# Modulations of blood biochemical parameters of golden mahseer, Tor putitora following exposures to single and mixed organophosphate

# Modulations of blood biochemical parameters of golden mahseer, *Tor putitora* following exposures to single and mixed organophosphate

**Prabesh Singh Kunwar [1,2]*, Amit Kumar Sinha[3], Gudrun De Boeck[4], Kumar Sapkota[1,*]**

[1]Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

[2]Central Fisheries Promotion and Conservation Centre, Balaju, Kathmandu, Nepal

[3]Department of Aquaculture and Fisheries, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, 71601, AR, USA

[4]Systemic Physiological and Ecotoxicological Research, Department of Biology, University of Antwerp, Groenenborgerlaan 171, BE-2020 Antwerp, Belgium

**Running title: Pesticide mediated biochemical alteration in *Tor putitora***

* Corresponding authors:

E-mail: prabeshkunwar@yahoo.com (P.S. Kunwar); kumar.sapkota@cdz.tu.edu.np (K. Sapkota)

**Abstract**

Increasing pesticide application is a serious threat to human health and biodiversity. In nature, pesticides prevail in mixtures; therefore the joint effects of pesticides should be taken into consideration due to their priority in toxicity research when aiming at realistic evaluations. In this study, individual and mixture toxicity of the commonly used organophosphate insecticides-chlorpyrifos and dichlorvos was explored. Healthy and clinically active juveniles of golden mahseer (*Tor putitora*) were exposed to sub-lethal doses (10% of the 96 h-$LC_{50}$) of the chlorpyrifos, dichlorvos, and their mixture. Blood sampling was conducted after 24 h and 96 h of exposure, followed by a 1 week recovery period. Among the examined biochemical parameters; blood glucose in dichlorvos treatment; alanine aminotransferase and alkaline phosphatase in chlorpyrifos and dichlorvos treatments; and aspartate aminotransferase and urea in mixture pesticide treatments were elevated. In contrast, blood albumin and triglycerides were diminished in mixture pesticide treatments. Vital organs like kidney and liver of the tested animals were compromised to different magnitudes in different pesticide treatments. Kidney was found to be more sensitive than liver in terms of pesticide toxicity during this short exposure experiment. This study revealed that most of the biomarkers were mainly affected at a later exposure phase (after 96 h) and steadily recovered during the depuration period.

**Keywords:** Albumin, Chlorpyrifos, Dichlorvos, Glucose, Liver

## 1. Introduction

The use of pesticides began a long time ago (before 2000 BC) to protect crops from pests. However, their application was intensified since 1940 with the emergence of synthetic pesticides (Garcia et al., 2012). From 1990 to 2018, the global pesticide application raised from 2,299,979 tonnes to 4,122,334 tonnes. During the same period, it was increased from 60 tonnes to 574 tonnes in Nepal (FAOSTAT, 2021). The government authority of Nepal reported insecticides as the second major group of pesticides imported and formulated in Nepal, after fungicide. But, in terms of monetary value insecticide is the major group possessing 51.65% of the total national investment in pesticides (PQPMC, 2019).

Despite the pesticide's role in disease control and production enhancement of agro crops, the harmful effects of pesticides to the environment, non-targeted biota and human health cannot

be ignored. Dogan and Can (2011) also stated that in some situations, it is a practical method, however the benefits of pesticides are not derived without their negative consequences. It is unfortunate that only a small portion (<0.1%) of the applied pesticide reach their target pests while the majority (>99.9%) of it remains in the environment, contaminating the atmosphere, soil, and water (Pimentel, 1995). In this way, non-target aquatic organisms, which are of great economic importance to humans, are distressed by the haphazard application of pesticides and their discharge into the aquatic systems through leaching, agricultural runoff, precipitation, spray drift, improper disposal, irrigation waters, and industrial effluent (Adhikari et al., 2004; Sunanda et al., 2016; Wang et al., 2013).

Golden mahseer (*Tor putitora*) are important freshwater cyprinid fishes, which are naturally distributed in the trans-Himalayan region of Nepal, Pakistan, India, Myanmar and South-east Asia including Thailand, Lao PDR, Cambodia, Vietnam, southern China, Peninsular Malaysia, Borneo, Sumatra and Java (Ingram et al., 2005). They are highly valuable species both for game and food fisheries (Ingram et al., 2005). Because of the delicacy and sports value, golden mahseer is a highly prioritized freshwater cyprinid fish in Nepal, and it is also considered as a flagship species of this country (Swar, 2002). In general, fish are a good indicator for water quality assessment (Velmurugan et al., 2007); therefore, many studies have been performed worldwide addressing pesticide toxicity and their potential impact on fish, but golden mahseer has received little attention. There is an alarming decline of *Tor* populations from the natural environment due to various causes like environmental degradation, pollution and overfishing. However, one of the major causes of their population decline is water pollution (Yousafzai et al., 2008). Jha et al. (2018) estimated more than 50% of their population decline in the last 21 years. Water pollution not only alters fish community structure but also facilitates the establishment of invasive species (Gomes-Silva et al., 2020).

