

Experimental And Molecular Docking Approach To Study The Pesticide-Induced Changes In Growth, Metabolic Function And Health Biomarkers Of Freshwater Fish

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ABSTRACT

This study assessed the sublethal effects of profenofos, a commonly used organophosphate pesticide in Pakistan, on growth, metabolic function, oxidative stress, hematological biomarkers, and molecular interactions in the freshwater fish *Labeo rohita*. Fish were exposed for 28 days to three sublethal concentrations of profenofos (1%, 5%, and 10% of the 96-h LC₅₀), followed by a 14-day recovery period. Growth performance declined significantly in medium- and high-dose groups, with reduced specific growth rate and condition factor, alongside increased feed conversion ratio and hepatosomatic index, reflecting impaired nutrient utilization and hepatic stress. Metabolic assays revealed glycogen and protein depletion with elevated ALT and AST, indicating hepatocellular damage. Antioxidant responses showed initial activation of SOD, CAT, and GPx at lower doses, but inhibition under higher stress, while lipid peroxidation and reduced GSH/GSSG ratios confirmed oxidative imbalance. Hematological disruptions included declines in RBC, Hb, and Hct, with increased WBC counts, hypoalbuminemia, and elevated glucose and triglycerides, suggesting systemic physiological stress. To complement experimental findings, molecular docking analysis using modeled fish enzymes (ALT, AST, SDH, LDH-A, SOD1, CAT) demonstrated strong binding of profenofos to catalytic residues, with docking scores ranging from -5.5 to -7.9 kcal/mol. These interactions supported observed enzyme inhibition and oxidative stress. Overall, profenofos exposure suppressed growth, disrupted metabolism, induced oxidative stress, and impaired hematological health in *L. rohita*, highlighting the value of integrating biomarker assays with molecular docking for ecotoxicological monitoring of pesticide pollution in freshwater ecosystems.

Keywords: Profenofos, *Labeo rohita*, Oxidative Stress, Hematology, Biomarkers, Freshwater Ecotoxicology, Molecular Docking

Introduction

Anthropogenic pollution to the freshwater ecosystem is very sensitive since pesticides have been made one of the persistent and widespread pollution agents (Sumudumali & Jayawardana, 2021). The intensification of agriculture has led to severe intensifications in the use of pesticides, and huge amounts of the latter eventually also contaminate water bodies using the surface runoff, leaching, or aerial drift mechanisms (Wato et al., 2020; Zubairi et al., 2021). Upon entering into aquatic environment, the pesticides are taken up by non-target organisms, including fish, and cause sublethal adverse effects that may affect growth, reproduction, and survival (Kadiru et al., 2022; Akhter et al., 2024). Since fish are used both as the source of dietary protein by humans and as sentinel species to track the health of the environment, it is essential to comprehend the toxicological consequences of having been exposed to a pesticide (Ray & Shaju, 2023).

Many families of pesticides, like organophosphates, pyrethroids, carbamates, and herbicides (glyphosate), have been reported to disrupt fish physiology by causing oxidative stress, neurotoxicity, and endocrine disruption (Srivastava et al., 2016). Chlorpyrifos and diazinon are organophosphates that also inhibit acetylcholinesterase (AChE) and interfere with neuromuscular transmission, leading to behavioral dysfunctions and dysregulation of various metabolic activities (Mostafalou & Abdollahi, 2023; Chen et al., 2024). Growth inhibition, compromised swimming, and disrupted antioxidant defense systems are linked to such pyrethroids as cypermethrin and lambda-cyhalothrin (Ali, 2012; Ravula & Yenugu, 2021). Carbamates, like carbaryl and carbofuran, have the same effect on the functioning of the nervous system and cause an oxidative imbalance, which has implications on the state of energy metabolism and tissue structure (Moreira et al., 2022). Of growing concern are herbicides, especially glyphosate, which has been reported to be genotoxic as well as immunotoxic to a variety of freshwater species (Annett et al., 2014; Peillex & Pelletier, 2020; Martins-Gomes et al., 2022).

In addition to acute lethality, long-term exposure to pesticides causes complicated physiological changes that are solid biomarkers of stress (Sule et al., 2022). The shifts in antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), lipid peroxidation, hematological parameters, and endocrine hormones are regularly noted as the markers of the enzymes sensitive to toxic influence (Carmo de Carvalho e Martins et al., 2022). They are biochemical and molecular endpoints that most of the time occur before the visible alteration of growth and histopathological tissue injury, thus providing early warnings of environmental pollution (Soare et al., 2019).

Along with emerging evidence, there are large gaps to be filled in relation to the relationship between biochemical responses using biomarkers and long-term growth and health effects of exposures that are environmentally realistic (de Paula Nunes et al., 2025). Most of the literature has dealt with individual pesticides in controlled laboratory experiments, whereas in natural freshwaters, there are likely to be multicomponent mixtures and chronic exposure to fish (Saravanan & Vidhya, 2025). Moreover, intertaxonomic variation in sensitivity and adaptive routes has received

little attention and makes it difficult to generalize outcomes among economically and ecologically important taxa.

Thus, combined research utilizing growth performance, metabolism, and health biomarkers gives us a clear picture of assessing the stress indices induced by the pesticides on freshwater fish. Not only do such studies enhance our mechanistic understanding of the action of toxicants, but they also provide greater confidence in models that predict ecological risk and in approaches to pesticide management in a sustainable way.

Materials and Methods

Experimental Fish and Acclimation

Juvenile *Labeo rohita* (mean weight 20-30 g; length 12-14 cm) were bought in an approved hatchery and acclimatized in glass aquaria with dechlorinated and aerated tap water after 14 days. Its water conditions were regulated at a temperature of 26 $\pm$ 1°C, pH of 7.4 $\pm$ 0.2, dissolved oxygen of more than 6 mg L⁻¹, and total hardness of 120 to 180 mg CaCO₃ L⁻¹ at a 12:12 h light-dark photocycle. Fish acclimation continued, and throughout the experiment they were fed with a commercial pellet diet at 3 percent of the fish body weight per day as two equal portions of food. The Institutional Animal Ethics Committee accepted all the procedures of the experiment, and fish were anesthetized using MS-222 (100 mg/L) during handling and euthanized using 300 mg/L of MS-222 at the sampling time.

Test Chemical and Stock Solution Preparation

Profenofos (analytical grade, 98%) was chosen as the test chemical because it is broadly used in agriculture in the country and can be easily detected in freshwater systems. A stock solution of 1 g L⁻¹ was made in HPLC-grade acetone kept at 4°C in darkness until used. Working concentrations were made each day by suitable dilution in test water. Solvent control containing acetone volume proportion (with a concentration of not more than 0.01% (v/v)) was added to each experimental setup.

Acute Toxicity and Dose Selection

An initial assessment of acute toxicity was conducted to discover the 96-h LC₅₀ of profenofos. In the case of fish, the geometric series of concentrations were displayed, with single reloads of the pesticide every 24 h and an 80-percent water change of silhouettes under static-renewal conditions using seven fish at each concentration (n = 7 at each concentration). The mortality was measured at 24, 48, 72, and 96 h, and the LC₅₀ was calculated by using probit analysis. According to the received LC 50 value, three sublethal levels at the level of about 1, 5, and 10 percent of LC 50 were chosen to be utilized in the chronic exposure study.

Chronic Exposure Design

This chronic toxicity experiment took place by testing a 28-day exposure period and a 14-day recovery period in clean water. Five control groups were arranged, which included the control, solvent control, and three different levels of profenofos (low,

medium, and high). These were repeated three times in three aquaria with 10 fish in each tank; each tank was arranged randomly in order to reduce positional effects. The aquaria (150-200 L) were well aerated, and their fronts were partially covered with a lid to decrease volatilization and then kept at equal environmental conditions to those of acclimation. Enough water was changed every day (80%) and with a new mixture of profenofos solutions. The sampling was done on days 0, 7, 14, 28, and 42.

Growth Performance and Condition Indices

Before recording the individual body weight and length, individual animals were fasted 24 h at every sampling. Standard equations were used to calculate specific growth rate (SGR), feed conversion ratio (FCR), and Fulton condition factor (K). Hepatosomatic index (HSI) and gonadosomatic index (GSI) were measured as somatic indices using dissected tissues of subsampled fish (n = 6 per tank).

Tissue Collection and Processing

The caudal vein was lanced with heparinized syringes. The hematological analyses have used a portion of the whole blood, and the rest of the sample was centrifuged at 3000 g at 4 degrees Celsius for a duration of 10 min to isolate the serum that was later stored at minus 20 degrees Celsius to conduct biochemical analysis. Aliquots of liver, gill, and muscle tissues were taken, washed in ice-cold phosphate-buffered saline (pH 7.4), dried on paper towels, and snap-frozen in liquid nitrogen. Organs were preserved at minus 80 degrees Centigrade before being analyzed. In cases involving biochemical assays, 10% tissue homogenates were homogenized with the phosphate buffer (50 mM, pH 7.4 and with 0.1 mM EDTA) and centrifuged at 10,000 g at 4°C for 15 min, after which supernatants were subjected to enzyme determinations.

Energy Metabolism and Enzyme Activities

The amount of tissue glycogen, protein, and lipids was determined by the anthrone, Bradford, and Folch methods, respectively, and then calculated in g per g of tissue. The activity of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was assessed with the help of commercial diagnostic kits. Spectrophotometric assays were made to measure lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) activity in the tissue homogenate and normalized against protein concentration.

