RESEARCH PAPER

TOXICOLOGICAL ASSESSMENT OF CHRONIC POLYCHLORINATED BIPHENYLS (PCBs) BIOACCUMULATION IN *LABEO ROHITA*: A HISTOPATHOLOGICAL APPROACH

Sopan Ainar[1], Vandana Bhavare[2]

[1,2]*Department of Zoology, Sangamner Nagarpalika Arts, D. J. Malpani Commerce and B. N. Sarada Science College, Ghulewadi Tal: Sangamner, Dist: Ahilyanagar-422605*
(Affiliated to Savitribai Phule Pune University, Pune M.S. India-410007)

ABSTRACT
Polychlorinated Biphenyls (PCBs) are highly carcinogenic chemical compounds banned in India and some other countries, used in industrial and consumer products. PCBs have devastating effects on ecological balance of the recipient environment and a diversity of organisms. Water pollution by PCBs is a global concern which represents a growing threat to the aquatic animals and also human beings. Fish are known for their low susceptibility to PCBs toxicity. Since toxicity is based on the effect that a toxicant produces at a target organ within an organism, establishing the relationship between the concentration of substance at the target organ and the subsequent toxic effect can provide tool for predicting toxicity. To investigate chronic Polychlorinated biphenyls sublethal effects to *Labeo rohita* (3 treatments) were determined. *Labeo rohita* belonging to the family Cyprinidae, weighing 25-30g procured from local fish dealer. Fish were acclimatized for a week at holding temperature of 18-22^{0}C. Chronic PCBs Lc50 wass 0.90 ppm for 28 days, PCBs deposition in tissues had increased significantly with dose and exposure duration dependent manner. Several histological alterations were noted in the organs like Gills, Gonads, Liver and Kidney of all treated compared to control group. Hence *Labeo rohita* found less susceptible to PCBs toxicity. To gain insight into the mechanisms of the low susceptibility of *Labeo rohita*, we aim to evaluate this research.

Keywords: Chronic, Sublethal, Susceptibility, Biomarker, PCBs, Lc50, *Labeo rohita*
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INTRODUCTION:

With the increasing human population and urbanization has put enormous pressure on natural resources. The rapid growth of the human population and industrialization in recent years has led to increased waste and pollution emissions into the environment [11]. Contamination of water bodies by harmful substances from sources like industrial discharge, agricultural runoff, and urban runoff, is a critical environmental issue degrading ecosystems, harming aquatic life, stemming from these developments has become a longstanding and pressing concern for humanity [20]. Posing severe health risk to human, necessary urgent research into emerging contaminants and advanced treatment solution for sustainable management [12].

Global production reached 1.3 million tons between 1965 and 1974, with China producing about 10,000 tons before the ban [21]. Some studies have reported the occurrence and distribution of PCBs in rivers worldwide including the Johannesburg River, South Africa. In India, assessed ecotoxicological PCBs risk of Yamuna River sediments [9], Investigated PCBs in the Hooghly and Brahmaputra, linking industrial sources to contamination [4], first comprehensive study on legacy POPs (including PCBs) in Kerala and Tamil Nadu rivers (Periyar, Bharathappuzha, Adyar & Cooum [5], focused on low-chlorinated PCBs in the Ganga River (Kanpur/ Prayagraj) and Examined PCBs distribution in Thane Creek sediments, Mumbai.

Surface water quality concerns are growing due to an increase in various persistent organic pollutants (POPs) found in environmental media such as soil, biota, dust, and air. Among these, Polychlorinated biphenyls (PCBs) are notable contributors to the rise in pollution levels in water [2]. PCBs persist in the environment, posing significant threats due to their high lipophilicity, which facilitates biomagnifications in the food chain, leading to serious health effects in humans, including carcinogenicity, immune suppression, reproductive impairment, respiratory issues, and neurobehavioral changes [1]. Continuous monitoring and assessment of

**Author for Correspondence: Dr. Vandana Bhavare*

their ecotoxicological impacts are essential. As per the studies, there are significant gaps in terms of seasonal distribution, coverage, and risk assessment.

