



## Biochemical Responses of Protein and Lipid Levels to Polychlorinated Biphenyls (PCBs) Chronic Bioaccumulation in *Labeo rohita*

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### ABSTRACT

Polychlorinated Biphenyls (PCBs) are known to accumulate in fat rich tissues due to their lipophilic characteristics, leading to bioconcentration and biomagnifications in the food chain. This accumulation poses risk to human health and the environment. The bioaccumulation of Polychlorinated Biphenyls in *Labeo rohita* can significantly impact their protein and lipid concentrations. The current research investigated how PCBs impact on protein and lipid levels in *Labeo rohita* under laboratory conditions. *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°C. Chronic PCBs Lc50 is 0.90 ppm for 28 days, PCBs deposition in tissues had increased significantly with dose and exposure duration dependent manner. The Lowry method was used to measure total protein concentrations, while lipid content was determined through Folch extraction method. The study results indicated that biochemical components showed a significant decrease which linked to increasing dosages of the treatment. The control group showed protein levels of 6.03 mg/g which decreased to 3.51 mg/g at 0.90 ppm (Lc50) while lipid levels dropped from 13.06 mg/g to 9.81 mg/g. (Lc50). The study reveals that, the PCBs exposure causes metabolic stress which results in the depletion of necessary biochemical reserves. The study demonstrates that *Labeo rohita* protein and lipid levels function as reliable biomarkers to evaluate how aquatic ecosystems experience toxicological effects and environmental contamination.

**Keywords:** Polychlorinated Biphenyls (PCBs), Protein, Lipid, depletion, *Labeo rohita*, Lowry's Method, Folch Method, Biochemical Changes, Biomarker.

### INTRODUCTION

Industrial discharge, agricultural runoff, sewage effluents and persistent organic pollutants create environmental pollution which damages aquatic ecosystems [7]. Water bodies function as chemical storage areas which cause toxic substances to build up in aquatic life forms through bioaccumulation and biomagnifications [4]. Fish populations face high risks from water pollution because harmful substances enter their bodies through gills and body tissues. The presence of heavy metals and PCBs in the environment interrupts metabolic functions and enzyme activity and depletes energy reserves [12]. The environment suffers from long-term exposure which causes growth and reproductive and survival problems that lead to a decline in aquatic biodiversity and ecosystem stability. Polychlorinated Biphenyls (PCBs) are synthetic chlorinated hydrocarbons which manufacturers used in electrical equipment and cooling systems and transformers until many countries prohibited their use [23]. These substances maintain resistance to degradation because of their highly stable chemical structure and their ability to dissolve in fats which enables them to remain in aquatic environment for extended periods. PCBs build up in fish tissues because of their fat-soluble properties and they spread through food chains which results in increased PCBs concentration in higher trophic levels. Oxidative stress together with endocrine disruption and immune suppression and metabolic disturbances occur because of ongoing exposure to the substance [13]. PCBs function as probable human carcinogens which continue to contaminate the environment because of existing pollution control measures. Fish tissues primarily contain proteins and lipids, which are essential biochemical constituents. Proteins serve as structural components, enzymes, hormones, and transport molecules, which are essential for sustaining growth and metabolic processes. Lipids serve as primary energy stores, essential parts of cell membranes, and steroid hormone production materials. The concentration of proteins and lipids differs according to species, age, nutritional factors, and environmental conditions. Under toxic stress conditions, the metabolic processes of organisms lead to a reduction of their biochemical components because their energy requirements increase [9]. Polychlorinated biphenyls (PCBs) cause significant cellular and biochemical alterations in fish *Labeo rohita* due to their persistent nature and ability to accumulate in tissues [1]. Aquatic organisms undergo toxic stress assessment through the use of protein and lipid measurements as biochemical biomarkers. Protein concentration changes provide information about changes in metabolic activity and enzyme function and tissue damage extent. The body uses energy reserves through lipid content reduction when exposed to pollutants. PCBs exist as lipophilic substances which enable them to interact with lipid metabolism processes while they build up in fatty body tissues [3].

