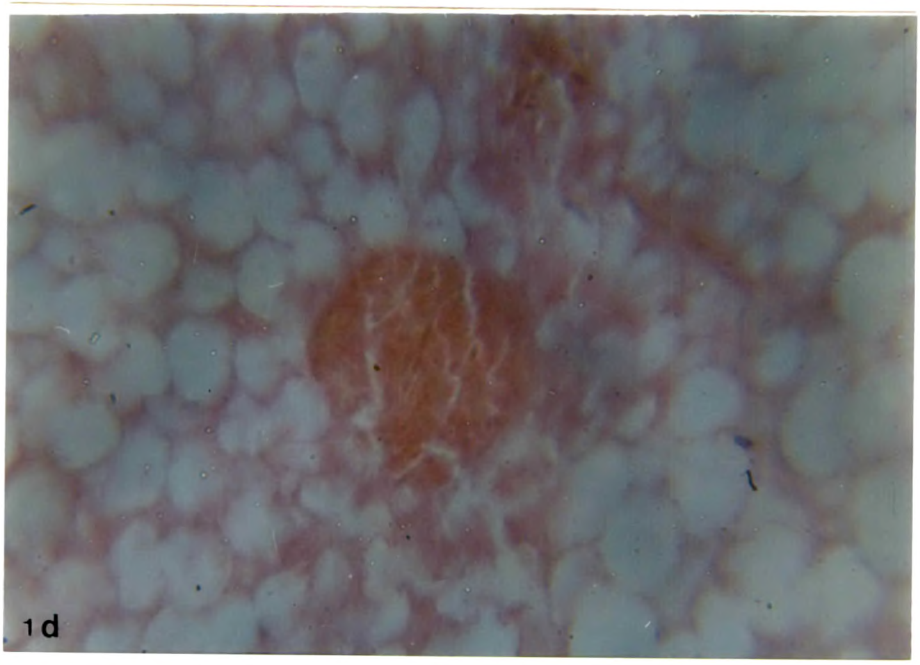

1a
1b
1c

PLATE II: Photomicrographs of sections of liver and gill of <u>B.dussumieri</u>

Figure 1d: Enlarged version of the section of liver of polluted fish (X 690) note the well formed erythropoietic nodule.

Figure 2a: Section of gill of control fish showing normal looking central rachis and lateral lamellae (X160)