Polychlorinated biphenyls (PCBs) cause significant cellular and biochemical alterations in fish *Labeo rohita* due to their persistent nature and ability to accumulate in tissues. This severe histopathological alteration in major organs such as the Gills, Gonads, Liver and Kidney. Structural damage in gill's includes epithelial hyperplasia with lamellar fusion, edema increased mucus production, and general necrosis, liver changes involve cytoplasmic vacuolization, cell fusion, blood congestion, and the presence of melanomacrophage aggregates, kidney changes include renal tubules degeneration, vacuolation of interstitial tissue, and deformation of cell nuclei are observed [18].

While there are studies on PCB's contamination worldwide, this research specifically highlights western India, an area that has not received adequate attention despite its economic importance. The findings from this report will provide a vital reference for future environmental monitoring and risk assessments regarding PCBs in rivers and lakes, particularly within Maharashtra State India.

MATERIALS AND METHODS:

A group of healthy, disease-free 3 months old male *Labeo rohita* 25-30g in weight, 9-10 cm in length were procured from local fish dealer. Fishes were acclimatized for 1 week at laboratory conditions, were fed with commercial aquarium food. Laboratory water analyzed for different physico-chemical parameters [3]. Aquarium water was maintained as natural habitats of the fish, no fish mortality recorded while acclimatization. Polychlorinated Biphenyls (PCBs) Lc50 for 96 hrs was statistically determined [7] and predicted bearable PCBs concentration for chronic exposure. Well acclimatized fishes showing no signs of stress were selected and divided into four groups, exposed to PCBs toxicant. The first group served as a control and remaining three were experimental. A dose of 0.30 ppm, 0.60 ppm, 0.90 ppm and 1.20 ppm of PCBs was administered to experimental group daily for 28 days. The experiment was carried out in triplicate; the fish were kept in glass aquarium, containing 10 fish/ 40 Lit. Test medium with aeration. The test solutions were renewed once after 24hrs [19].

After completions of experiment 4 fish from each group were anesthetized with MS-222 (Sigma) [17] sacrificed and decapitated both control and experimental group. Tissues were fixed in aqueous Bouins fixative for 24 hrs. with a change after 24 hours. Fixed tissues were washed with 70% ethanol and dehydrated further through 80% and ethanol and cleared in Xylene. The tissues were embedded in paraffin wax and 5µm longitudinal sections were obtained on microtome, placed on slides then dried in oven at 370C overnight. Sections were stained with Haematoxylin and Eosin; slides were dehydrated in graded ethanol, cleared in Xylene and mounted with DPX [6] to observe the architecture of Gills, Gonads, liver and Kidney of both groups. Stained slides were photographed and observed under microscope.

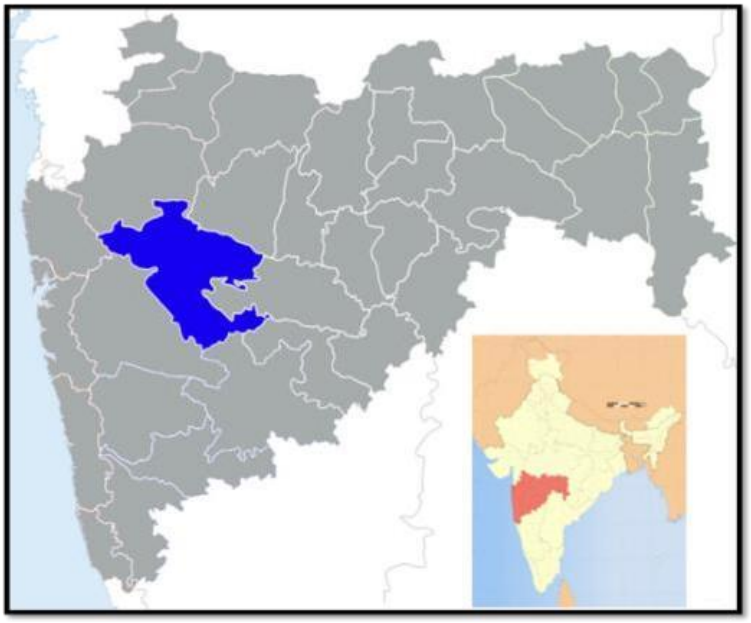


Fig: Map of Dnyaneshwar Sagar, Tal- Rahuri Dist- Ahemadnagar, M.S. India.