Among the different groups of pesticides, organophosphate-based formulations are the most commonly used pesticides for crop treatment and fish farming operations (El Nahas et al., 2017; Kafle et al., 2015; Rao et al., 2017; Varó et al., 2007). Chlorpyrifos and dichlorvos are widely used organophosphate pesticides worldwide (Ali et al., 2009; Sun et al., 2015; Ural and Çalta, 2005), evident by traces of their residues in water, soil, sediments, and in wild and cultured fish (Akoto et al., 2016; Kafle et al., 2015; Nag et al., 2020; Singh et al., 2015; Sun and Chen, 2008). Therefore, they are a serious source of water pollution, and their toxicity

mechanisms on important species must be elucidated if targeted conservation measures are to be taken.

The toxic effects of pesticides vary when treated individually or in combination. The combined effects of pesticide toxicity can be additive (an effect produced by mixture pesticides is exactly equal to the sum of individual pesticide's effects), synergistic (an effect caused by mixture pesticides is higher than the sum of its individual pesticide's effect), or antagonistic (an effect caused by mixture pesticides is less than the sum of its individual pesticide's effect). The joint effects of the same pesticide combination can also be different indicating species specific action of pesticide in fish (Kunwar et al., 2021a, 2021b). In the aquatic system, pesticides arise from various sources and are oftentimes present in the mixtures; therefore evaluation of the joint effects of pesticides becomes more realistic than the individual pesticide toxicity.

In aquatic animals, including fish, any kind of waterborne pollution is easily reflected in the circulatory system (Ismail et al., 2017); therefore, blood parameters can serve as important bioindicators of aquatic pollution as well as of the health status of the fish (Bhatnagar et al., 2017; Öner et al., 2008; Saravanan et al., 2011; Vosylienė, 1999). In one of the few studies on blood parameters of golden mahseer, the effects of synthetic pyrethroid- cypermethrin on RBC and WBC counts were documented (Ullah et al., 2015). Yousafzai et al. (2008) elucidated changes in haemoglobin (Hb), packed cell volume (PCV), blood cells count, blood total protein, cholesterol, glucose, glutamate oxaloacetate transaminase (GOT), glutamate oxaloacetate transaminase (GPT) and creatine phosphokinase (CPK) in golden mahseer sampled from the polluted natural waters but no literature on blood biochemical effects induced by organophosphate pesticides could be found for this species. Therefore this research aims to understand the toxic effect of the representative organophosphate insecticide chlorpyrifos, dichlorvos and their mixture on biochemical parameters like blood glucose, total protein, albumin, globulin, triglycerides, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of golden mahseer. These are key parameters for the assessment of stress, energy metabolism as well as functional status of kidney and liver in animals.

## 2. Materials and Methods

### 2.1. Acclimatization of the test animals

Healthy juveniles of golden mahseer (exact weight mentioned in section 2.2) were collected from the Fisheries Development Centre, Kulekhani, Makawanpur, Nepal and transported in oxygen packed plastic bags to Central Fisheries Promotion and Conservation Centre, Kathmandu, Nepal. Fish were stocked in 350 L indoor glass aquaria equipped with individual water recirculation and aeration systems. Fish were regularly fed ad libitum with commercial pellet feed (Sreema feed Pvt. Ltd., India) containing 32% protein. Uneaten food and fecal matters were removed with the help of a scoop net and siphon. Water filters, pumps, pipes and air stones were regularly cleaned. Everyday approximately half of the aquarium water was exchanged with freshwater to maintain optimum water quality. Water temperature, pH, dissolved oxygen, total ammonia, hardness, $Na^+$, $K^+$ and $Cl^-$ ranged between 23.5 - 25.2 °C, 7.6 - 7.8, 5.7 -6.6 mg/L, 0.20 - 0.26 mg/L, 51 -58 mg $CaCO_3$/L, 1.13 - 1.25 mmol/L, 0.06 -0.10 mmol/L and 0.52 - 0.64 mmol/L, respectively. Fish were acclimatized for two weeks in these conditions before using them for the experiment. This research followed ethical guidelines as approved by the ethical review board of Nepal Health Research Council (Ref. No. 1215), Government of Nepal.

### 2.2. Pesticide exposure and depuration

Acclimatized fish were weighed and transferred to 35 L small glass aquaria. Each aquarium accommodated only one fish at a time. The average weight of the fish used in this experiment was 53.12 ± 4.13 g (mean ± SD). Fish were left undisturbed overnight with continuous aeration. Commercial grade pesticides were selected for exposure. For dichlorvos, G-VAN (80%; Greenriver Industry Co., Ltd., ShenZhen, China) and for chlorpyrifos, Dursban (20%; Dow Agro Sciences Pvt. Ltd., India) were used. These products are legally registered by the Plant Quarantine and Pesticide Management Centre, government of Nepal for marketing and application.