Figure 2b: Section of a gill of polluted fish. Note the hypertrophied lamellae and blood cell filled central rachis.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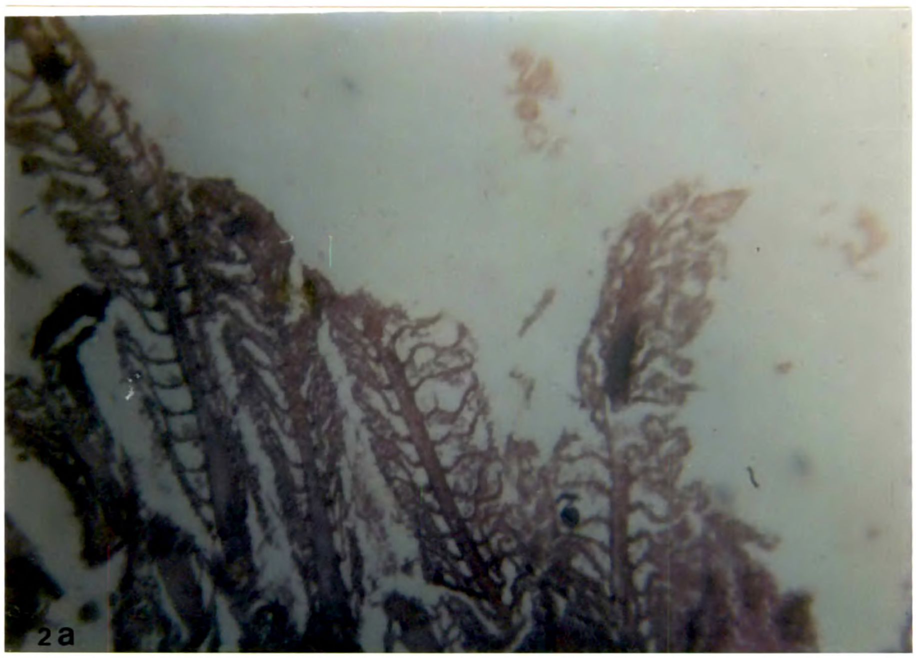
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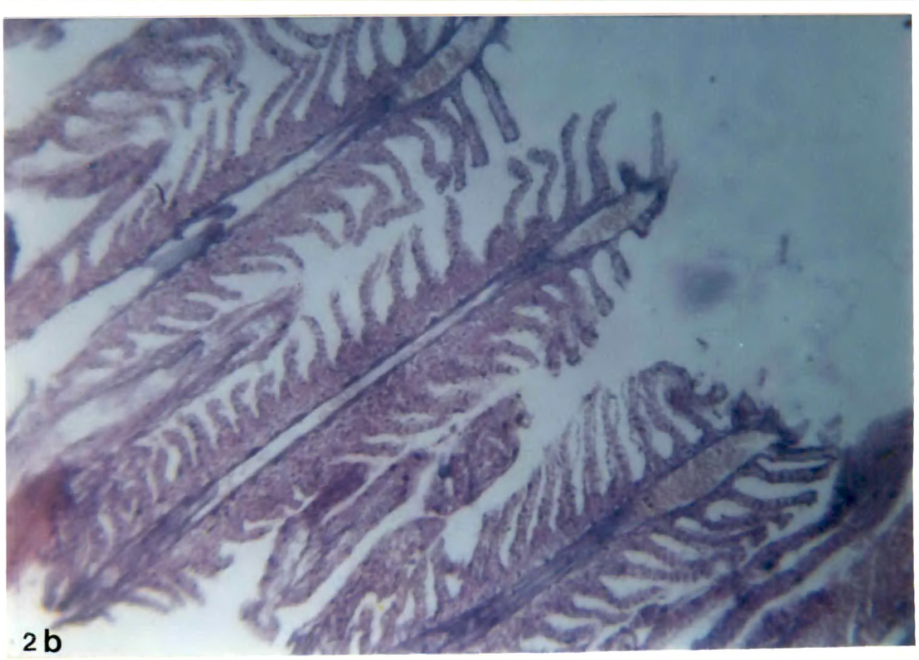
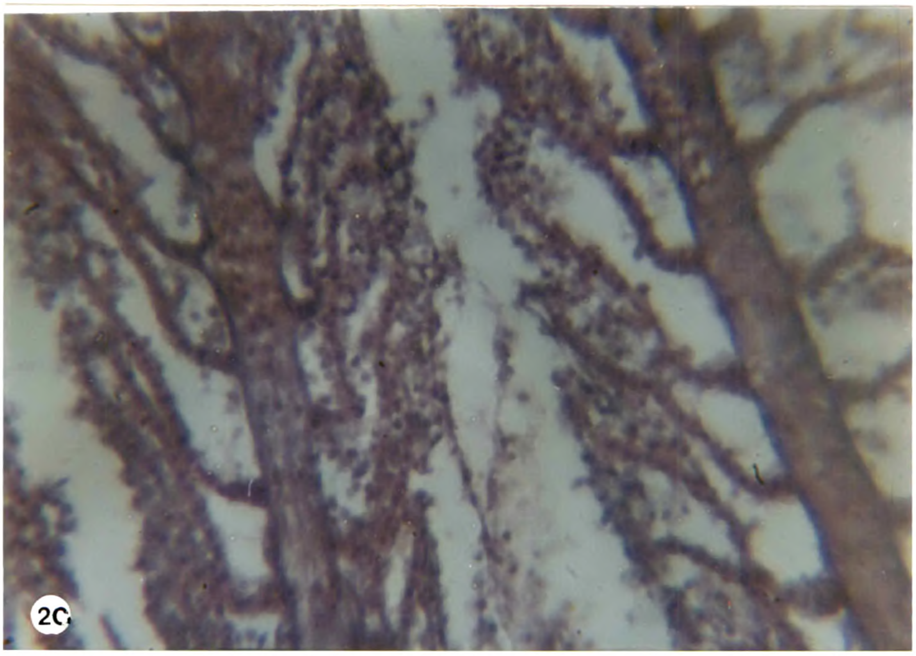

2b

PLATE III: Photomicrographs of sections of gill of *B. dussumieri*.

Figure 2c: Englarged version of the gill of a control fish, showing normal looking rachis and lateral lamellae (X690)