The assessment of protein and lipid levels in *Labeo rohita* fish enables researchers to measure pollutant effects and determine environmental health conditions.

*Labeo rohita* (Rohu) is one of the most important Indian major carps which South Asian countries have established as their primary fishery species. The species serves as an ideal model organism for toxicological research because of its economic value and environmental impact [8]. The species serves as an indicator of freshwater system health because it exists in freshwater environments. The research study found Rohu to be an appropriate research model because its physiological functions and biochemical makeup are well documented [14]. The species functions as a bioindicator for aquatic toxicology studies because it shows high sensitivity to PCBs and handles bioaccumulation of hazardous substances. While there are studies on PCBs contamination worldwide, [15] 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

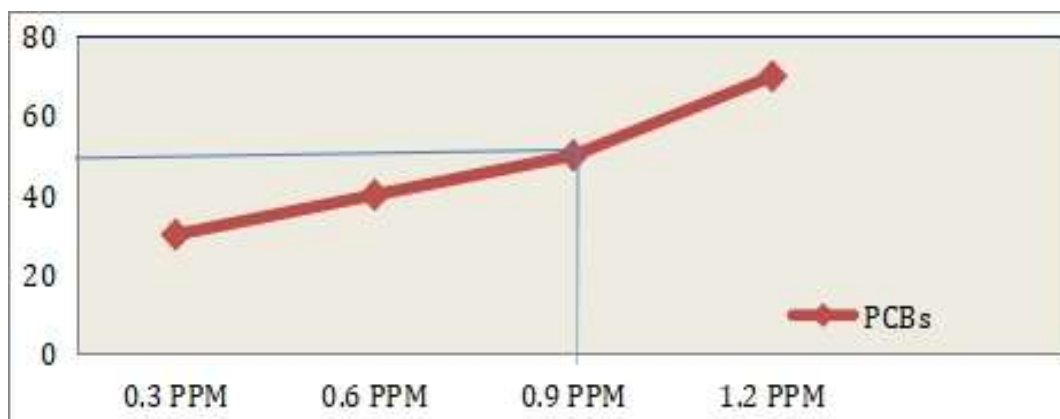
Groups of healthy, disease-free 3 months old *Labeo rohita* 25-30g in weight, 9-10 cm in length were procured from local fish dealer. Fishes were acclimatized for 1 week at laboratory conditions, were fed with commercial aquarium food. Laboratory water analyzed for different physico-chemical parameters [2] [3]. Aquarium water was maintained specific temperature and air flow as natural habitats of the fish, no fish mortality recorded while acclimatization. PCBs Lc50 for 96 hrs. was statistically determined [5] and predicted bearable PCBs concentration for chronic exposure. Well acclimatized fishes showing no signs of stress were selected and divided into five groups, exposed to PCBs toxicant. The first group served as a control and remaining four were experimental. A dose of 0.30 ppm, 0.60 ppm and 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 [22]. After completions of experiment 4 fish from each group were anesthetized with MS-222 (Sigma) [20]. Sacrificed and tissues were collected from both control and experimental group. Tissues were homogenized in 1 N NaOH and centrifuged at 5000 RPM for 10 minutes, and the supernatant was collected for biochemical analysis. Total protein was estimated according to the Lowry method [11] [19], which relies on protein reactions with copper sulfate in an alkaline solution, followed by testing with Folin–Phenol reagent. The measurement at 650 nm used BSA as standard to determine total protein according to the Lowry method, which needed proteins to react with copper sulfate in an alkaline solution followed by testing with Folin–Phenol reagent. The Folch extraction method [6] [17] used to extract lipids, which they reported as milligrams per gram of tissue weight. The researchers used statistical methods to evaluate their gathered data, which they used to determine significance when results reached  $p < 0.05$ .