In total, 24 aquaria of 35 L capacity each were used for this sub-lethal exposure. Each aquarium accommodated three fish for the test. On the day of exposure, working solutions of pesticides were freshly prepared in distilled water and the calculated volume of the stock solution was added to reach the sub-lethal concentrations of chlorpyrifos- 0.075 mg/L, dichlorvos- 1.29 mg/L and their mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L) in the test chamber. The

selected concentrations represent 10% of the determined 96 h-$LC_{50}$ of chlorpyrifos and dichlorvos to golden mahseer (Kunwar et al., 2021b). There were 6 replicates for each pesticide treatment and for the control. The test was conducted in semi-static condition and the test solution was renewed periodically (Shirdel et al., 2020). After accomplishing the 96 h exposure, the fish were left undisturbed in the same aquaria for one further week in the pesticide free water to evaluate their recovery from the pesticide effects.

### 2.3. Serum collection

Blood sampling was done three times- after 24 h and 96 h of pesticide exposure, and 1 week of depuration period. Fish were gently removed from aquaria and anesthetized in clove oil. The anesthetized fish were wrapped with a wet towel and blood was drawn from the caudal vein using a 3 mL syringe. The blood collected in eppendorf tubes was left for 30 minutes to clot properly (Tuck et al., 2009). Later, it was centrifuged for 10 minutes at 3,000 rpm to separate serum from blood cells (Zahran et al., 2018). Transparent serum was transferred to clean eppendorf tubes and stored at -30 °C for later biochemical measurements.

### 2.4. Biochemical measurements

Prior to the biochemical tests, serum samples were properly thawed at room temperature. Glucose was determined by the oxidase (GOD) and peroxidase (POD) method (Eco-pak glucose kit, Accurex Biomedical Pvt. Ltd., India). Total protein was determined by the Biuret method using a total protein kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India). Albumin was determined by the Bromocresol Green (BCG) method using an albumin kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India). Globulin was estimated by subtracting albumin from total protein (Qureshi et al., 2016). Urea was determined by the Glutamate Dehydrogenase (GLDH) Kinetic method using a urea kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India). Creatinine was estimated by a modified Jaffe's Kinetic method using a creatinine kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India). Triglycerides were measured by the Glycerophosphate-Oxidase (GPO)/PAP method using a triglycerides kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India). Alanine aminotransferase (ALT or ALAT) activity was assayed by a modified IFCC method using a calkine serum glutamic-pyruvic transaminase (SGPT)/ALAT kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India); Aspartate

aminotransferase (AST or ASAT) activity was quantified by a modified IFCC method using a calkine serum glutamic oxaloacetic transaminase (SGOT)/ASAT kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India) and alkaline phosphatase (ALP or ALKP) activity was determined by the p- Nitrophenylphosphate (pNPP) kinetic method using a calkine ALP kit (Coral Clinical systems, Tulip Diagnostics Pvt., Ltd., India).

### 2.5. Statistical analysis

The normality of the data and homogeneity of variances was assessed by Shapiro-Wilk and Levene's test, respectively. Mean difference among the various treatment groups was analyzed by one-way ANOVA followed by Post Hoc tests. Whenever the data were not normally distributed, non parametric Kruskal-Wallis test with multiple pair comparison was applied. All analyses were done using SPSS version 20.

## 3. Results

### 3.1. Blood glucose

In general, there was a numerically increasing trend of blood glucose in single pesticide treatments but not in mixture groups. However, the elevation was significant ($P < 0.001$) only in dichlorvos treated fish after 24 h of exposure. When the glucose level was compared among the same pesticide treated fish during different time intervals, a significant recovery ($P < 0.001$) of glucose level was observed after 96 h and 1 week recovery (WR) compared to the 24 h measurement in dichlorvos treated fish (Fig.1). The compiled data (all pesticide treatments as an exposed group) analysis revealed that although blood glucose level was increased by pesticide exposure, significant elevation ($P < 0.05$) compared to control was observed only during the early sampling time (24 h). The elevated blood glucose significantly recovered ($P < 0.01$) at successive sampling intervals- 96 h and 1 WR (Table 1).

### 3.2. Blood proteins

Regardless of the pesticide treatments, exposure time or depuration phase, no significant effect on blood total protein and globulin was found (Fig. 2 and 4). However, there was a declining trend for the mixture, especially at 96 h, which recovered after 1 week of depuration. In general,

there was also a declining trend of the blood albumin with pesticide exposure but the difference was significant ($P < 0.01$) only at 24 h of exposure compared to its control level. The diminished albumin at 96 h in mixture group was significantly recovered ($P < 0.05$) during the depuration period (Fig. 3).