Figure 2d & 2e: Englarged versions of the gill of a polluted fish. Note the RBC filled central rachis and lateral lamalle with hypertrophied cells and even presences of RBCs (X690).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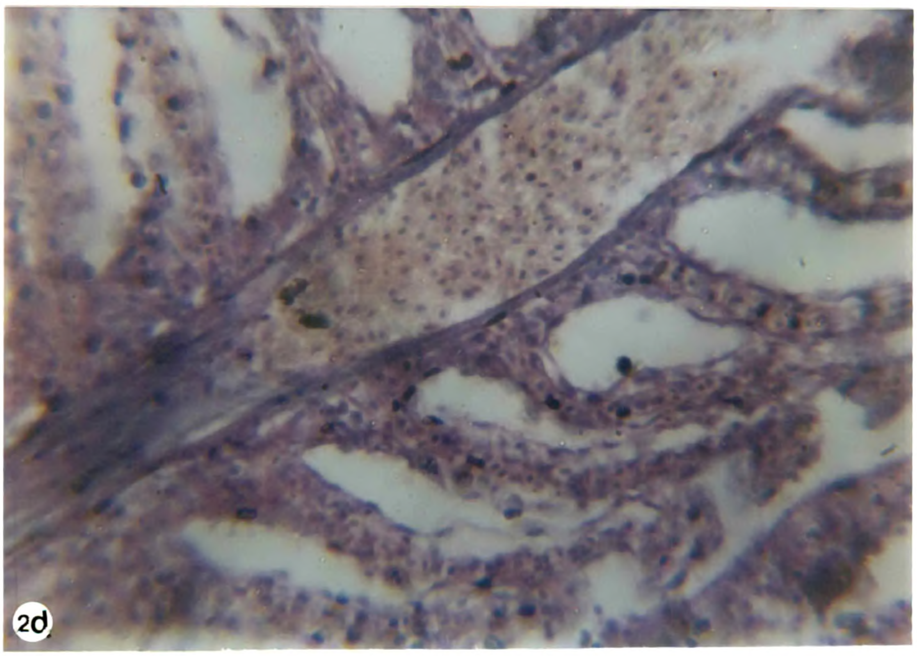
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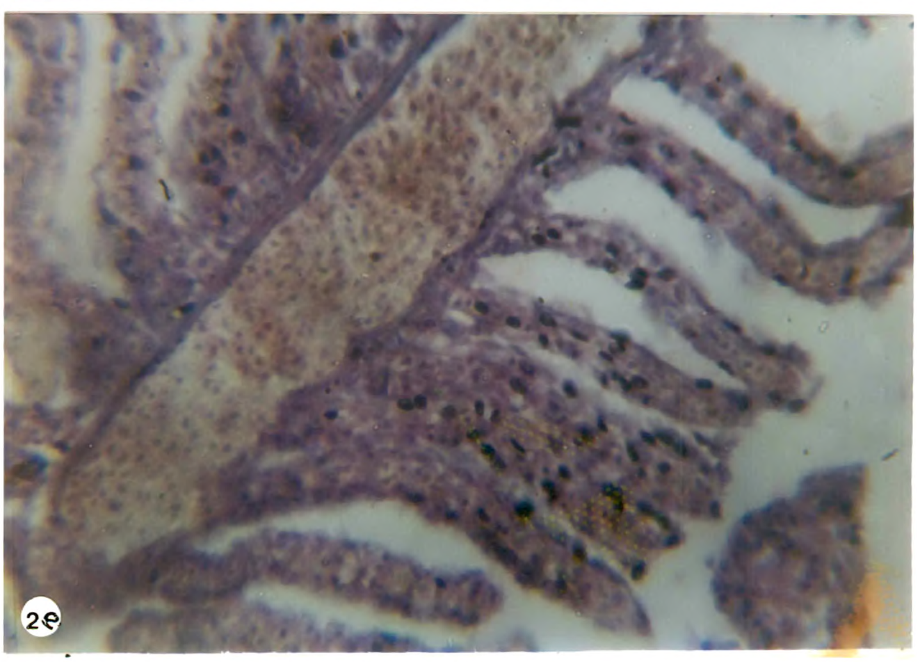

2e

channels in the lamellae were filled with RBC. Some of the lamellar epithelial cells also depicted pycnotic nuclei (Figs- 2b,d,e).

INTESTINE

The intestine of control fishes showed a normal picture with numerous well formed mucosal folding and prominent mucosal epithelium (Fig- 3a). The intestine of polluted fishes showed reduced and loosely arranged mucus foldings and in general the muscosal epithelial cells were hypertrophied with heavy infiltration of leucocytes in the submucosal area. Mucus cells in the epithelium seemed to be loosing structure and becoming degenerate (Figs- 3b,c).

MUSCLE

The muscle of non polluted fishes showed normal looking fasciculi and muscle fibres (Fig- 4a). The muscle of polluted fishes did not show any serious histological lesions though the muscle fibres and fasciculi in general seemed to be hypertrophied.(Fig- 4b).

OVARY

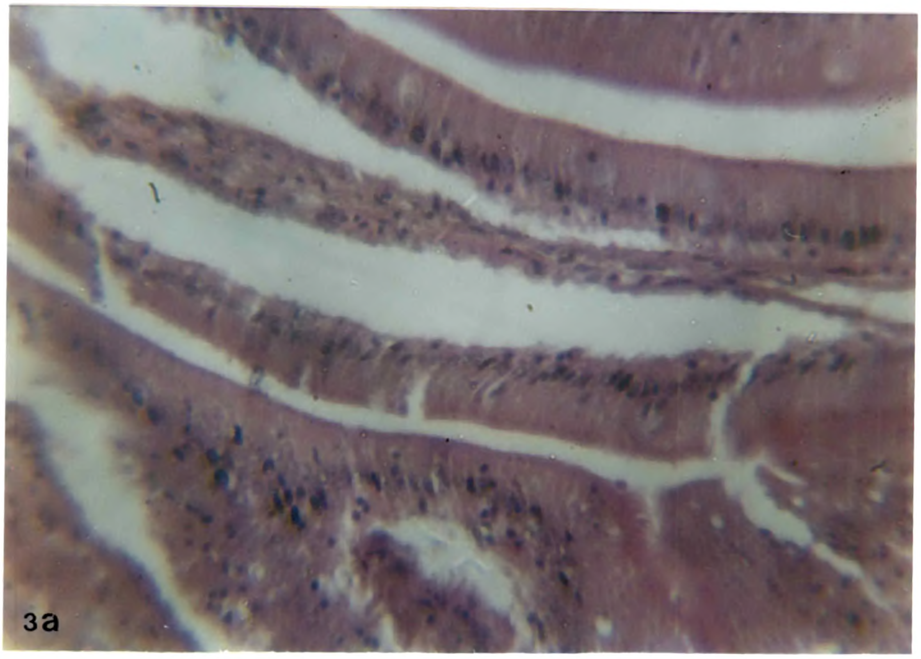
The ovary of normal fishes showed oocytes in various stages of vitellogenesis and few atretic oocytes and interstitial cells (Fig- 5a-c). The ovary of polluted fishes showed

PLATE IV : Photomicrographs of sections of intestine of _B.dussumieri_.