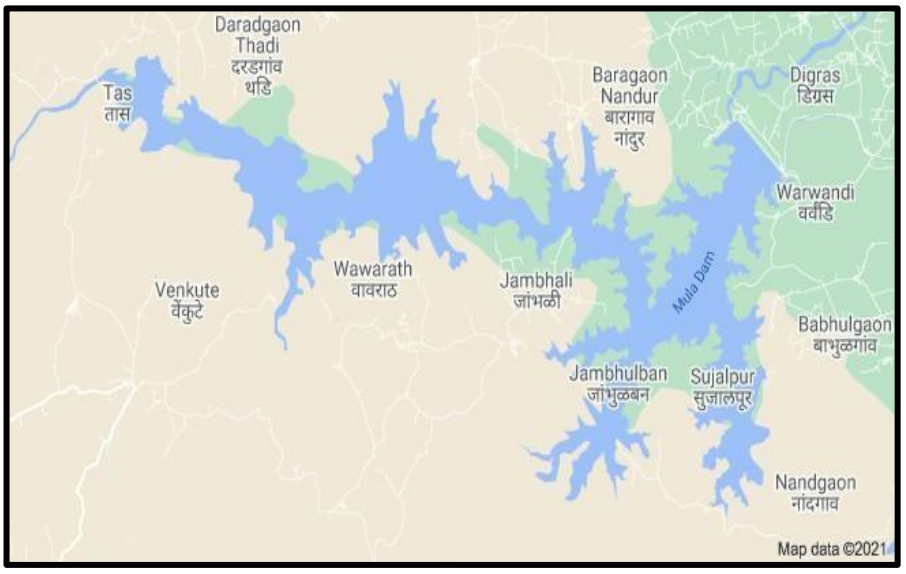


Fig: Dnyaneshwar Sagar, Tal- Rahuri Dist- Ahemadnagar, M.S. India.

RESULT AND DISCUSSION:

3.1: Physicochemical Parameters: Physicochemical parameters of laboratory water were tested; The water parameters were analyzed, Temperature 18-22^{0}C, pH 7.0-7.5, dissolved oxygen 6.8- 7.0 mg/l and total hardness 280 -300 mg/l.

3.2: Lc50: The 50% mortality was observed within 28 days, Lc50 value calculated by graphic method to be 0.90 ppm of PCBs (Table 3.2.1 and Fig. 3.2.1)

PCBs Conc. (ppm)	Dead Animal	Total Animal	% Mortality	Probate Mortality
Control	**0**	**10**	**0.0**	**0.0**
0.30	**3**	**10**	**30**	**4.48**
0.60	**4**	**10**	**40**	**4.75**
0.90	**5**	**10**	**50**	**5.00**
1.20	**7**	**10**	**70**	**5.52**