Data for total protein, albumin and globulin were also analyzed by compiling different pesticide treatments as exposed group and compared with their respective controls but the differences always remained insignificant. However, the decreasing trend was again observed in pesticide exposed fish at both 24 h and 96 h of exposure and recovered during the depuration phase (Table 1).

### 3.3. Blood triglycerides

There was a clear declining trend of blood triglycerides in all pesticide treatments compared to control, but a sharp significant drop ($P < 0.001$) was observed only in mixture pesticide group after 96 h of exposure. When the triglyceride level was compared among the same pesticide treated fish during different time intervals, a significantly lower ($P < 0.05$) level was observed after 96 h in mixture pesticide treated fish compared to its 24 h of measurement (Fig. 5). The compiled data analysis revealed that pesticide exposure reduces the triglycerides level significantly ($P < 0.05$) at the later phase of the exposure i.e. 96 h (Table 1).

### 3.4. Blood urea and creatinine

Blood urea was stable in response to chlorpyrifos exposure. However, there was an increasing trend of urea levels in dichlorvos and mixture pesticide exposure, but a significant elevation ($P < 0.01$) was found only at 96 h in the mixture pesticide treated fish. The increasing trend for blood creatinine was also noticed by the pesticide treatments but all these increments were insignificant. The elevated urea and creatinine dropped to the control level during one week depuration period (Fig. 6 and 7). Statistical analysis also revealed no significant difference in both parameters among the same pesticide treatments over the whole experimental period (Figs. 6 and 7). The same was true for compiled data analysis, although exposed groups showed numerically higher urea and creatinine compared to their respective controls (Table 1).

### 3.5. Alanine aminotransferase (ALT)

Blood ALT level increased significantly compared to the control after 96 h of exposure ($P < 0.01$) in both chlorpyrifos and dichlorvos treated fish. The elevated ALT levels restored after 1 week of recovery period showing significantly lower ALT levels in chlorpyrifos ($P < 0.01$) and dichlorvos ($P < 0.05$) treated fish after 1 week of recovery period compared to their 96 h counterparts (Fig. 8). The compiled data analysis also revealed increased ALT levels in exposed group after 96 h ($P < 0.01$) which significantly dropped ($P < 0.001$) after 1 week of recovery period (Table 1).

### 3.6. Aspartate aminotransferase (AST)

Blood AST level tended to be increased by all pesticide treatments but significantly higher AST levels compared to respective controls were observed only in mixture pesticide treatment after 24 h ($P < 0.01$) and 96 h ($P < 0.001$) of exposure. Among the same pesticide treatments over the whole experimental period, significantly elevated ($P < 0.01$) AST was observed in mixture group after 96 h compared to the 24 h, and significantly lower ($P < 0.001$) AST was observed after 1 week recovery compared to 96 h observation (Fig. 9). The average blood AST in exposed group (compiled) were not significant from their control levels; but after 1 week of recovery period, blood AST in exposed group was significantly lower ($P < 0.01$) compared to 96 h of observation in the same treatment group (Table 1).

### 3.7. Alkaline phosphatase (ALP)

Blood ALP was elevated significantly compared to control values after 96 h of exposure in chlorpyrifos ($P < 0.001$) and dichlorvos ($P < 0.01$) pesticide groups. These elevations sharply declined again to values close to the control level after 1 week of recovery period. Among the same pesticide treatments, chlorpyrifos treated fish exhibited significantly higher ALP at 96 h ($P < 0.05$) compared to 24 h values which became significantly lower again compared to this 96 h value in chlorpyrifos ($P < 0.001$) and dichlorvos ($P < 0.01$) treated fish after 1 week of recovery (Fig. 10). The average blood ALP (compiled) data analysis showed significant elevation ($P < 0.01$) by pesticide exposure compared to control after 96 h which was also significantly higher ($P < 0.05$) than 24 h ALP measurement of the same exposed group. This elevated ALP significantly dropped again ($P < 0.001$) reaching control levels after one week of recovery period (Table 1).

## 4. Discussion

Chlorpyrifos is a pesticide extensively applied in agriculture and domestic operations (Ali et al., 2009; Halappa and David, 2009; Sun et al., 2015). Similarly, dichlorvos is also a commonly used pesticide (Das, 2013; Mennear, 1998; Sun et al., 2015; Ural and Çalta, 2005). It is one of the main chemical agents used against fish ectoparasites (Das, 2013; Varó et al., 2007). The majority of the applied pesticides are released into the environments that ultimately reach the aquatic systems which can alter the biochemical parameters of the aquatic animals.