## RESULT AND DISCUSSION

**3.1: Lc50:** The 50% mortality was observed within 28 days, Lc50 value calculated by Probate Mortality and Graphic method to be 0.90 ppm of PCBs (Table 3.1.1 and Fig. 3.1.1)

**Table 3.1.1:** % and Probate Mortality of PCBs in *Labeo rohita* (0.90 ppm)

PCBs (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



**Fig. 3.1.1:** PCBs Lc50 in *Labeo rohita* (0.90 ppm)

### 3.2: Total Protein Concentration:

#### 3.2.1: Protein Concentration in Control:

Chronic bioaccumulation of PCBs exposure causes a significant decrease which linked to increasing dosages of the treatment in *Labeo rohita*. The control group provides baseline biochemical values for comparison with treated groups. The Protein concentration is expressed in mg/g tissue weight.

**Table 3.2.1:** Total Protein Concentration of Control Group in *Labeo rohita*

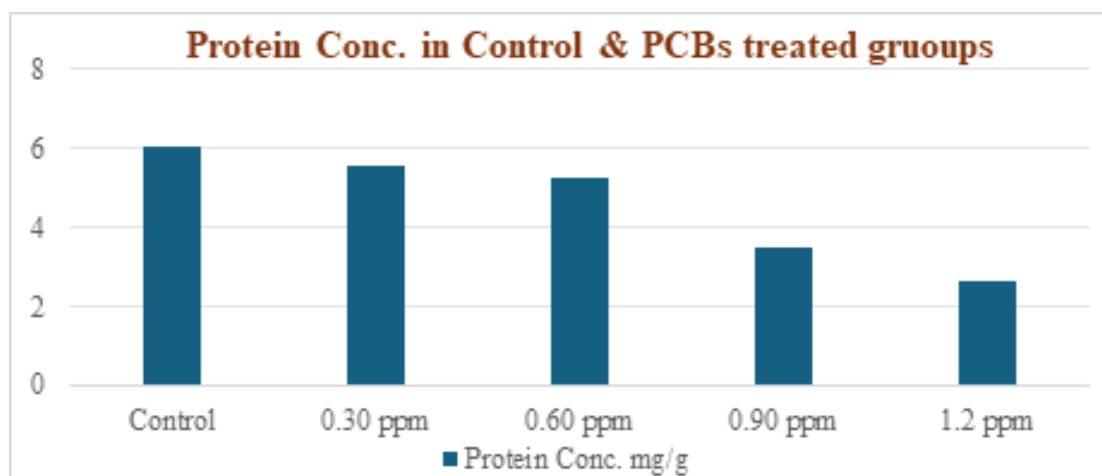
Fish Sample	Absorbance (660 nm)	Protein Conc. (mg/g)
C1	0.82	6.10
C2	0.80	5.95
C3	0.84	6.20
C4	0.79	5.88
C5	0.83	6.02
Mean $\pm$ SD	—	6.03 $\pm$ 0.12 mg/g

The control group displayed an average protein concentration of 6.03  $\pm$  0.12 mg/g which demonstrated that the unexposed fish maintained stable metabolic and physiological states.

#### 3.2.2: Dose-Dependent Changes in Protein Levels:

**Table 3.2.2:** Total Protein Concentration of Control & PCBs treated Groups in *Labeo rohita*

PCBs Conc. (ppm)	Protein Conc. (mg/g)	% Decrease Compared to Control
Control (0.00)	6.03	—
0.30 ppm	5.53	8.29%
0.60 ppm	5.22	13.43%
0.90 ppm	3.51	41.79%
1.20 ppm	2.66	55.89%



**Fig. 3.2.1:** Total Protein Concentration of Control and PCBs treated Groups in *Labeo rohita*.

The results show that total protein levels decrease in direct correlation to increasing PCBs concentration. The results group control indicated 6.03 mg/g protein but exposure to 0.30 ppm and 0.60 ppm caused moderate reductions of 8.29% and 13.43% respectively. The study found a significant drop in metabolic function at 0.90 (Lc50) ppm which indicated a decrease of 41.79% and at 1.20 ppm which showed a decrease of 55.89% to lethal metabolic stress.