Table 3.2.1: % and Probate Mortality of PCBs in *Labeo rohita*

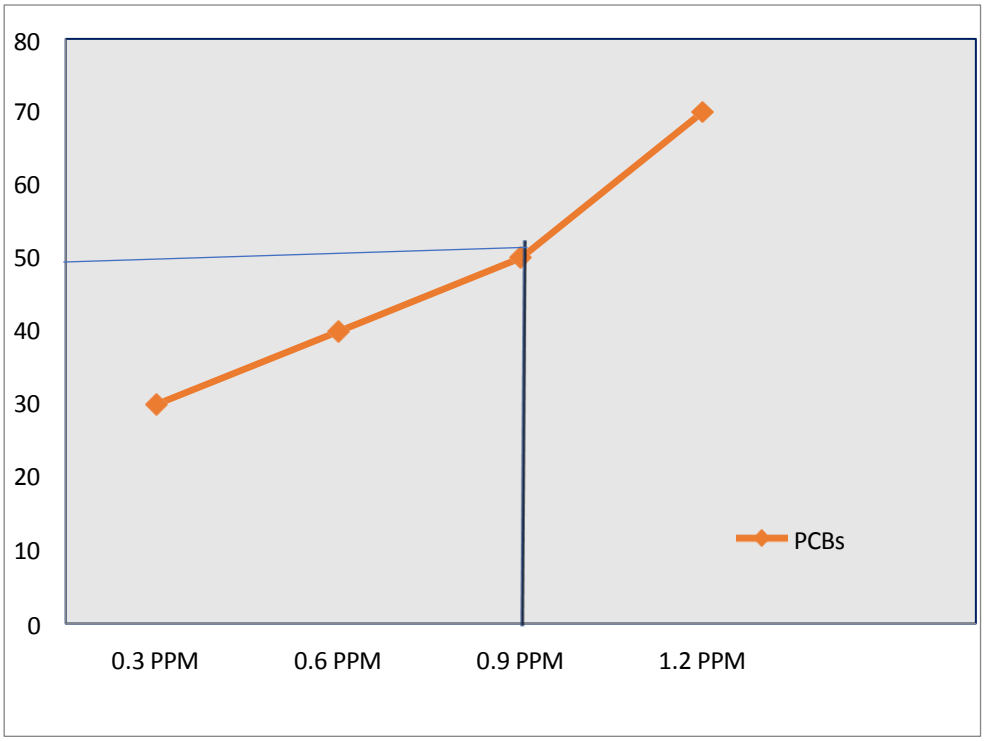


Fig. 3.2.1: Lc50 value for PCBs in *Labeo rohita*

3.3: Histopathological Alteration:

Chronic bioaccumulation of PCBs exposure causes several structural abnormalities in the Gills, Gonads, Liver and Kidney of *Labeo rohita* [14]

3.1.1 : Gill:

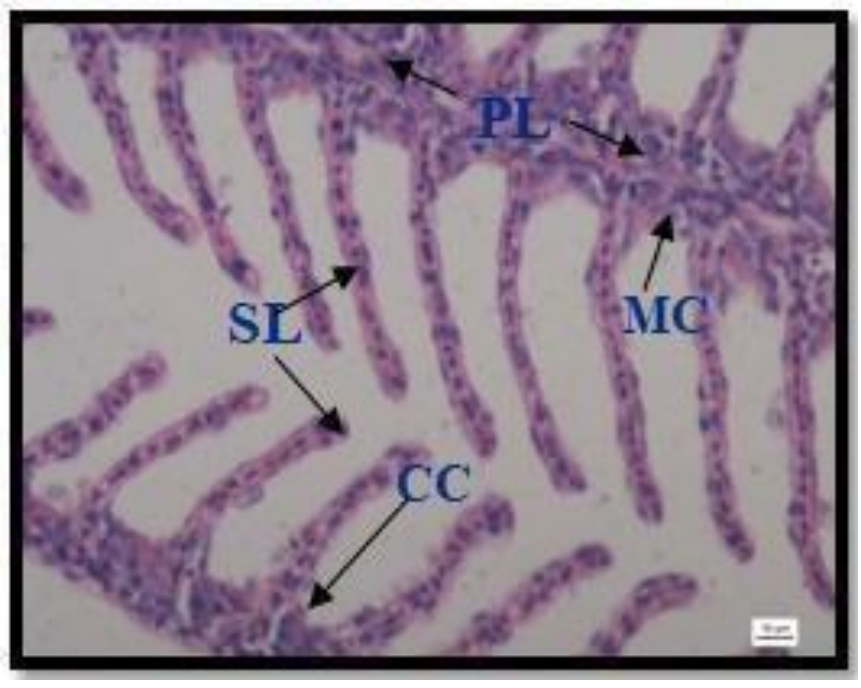
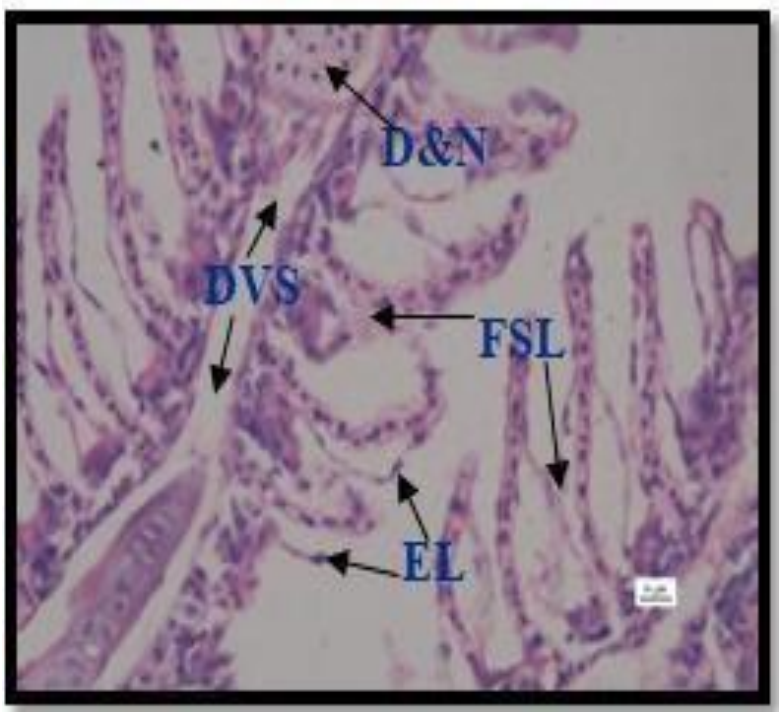


