

SYNERGISTIC EFFECTS OF POLYCHLORINATED BIPHENYLS AND CADMIUM ON OXIDATIVE STRESS, ANTIOXIDANT ENZYMES AND IMMUNITY IN *LABEO ROHITA*

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ABSTRACT

Environmental pollutants, including cadmium (Cd) and polychlorinated biphenyls (PCBs), are widely distributed in the environment. These pollutants are highly toxic to living organisms and cause serious damage to both terrestrial and aquatic ecosystems. The present study was designed to determine the effects of combined exposure to PCBs and Cd on the freshwater fish *Labeo rohita*. A total of 80 *L. rohita* were randomly divided into four groups (n = 20/group): control, PCB-treated (10 µg/L), Cd-treated (1 mg/L) and PCB + Cd co-treated (10 µg/L + 1 mg/L). The experiment was conducted for 21 days. The results revealed that exposure to PCBs and Cd alone caused significant damage in fish. However, greater damage was observed in the PCB + Cd co-treated group compared with the Cd-treated and PCB-treated groups. Co-administration of PCBs and Cd increased the levels of reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), while decreasing the activities of antioxidant enzymes. Furthermore, Cd and PCB exposure disturbed the hematological parameters of *L. rohita*. Increased levels of hepatic enzymes, including AST, ALT and ALP, were also observed in the blood, indicating liver damage. In addition, Cd and PCB exposure reduced fish immunity by lowering lysozyme activity, phagocytic activity, IgM and ACP levels. The findings demonstrated that combined exposure to PCBs and Cd caused severe toxic effects in *L. rohita*, suggesting that these pollutants become more harmful to aquatic organisms, especially freshwater fish, when present together.

1. INTRODUCTION

Aquatic ecosystems today continue to be subjected to unparalleled stress upon contact with a combination of new and old pollutants, posing a threat to both fish and human populations. Among such polluters of utmost concern continue to be Polychlorinated Biphenyls (PCBs) and the heavy metal cadmium (Cd) (Sattar *et al.*, 2025).

PCBs continue to be a family of synthetic organic chemicals widely used across a wide range of applications owing to thermal stability and persistence until they were prohibited owing to persistence, bioaccumulative nature, and documented toxicity (Feng *et al.*, 2016). Likewise, cadmium, a preventable and highly toxic heavy metal contaminant, finds entry into aquatic ecosystems through effluents originating across industries and agricultural runoff (Naderi *et al.*, 2020). The two contaminants have the potential to become encapsulated in the tissues of aquatic organisms and rise up the aquatic food chain, which

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presents a high risk to the integrity of ecosystems. Aquatic ecosystems have often been evaluated simply by the use of fish species to provide a form of bioindicator to measure the integrity of aquatic systems and associated toxicologic effects on living organisms in which enzymatic activity (e.g. lactate dehydrogenase (LDH), succinic dehydrogenase (SDH), creatine kinase (CK) and alkaline phosphatase (ALP) remain a sort of pollution biomarker in waters (Adamovsky et al., 2024).

Previous studies have showed that polychlorinated biphenyls (PCBs) and cadmium cause toxic effects of to fish physiology. Studies have shown that PCBs are able to exhibit a wide range of adverse effects concerning reproductive injury, neurotoxicity, and immunotoxicity predominantly through the ROS pathway and subsequent oxidative stress (Feng et al., 2016). Likewise, cadmium exposure has been reported to cause extreme oxidative stress, which results in disruption of cellular homeostasis and damage to lipids, proteins, and DNA. Cadmium may also interfere with essential metabolic functions, including the generation of antioxidant enzymes (Chen et al., 2019). To a large extent, these solitary pieces of evidence have already determined a definite correlation with these pollutants and fish bodily dysfunction, largely in connection with key organs of gills and liver (Davis et al., 2020).