### 3.3: Total Lipid Concentration:

Chronic bioaccumulation of PCBs exposure causes a significant decrease which linked to increasing dosages of the treatment in *Labeo rohita* [16]. The control group provides baseline biochemical values for comparison with treated groups. The lipid concentration is expressed in mg/g wet tissue weight.

**Table: 3.3.1:** Total Lipid Concentration of Control Group in *Labeo rohita*

Sr. No.	Sample No.	Tissue Weight (g)	Lipid Concentration (mg/g)
1	C1	1.0	13.10
2	C2	1.0	12.95
3	C3	1.0	13.20
4	C4	1.0	12.88

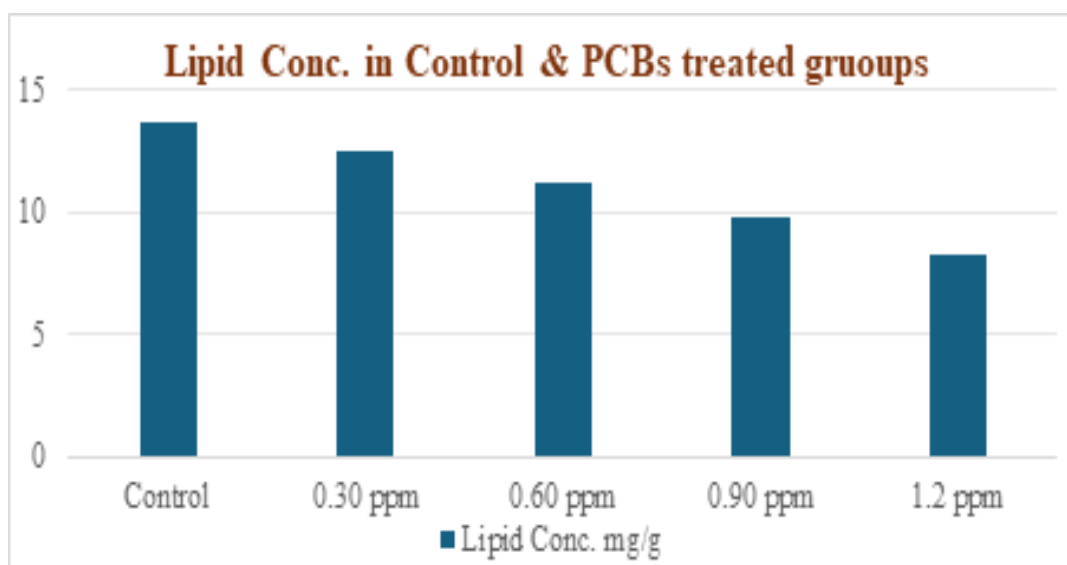
5	C5	1.0	13.17
Mean ± SD	—	—	13.06 ± 0.14 mg/g

The control group of *Labeo rohita* showed a mean total lipid concentration of 13.06 ± 0.14 mg/g which demonstrated that metabolic activity remained stable while the physiological condition stayed normal during the time period without PCBs exposure.

• **Dose-Dependent Changes in Lipid Levels:**

**Table 3.3.2:** Total Lipid Concentration of Control & PCBs treated Group in *Labeo rohita*

PCBs Conc. (ppm)	Lipid Concentration (mg/g)	% Decrease Compared to Control
Control (0.00)	13.06	—
0.30 ppm	12.53	4.06%
0.60 ppm	11.22	14.09%
0.90 ppm	9.81	24.89%
1.20 ppm	8.27	36.67%