Fig. 3.3.1. A: Control (Untreated)



B: 0.90 PPM (treated) Lc50 group

Untreated group shows normal gill structure, Primary Lamellae (PL), Secondary Lamellae (SL), Chloride Cell (CC) but treated group shows alteration like Dilatation of the venous sinus observed at the Middle of the primary lamellae (DVS); Complete fusion of Secondary lamellae (FSL), epithelial lifting (EL), Degeneration and Necrosis (D & N).

3.1.2 : Gonad:

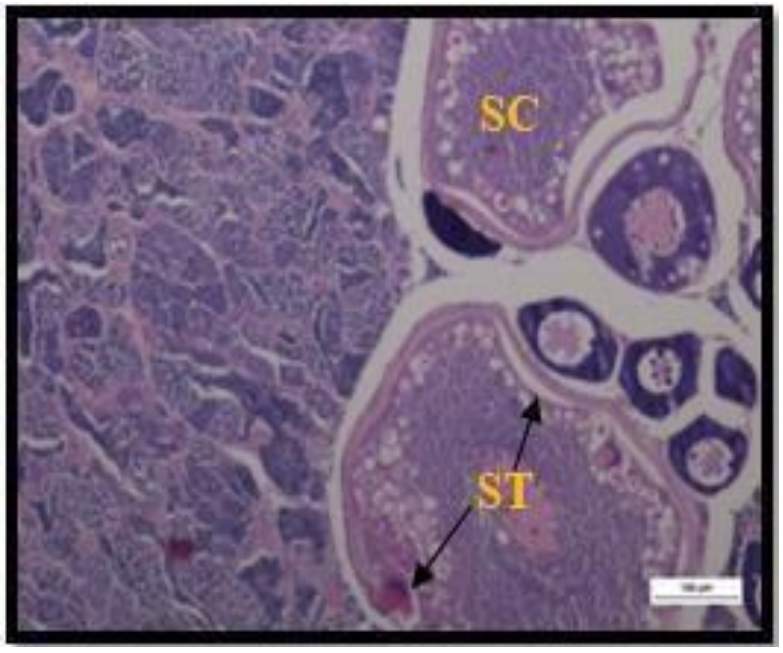
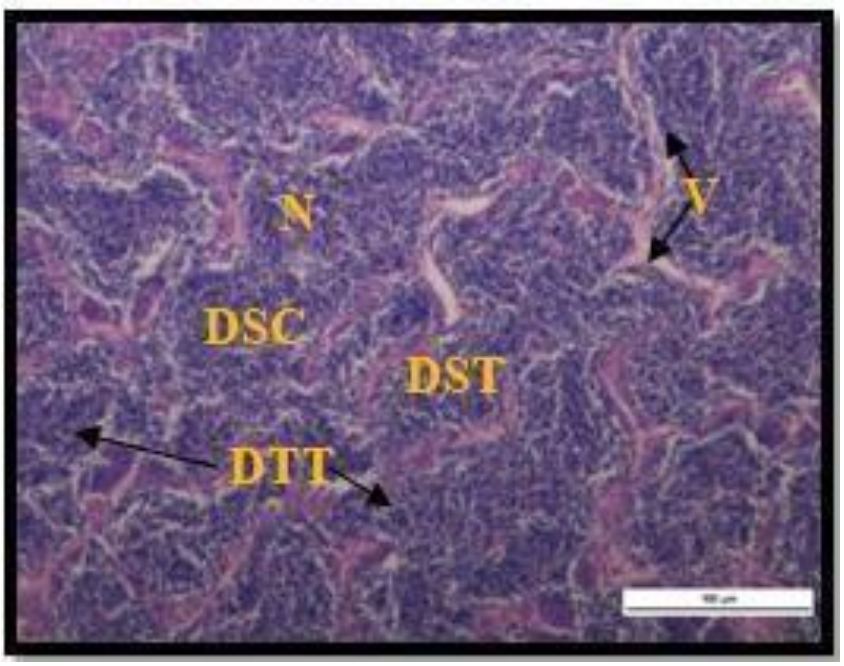