Water organisms are denied an opportunity to experience a monotypic vacuum of toxins but a blend of pollutants that may or may not be synergistic or antagonistic (Zou et al., 2022). Co-exposure of cadmium and polychlorinated biphenyls (PCBs) is a topical and least researched problem because both substances are found together in most polluted water bodies. The question of whether the interaction of two such pollutants that are known to cause oxidative stress, through various different mechanisms, is larger than the combined effect of the two chemicals is of critical importance. Such synergies can also circumvent native protective mechanisms within an organism and have more dramatic and rapid toxicological effects (Lee et al., 2023). In the present study, the synergistic impact of co-exposure of PCBs and cadmium has been evaluated on the freshwater fish of commercial and ecological importance, *Labeo rohita*. By evaluating key oxidative stress parameters,

antioxidant enzyme activities, and lipid profile changes of the species, the combined toxicological responses of the two pollutants have been investigated. The findings of the current research will significantly contribute to the understanding of combined effects of persistent organic pollutants and heavy metals, and hence a more realistic estimation of environmental hazards and a contribution to the regulation of more efficient water quality management strategies to ensure aquatic biodiversity and food security.

2. MATERIALS AND METHODS

2.1. Ethical approval

All methods used in the experiments were found to be acceptable to the Animal Research Ethics Committee (GCUF/ERC/731). Fish that are caught should be handled in a manner that avoids causing pain, distress, suffering, and the loss of external mucus or scales inadvertently. Handling time and experimental procedure should be kept to a minimum to avoid causing unnecessary stress and prolonged exposure (Bennett et al., 2016).

2.2. Chemicals

Chemicals Polychlorinated Biphenyls (PCBs) and Cadmium (Cd) were purchased from Sigma Aldrich (Shanghai, China). All other chemicals used in the experiment were of analytical grade.

2.2. Experimental Fish and Acclimatization Protocols

80 *L. rohita* adults were bought from a local fish market in Faisalabad, Pakistan. The fish were kept at regular laboratory conditions at Government College University, Faisalabad, Pakistan for three weeks of acclimatization. The fish were fed twice daily with 2 % of the body weight. A random sub-sample of 20 fish (13.7 ± 1.7 cm body length, 55.6 ± 7.4 g body weight) were chosen after acclimatization for the study by the stratified random sampling method to represent the population over the size range. This method was used to ensure the chosen fish are representative of the entire population.

2.4. Experimental design

During the acclimatization period, the fish were randomly distributed in four glass aquaria of 130 liters each with a capacity to hold a density of 20 fish per

tank. The aquaria were allocated to the control group, PCB-treated (10 µg/L), Cd-treated (1mg/L) and PCB+Cd (10 µg/L+1mg/L) treated group. The experiment was conducted for 21 days, and the fish were anesthetized and sacrificed on completion of the experiment. Blood was drawn in blood vials and stored at -20°C for analysis. The liver was dissected and stored in an ice box, kept at -20°C for further biochemical assessment. Blood samples were drawn for estimation of biochemical parameters.

2.3. Hematological analysis:

Hematology analyses were performed to examine various parameters of the blood using XQ-320™ Automated Hematology Analyzer (Sysmex, Japan). Parameters of basic hematology were measured such as red blood cell (RBC) count, white blood cell (WBC) count, platelet (PLT) count, Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) and hematocrit (HCT) percentage and hemoglobin (Hb) level.

2.4. Antioxidant and Oxidative Stress

For oxidant and other markers estimation, blood tissues were homogenized and supernatant collected and put at -80°C. ROS levels were estimated after Hayashi *et al.* (2007). Levels of GSH and TBARS were estimated after Iqbal *et al.* (1996). Activities of SOD and CAT in homogenate were estimated after Winterbourn *et al.* (1975) and Aebi *et al.* (1972) methodologies, respectively. Further, activity of POD was estimated after Chance and Maehly (1955) methodology.

2.5. Hepatic enzymes:

Levels of hepatic enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP) were estimated having used commercially available reagents supplied by RANDOX Limited.

2.6. Immunological Parameters:

Phagocytic activity, lysozyme activity, and Immunoglobulin M (IgM) activity were determined according to protocols prepared by Ellis (1990), Cai *et al.* (2004), and Sharma *et al.* (2010). Additionally, Alternative complement pathway (ACP) activity was determined using a procedure developed by Yano (1992).

2.7. Statistical analysis

The results are presented as Mean ± SEM. SPSS (v 26) was used for statistical analysis and generating graphs. One-way ANOVA followed by Tukey's test were applied to compare the groups. $P < 0.05$ was considered significant.