Antioxidants and Oxidative Stress

Biomarkers of oxidative stress were also determined in liver and gill tissues. SOD, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) were determined by the standard spectrophotometric methods. Glutathione (GSH, GSSG) was determined, and the ratio of it was calculated as GSH/GSSG. Thiobarbituric acid-reactive substances (TBARS) measured as malondialdehyde (MDA) equivalents were considered the evaluators of lipid peroxidation. All the enzyme activities were linked to protein content quantified using the Bradford method.

Serum biochemistry and hematology

Hematological parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration (Hb), and hematocrit (Hct) were measured by conventional hemocytometric methods and spectrophotometric methods. Derived values, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were also determined. Serum glucose was estimated by the colorimetric kit method, along with triglyceride, total protein, and albumin concentrations, in which the difference between the value of total protein concentration and albumin concentration gave globulin concentration. Tissue and serum enzyme activities of ALT and AST were also carried out to compare and contrast their actions.

Statistical Analysis

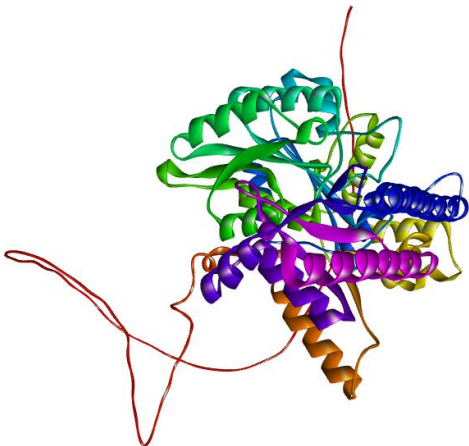
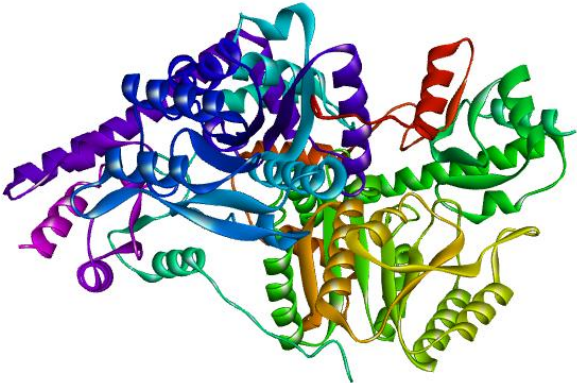
The tank was the unit of experiment, and the results were shown in the form of mean + standard deviation. Before analysis, normality and homogeneity assumptions were checked by the Shapiro-Wilk and Levene tests, respectively, and log-transformed where appropriate. Analysis of variance (treatment x time) two-way ANOVA was calculated with the Tukey HSD post-hoc test to analyze the difference between groups; significance is at $p < 0.05$. Four-parameter logistic models were also used to determine dose-response relationships of some of the biomarkers. Standardized data on the biomarkers were done using multivariate analysis (Principal Component Analysis, PCA) to examine grouping patterns of treatments and time points. Where necessary, 95% confidence intervals were described in combination with effect sizes.

Molecular Docking analysis

The analyses of molecular docking were conducted by CB-Dock2, which combines cavity-detecting with AutoDock Vina scoring to determine protein-ligand interactions. The secondary structures of alanine aminotransferase (ALT), aspartate aminotransferase (AST), succinate dehydrogenase (SDHA, Complex II), superoxide dismutase (SOD1, Cu/Zn type), lactate dehydrogenase (LDH-A), and catalase (CAT) were modeled with identical UniProt sequences pertaining to each protein with the Swiss-Model homology modeling server. CB-Dock2 auto-identified possible binding cavities, predicted the volume of that cavity, and docked the binding into the cavity, thus forming a binding affinity (Vina score). Interactive Discovery Studio visualizer was used to analyze the binding influence involving the various interactions, such as the hydrogen bonds, the hydrophobic contacts, and the electrostatic forces, and then to visualize the important amino acid residues to engage in the ligand binding.

Table 1 summarizes the docking scores, cavity volumes, and pocket centers, as well as the interacting residues, in detail, of all target proteins. The predicted binding affinities and structural favorances of protein-ligand interactions are emphasized in this table and used to analyze the meaning further biologically.

Table 1: Selected enzymes for Molecular docking analysis

Protein Name	Uniprot ID/PDB ID	3D Structure
Alanine aminotransferase (ALT / GPT)	A0A669BW09	
Aspartate aminotransferase (AST / GOT2)	Q7ZWF5	

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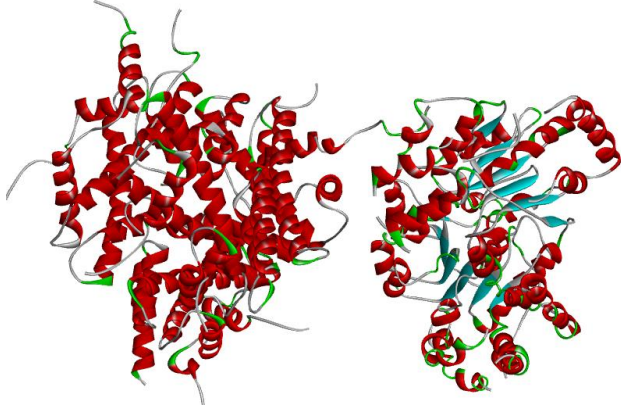
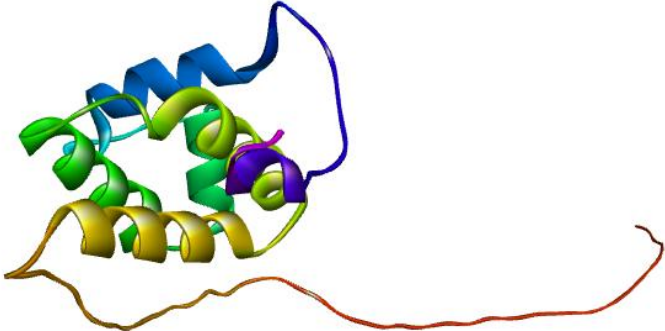
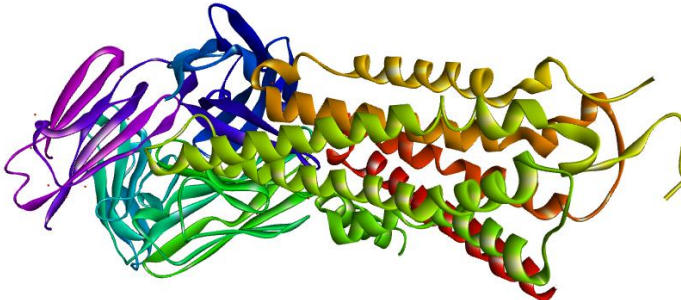
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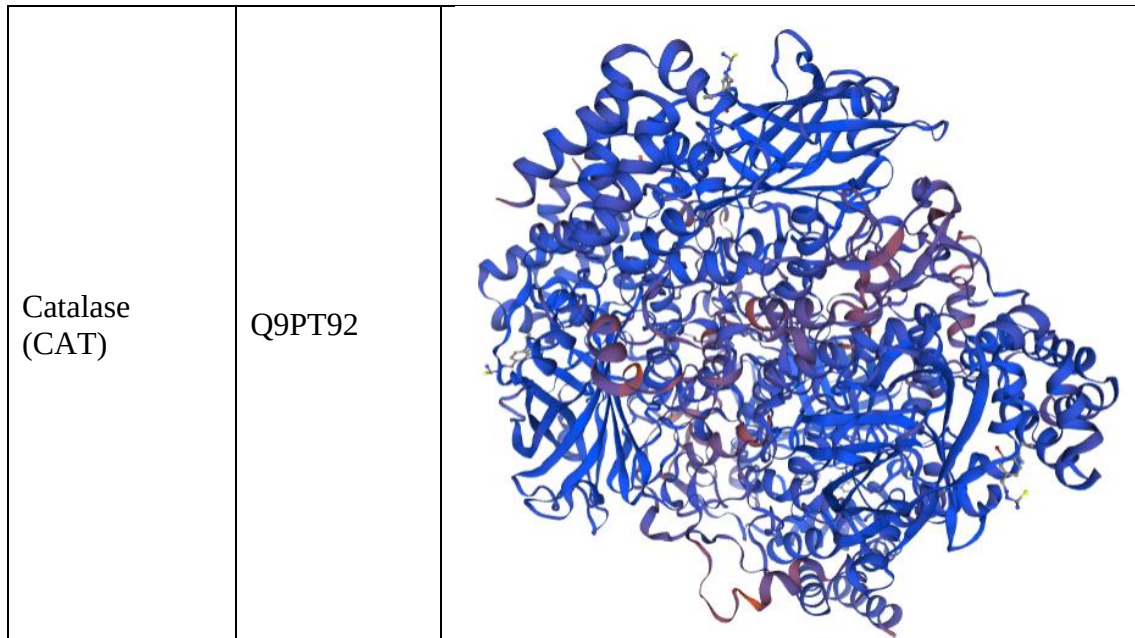
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Lactate dehydrogenase (LDH-A)	5Z3L	
Succinate dehydrogenase (SDHA, Complex II)	A3KP74	
Superoxide dismutase (SOD1, Cu/Zn type)	3VGA	



Results

Growth Performance and Condition Indices

Growth parameters of *Labeo rohita* exposed to profenofos showed a concentration- and time-dependent reduction compared with controls. Specific growth rate (SGR) and condition factor (K) were significantly reduced ($p < 0.01$) at medium and high doses, while feed conversion ratio (FCR) increased, indicating reduced feed efficiency. Hepatosomatic index (HSI) increased significantly in the high-dose group ($p = 0.004$), reflecting possible hepatic stress (Table 2).