Figure 3a: Intestine of control fish showing prominent villus foldings. Note the prominent mucosal epithelium with scattered goblet cells (arrows) (X 690)

Figure 3b: Intestine of polluted fish showing reduced mucosal folding and hypertrophied cells (X160)

Figure 3c: Enlarged version of the same, showng hypertrophied cell, loss of mucous cell , and invasion of lymphocytes (L) (X690).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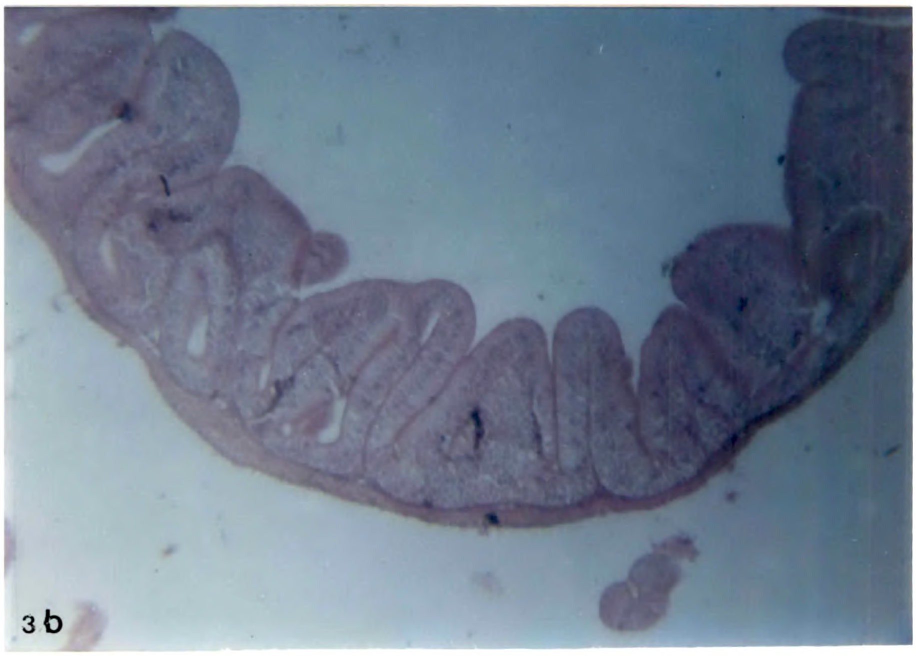
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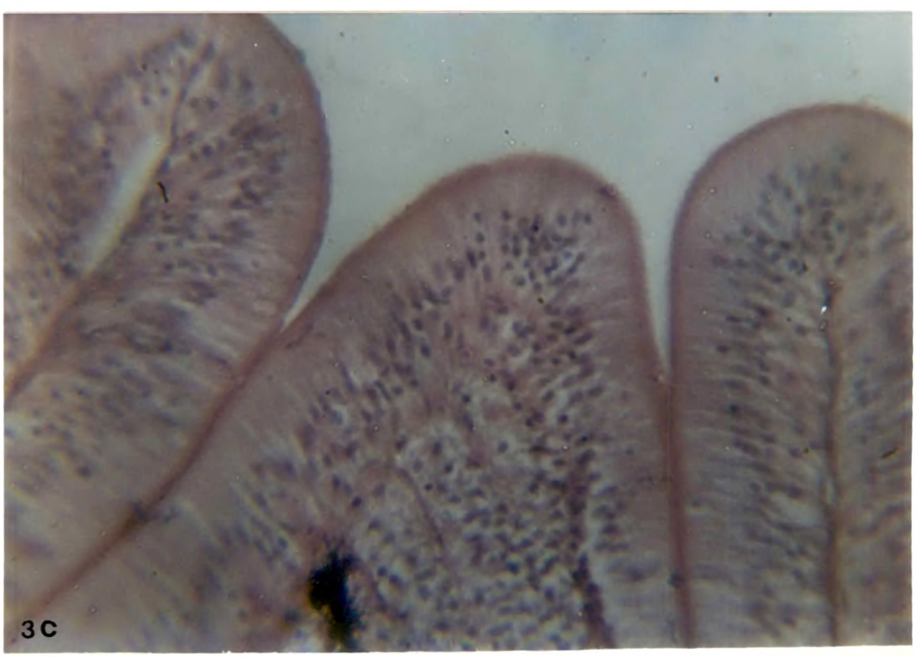
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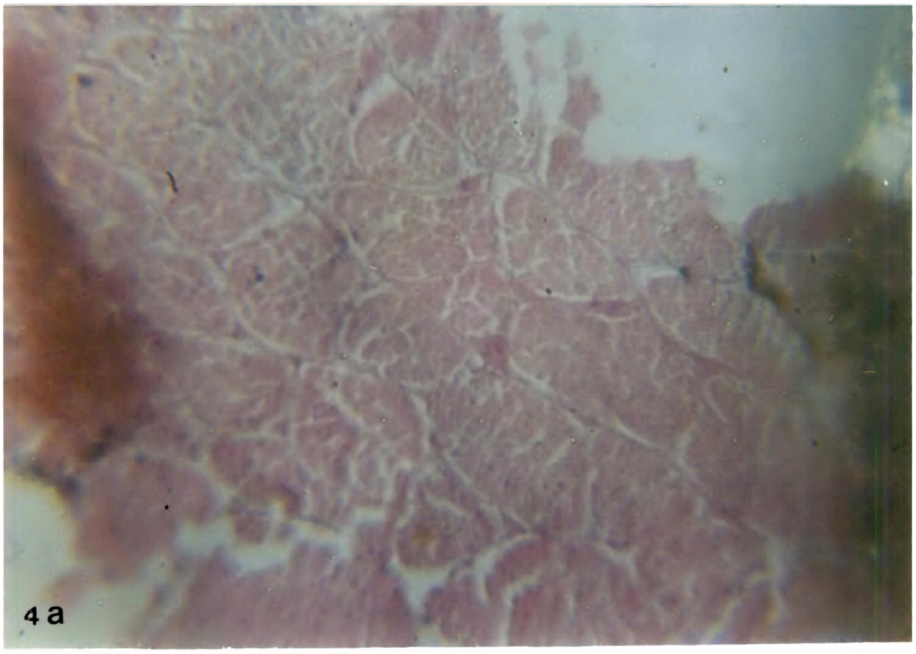
PLATE V: Photomicrographs of sections of muscles of *B.dussumieri*.