3. RESULTS

3.1. Hematological Parameters

The results showed that RBCs, Hb, MCV, MCH, and HCT were lowered, and WBCs and PLT were increased significantly ($P < 0.05$) in PCB, Cd and PCB + Cd co-treated groups as compared to the control. Moreover, it was revealed that PCB + Cd co-administration brought the most disturbances in the mentioned hematological parameters, that is evident from their significant ($P < 0.05$) differences from the PCB and Cd alone treated groups. Thereby predicting that PCB + Cd co-treatment was the most toxic to the fish. Table 1 shows the impact of PCB and Cd treatments on hematological parameters of *L. rohita*.

3.2. Oxidative and antioxidant parameters

Table 2 demonstrates the effect of Cd and PCB on oxidative stress markers and antioxidant enzymes of *L. rohita*. It was observed that Cd and PCB treatment substantially ($P < 0.05$) decreased the activities of antioxidant enzymes and increased the levels of TBARS and ROS. Moreover, the disturbances in these parameters were markedly increased ($P < 0.05$) further in PCB + Cd co-treated fish. This suggests that PCB + Cd co-treatment increased the oxidative stress in *L. rohita*.

3.3. Hepatic enzymes

It was observed that Cd and PCB treatment resulted in a significant ($P < 0.05$) levels of hepatic enzymes as compared to the control. Moreover, the highest levels of hepatic enzymes were found in PCB + Cd co-treated group that shows significantly higher levels of these enzymes as compared to PCB and Cd alone treated groups. These results predict that PCB + Cd treatment significantly damaged the hepatic tissues of *L. rohita*. Figure 1 shows the impact of PCB and Cd on the levels of hepatic enzymes.

3.4. Immune parameters

Figure 2 demonstrates the impact of Cd and PCB treatment on immune parameters of *L. rohita*. It was revealed from our results that PCB and Cd treatment notably ($P < 0.05$) lowered the activities of immune parameters. Similarly to other parameters, the most disturbance in immune parameters was also brought by PCB + Cd co-treatment, that is evident from significantly ($P < 0.05$) activities of immune parameters in combine treated group as compared to Cd and PCB alone treated groups.

4. DISCUSSION

The current study was designed to investigate the synergistic effects of co-exposure of Polychlorinated Biphenyls (PCBs) and cadmium (Cd) on the health of the freshwater fish, *L. rohita*. The findings from the hematological, oxidative stress, hepatic, and immune parameters all point towards the fact that the interactional effect of PCB and Cd exposure is more harmful compared to the effect of each pollutant singularly. The findings indicated that such pollutants have a synergistic effect, which emphasizes the underappreciated dangers of co-contamination in aquatic environments.

The hematological profile indicated a considerable reduction in red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (HCT) in all the treated groups relative to the control, the biggest reduction found in the PCB + Cd co-treated group (Table 1). This means that there is an anemic condition, which is a normal physiological response to exposure to unhealthy chemicals. The decrease in these parameters correlates with a decrease in the capacity of blood to perform the oxygenation process, leading to hypoxia in the tissues and the consequent metabolic disturbances (Almahallawi et al., 2024). The further rise in the amount of white blood cell (WBC) and platelet (PLT) indicates an acute inflammation and stress reaction as the fish immune system is combating against an adverse challenge. Increased toxicity as a result of synergist effect is further justified by the fact that both combined treatment groups had maximum changes (Abalaka, 2013).

The findings are also confirmed by the results on markers of oxidative stress and the activity of

antioxidant enzymes. Interestingly, all the treatment groups had very high levels of the reactive oxygen species (ROS) and TBARS, which are signs of lipid peroxidation (Ahmed et al., 2025). This observation results in an assumption that not only polychlorinated biphenyls (PCBs), but also cadmium (Cd) is a toxicant, and oxidative damage is a well-investigated toxicity mode. In addition to this change in plasma glutathione levels, the activity of several antioxidant enzymes, including peroxidase (POD) and superoxide dismutase (SOD), was also reduced (Ghazanfar et al., 2018). This effect suggests that an internal defense mechanism against antioxidants had been disturbed by extreme oxidative stress caused by the contaminants (Bukowska, 2004). The majority of the imbalances previously observed in two sets of co-treatments were reproduced, thereby contributing to the demonstration of two-pollutant synergies.