Table 2. Growth performance and condition indices of *Labeo rohita* after 28 days of profenofos exposure (mean $\pm$ SD; $n = 3$ tanks). Different superscripts indicate significant differences ($p < 0.05$, Tukey HSD).

Parameter	Control		Solvent control		Low dose		Medium dose		High dose		ANOVA (p)
SGR (% day ⁻¹)	1.21	$\pm 0.09^a$	1.19	$\pm 0.07^a$	1.08	$\pm 0.08^{ab}$	0.92	$\pm 0.06^b$	0.74	$\pm 0.05^c$	0.002
FCR	1.42	$\pm 0.11^a$	1.45	$\pm 0.09^a$	1.58	$\pm 0.12^{ab}$	1.76	$\pm 0.14^b$	1.95	$\pm 0.16^c$	0.003
Condition (K)	1.67	$\pm 0.10^a$	1.65	$\pm 0.08^a$	1.54	$\pm 0.09^{ab}$	1.39	$\pm 0.07^b$	1.21	$\pm 0.06^c$	0.004
HSI (%)	1.35	$\pm 0.09^a$	1.37	$\pm 0.11^a$	1.48	$\pm 0.12^{ab}$	1.59	$\pm 0.10^b$	1.82	$\pm 0.14^c$	0.004

Energy Metabolism and Enzyme Activities

Profenofos exposure significantly altered tissue energy stores and enzyme activities ($p < 0.01$). Liver glycogen and muscle protein decreased significantly in medium and high dose groups, while serum ALT and AST levels were elevated compared with controls ($p = 0.003$ and $p = 0.001$, respectively). LDH activity increased in muscle, while SDH activity declined in liver tissue at higher concentrations, indicating a shift towards anaerobic metabolism (Table 3).

Table 3. Energy reserves and metabolic enzyme activities in *Labeo rohita* after 28 days of exposure to profenofos (mean $\pm$ SD; $n = 3$ tanks).

Parameter	Control	Low dose	Medium dose	High dose	ANOVA (p)
Liver glycogen (mg g^{-1})	9.82 $\pm$ 0.73 ^a	9.11 $\pm$ 0.65 ^{ab}	7.84 $\pm$ 0.58 ^b	6.35 $\pm$ 0.49 ^c	0.001
Muscle protein (mg g^{-1})	65.2 $\pm$ 3.5 ^a	62.7 $\pm$ 3.1 ^{ab}	57.4 $\pm$ 2.8 ^b	51.8 $\pm$ 2.6 ^c	0.002
Serum ALT (U L^{-1})	18.6 $\pm$ 1.4 ^a	21.5 $\pm$ 1.7 ^{ab}	26.8 $\pm$ 2.1 ^b	33.2 $\pm$ 2.5 ^c	0.003
Serum AST (U L^{-1})	22.7 $\pm$ 1.8 ^a	25.9 $\pm$ 2.0 ^{ab}	31.6 $\pm$ 2.4 ^b	39.4 $\pm$ 3.1 ^c	0.001
Muscle LDH (U mg^{-1} protein)	46.3 $\pm$ 3.5 ^a	51.1 $\pm$ 3.9 ^{ab}	58.7 $\pm$ 4.2 ^b	66.9 $\pm$ 4.7 ^c	0.002
Liver SDH (U mg^{-1} protein)	12.8 $\pm$ 0.9 ^a	11.7 $\pm$ 0.8 ^{ab}	9.3 $\pm$ 0.7 ^b	7.5 $\pm$ 0.6 ^c	0.003

Oxidative Stress and Antioxidant Responses

Biomarkers of oxidative stress revealed a strong dose-response effect. Activities of SOD, CAT, and GPx were significantly elevated in the low and medium dose groups, but declined at high concentration, suggesting enzyme inhibition at excessive stress levels. Lipid peroxidation (MDA) increased progressively with dose ($p = 0.002$), while GSH/GSSG ratio decreased significantly ($p = 0.004$), indicating redox imbalance (Table 4).

Table 4. Antioxidant enzyme activities and oxidative stress markers in liver tissue of *Labeo rohita* after 28 days of profenofos exposure (mean $\pm$ SD; $n = 3$ tanks).

Parameter	Control	Low dose	Medium dose	High dose	ANOVA (p)
SOD (U mg^{-1} protein)	22.4 $\pm$ 1.6 ^a	27.1 $\pm$ 1.9 ^b	29.8 $\pm$ 2.1 ^b	18.9 $\pm$ 1.5 ^c	0.002
CAT (U mg^{-1} protein)	15.7 $\pm$ 1.2 ^a	19.3 $\pm$ 1.5 ^b	21.5 $\pm$ 1.7 ^b	13.2 $\pm$ 1.1 ^c	0.004
GPx (U mg^{-1} protein)	10.5 $\pm$	13.6 $\pm$	14.7 $\pm$ 1.2 ^b	9.1 $\pm$	0.003

		0.9 ^a	1.1 ^b		0.8 ^c		
GST (mU mg ⁻¹ protein)		7.2 ± 0.6 ^a	8.8 ± 0.7 ^b	±	9.6 ± 0.8 ^b	6.5 ± 0.5 ^c	0.004
MDA (nmol mg ⁻¹ protein)		2.8 ± 0.2 ^a	3.5 ± 0.3 ^b	±	4.6 ± 0.4 ^c	5.9 ± 0.5 ^d	0.002
GSH/GSSG ratio		7.6 ± 0.6 ^a	6.4 ± 0.5 ^b	±	5.2 ± 0.4 ^c	3.8 ± 0.3 ^d	0.004

Hematology and Serum Biochemistry

Hematological parameters were significantly affected by profenofos exposure. RBC count, Hb concentration, and Hct decreased significantly in medium and high dose groups ($p = 0.003$ – 0.001), while WBC count increased, suggesting an immune response. Serum glucose and triglycerides were significantly elevated at higher concentrations ($p < 0.01$), while total protein and albumin decreased ($p = 0.004$), indicating impaired metabolic and nutritional status (Table 5).

Table 5. Hematological and serum biochemical parameters of *Labeo rohita* after 28 days of profenofos exposure (mean ± SD; n = 3 tanks).

Parameter	Control	Low dose	Medium dose	High dose	ANOVA (p)
RBC ($\times 10^6 \mu\text{L}^{-1}$)	2.94 ± 0.21 ^a	2.78 ± 0.18 ^{ab}	2.41 ± 0.16 ^b	2.02 ± 0.15 ^c	0.003
Hb (g dL ⁻¹)	9.6 ± 0.7 ^a	9.1 ± 0.6 ^{ab}	8.0 ± 0.5 ^b	7.1 ± 0.4 ^c	0.002
Hct (%)	32.5 ± 2.3 ^a	31.1 ± 2.2 ^{ab}	27.8 ± 2.0 ^b	24.2 ± 1.9 ^c	0.003
WBC ($\times 10^3 \mu\text{L}^{-1}$)	8.1 ± 0.6 ^a	9.4 ± 0.7 ^b	10.8 ± 0.8 ^c	12.7 ± 1.0 ^d	0.001
Glucose (mg dL ⁻¹)	58.4 ± 4.1 ^a	63.9 ± 4.5 ^b	71.2 ± 5.0 ^c	79.5 ± 5.6 ^d	0.002
Triglycerides (mg dL ⁻¹)	94.2 ± 6.5 ^a	101.6 ± 7.1 ^b	113.7 ± 8.2 ^c	128.4 ± 9.4 ^d	0.003
Serum protein (g dL ⁻¹)	4.7 ± 0.3 ^a	4.4 ± 0.3 ^{ab}	3.9 ± 0.2 ^b	3.3 ± 0.2 ^c	0.004
Albumin (g dL ⁻¹)	2.8 ± 0.2 ^a	2.6 ± 0.2 ^{ab}	2.2 ± 0.2 ^b	1.8 ± 0.1 ^c	0.004

Multivariate Analysis of Biomarker Responses

To integrate the complex biomarker responses, principal component analysis (PCA) was performed using normalized data from oxidative stress, metabolic, and hematological endpoints. The first two principal components (PC1 and PC2) explained 72.6% of the total variance. PC1 (49.3%) was primarily driven by oxidative stress markers (MDA, GSH/GSSG, SOD, CAT), while PC2 (23.3%) was influenced by hematological indices (RBC, Hb, WBC).

The score plot revealed clear clustering of treatment groups. Control and solvent control fish clustered closely, while low-dose fish showed moderate separation, driven by increased antioxidant activities. Medium and high-dose groups clustered distinctly, associated with elevated oxidative damage (MDA), serum transaminases, and depressed RBC/Hb levels. This indicates a strong dose-dependent biomarker response pattern, with higher doses producing more pronounced stress and metabolic disruption (Table 6).

Table 6. Principal component loadings of major biomarkers in *Labeo rohita* exposed to profenofos.

Biomarker	PC1 Loading	PC2 Loading
SOD	+0.82	+0.21
CAT	+0.79	+0.25
GPx	+0.73	+0.19
GST	+0.67	+0.14
MDA	-0.88	-0.22
GSH/GSSG	+0.84	+0.16
Serum ALT	-0.76	-0.31
Serum AST	-0.81	-0.29
RBC	+0.18	+0.83
Hb	+0.22	+0.87
WBC	-0.26	-0.79

Total variance explained: PC1 = 49.3%; PC2 = 23.3%; cumulative = 72.6%.