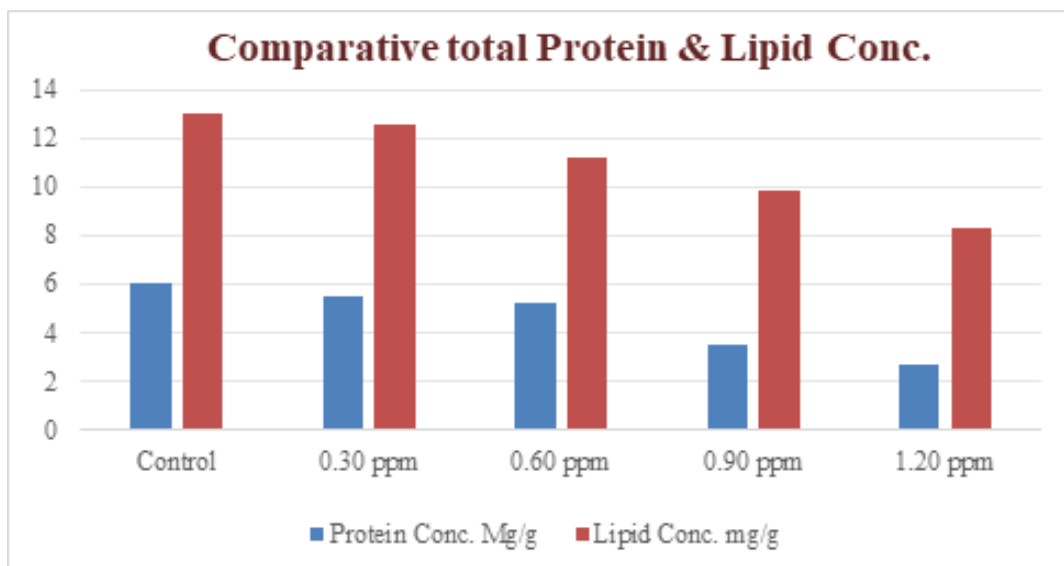
**Fig. 3.3.1:** Total Lipid Concentration of Control and PCBs treated Group in *Labeo rohita*.

The total lipid concentration in *Labeo rohita* showed a clear dose-dependent decline with increasing PCBs concentrations through the experimental design which required 28-day exposure testing. The control group recorded 13.06 mg/g, while treated groups showed progressive reduction: 12.53 mg/g, 11.22 mg/g, 9.81 mg/g, and 8.27 mg/g in 0.30, 0.60, 0.90 (Lc50) and 1.20 ppm respectively.

**3.4: Comparative Analysis of Protein and Lipid Alterations:**

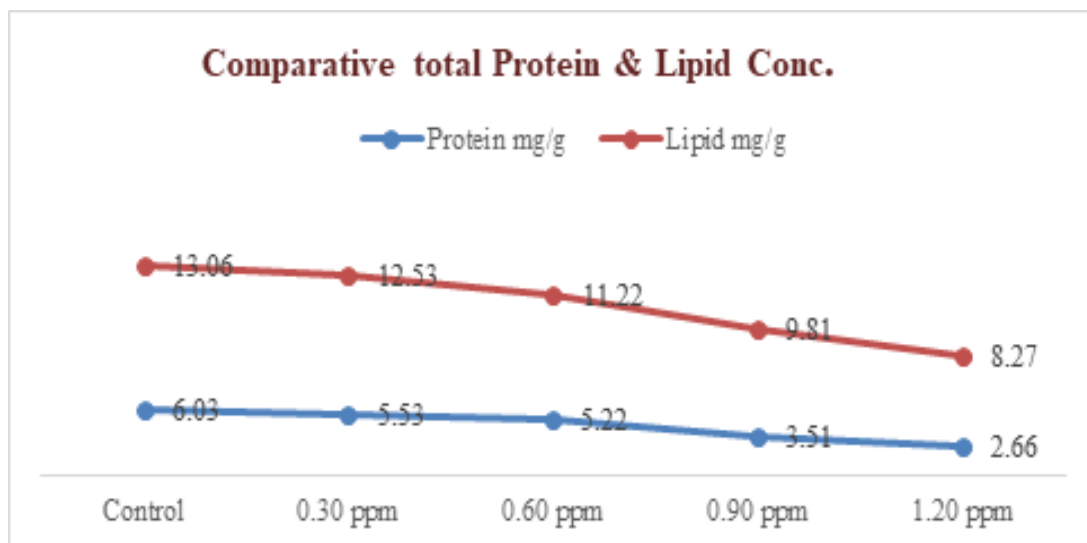
**Table 3.4.1:** Total Protein and Lipid Concentration in *Labeo rohita*