Fig: 3.3.2.A: Control (Untreated)



B: 0.90 PPM (treated) Lc50 group

Untreated group shows normal gonad structure, Seminiferous tubules (SC), spermatogenic cells (SC) but treated group shows alteration like Degeneration of seminiferous tubules (DST), Reduction of spermatogenic cells, Vacuolization in germinal epithelium (V), Necrosis of germ cells (N), and Disorganization of testicular tissue (DTT).

3.1.3 : Liver

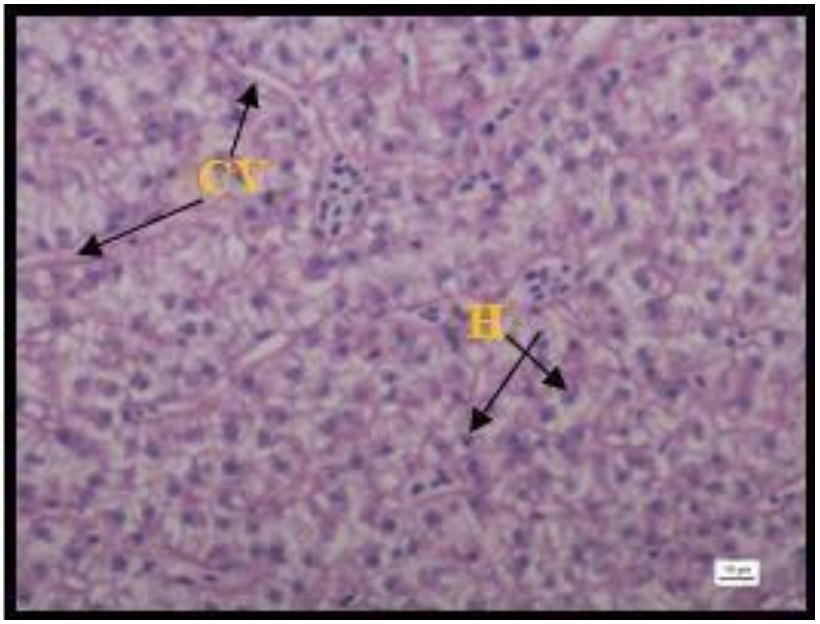
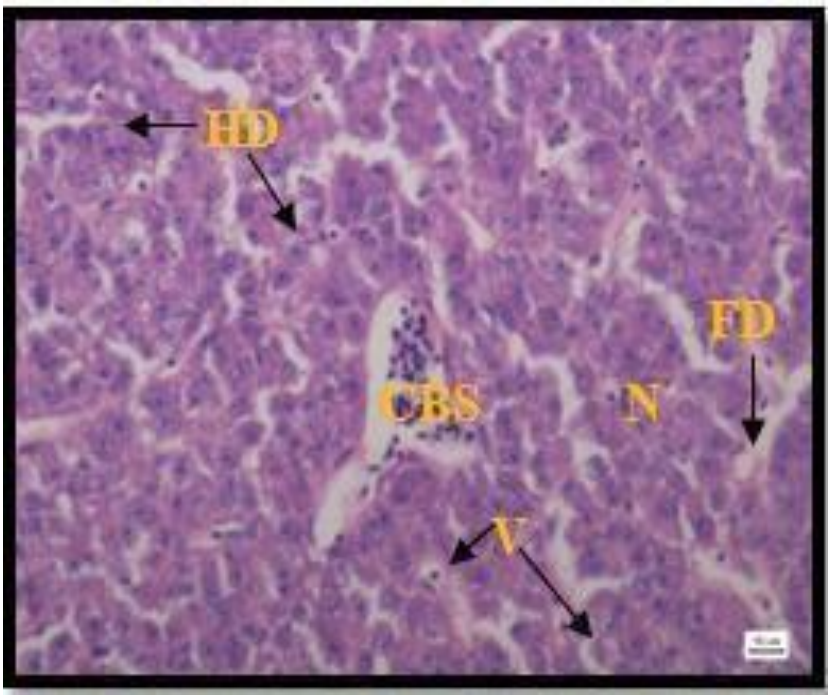