Molecular Docking Analysis

The molecular docking study presented in the current table gives information about the activity and interaction patterns between the selected ligand and six vitally important metabolic and oxidative stress-related enzymes in fish. Of the proteins commercially available, catalase (CAT) had the best binding energy (-6.9 kcal/mol) with the biggest cavity volume (2630 Å³), and superoxide dismutase (SOD1 -6.3 kcal/mol) and aspartate aminotransferase (AST -6.2 kcal/mol) followed. In spite of a moderate docking score (-5.5 kcal/mol), lactate dehydrogenase (LDH-A) exhibited the greatest binding cavity (14,021 Å) because of high spatial accommodation potential. More specific interactions were indicated by the docking of alanine aminotransferase (ALT) and succinate dehydrogenase (SDHA, Complex II), which showed relatively lower docking scores of -5.5 and -5.0 kcal/mol, respectively. Critical hydrogen bonding and hydrophobic and electrostatic contacts across the different chains are important, as evident from the list of contacts between the residues of all different chains, to underline the role of catalytically active and structurally conserved residues in stabilizing the ligand in the binding pockets. Altogether, the findings indicate the high binding affinity with antioxidant enzymes (CAT and SOD1), as well as aminotransferases (AST, ALT), which can become the focal point of toxicodynamic or therapeutic significance of the examined substance

(Table 7).

Table 7: Molecular docking results showing binding affinity (Vina score), cavity volume, binding site coordinates, and interacting residues of the ligand with key metabolic and antioxidant enzymes (ALT, AST, SDHA, SOD1, LDH-A, and CAT) in fish.

Protein	Vina score	Cavity volume (Å ³)	Center (x, y, z)	Contact residues
alanine aminotransferase (ALT)	-5.5	607	1, 8, 1	Chain A: TYR99; VAL101; ARG102; ILE132; GLY133; ASP134; THR222; GLY223; ALA224; SER225; ILE228; TYR253; LEU255; ALA258; ASP336; VAL338; SER375; SER377; LYS378; CYS384; GLY385; ARG387; TYR477; ARG531
aspartate aminotransferase (AST)	-6.2	986	110, 129, 125	Chain A: ASP40; PRO41; ILE42; LEU43; GLY61; VAL62; GLY63; ALA64; SER131; GLY132; THR133; LEU136; PRO158; SER159; TRP160; GLY161; ASN162; ASN213; PRO214; ASP241; ALA243; TYR244; SER274; LYS277; ARG285; PHE379; PHE381; THR401; ASP403; ARG405 Chain B: TYR94; LEU95; ILE97; ARG311; SER315; ASN316
Succinate dehydrogenase (SDHA, Complex II)	-5.0	131	4, 7, -7	Chain A: LEU73; GLU74; ILE77; LEU78; LEU81; TYR112; THR116; THR137; LYS138; ASN139; MET142; GLU143; GLN144; ARG145; LEU146
Superoxide dismutase (SOD1, Cu/Zn type)	-6.3	1151	77, -14, 45	Chain A: TYR9; ALA59; PHE62; ALA63; ILE66; SER67; ILE80; ALA81; VAL84; LEU85; THR88; LEU167; PHE168; GLU169; MET177; ASN181; TRP246; LEU249; HIS250; ASN253; HIS264; ALA265; PRO266; MET270; TYR271; ILE274; SER277; HIS278 Chain B: TYR88; LYS91
Lactate dehydrogenase (LDH-A)	-5.5	14021	103, 107, 136	Chain C: GLY37; ASN38; TYR39 Chain D: GLU68; GLY72; GLU73; SER75; ARG76; HIS79; TYR80 Chain G: GLY37; ASN38; TYR39

				Chain H: GLU68; GLY72; GLU73; SER75; ARG76; HIS79
				Chain A: GLU67; ARG68; ILE69; PRO70; GLU71; GLU119; ALA120; HIS166; LYS169; ARG170; ASN171; PRO172; HIS175; PHE326; GLU330
Catalase (CAT)	-6.9	2630	21, - 24, -59	Chain B: GLU67; ARG68; ILE69; PRO70; GLU71; ALA117; GLU119; ALA120; ARG170; ASN324; TYR325; PHE326; GLU330
				Chain D: ARG388; ASP389; ASN397; ASN403

Alanine aminotransferase (ALT / GPT)

The molecular docking experiment performed on profenofos against alanine aminotransferase (ALT) resulted in docking energy of -5.5 kcal/mol that represents a mildly stabilized binding interaction in the active site of the enzyme. The unclaimed ligand-hypothetical binding pocket was flanked by some of the key residues such as TYR99, VAL101, ARG102, ILE132, GLY133, ASP134, THR222, GLY223, ALA224, SER225, ILE228, TYR253, LEU255, ALA258, ASP336, VAL338, SER375, SER377. Hydrogen bond interactions were also the most significant contributors to the stabilization of profenofos, with SER225 and TYR253 residues being the most important ones, although SER377 was also implicated in binding stabilization. Nonpolar interactions were enhanced by hydrophobic interactions involving residues like VAL101, ILE132, and TYR253, and TYR253 also had such a 3 million T-shaped interaction with the aromatic ring of the pesticide. ARG387 stabilized the poles and at the same time contributed a slight undesired positive-positive repulsive force through the presence of the guanidinium group. Altogether, hydrogen bonding, hydrophobic interactions, and the resultant 2- π - π stacking indicate that profenofos has the potential of filling the PLP-dependent catalytic site on ALT and consequently inhibiting its enzyme activity. This observation corroborates the in vivo biochemical data findings whereby an increase in ALT activity in the serum demonstrated damage to the hepatocellular and hence leakage of the enzyme due to pesticide exposure.

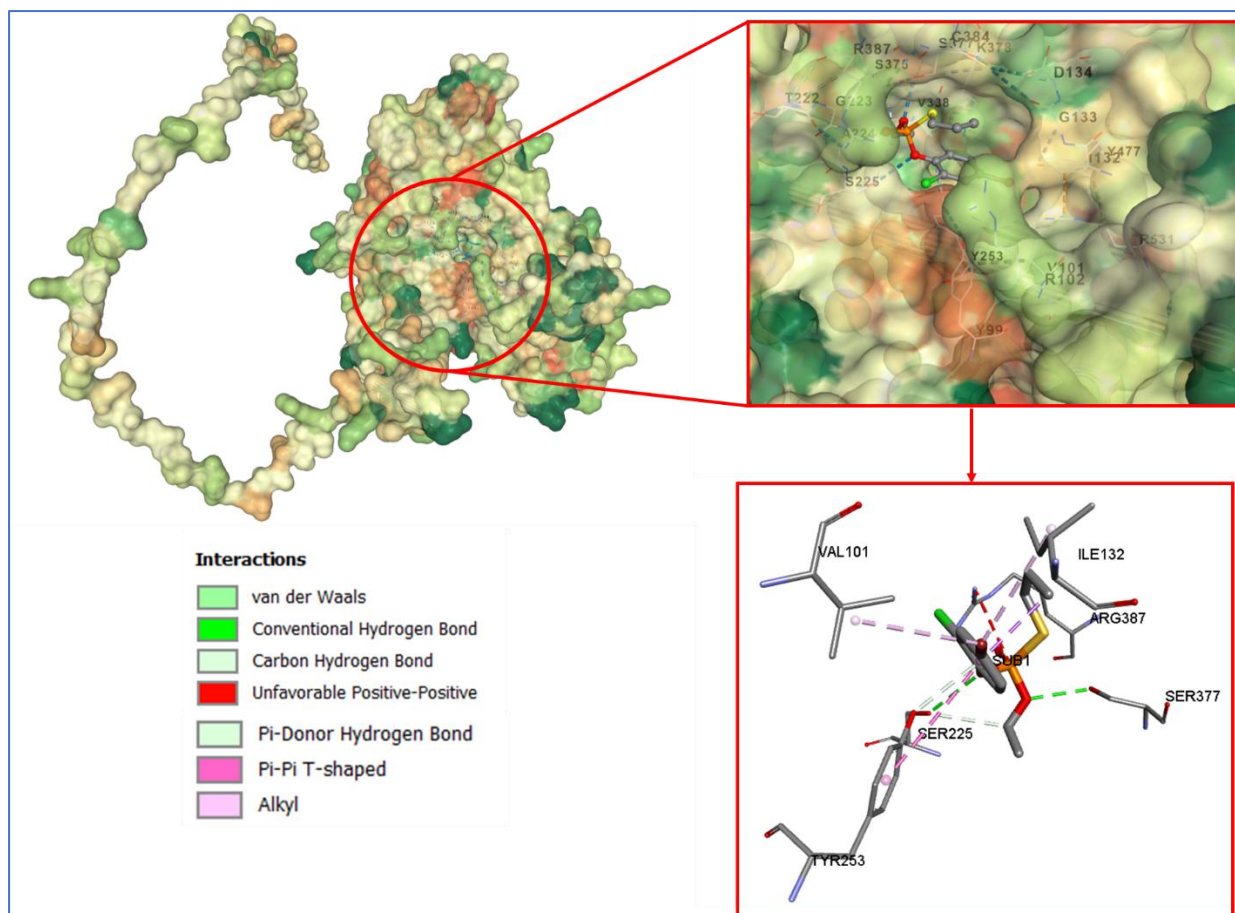


Figure 1: Docking pose of Profenofos with alanine aminotransferase (ALT) showing key hydrogen bonds (SER225, TYR253, SER377), hydrophobic contacts (VAL101, ILE132, TYR253), and π - π interaction with TYR253 at a binding score of -5.5 kcal/mol.