Biochemical parameters are used as important biomarkers in toxicity research. Any deviation in biochemical parameters indicates some sort of disturbance in the animal's homeostasis and could potentially deteriorate its health. Blood glucose is one of the bioindicators that, together with plasma cortisol, is extensively studied as environmental stress indicator (Banaee et al., 2013; Bhatnagar et al., 2017; Dogan and Can, 2011; Koul et al., 2007; Medda, 1993; Ramesh and Saravanan, 2008; Saravanan et al., 2011). In common carp (*Cyprinus carpio*), Hatami et al. (2019) reported an increment in the glucose level in response to pesticide mediated stress. Significant elevation of blood glucose is due to gluconeogenesis which fuels the energy for the increased metabolic demands to cope with stressors including pesticides (Bhatnagar et al., 2017; Ramesh and Saravanan, 2008; Saravanan et al., 2011). Moreover, in previous studies depletion of liver glycogen in response to pesticide exposure was coupled with increased glucose in circulation, suggesting enhanced glycogenolysis as a strategy to meet the energy requirements (Ezike et al., 2017; Narra et al., 2015). In our experiment, glucose levels tended to be elevated by both single pesticide exposures, even though this was only significant for dichlorvos. Increased glucose levels due to pesticide exposures have been observed before, e.g. due to exposure of chlorpyrifos (Banaee et al., 2013; Ramesh and Saravanan, 2008) and lindane (Saravanan et al., 2011) to common carp, chlorpyrifos to mrigal (*Cirrhinus mrigala*; Bhatnagar et al., 2017), and dimethoate to rainbow trout (*Oncorhynchus mykiss*; Dogan and Can, 2011). We speculate that the high glucose levels observed in our experimental animals was due to elevated glycogenolysis together with gluconeogenesis. The reducing trend of triglyceride levels in pesticide exposed golden mahseer, with a significant impact in the mixture group, might offer an additional indication for the switch towards another metabolic pathway (gluconeogenesis) which is activated to provide energy during these exposures. Similar to our observation, Hatami et al. (2019) reported a decreased level of triglycerides in chlorpyrifos exposed common carp. In our

experimental organism, there seemed no considerable contribution of protein in energy production. No reduction of overall protein in pesticide exposed fish indicates that protein metabolism was not severely affected by pesticide exposure. Likewise, no effect of chemical stress was documented on the blood protein of common carp and rainbow trout (De Smet and Blust, 2001; Velisek et al., 2006). Animals prioritize oxidation of carbohydrates and fat for energy production, therefore utilizing protein as an energy source comes only at critical conditions. Probably such situation did not prevail in the present case during the short term observation period (96 h). Moreover, the sub-lethal doses used in our experiment might have been too low to cause a significant effect in protein catabolism.

Enzymatic profiles of ALT, AST and ALP were measured to evaluate the liver health of animals. These enzyme activities were elevated after pesticides exposure. Increased blood ALT, AST and ALP were also found in other fish species in response to pesticides (Banaee et al., 2013; Ghaffar et al., 2015; Jaffer et al., 2017; Koul et al., 2007; Medda, 1993). The high level of these three enzymes observed in golden mahseer could be due to hepatic cell damage as described in other studies on fish (Banaee et al., 2013; Deka and Mahanta, 2015; Ghelichpour et al., 2017; Jaffer et al., 2017). The histopathological lesions such as congestion, cytoplasmic degeneration, hypertrophy, necrosis, nuclear degeneration, pyknosis, sinusoids dilation and congestion, and vacuolar degeneration were reported in the liver tissue of nonylphenol exposed fish (Shirdel et al., 2020).

Albumin and globulins are major components of total protein. There was a clear decreasing trend of albumin, although it was significant only in the mixture group at 24 h. There was no distinct trend for globulin. Decreasing blood albumin and globulin was reported in sub-lethal exposure of chlorpyrifos to mrigal (Bhatnagar et al., 2017) but insignificant effects on these proteins were also reported in rainbow trout (Velisek et al., 2006). Albumin is synthesized by the liver while globulins are produced both by the liver and immune system (plasma cells). Therefore, reduction in albumin in our experiment could be attributed to the liver impairment, also supported by high ALT, AST and ALP, leading to insufficient albumin production.

Blood urea and creatinine levels are commonly used signs for kidney function. Urea is the chief end product of protein metabolism while creatinine is an anhydride of creatine present in muscles (Jyothi and Narayan, 2000). Production of creatinine is generally more stable than any other excretory product which makes it a more reliable and strong biomarker for accessing

renal function (Jyothi and Narayan, 2000). The observed increasing trend of urea, although significant only in mixture group at 96 h, and the similar trend of creatinine observed in our study corroborates with the findings in Singhi (*Heteropneustes fossilis*; Shaikh and Gautum, 2014) and common carp (Jaffer et al., 2017) exposed to dichlorvos and chlorpyrifos, respectively. When the excretory organs (kidney) cannot function properly, high urea and creatinine build up in the blood which might have occurred in golden mahseer during this experiment. Our postulation is also supported by histopathological alteration like Bowman's space increase, congestion, glomerular degeneration, increment of tubule diameter, melanomacrophages centers, necrosis, and tubule degeneration of kidney tissue in nonylphenol-exposed fish (Shirdel et al., 2020).