Figure 4a: Muscle of control fish showing muscle fasciculi and fibres (X690)

Figure 4b: Muscle of polluted fish showing hypertrophied fasciculi and fibres (X690)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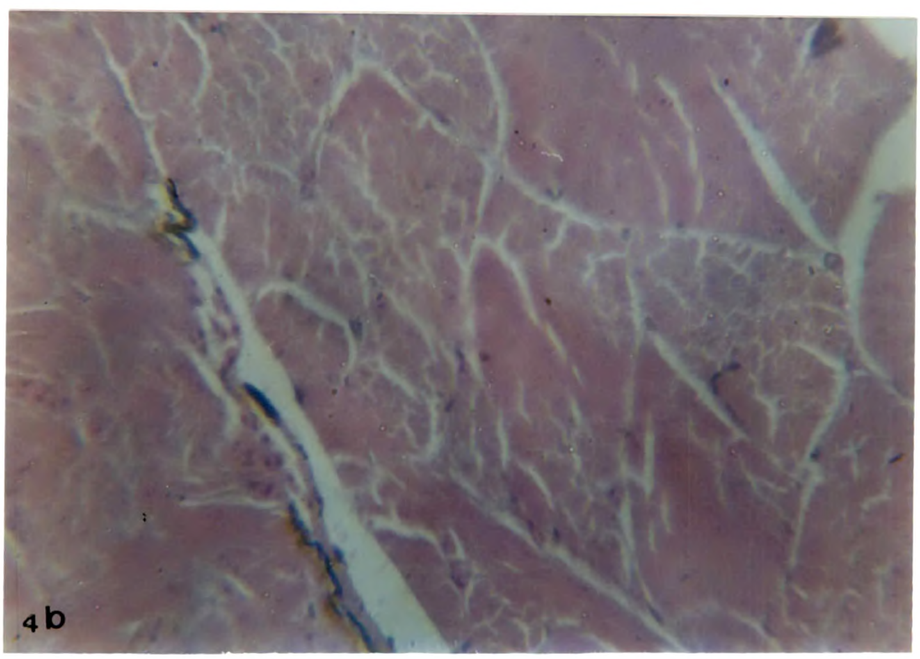
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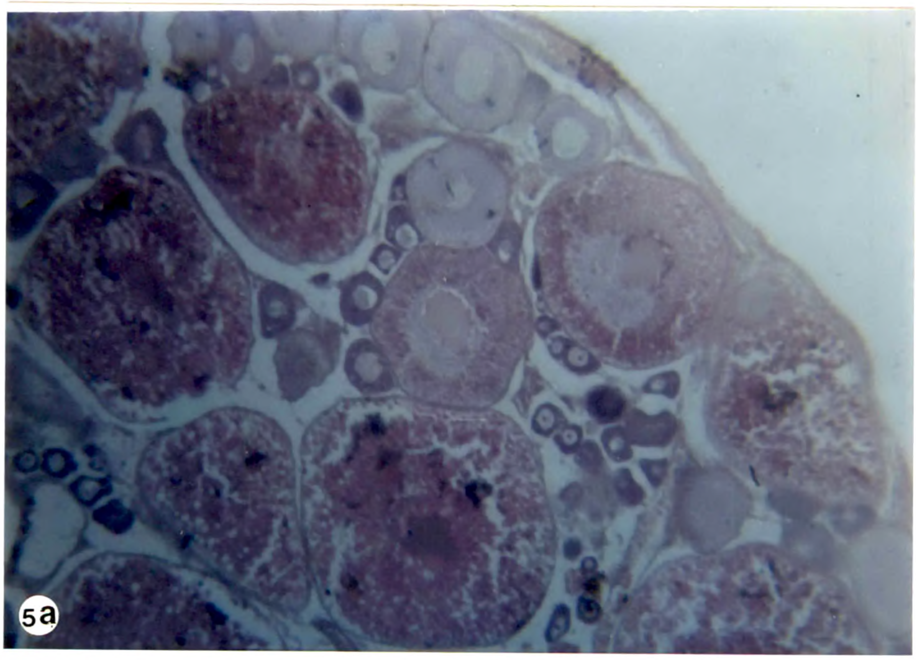
PLATE VI: Photomicrographs of sections of ovary of *B.dussumieri*.