Some symptoms of hepatic injury include an increase in the levels of hepatic enzymes, such as alkaline phosphatase (AP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), which appeared in this exposed fish (Abdel-Tawwab et al., 2018). Since it is the largest organ that performs the detoxification process, high concentrations in the blood are definite signs of hepatocellular dysfunction. Hepatotoxic effects were largely observed when the checkerboard assays were co-exposed to polychlorinated dibenzenedicarboxylic (PCBs) and cadmium (Cd), with the co-exposure of the two components recording the highest hepatotoxic effect (Jamil et al., 2024). This finding was a reward because it has been established that the liver is mostly susceptible to free radicals' exploitation.

Lastly, the parameters of immunity, i.e. lysozyme, phagocytic activity, immunoglobulin M (IgM) and alternative complement pathway (ACP), were shown to be highly suppressed in the groups of treatment (Chakrabarti et al., 2014). This type of immunosuppression exposes fish to infections and other diseases. Both PCB and cadmium control showed the most aggressive of immune responses and consequently, display the extreme danger of poor health that follows all such synergistic poisons (Yasin et al., 2025). Finally, the findings of this paper present valuable information regarding the toxicity effects of co-contamination. PCB and cadmium together cause a

stronger effect on the physiological, biochemical, and immunological integrity of *Labeo rohita* compared to the effect of an individual pollutant (Giri *et al.*, 2016). These results may indicate that current environmental regulations should undergo a review to accommodate these multidimensional interactions of contaminants within water systems.

5. CONCLUSION

Finally, the co-treatment with Cd and PCB caused serious damages in *L. rohita*. Treatment with Cd and PCB decreased antioxidant enzymes, immunity indices and augmented oxidative stress and hepatic parameters. In addition, they also disrupted the hematological parameters of fish. This is an indication that Cd and PCB are highly toxic to the aquatic life. As such, efforts must be made to minimize the amount of pollution in the water that is the primary source of exposure of these chemicals to living organisms.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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Table 1. Impact of PCB and Cd on hematological parameters of *L. rohita*.

Parameters	Groups			
	Control	PCB	Cd	PCB + Cd
RBCs ($10^6/\mu\text{L}$)	2.17 $\pm$ 0.10 ^a	1.58 $\pm$ 0.09 ^b	1.25 $\pm$ 0.07 ^b	0.86 $\pm$ 0.06 ^c
WBCs ($10^3/\mu\text{L}$)	10.21 $\pm$ 0.19 ^d	13.81 $\pm$ 0.15 ^c	16.92 $\pm$ 0.25 ^b	25.17 $\pm$ 0.24 ^a
PLT ($10^3/\mu\text{L}$)	31.39 $\pm$ 0.42 ^d	37.14 $\pm$ 0.39 ^c	40.94 $\pm$ 0.35 ^b	47.27 $\pm$ 0.49 ^a
Hb (g/dL)	6.09 $\pm$ 0.10 ^a	4.99 $\pm$ 0.18 ^b	3.62 $\pm$ 0.12 ^c	2.21 $\pm$ 0.12 ^d
MCV (fL)	153.19 $\pm$ 1.07 ^a	132.99 $\pm$ 0.74 ^b	110.36 $\pm$ 1.68 ^c	94.95 $\pm$ 1.34 ^d
MCH (Pg)	49.34 $\pm$ 0.43 ^a	44.02 $\pm$ 0.37 ^b	39.88 $\pm$ 0.43 ^c	25.22 $\pm$ 0.62 ^d
HCT (%)	15.25 $\pm$ 0.13 ^a	12.00 $\pm$ 0.22 ^b	10.14 $\pm$ 0.20 ^c	7.87 $\pm$ 0.16 ^d

Mean $\pm$ SEM values of RBCs ($10^6/\mu\text{L}$), WBCs ($10^3/\mu\text{L}$), PLT ($10^3/\mu\text{L}$), Hb (g/dL), MCV (fL), MCH (Pg) and HCT (%) showing different superscripts are significantly ($P < 0.05$) different from each other.