The highest impact on renal function seems to be in the mixture pesticide group after 96 h of exposure. At that sampling time and treatment group blood glucose, total protein, albumin, globulin and triglycerides were noted the lowest over the whole experimental period, but the reduction was significant only for the triglycerides. The stress indicator glucose, which is expected to be higher during pesticide treatment throughout the exposure, surprisingly remained lower than the control at this point. Blood urea and AST were also highest in this group. This clearly indicates that fish kidney was significantly affected by chlorpyrifos and dichlorvos mixture at this particular sampling time (96 h). Most of the measured biochemical parameters could have been diminished due to their loss from the damaged kidneys whereas the highest urea level could have been due to the compromised filtration capacity of the excretory organ (kidney). Similarly, the highest blood AST was contributed most probably to renal cell damage. AST is not a specific hepatic enzyme (Ghelichpour et al., 2017); and high AST activity in common carp kidney was reported by De Smet and Blust (2001) which also supports this notion.

Elevation of blood ALP occurs when there are hepatobiliary problems (Ghelichpour et al., 2017) and ALT is also a liver specific enzyme. Significantly high ALP and ALT were found after 96 h with individual pesticide exposures whereas it was not significant in the mixture, indicating that hepatic cells were more sensitive to the individual pesticides compared to the mixture pesticides treatment. Blood AST was significantly elevated in the mixture pesticide group but not in individual pesticide groups which indicates renal cells were more sensitive to mixture pesticides compared to individual pesticide treatments. Significant elevation of urea and AST, significant reduction of triglycerides, and elevating trend of creatinine signifies renal cells were highly

affected by the pesticides. At the same time, despite high AST, ALT and ALP levels, the protein metabolism of the fish remained unchanged which indicate liver tissues of the fish were less affected by such treatments.

In this study, the biochemical modulation exhibited by fish was noticeable even at sub-lethal concentrations of pesticides. From this, we can envisage the potential impact of such chemicals at higher concentrations that can be linked to mass mortalities of fish in natural water systems (Polidoro and Morra, 2016; Sabra and Mehana, 2015).

The time-wise data analysis revealed pesticide exposure was more stressful to fish in the beginning and gradually dropped, and the animals adapted to the changing environmental condition. However, the effects of pesticides on the liver and kidney were more prominent at the later phase of exposure (96 h) indicating that it might have taken some time to inflict such impairment through the circulatory system. Adhikari et al. (2004) documented that blood parameters in rohu (*Labeo rohita*) were improved by slowly eliminating pesticides when they were transferred to freshwater. Similar results were found after one week depuration period in our experimental animals, where most of the deviated biochemical parameters tended to be stabilized exhibiting recovery signs from pesticide effects. This indicates pesticide detoxifying enzymes were induced in our test species causing the elimination of pesticides from their body. In addition, the induction of the detoxifying enzyme and depuration was found proportional to the concentration of the toxicants (Ikpesu, 2013).

## 5. Conclusion

Biochemical parameters of golden mahseer were affected by sub-lethal exposure of chlorpyrifos, dichlorvos and their mixture. Fish exhibited stress response in the early exposure phase but the effects on vital organs were prominent at the later phase of the experiment. The liver was found to be more sensitive to individual pesticides, and kidney to mixture pesticide treatments. Detrimental effects of pesticides were more severe on the kidney than the liver during this short term sub-lethal exposure. However, the fish recovered from such toxic effects during the one week depuration period.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgements**

We sincerely acknowledge Central Fisheries Promotion and Conservation Center, Balaju, Kathmandu, Nepal for allowing research facilities. We thank Mr. Mohan Kumar Yadav and Ajaya Kunwar for their help in the acquisition of the test animals, Mr. Suresh Khadka for helping in serum collection and Mr. Suraj Shrestha for providing support in biochemical analysis.

**References**

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C.T., Ayyappan, S., 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost , *Labeo rohita* ( Hamilton ) 58, 220–226. https://doi.org/10.1016/j.ecoenv.2003.12.003.

Akoto, O., Azuure, A.A., Adotey, K.D., 2016. Pesticide residues in water, sediment and fish from Tono Reservoir and their health risk implications. Springerplus 5, 1–11. https://doi.org/10.1186/s40064-016-3544-z.

Ali, D., Nagpure, N.S., Kumar, S., Kumar, R., Kushwaha, B., Lakra, W.S., 2009. Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* ( Bloch ) using micronucleus assay and alkaline single-cell gel electrophoresis. Food Chem. Toxicol. 47, 650–656. https://doi.org/10.1016/j.fct.2008.12.021.

Banaee, M., Haghi, B.N., Ibrahim, A.T.A., 2013. Sub-lethal toxicity of chlorpyrifos on Common carp, *Cyprinus carpio* (Linnaeus, 1758): Biochemical response. Int. J. Aquat. Biol. 1, 281–288.