Figure 5a: Ovary of control fish showing oocytes of various stages of development, intense yolk deposition and also interstitial cells (X160)

Figure 5b: Ovary of a polluted fish showing hypertrophied oocytes with reduced yolk depostion and interstitial cells (X160).

Figure 5c: Englarged version of the ovary of control fish. Note the fully yolk laden oocytes with closely packed yolk droplets and interstitial cells (X690)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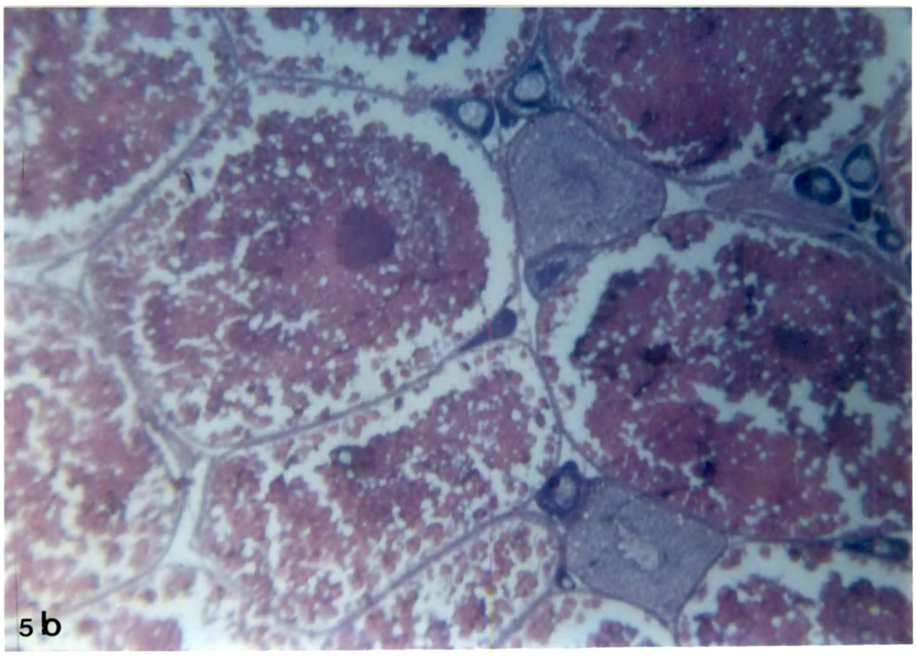
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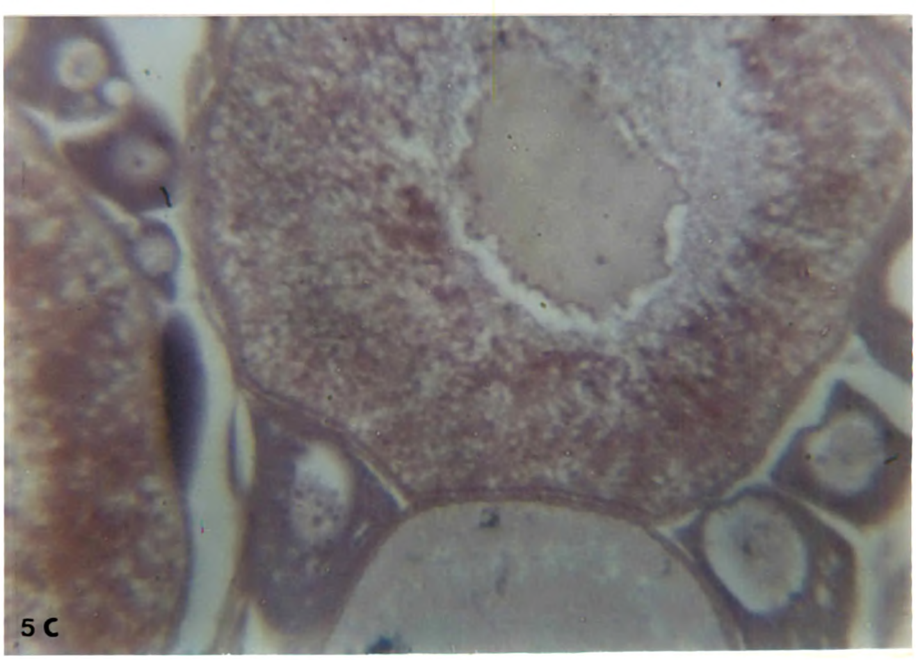
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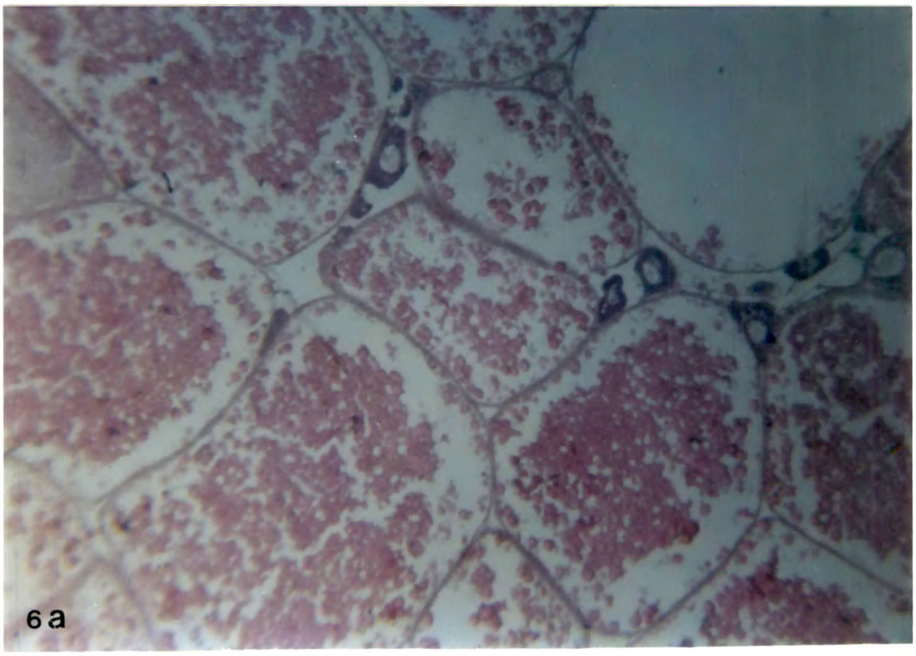
PLATE VII: Photomicrographs of sections of ovary of *B.dussumieri*.