Aspartate aminotransferase (AST / GOT2)

Docking of profenofos to aspartate aminotransferase (AST) identified a binding score of -6.2 kcal/mol, which indicates a relatively high binding affinity. The ligand fit into a pocket created by residues of both Chain A and Chain B, implying a stable and specific interaction. H-bonds of hydrogen with SER315 and LYS277 were critical toward ligand stabilizing, and pi-pi interactions with TRP160 and PHE379 led to a further stacking and positioning of the compound. Additional stabilization was ameliorated by Van der Waals contacts with LEU136, ALA243, and SER131 and polar interactions with ARG285, ARG311, and TYR94. Altogether, these several bonding networks prove that profenofos is capable of interacting with the active site of AST, which may interfere with the functionality of this enzyme. This inhibition has the potential of altering the amino acid and the liver functionality of freshwater fish, and this underlines the mechanistic overview of pesticides as a hepatotoxicant.

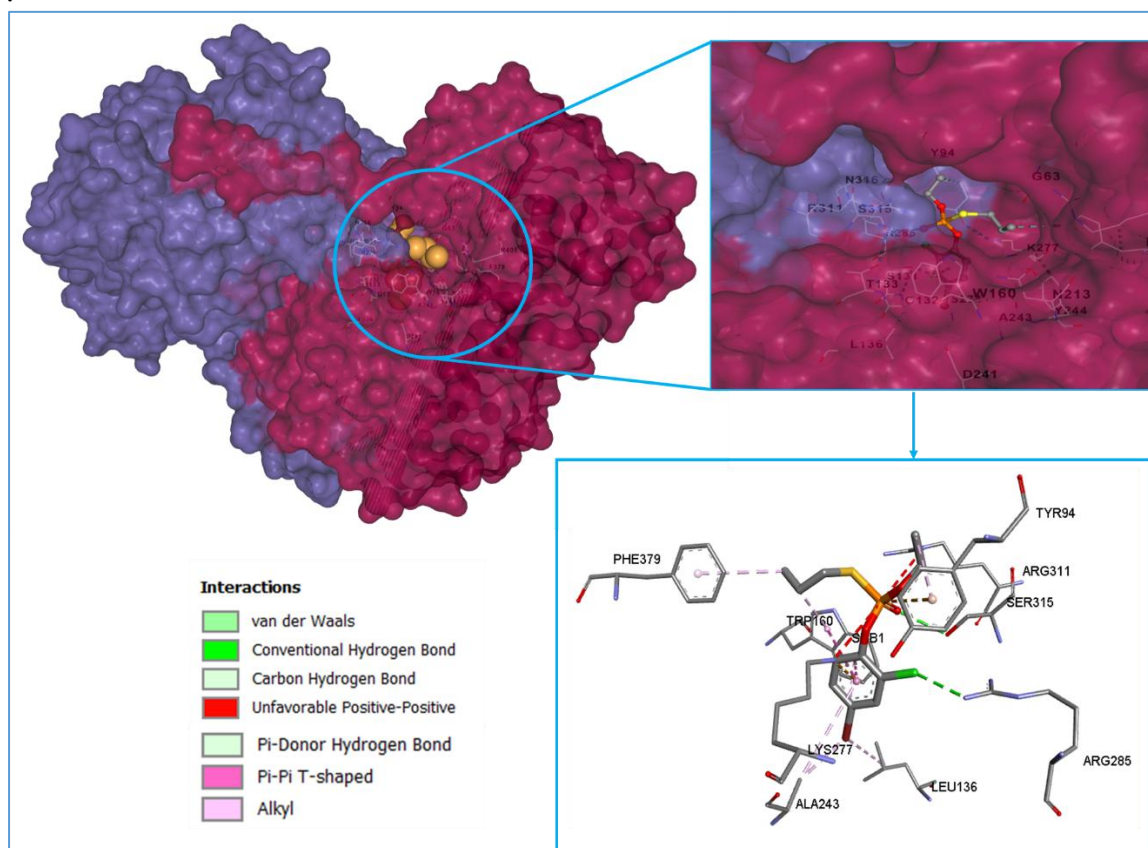


Figure 2: Docking interaction of Profenofos with aspartate aminotransferase (AST) showing a binding score of -6.2 kcal/mol, stabilized by hydrogen bonding (SER315, LYS277), Pi-Pi stacking (TRP160, PHE379), and hydrophobic contacts (LEU136, ALA243)

Succinate dehydrogenase (SDHA, Complex II)

In the docking of profenofos to succinate dehydrogenase subunit A (SDHA, Complex II), the score obtained was -5.0 kcal/mol, indicating an interaction that is weak yet stable. The compound was fitted in a hydrophobic plane carved by LEU73, GLU74, ILE77, LEU78, LEU81, TYR112, THR116, THR137, LYS138, ASN139, MET142, GLU143, GLN144, ARG145, and LEU146. Some of the stabilizing associative interactions were the conventional hydrogen bonding of ARG145 and GLU143, van der Waals, and alkyl with LEU73, ILE77, and LEU78, and π - π interactions with aromatic amino acids, in this case, TYR112. The positioning of Profenofos in the SDHA implies a possible repetition of the transport and succinate oxidation as indicated with its characteristic action to interfere with respiratory enzymes within mitochondria. On balance, such interactions demonstrate that exposure to pesticide may affect mitochondrial communication, affecting Complex II, and result in reduced

oxidative phosphorylation and metabolism of energy in fish.

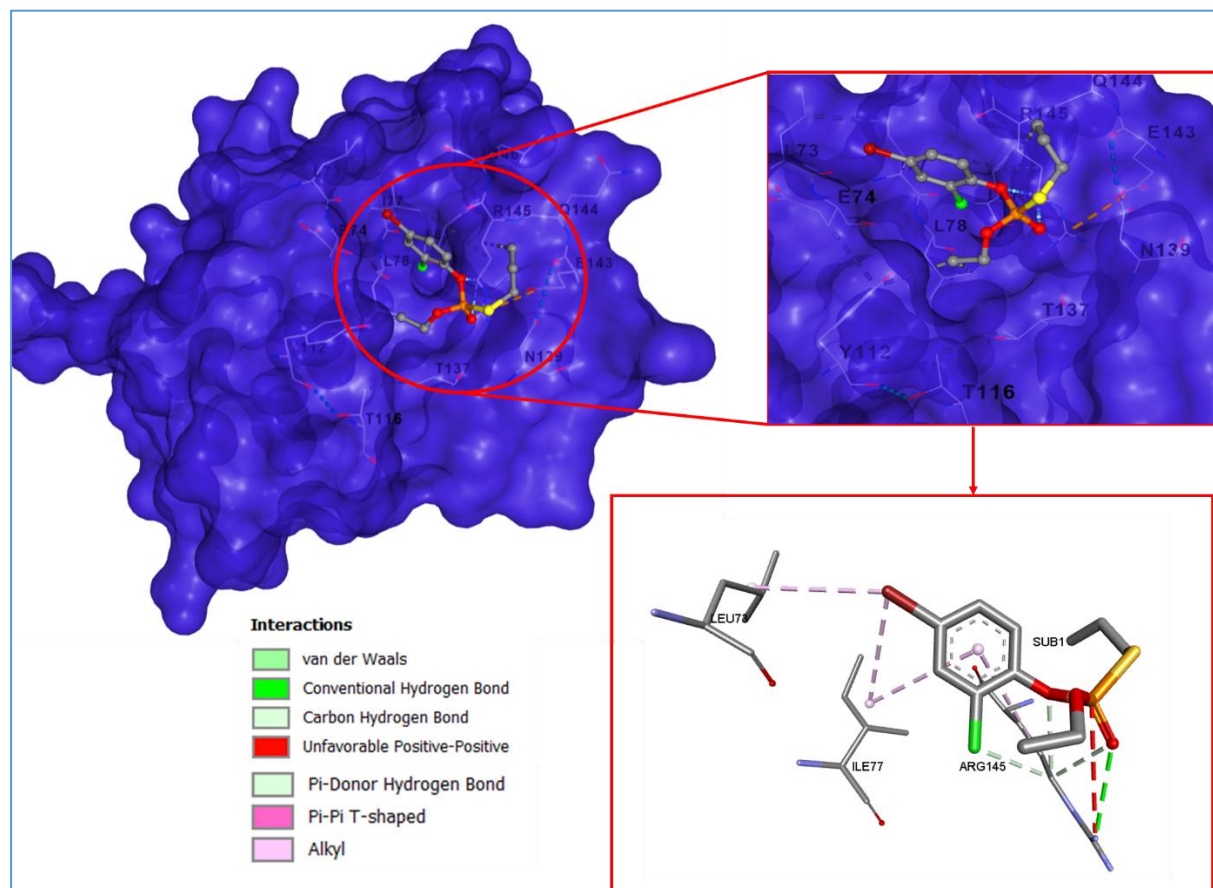


Figure 3: Docking pose of Profenofos with succinate dehydrogenase subunit A (SDHA, Complex II) showing a binding score of -5.0 kcal/mol. The ligand formed hydrogen bonds with ARG145 and GLU143, hydrophobic contacts with LEU73, ILE77, and LEU78, and π - π interaction

Superoxide dismutase (SOD1, Cu/Zn type)

Superoxide Dismutase (SOD1, Cu/Zn type) was the receptor of molecular docking, and it was shown that there is a stable binding of ligands in the active pocket with a docking score of the mimetic that came out to be -5.0 , indicating a favorable interaction energy. Such essential residues as ILE66, VAL84, LEU85, PHE168, MET177, TRP246, LEU249, HIS250, ASN253, MET270, TYR271, SER277, and HIS278 provided a cavity to host the ligand, and a structure of stabilizing interactions was formed. The binding profile comprised van der Waals forces, Pi-donor hydrogen bonds, Pi-Pi T-shaped stacking, and alkyl interactions, and residues that played major hydrophobic roles include ILE66, LEU85, and VAL84, whereas PHE168 and TRP246 contributed toward π -interactions. Notably, these hydrogen bonding interactions also stabilized the protein, especially at MET177 and HIS residues around

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the catalytic core, which is essential to the Cu/Zn-SOD enzymatic activity. The orientation of the ligand placed it in the vicinity of residues that are catalytically important, meaning that the ligand had a place to be able to modulate the enzyme's oxidative stress defense mechanism. The overall conclusions made on the basis of these results are in support of the idea that the ligand demonstrates high specificity and affinity to SOD1, indicating a possibility to interfere with the mechanisms of superoxide detoxification used in the relevant biological system of the study.