Bhatnagar, A., Cheema, N., Yadav, A.S., 2017. Alterations in haematological and biochemical profile of freshwater fish, *cirrhinus mrigala* (Hamilton) exposed to sub-lethal concentrations of chlorpyrifos. Nat. Environ. Pollut. Technol. 16, 1189–1194.

Das, S., 2013. A Review of Dichlorvos Toxicity in Fish. Curr. World Environ. 8, 143–149. https://doi.org/DOI : http://dx.doi.org/10.12944/CWE.8.1.08.

De Smet, H., Blust, R., 2001. Stress responses and changes in protein metabolism in carp *Cyprinus carpio* during cadmium exposure. Ecotoxicol. Environ. Saf. 48, 255–262. https://doi.org/10.1006/eesa.2000.2011.

Deka, S., Mahanta, R., 2015. Effect of Dichlorvos on hepato-renal functions of Indian catfish *Heteropneustes fossilis* (Bloch). Int. J. Adv. Res. 3, 1112–1119.

Dogan, D., Can, C., 2011. Hematological, biochemical, and behavioral responses of *Oncorhynchus mykiss* to dimethoate. Fish Physiol. Biochem. 37, 951–958. https://doi.org/10.1007/s10695-011-9492-1.

El Nahas, A.F., Abdel-Razek, M.A.S., Helmy, N.M., Mahmoud, S., Ghazy, H.A., 2017. Impaired antioxidant gene expression by pesticide residues and its relation with other cellular biomarkers in Nile Tilapia (*Oreochromis niloticus*) from Lake Burullus. Ecotoxicol. Environ. Saf. 137, 202–209. https://doi.org/10.1016/j.ecoenv.2016.12.006.

Ezike, C.O., Echor, F.O., Ajima, M.N.O., Maria, V.L., 2017. Butrylacetycholinesterase Activity in Liver and Plasma, Liver Glycogen and Plasma Glucose Content, Haematology and Behaviour of Clariid Catfish *Clarias Gariepinus* to Dichlorvos. Int. J. Adv. Fish. Aquat. Sci. 3, 90–105. https://doi.org/10.23953/cloud.ijafas.332.

FAOSTAT, 2021. Food and Agriculture Organization of the United Nations, http://www.fao.org/faostat/en/#home.

Garcia, F.P., Ascencio, S.Y.C., Oyarzun, J.C.G., Hernandez, A.C., Alavarado, P.V., 2012. Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks. J. Res. Environ. Sci. Toxicol. 1, 279–293.

Ghaffar, A., Hussain, R., Khan, A., Abbas, R.Z., 2015. Hemato-biochemical and genetic damage caused by triazophos in fresh water fish, *Labeo rohita*. Int. J. Agric. Biol. 17, 637–642. https://doi.org/10.17957/IJAB/17.3.14.1016.

Ghelichpour, M., Taheri Mirghaed, A., Mirzargar, S.S., Joshaghani, H., Ebrahimzadeh Mousavi, H., 2017. Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of *Cyprinus carpio* (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb. Aquac. Res. 48, 5666–5676. https://doi.org/10.1111/are.13390.

Gomes-Silva, G., Cyubahiro, E., Wronski, T., Riesch, R., Apio, A., Plath, M., 2020. Water pollution affects fish community structure and alters evolutionary trajectories of invasive guppies (*Poecilia reticulata*). Sci. Total Environ. 730.

https://doi.org/10.1016/j.scitotenv.2020.138912.

Halappa, R., David, M., 2009. Behavioural responses of the freshwater fish, *Cyprinus carpio* (Linnaeus) following sublethal exposure to chlorpyrifos. Turkish J. Fish. Aquat. Sci. 9, 233–238. https://doi.org/10.4194/trjfas.2009.0218.

Hatami, M., Banaee, M., Haghi, B.N., 2019. Sub-lethal toxicity of chlorpyrifos alone and in combination with polyethylene glycol to common carp (*Cyprinus carpio*). Chemosphere 219, 981–988. https://doi.org/10.1016/j.chemosphere.2018.12.077.

Ikpesu, T., 2013. Depuration Technique of Xenobiotics with Reference to Accumulation and Elimination of Paraquat Dichloride in *Clarias Gariepinus*. Iran. J. Toxicol. 894–899.

Ingram, B., Sungan, S., Gooley, G., Sim, S.Y., Tinggi, D., De Silva, S.S., 2005. Induced spawning, larval development and rearing of two indigenous Malaysian mahseer, *Tor tambroides* and *T. douronensis*. Aquac. Res. 36, 1001–1014. https://doi.org/10.1111/j.1365-2109.2005.01309.x.

Ismail, M., Ali, R., Shahid, M., Khan, M.A., Zubair, M., Ali, T., Mahmood Khan, Q., 2017. Genotoxic and hematological effects of chlorpyrifos exposure on freshwater fish *Labeo rohita*. Drug Chem. Toxicol. 1–5. https://doi.org/10.1080/01480545.2017.1280047.