Table 2. Impact of Cd and PCB on oxidative and antioxidant parameters.

Parameters	Groups			
	Control	PCB	Cd	PCB + Cd
ROS (units/mg protein)	0.95 $\pm$ 0.06 ^d	3.48 $\pm$ 0.14 ^c	4.05 $\pm$ 0.10 ^b	7.73 $\pm$ 0.15 ^a
TBARS (nmol/TBARS formed/mg protein/min)	30.04 $\pm$ 0.19 ^c	37.92 $\pm$ 0.32 ^b	39.01 $\pm$ 0.29 ^b	48.16 $\pm$ 0.38 ^a
POD (units/min)	4.59 $\pm$ 0.16 ^a	3.59 $\pm$ 0.17 ^b	3.35 $\pm$ 0.11 ^b	2.14 $\pm$ 0.14 ^c
SOD (units/mg protein)	12.55 $\pm$ 0.11 ^a	10.48 $\pm$ 0.26 ^b	9.63 $\pm$ 0.19 ^b	8.18 $\pm$ 0.16 ^c
GSH (mmol/g tissue)	6.05 $\pm$ 0.15 ^a	4.77 $\pm$ 0.12 ^b	3.89 $\pm$ 0.07 ^c	2.83 $\pm$ 0.06 ^d
CAT (units/min)	6.14 $\pm$ 0.09 ^a	5.18 $\pm$ 0.08 ^b	4.74 $\pm$ 0.08 ^c	3.77 $\pm$ 0.06 ^d

Mean $\pm$ SEM values of ROS, TBARS, POD, SOD, GSH, and CAT showing different superscripts are significantly ($P < 0.05$) different from each other.

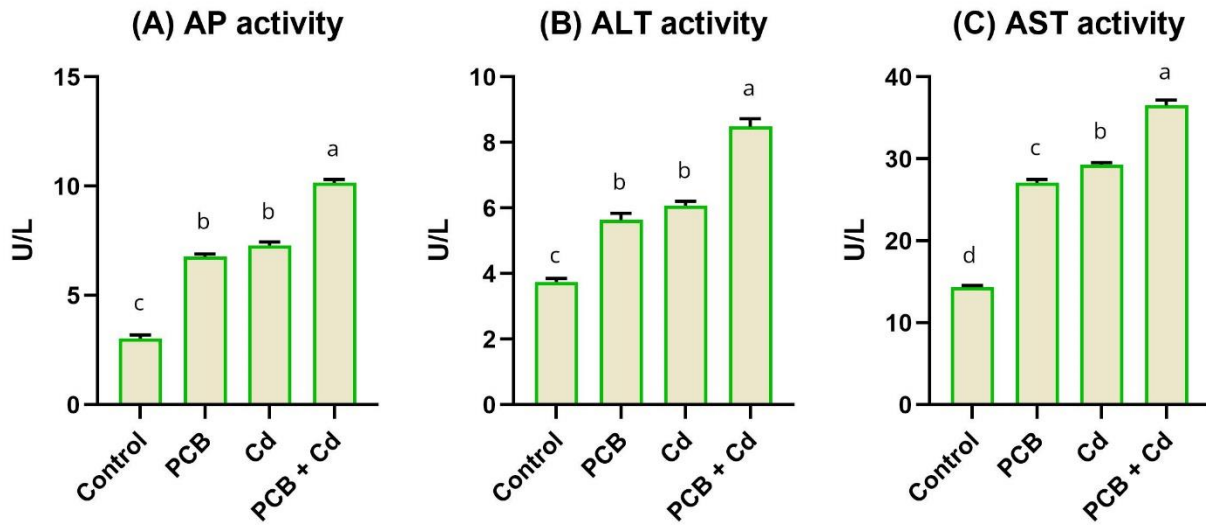
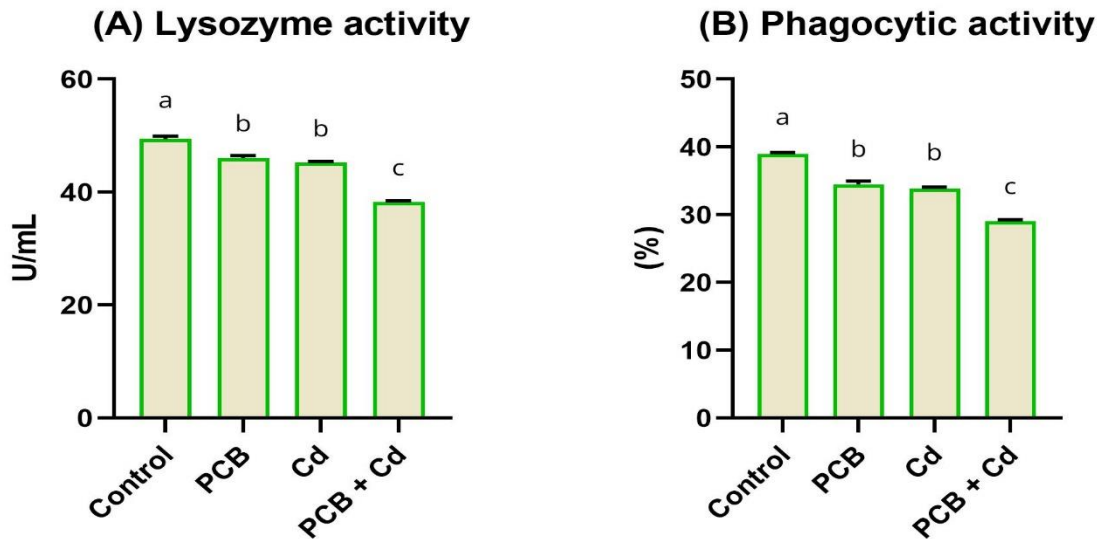