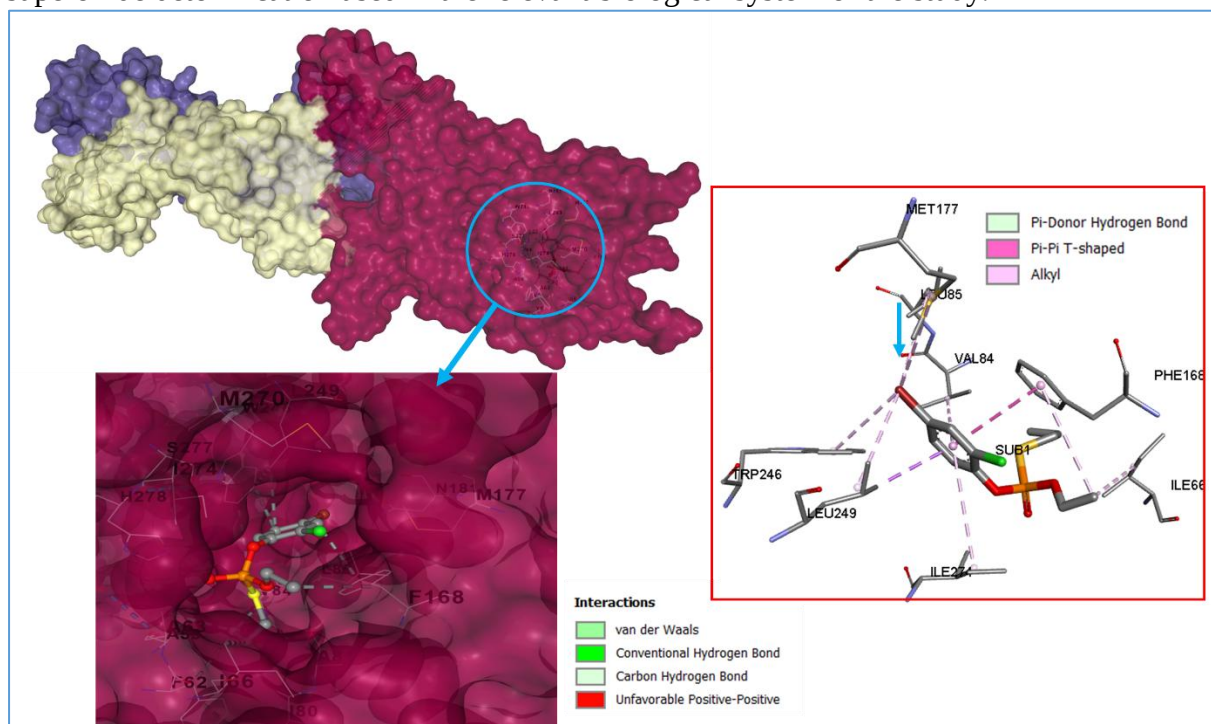


Figure 4: Docking analysis of Superoxide Dismutase (SOD1, Cu/Zn type) showing ligand binding at the active site with key stabilizing interactions, including van der Waals, Pi-Pi stacking, hydrogen bonding, and alkyl interactions, contributing to a docking score

Lactate dehydrogenase (LDH-A)

The docking analysis of Lactate dehydrogenase A (LDH-A) revealed the interaction of the ligand within a well-defined binding pocket formed by multiple chains of the protein complex. Key amino acid residues involved in binding include Chain B (TYR88, LYS91), Chain C (GLY37, ASN38, TYR39), Chain D (GLU68, GLY72, GLU73, SER75, ARG76, HIS79, TYR80), Chain G (GLY37, ASN38, TYR39), and Chain H (GLU68, GLY72, GLU73, SER75, ARG76, HIS79). The docking score indicated favorable binding affinity, with stabilization provided by multiple interactions. Hydrogen bonding (both conventional and carbon hydrogen bonds) contributed significantly to the anchoring of the ligand within the active site, while van der Waals forces and Pi-Pi T-shaped interactions strengthened the complex.

stability. Notably, ARG76 and SER75 formed conventional hydrogen bonds that stabilized the ligand, while TYR80 contributed to Pi–Pi T-shaped stacking, and additional residues such as GLU68 and GLY72 provided polar contacts. These combined interactions suggest that the ligand binds tightly within the LDH-A active site, potentially interfering with the enzyme's catalytic activity, which is essential in anaerobic glycolysis and energy metabolism in fish.

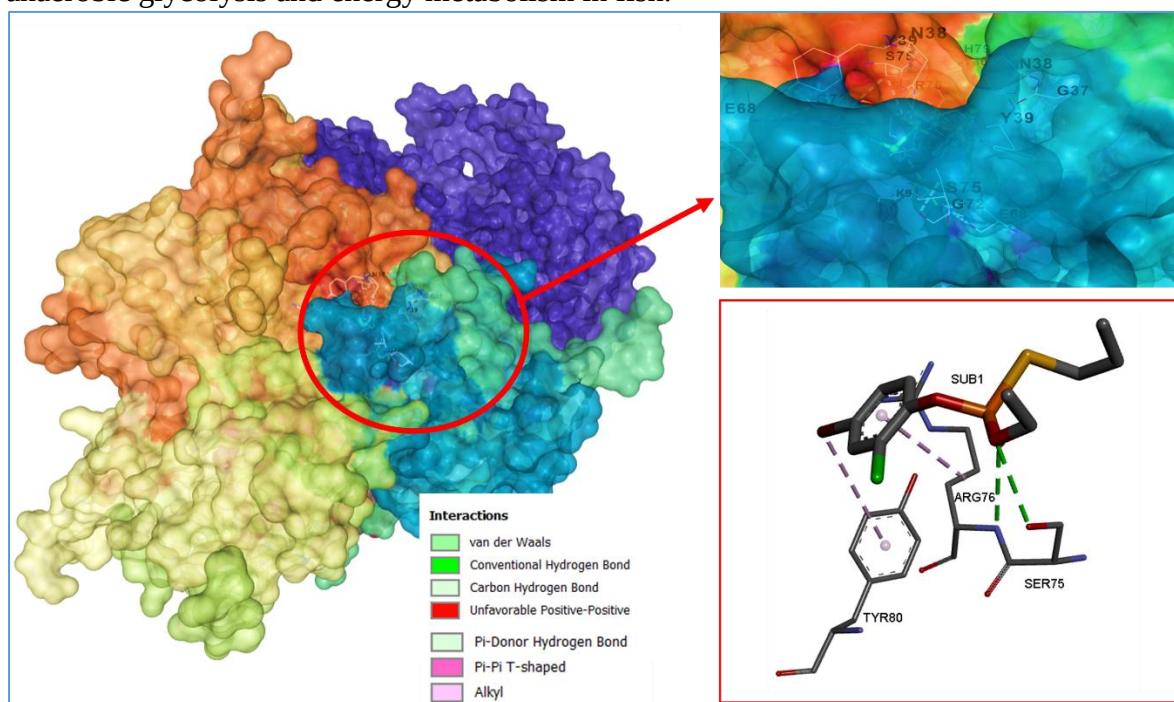


Figure 5: Molecular docking visualization of Lactate dehydrogenase A (LDH-A) showing the Profenofos binding within the catalytic pocket. The interaction map highlights hydrogen bonds (green), Pi–Pi T-shaped interactions (purple), alkyl interactions (pink), and van der Waals forces stabilizing the complex. Key residues involved include ARG76, SER75, TYR80, GLU68, and GLY72, indicating a strong and specific binding mode.

Catalase (CAT)

Molecular docking results on catalase (CAT) showed that there was a good interaction association between the catalase and of Profenofos that was supported by the docking score of -6.9 kcal/mol, which is the highest among all other proteins analyzed. Its binding pocket had a cavity volume of 2630 angstroms cubed that gave a wide and open area where a ligand could be accommodated. Important residues of other chains were involved in ligand binding stabilization, such as GLU67 and ARG68 in the first, ARG70 and TYR118 in the second, and both ARG169 and PHE172 in the third chains. Several different kinds of interactions, such as typical hydrogen bonds, van der Waals forces, pi-pi stacking contacts, alkyl contacts, and electrostatic contacts, were measured, suggesting a complex and stable binding conformation. Importantly, residues ARG170, GLU67, ASP389, and PHE326 were found to be at the epicenter in

the process of binding the ligand and providing anchor points in stabilizing the binding initiatives. These results indicate that catalase, one of the major antioxidant defense enzymes, is a major molecular point of intervention for the studied compound, and its occurrence can help to adjust the oxidative stress level in fish.