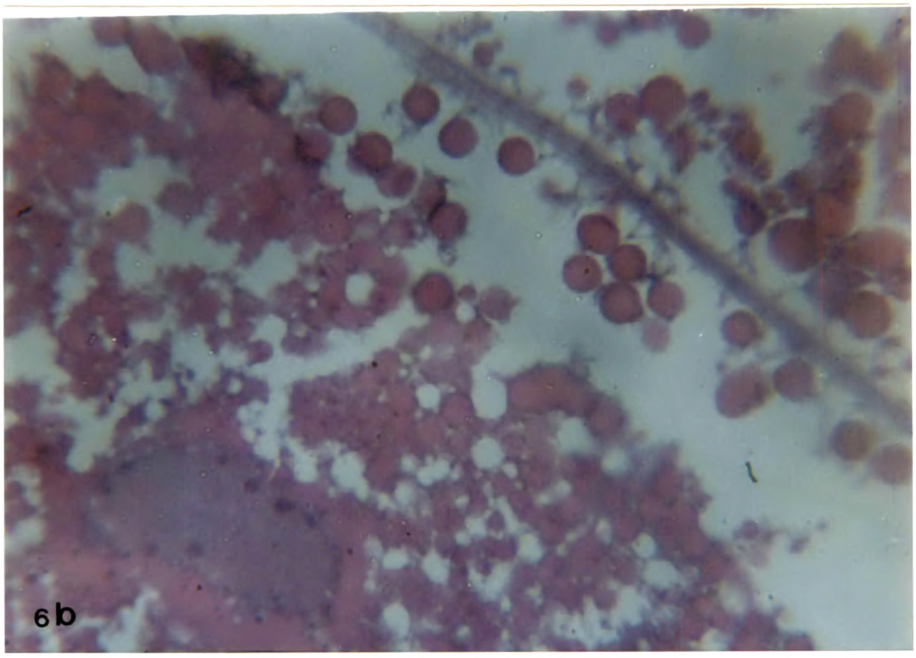
Figure 6a: Ovary of a polluted fish showing oocytes with reduced yolk deposition varying from loosely packed moderate amounts to total absence (X160)

Figure 6b: Enlarged version of the same. Note the loosely packed and minimal droplets in two adjacent oocytes (X 690)

Figure 6c: Another version of same showing oocytes with sparse yolk droplets and degenerating interstitial cells (X 690).