Figure 1. Impact of PCB and Cd on the activities of (A) AP, (B) ALT and (C) AST. Different superscripts above the error bars show that the values are significantly ($P < 0.05$) different from each other.



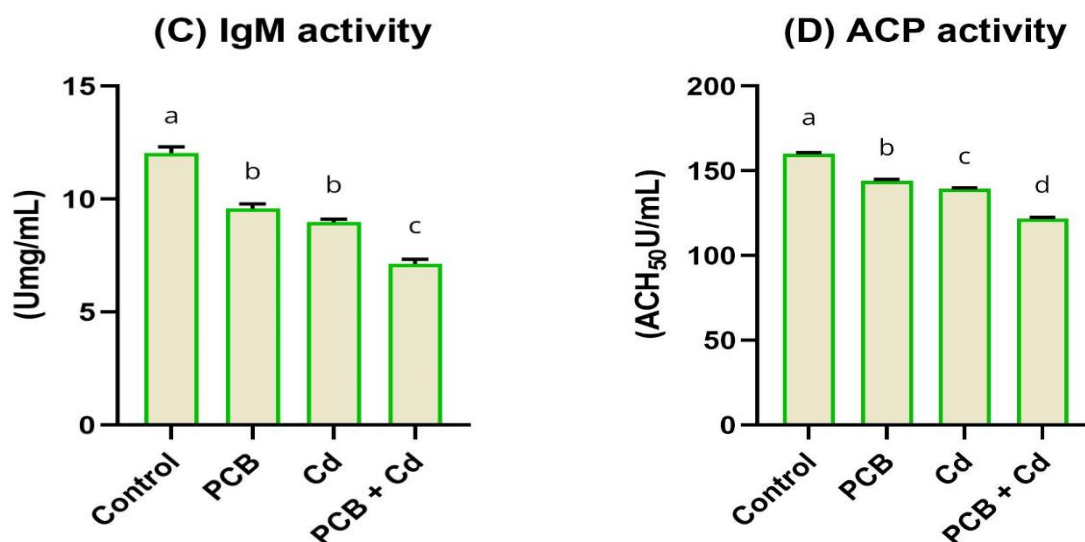


Figure 2. Impact of PCB and Cd on (A) Lysozyme activity, (B) Phagocytic activity, (C) IgM activity and (D) ACP activity. Different superscripts above the error bars show that the values are significantly ($P < 0.05$) different from each other.