Fig: 3.3.3.A: Control (Untreated)



B: 0.90 PPM (treated) Lc50 group

Untreated group shows normal liver structure Central Vein (CV), Hepatocytes (H) but treated group shows alteration like Hepatocellular degeneration (HD), Vacuolization of hepatocytes (V), Necrosis of liver cells (N), Congestion of blood sinusoids (CBS), Fatty degeneration (FD).

3.1.4 : Kidney:

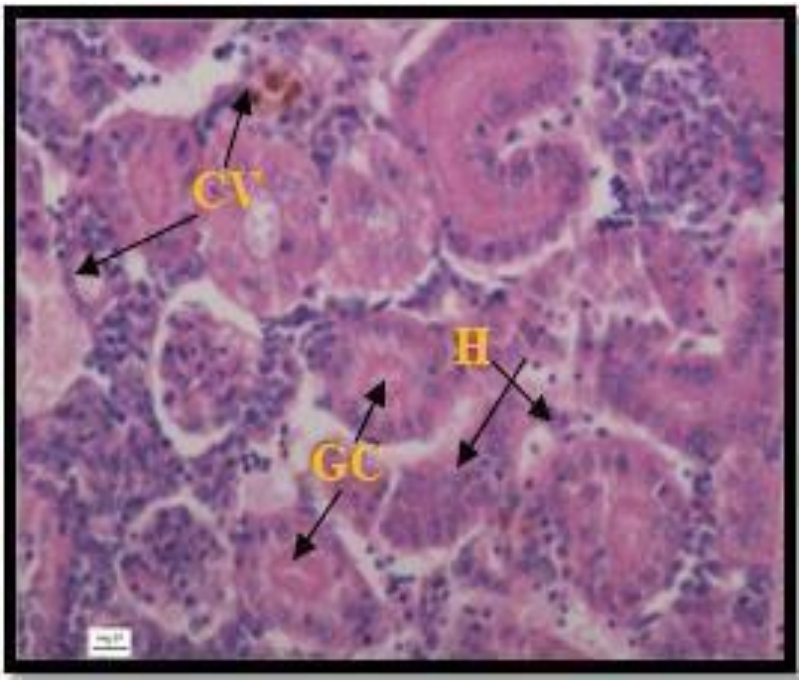
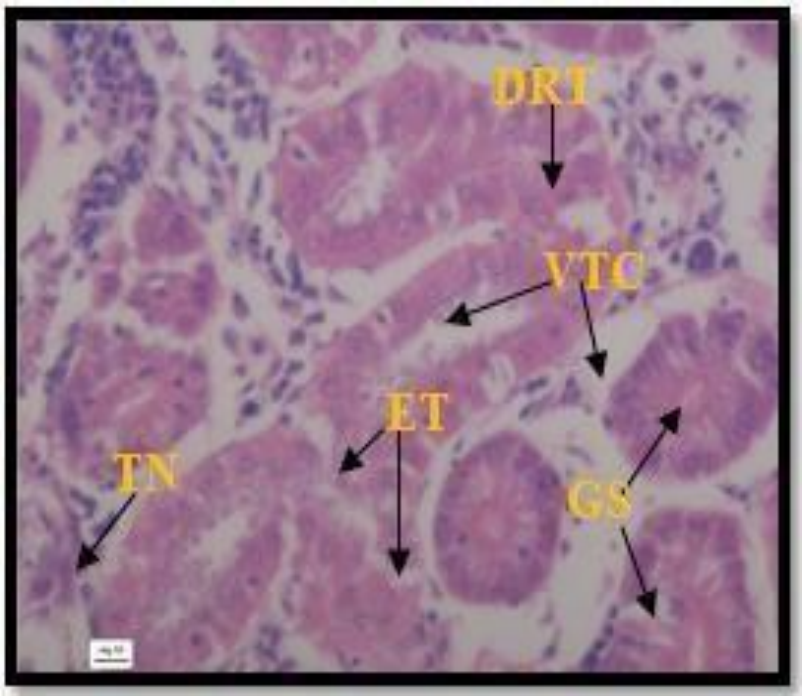