PCBs Conc. (ppm)	Protein (mg/g)	% Decrease in Protein	Lipid (mg/g)	% Decrease in Lipid
Control (0.00)	6.03	—	13.06	—
0.30 ppm	5.53	8.29%	12.53	4.06%
0.60 ppm	5.22	13.43%	11.22	14.08%
0.90 ppm	3.51	41.79%	9.81	24.88%
1.20 ppm	2.66	55.88%	8.27	36.68%



**Fig. 3.4.1:** Comparative Protein and Lipid Concentration in *Labeo rohita*.

The data clearly show that *Labeo rohita* experiences a decrease in protein and lipid levels when exposed to higher PCBs concentrations. The protein content showed a gradual decline from 6.03 mg/g in control to 2.66 mg/g at 1.20 ppm (55.88% reduction). The lipid levels decreased from 13.06 mg/g to 8.27 mg/g (36.68% reduction) following the same declining pattern. The higher concentrations of 0.90 (Lc50) and 1.20 ppm showed more significant decline because they caused extreme metabolic distress.



**Fig. 3.4.2:** Comparative total Protein and Lipid Conc. in *Labeo rohita*.

The graphical trend would show a steady downward slope in both protein and lipid levels as PCBs concentration increases. The actual protein depletion shows stronger effects than the lipid reduction which indicates that the body undergoes increased proteolysis during the toxic stress period. The findings demonstrate that protein and lipid levels in *Labeo rohita* function as dependable biochemical indicators for assessing PCBs-induced toxicity and environmental pollution.

The present study shows that *Labeo rohita* fish experienced significant biochemical changes after 28 days of PCBs exposure which specifically affected their protein and lipid metabolism. The total protein levels showed a decrease from 6.03 mg/g in control to 3.51 mg/g at 0.90 ppm (Lc50) which corresponds to a reduction of 41.79% because the toxic stress condition caused the fish to lose their ability to produce proteins while their bodies underwent increased protein breakdown. PCBs create oxidative stress in organisms which results in the destruction of structural and functional proteins through the impairment of their enzyme systems [10]. The lipid content dropped from 13.06 mg/g in control to 9.81 mg/g. (Lc50) which represents a 24.88% decrease because the fish needed to use proteins as an energy source during toxic stress situations while it stores lipids as its primary energy reserve and uses them when faced with extended stress. The decline of protein and lipid levels will result in negative effects on growth, reproduction, immune system function and general body performance [21]. The decline of protein and lipid levels will result in negative effects on growth reproduction immune system function and general body performance. PCBs act as lipophilic substances which disrupt lipid metabolic processes by depleting essential biochemical substances [18]. The comparative analysis

demonstrates that PCB exposure induces biochemical changes which occur at different levels based on the concentration of the substance. The higher proteolysis rate during stress conditions resulted in greater protein reduction when compared to lipid loss. The study results indicate that protein and lipid measurements function as accurate biochemical indicators for evaluating PCB toxicity in aquatic life forms.

## CONCLUSION

Biochemical responses of protein and lipid concentrations from the present study indicates that, the *Labeo rohita* fish exposed to Polychlorinated Biphenyls (PCBs) for 28 days experienced dose-dependent decreases in total protein and lipid levels. Protein levels declined from 6.03 mg/g which decreased to 3.51 mg/g at 0.90 ppm (Lc50) while lipid levels dropped from 13.06 mg/g to 9.81 mg/g. (Lc50), indicating severe metabolic disturbance. The protein and lipid content serve as dependable biochemical metrics for assessing toxic stress conditions. The lipophilic nature of PCBs together with their persistent pollution properties enables these substances to build up within aquatic an animal which creates major environmental dangers through food chain contamination. The results reveal that, water bodies require ongoing monitoring through biochemical biomarkers while additional long-term research should examine molecular mechanisms together with bioaccumulation patterns and environmental management approaches to decrease PCBs pollution and safeguard aquatic species.

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