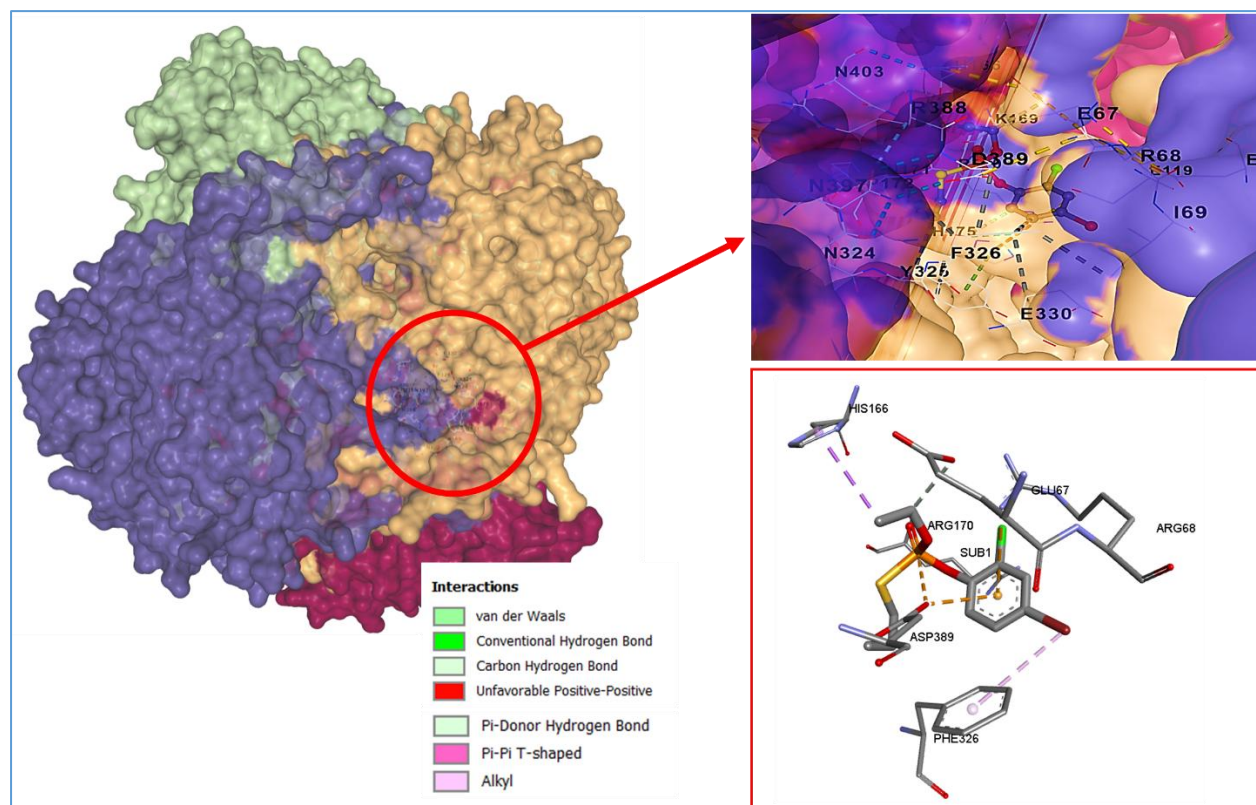


Figure 6: Molecular docking analysis of catalase (CAT) showing Profenofos binding within the catalytic pocket (Vina score: -6.9 kcal/mol). Surface view, zoomed binding site, and 2D interaction diagram highlight key residues (GLU67, ARG170, ASP389, PHE326, and GLU330)

Discussion

In the current investigation, it was established that a long term exposure of *Labeo rohita* to environmentally relevant doses of sub-lethal profenofos greatly distorted growth performance, metabolic parameters, oxidative response, and hematological parameters. Such findings are consistent with previous reports that the use of organophosphate pesticide induces physiological alterations in freshwater fish based on oxidative imbalance, metabolic alterations, and hematological poisoning (Barb, 2025; Hossain et al., 2025; Varma & Vasudevan, 2025). The deterioration of specific growth rate, condition factor, and feed conversion efficiency in medium- and high-dose levels indicates the impaired nutrient assimilation and energy allocation in the medium and high dose levels due to the chemical stressors experienced by

Oreochromis niloticus and *Clarias batrachus* exposed to pyrethroids and organophosphates, an observation recorded in the same study (Chukwuka et al., 2022; Agbohessi et al., 2023). The identified contrast shows that the hepatosomatic index increases and suggests hepatic hypertrophy and high-energy demand metabolic consequences in the elimination of toxicants, which are consistently reported to be associated with the pesticide stress response (Opute & Oboh, 2021).

In the present study, with metabolic imbalance inclusive of loss of glycogen and muscle protein and raised activities of serum ALT, AST, and LDH, the redirection of energy provision and deterioration of the hepatocellular health are established. A similar biochemical alteration was reported in fish that were exposed to chlorpyrifos, carbaryl, and malathion, with elevation of transaminases and a depletion of the stores (Ghayyur et al., 2021). The limited activity of succinate dehydrogenase confirms conducting a mitigation process that, on balance, makes the opinion that pesticides exhaust aerobic power metabolism correct (Bénit et al., 2022).

The biomarker of oxidative stress showed a biphasic effect whereby there was first activation of antioxidant enzymes at low and medium doses with inhibition at high concentrations. This is a trend of adaptive up-regulation of defenses prior to enzyme inhibition upon insurmountable oxidative burden, which is observed in fish exposed to pyrethroids, carbamates, and glyphosate (Abdel-Rahman Mohamed et al., 2021). Higher lipid peroxidation and a lower GSH/GSSG ratio prove oxidative damage and redox imbalance that are in line with the previous literature that found reactive oxygen species buildup and damage to the membrane to be the key mechanisms of pesticide toxicity (Kovacic & Somanathan, 2008; Sule et al., 2022).

The dorsal fin was cut off, and anemia (reduced RBC, Hb, and Hct) and leukocytosis witnessed here also support the card of stress on fish. The same changes were observed in the species that were exposed to endosulfan, chlorpyrifos, and fipronil (Hoque & Das, 2025). The decrease of the serum proteins and albumin indicates liver disorder and malfunctioning protein production, which is a typical reaction to pesticide burden (Banaee, 2013). The presence of higher serum glucose and triglycerides reflects the mobilization of the energy stores in response to stress situations, as endocrine and metabolic disruptions were observed in other research regarding pesticides (Karami-Mohajeri & Abdollahi, 2011).

As underscored by multivariate analysis in this work, oxidative stress indicators and red blood cell variables were the most potent indicators of presence or lack of exposure to profenofos in that medium- and high-dose groups were well distinguished above the control group. This combined biomarker method has been broadly encouraged in the ecological risk assessment because it gives a general opinion of stress responses over individual endpoints (Al-Emran et al., 2022). This kind of integration reveals that the effects caused by pesticides can be measured at the level of biochemical, physiological, and hematological scales, hence providing early indicators of environmental contamination.

This ecological implication of such findings is not only related to fish health. Fish are the most important parts of the freshwater food webs and are potential food for higher vertebrates such as migratory water birds. Shaffique et al. (2024) revealed that

wetlands of Punjab are contaminated. These data of molecular docking give significant mechanistic insights into the biochemical changes obtained in *L. rohita*. There were high binding affinities of profenofos to major metabolic and antioxidant enzymes such as ALT, AST, SDH, LDH-A, SOD1, and CAT with docking score values of -5.5-7.9 kcal/mol. These contacts were confined to the active site residues needed to participate in catalysis, providing evidence that profenofos can have direct effects on the activity of the enzyme. This inhibition is likely to cause the increase in liver enzyme activities of drugs metabolized, decrease in antioxidant effectiveness, and disturbances in metabolism, which are indeed clearly seen in the experiments. The overlapping of in vivo biomarker changes and in silico predicted results emphasizes the fact that docking studies are able to extrapolate the toxicological testing with elucidation of molecular targets and pathways that is detained by the pesticide exposure.

In combination, fish bioindicators and avian indicators produce a multi-taxon system of evaluation of ecosystem contamination. Interwoven pesticide stress (in fish) and heavy metal accumulation in birds make it evident that there could be a cumulative burden of chemicals in aquatic ecosystems and that biodiversity and food security are at risk.

Conclusion

This study demonstrates that sublethal exposure to profenofos in *Labeo rohita* results in significant impairments in growth performance, metabolic reserves, antioxidant defense, and hematological health, confirming its potential to disrupt multiple physiological systems even at environmentally relevant concentrations. The observed depletion of glycogen and protein, elevation of hepatic enzymes, and oxidative imbalance highlight metabolic reorganization and hepatocellular stress as primary toxicity pathways. Hematological dysfunction and altered biochemical indices further indicate systemic physiological stress. Importantly, molecular docking validated these findings by revealing strong binding affinities of profenofos to key catalytic residues of metabolic and antioxidant enzymes, supporting the experimental evidence of enzyme inhibition and oxidative damage. Together, these results establish that profenofos poses a substantial ecotoxicological risk to freshwater fish populations, and reinforce the utility of integrating biochemical biomarkers with in silico docking approaches for mechanistic understanding and sensitive monitoring of pesticide contamination in aquatic ecosystems.