Fig: 3.3.5. A: Control (Untreated)



B: 0.90 PPM (treated) Lc50 group

Untreated group shows normal arrangement of kidney cells and renal tubules but treated group shows alteration like Degeneration of renal tubules (DRT), Tubular necrosis (TN), Vacuolization of tubular cells (VTC), Glomerular shrinkage or degeneration (GS).

Structural abnormalities in gills (Fig. 3.3.1.B) show Epithelial hyperplasia (thickening of the gill epithelium due to excessive cell proliferation), Lamellar fusion (adjacent secondary lamellae fuse together), Epithelial lifting (detachment of epithelial cells from the basement membrane), Degeneration and necrosis of epithelial cells [8]. Structural damage in Gonads (Fig. 3.3.2.B) shows degeneration of seminiferous tubules (the tubular structure responsible for sperm formation becomes damaged), Reduction of spermatogenic cells (decreased number of spermatogonia, spermatocytes, and spermatids), Vacuolization in germinal epithelium (formation of empty spaces within cells due to toxic stress). Necrosis of germ cells (death of sperm producing cells). Disorganization of testicular tissue (irregular arrangement of cells inside seminiferous tubules) leads to decreased sperm production and abnormal gamete development [15]. The liver is the main organ responsible for detoxification, metabolism, and storage of lipids. Structural damage (Fig. 3.3.3.B) shows Hepatocellular degeneration (damage and swelling of hepatocytes). Vacuolization of hepatocytes (formation of Vacuoles due to lipid accumulation and cellular stress). Necrosis of liver cells (death of hepatocytes caused by toxicity), Disruption of hepatic cords (irregular arrangement of liver cells around sinusoids). Congestion of blood sinusoids (accumulation of blood in liver vessels) and Fatty degeneration (steatosis) (excessive lipid deposition in liver tissue) [16]. The kidney in fish plays a vital role in excretion, osmoregulation, hematopoiesis, and detoxification, making it highly sensitive to long-term exposure to PCBs. Structural damage (Fig. 3.3.4.B) shows Degeneration of renal tubules (structural damage to kidney tubules responsible for filtration and re-absorption), Tubular necrosis (death of tubular epithelial cells), Vacuolization of tubular cells (formation of vacuoles due to cellular stress and lipid accumulation), Glomerular shrinkage or degeneration (damage to the filtration units of the kidney), Congestion of blood vessels (accumulation of blood within renal tissues) and Interstitial edema (fluid accumulation between kidney tissues) [13].

These structural alterations negatively affect Respiration, Reproduction, Digestion, Metabolism, Excretion and overall fish health in contaminated aquatic environments.

CONCLUSION:

The toxicological assessment from the present study indicates that the Polychlorinated Biphenyls (PCBs)

cause different degrees of injuries to the fish Gills, Gonads, Liver and Kidney. It is recommended to treat chemicals before discharging to the resources to avoid negative impact on fishes. The accumulation of Polychlorinated Biphenyls in their tissues is dose and time dependent, and therefore may be reflective the levels of PCBs in the environment. Polychlorinated Biphenyls presence in the aquatic environment is toxic, hence directly or indirectly harmful to the fish and human beings but their mode of action could be different. Fish Histopathology is a promising tool in the further development of aquatic toxicology, moreover; it may be useful for the mammalian toxicology and carcinogenesis. Zebra fish liver tumor possesses molecular similarities to human liver cancer, making a pathway for cancerogenous study using fish as a model animal. Histological biomarkers of toxicity in fish organs are useful indicator of environmental pollution.

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