6a



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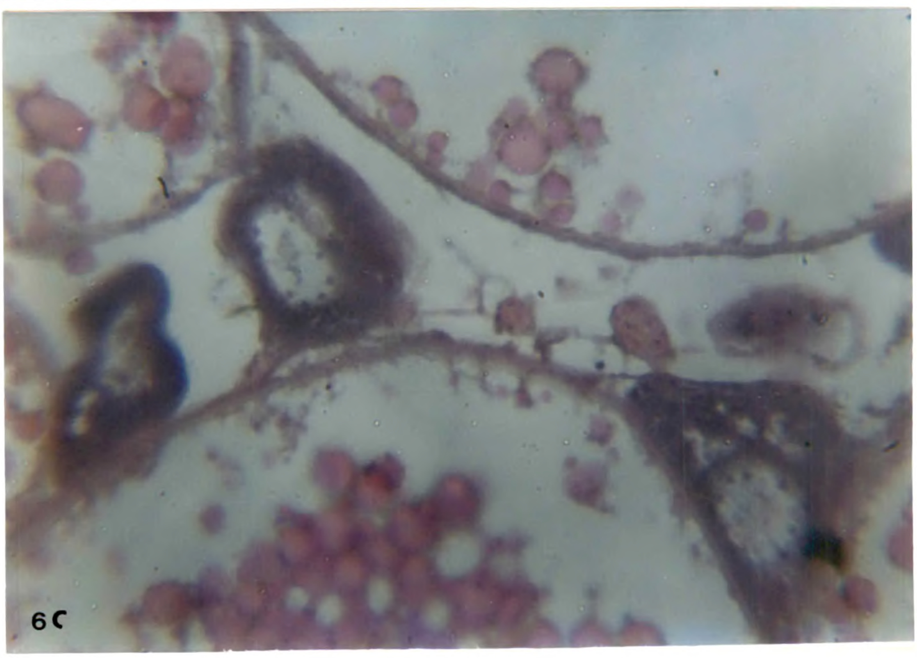

6c

distended vitellogenic oocytes and these hypertrophied oocytes showed sparse and loose vitellogenic deposits. The pre-vitellogenic oocytes and even the interstitial cells showed degenerative changes with nuclear hypertrophy, pycnosis and chromatin disintegration (Figs 6a c).

DISCUSSION

Heavy metals are receiving increasing attention in ecotoxicological research due to their increasing input into the environment, long term persistance, high toxicity to biota and their tendency to accumulate and show bio-magnification. Many histo-pathological studies on heavy metal toxicity on various tissues of fishes, especially gills have been carried out, as acute toxicity studies of individual metals in the laboratory. Varied histo-pathological lesions affecting different organs like gill, muscle, liver, intestine, brain, kidney and gonads have been documented by the above studies (Kothari *et al.*, 1983, Gearheart *et al.*, 1992, Thomas *et al.*, 1992, Kopper and Kulkarni, 1993, Ambrose *et al.*, 1994, Bhaskaran & Lall, 1994, Sonderva, 1994) The observations of the above studies cannot be compared logistically with the present one, as the current study involves a natural system with various pollutants, including metals with temporal fluctuations quantitatively and qualitatively. In such a system, the degree and mechanism of accumulation, uptake, elimination, tolerance, bio-magnification and even, the degree of toxicity, are all likely to be altered and unpredictable.

The present results show most devastating damage to the hepatic tissue with total fatty infiltration. This is easily understandable as many metals tested above have been shown to induce fatty liver degeneration. The presence of erythrocytes in nodular form and the aggregation of these cells in the sinusoids are suggestive of stimulated erythropoiesis. This may be relevant in the context of the previously reported reduced dissolved oxygen content of the Mahi estuary. The subtle hypertrophic alteration of the muscle is likely to impair the natural mobility and activity of the animals. Though most of the studies cited above have demonstrated severe damage to the gills even within short duration of exposure, in the present case, the gills did not seem to be that affected, though there were histopathological lesions in the lamellae, sporadic necrosis in the cells and heavy accumulation of lymphocytes. This could be mainly due to the possible antagonistic effect of different metals, and the ultimate attenuated toxic effect. Definite alterations in digestive and absorptive functions can be inferred from the noted alterations in the mucosal foldings and the structure of mucus cells. The histo-pathological alterations in the ovary also suggest reduced fecundity in such an environment as denoted by the highly distended oocytes with retarded, vitellogenic deposition and subtle architectural changes. The noted reduced vitellogenic contents of the oocytes might have some correlation with the observed extensive hepatic degeneration.

The effect of small quantities of various toxic materials on fish, although less apparent than certain fish kills, may be even more harmful. Therefore, it is difficult to speak about a maximum safe concentration of a toxic substance for fish. The sublethal effects can be considered to cover effects of all those concentrations which are not necessarily lethal for individuals even at prolonged exposures, but increase the population mortality, decrease its size or change its composition. This would include a group of effects which would be reflected on the growth rate, metabolism, reproduction or which impair the defence mechanism of fish, due to the deterioration of the environmental conditions in a water ecosystem by the changes of physical, chemical and biotic factors. The reduced size and number of *B.dussumieri* in the study area and, the histological appearance of their ovary noted in the present study, attest to the above. The decreased variety of fish fauna (hardly a couple of them surviving in skeletal numbers) in the Mahi estuary over the last one and a half decade, indicates the sublethal toxic effects of the pollutants discharged through the effluent channel. The relatively larger number of *B.dussumieri* may suggest their better tolerance and adaptation compared to other types of fish. It is likely that in due course of time, the Mahi estuary and the Gulf of Cambay would be completely denuded of all fish forms. The declining fish fauna could also be a reflection of the depletion of phyto and zooplanktons. Over all, the present study indicates destruction of the estuarine ecosystem at the Gulf of Cambay with the continued input of industrial effluents replete with metals.