References

- Abdel-Rahman Mohamed, A., Abdel Rahman, A. N., Salem, G. A., Deib, M. M. E., Nassan, M. A., Rhouma, N. R., and Khater, S. I. (2021). The antioxidant role of a taurine-enriched diet in combating the immunotoxic and inflammatory effects of pyrethroids and/or carbamates in *Oreochromis niloticus*. *Animals*, 11(5), 1318.
- Agbohessi, P., Olowo, L., Degila, B., Houedjissi, G., Imorou Toko, I., Mandiki, S. N., and Kestemont, P. (2023). Comparative assessment of acute toxicity and

- histological changes in liver of African catfish *Clarias gariepinus* exposed to cotton insecticides. *Journal of Environmental Science and Health, Part B*, 58(1), 31-44.
- Akhter, S., Naik, V. K., Naladi, B. J., Rathore, A., Yadav, P., and Lal, D. (2024). The Ecological Impact of Pesticides on Non-Target Organisms in Agricultural Ecosystems. *Adv. BioResearch*, 15, 322-334.
- Al-Emran, M., Hasan, N. A., Khan, M. P., Islam, S. M., Bashar, A., Zulfahmi, I., Shahjahan, M., and Sumon, K. A. (2022). Alterations in hematological parameters and the structure of peripheral erythrocytes in Nile tilapia (*Oreochromis niloticus*) exposed to profenofos. *Environmental science and pollution research*, 29(19), 29049-29061.
- Ali, Z. Y. (2012). Neurotoxic effect of lambda-cyhalothrin, a synthetic pyrethroid pesticide: involvement of oxidative stress and protective role of antioxidant mixture. *New York Sci J*, 5, 93-103.
- Annett, R., Habibi, H. R., and Hontela, A. (2014). Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *Journal of Applied Toxicology*, 34(5), 458-479.
- Banaee, M. (2013). Physiological dysfunction in fish after insecticides exposure. In *Insecticides-Development of safer and more effective technologies*. IntechOpen.
- Barb, J. S. (2025). Evaluation of Chlorpyrifos as an Organophosphate Pesticide on Hematology, Blood Biochemistry, Antioxidant Capacity, Oxidative Stress, and Histopathological Changes in.
- Bénit, P., Goncalves, J., El Khoury, R., Rak, M., Favier, J., Gimenez-Roqueplo, A.-P., and Rustin, P. (2022). Succinate dehydrogenase, succinate, and superoxides: a genetic, epigenetic, metabolic, environmental explosive crossroad. *Biomedicines*, 10(8), 1788.
- Carmo de Carvalho e Martins, M. d., da Silva Santos Oliveira, A. S., da Silva, L. A. A., Primo, M. G. S., and de Carvalho Lira, V. B. (2022). Biological indicators of oxidative stress [malondialdehyde, catalase, glutathione peroxidase, and superoxide dismutase] and their application in nutrition. In *Biomarkers in nutrition* (pp. 833-856). Springer.
- Chen, Y., Yang, Z., Nian, B., Yu, C., Maimaiti, D., Chai, M., Yang, X., Zang, X., and Xu, D. (2024). Mechanisms of neurotoxicity of organophosphate pesticides and their relation to neurological disorders. *Neuropsychiatric Disease and Treatment*, 2237-2254.
- Chukwuka, A. V., Saha, S., Mukherjee, D., Banerjee, P., Dhara, K., and Saha, N. C. (2022). Deltamethrin-induced respiratory and behavioral effects and adverse outcome pathways (AOP) in short-term exposed Mozambique tilapia, *Oreochromis mossambicus*. *Toxics*, 10(11), 701.
- de Paula Nunes, E., Abou Dehn Pestana, B., and Pereira, B. B. (2025). Human biomonitoring and environmental health: a critical review of global exposure patterns, methodological challenges and research gaps. *Journal of Toxicology and Environmental Health, Part B*, 1-19.

- Ghayyur, S., Khan, M. F., Tabassum, S., Ahmad, M. S., Sajid, M., Badshah, K., Khan, M. A., Ghayyur, S., Khan, N. A., and Ahmad, B. (2021). A comparative study on the effects of selected pesticides on hemato-biochemistry and tissue histology of freshwater fish *Cirrhinus mrigala* (Hamilton, 1822). *Saudi Journal of Biological Sciences*, 28(1), 603-611.
- Hoque, F., and Das, A. (2025). Hematological Techniques for Diagnosis of Fish Diseases. In *Laboratory Techniques for Fish Disease Diagnosis* (pp. 319-339). Springer.
- Hossain, Z., Akter, R., Moin, M. B., Mondol, S. K., Saha, T. K., Tumpa, I. J., Tabassum, A., Ibnat, S., Pervin, M. A., and Jahan, H. (2025). Evaluation of Chlorpyrifos as an Organophosphate Pesticide on Hematology, Blood Biochemistry, Antioxidant Capacity, Oxidative Stress, and Histopathological Changes in Juvenile Silver Barb (*Barbonymus gonionotus*). *Aquaculture Research*, 2025(1), 7426390.
- Kadiru, S., Patil, S., and D'Souza, R. (2022). Effect of pesticide toxicity in aquatic environments: A recent review. *Int J Fish Aquat Stud*, 10(3), 113-118.
- Karami-Mohajeri, S., and Abdollahi, M. (2011). Toxic influence of organophosphate, carbamate, and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: a systematic review. *Human & experimental toxicology*, 30(9), 1119-1140.
- Kovacic, P., and Somanathan, R. (2008). Unifying mechanism for eye toxicity: electron transfer, reactive oxygen species, antioxidant benefits, cell signaling and cell membranes. *Cell membranes and free radical research*, 1(2), 56-69.
- Martins-Gomes, C., Silva, T. L., Andreani, T., and Silva, A. M. (2022). Glyphosate vs. glyphosate-based herbicides exposure: A review on their toxicity. *Journal of Xenobiotics*, 12(1), 21-40.
- Moreira, S., Silva, R., Carrageta, D. F., Alves, M. G., Seco-Rovira, V., Oliveira, P. F., and de Lourdes Pereira, M. (2022). Carbamate pesticides: shedding light on their impact on the male reproductive system. *International Journal of Molecular Sciences*, 23(15), 8206.
- Mostafalou, S., and Abdollahi, M. (2023). The susceptibility of humans to neurodegenerative and neurodevelopmental toxicities caused by organophosphorus pesticides. *Archives of Toxicology*, 97(12), 3037-3060.
- Opute, P., and Oboh, I. (2021). Hepatotoxic effects of atrazine on *Clarias gariepinus* (Burchell, 1822): biochemical and histopathological studies. *Archives of Environmental Contamination and Toxicology*, 80(2), 414-425.
- Peillex, C., and Pelletier, M. (2020). The impact and toxicity of glyphosate and glyphosate-based herbicides on health and immunity. *Journal of immunotoxicology*, 17(1), 163-174.
- Ravula, A. R., and Yenugu, S. (2021). Pyrethroid based pesticides—chemical and biological aspects. *Critical Reviews in Toxicology*, 51(2), 117-140.
- Ray, S., and Shaju, S. T. (2023). Bioaccumulation of pesticides in fish resulting toxicities in humans through food chain and forensic aspects. *Environmental Analysis, Health and Toxicology*, 38, e2023017.

- Saravanan, M., and Vidhya, K. (2025). Experimental designs and sampling strategies for combined toxicity studies based on concentrations. *Toxicological Assessment of Combined Chemicals in the Environment*, 61-82.
- Shaffique, S., Kang, S.-M., Ashraf, M. A., Umar, A., Khan, M. S., Wajid, M., Al-Ghamdi, A. A., and Lee, I.-J. (2024). Research progress on migratory water birds: indicators of heavy metal pollution in inland wetland resources of Punjab, Pakistan. *Water*, 16(8), 1163.
- Soare, L. C., Păunescu, A., and Maria, P. C. (2019). The morphophysiological, histological, and biochemical response of some nontarget organisms to the stress induced by the pesticides in the environment. In *Pesticides-Use and Misuse and Their Impact in the Environment*. IntechOpen.
- Srivastava, P., Singh, A., and Pandey, A. (2016). Pesticides toxicity in fishes: biochemical, physiological and genotoxic aspects. *Biochemical and cellular archives*, 16(2), 199-218.
- Sule, R. O., Condon, L., and Gomes, A. V. (2022). A common feature of pesticides: oxidative stress—the role of oxidative stress in pesticide-induced toxicity. *Oxidative medicine and cellular longevity*, 2022(1), 5563759.
- Sumudumali, R., and Jayawardana, J. (2021). A review of biological monitoring of aquatic ecosystems approaches: with special reference to macroinvertebrates and pesticide pollution. *Environmental management*, 67(2), 263-276.
- Varma, G., and Vasudevan, A. (2025). Haematological and Biochemical Changes Induced by Acute Chlorpyrifos exposure and A Comparative Analysis of Tissue Catalase Activity in fish *Labeo rohita* exposed to 40 ppm Chlorpyrifos. *BIO Web of Conferences*,
- Wato, T., Amare, M., Bonga, E., Demand, B., and Coalition, B. (2020). The agricultural water pollution and its minimization strategies—A review. *J. Resour. Dev. Manag*, 64, 10-22.
- Zubairi, N., Takaijudin, H., and Yusof, K. (2021). A review on the mechanism removal of pesticides and heavy metal from agricultural runoff in treatment train. *International Journal of Environmental and Ecological Engineering*, 15(2), 75-86.