Jaffer, N.S., Rabee, A.M., Al-Chalabi, S.M.M., 2017. Biochemical and hematological parameters and histological alterations in fish *Cyprinus carpio* L. as biomarkers for water pollution with chlorpyrifos. Hum. Ecol. Risk Assess. 23, 605–616. https://doi.org/10.1080/10807039.2016.1261626.

Jha, B.R., Rayamajhi, A., Dahanukar, N., Harrison, A., Pinder, A., 2018. *Tor putitora*. The IUCN Red List of Threatened Species 2018: e.T126319882A126322226. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T126319882A126322226.en.

Jyothi, B., Narayan, G., 2000. Pesticide induced alterations of non-protein nitrogenous constituents in the serum of a fresh water cat fish, *Clarias batrachus* (Linn.). Indian J. Exp. Biol. 38, 1058–1061.

Kafle, B.K., Pokhrel, B., Shrestha, S., Raut, R., Dahal, B.M., 2015. Determination of Pesticide Residues in Water and Soil Samples From Ansikhola Watershed, Kavre, Nepal. Int. J. Geol. Earth Environ. Sci. 5, 119–127.

Koul, P.C., Mastan, S.A., Qureshi, T.A., 2007. Sub- lethal effect of Dichlorvos (DDVP) on certain biochemical parameters of *Channa gachua* (Ham.). J. Herb. Med. Toxicol. 1, 29–32.

Kunwar, P.S., Basaula, R., Sinha, A.K., De Boeck, G., Sapkota, K., 2021a. Joint toxicity assessment reveals synergistic effect of chlorpyrifos and dichlorvos to common carp (*Cyprinus carpio*). Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 246, 108975. https://doi.org/10.1016/j.cbpc.2021.108975.

Kunwar, P.S., Parajuli, K., Badu, S., Sapkota, B., Sinha, A.K., De Boeck, G., Sapkota, K., 2021b. Mixed toxicity of chlorpyrifos and dichlorvos show antagonistic effects in the endangered fish species golden mahseer (*Tor putitora*). Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 240, 108923. https://doi.org/10.1016/j.cbpc.2020.108923.

Medda, C., 1993. Haematological and biochemical alterations of *Catla Catla* fingerlings following sublethal exposutre to nuvan. J. Indian Fish. Assoc. 113–116.

Mennear, J.H., 1998. Dichlorvos: A regulatory conundrum. Regul. Toxicol. Pharmacol. 27, 265–272. https://doi.org/10.1006/rtph.1998.1217.

Nag, S.K., Saha, K., Bandopadhyay, S., Ghosh, A., Mukherjee, M., Raut, A., Raman, R.K., Suresh, V.R., Mohanty, S.K., 2020. Status of pesticide residues in water, sediment, and fishes of Chilika Lake, India. Environ. Monit. Assess. 192, 1–10. https://doi.org/10.1007/s10661-020-8082-z.

Narra, M.R., Rajender, K., Rudra Reddy, R., Rao, J.V., Begum, G., 2015. The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*. Chemosphere 132, 172–178. https://doi.org/10.1016/j.chemosphere.2015.03.006.

Öner, M., Atli, G., Canli, M., 2008. Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. Environ. Toxicol. Chem. 27, 360–366. https://doi.org/10.1897/07-281R.1.

Pimentel, D., 1995. Amounts of pesticides reaching target pests: Environmental impacts and ethics. J. Agric. Environ. Ethics 8, 17–29. https://doi.org/10.1007/BF02286399.

Polidoro, B.A., Morra, M.J., 2016. An ecological risk assessment of pesticides and fish kills in the Sixaola watershed, Costa Rica. Environ. Sci. Pollut. Res. 23, 5983–5991. https://doi.org/10.1007/s11356-016-6144-0.

PQPMC, 2019. List of Registered Pesticides and Pesticide Consumption Data. Plant Quarantine and Pesticide Management Centre, Ministry of Agriculture and Livestock Development. Kathmandu, Nepal.

Qureshi, I.Z., Bibi, A., Shahid, S., Ghazanfar, M., 2016. Exposure to sub-acute doses of fipronil and buprofezin in combination or alone induces biochemical, hematological, histopathological and genotoxic damage in common carp (*Cyprinus carpio* L.), Aquatic Toxicology. Elsevier B.V. https://doi.org/10.1016/j.aquatox.2016.08.012.

Ramesh, M., Saravanan, M., 2008. Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. Int. J. Integr. Biol. 3, 80–83.

Rao, J.C.S., Neelima, P., Rao, K.G., 2017. A review on the toxicity and other effects of Dichlorvos, an organophosphate pesticide to the freshwater fish. Biosci. Discov. 8, 402–415.

Sabra, F.S., Mehana, E.-S.E.-D., 2015. Pesticides Toxicity in Fish with Particular Reference to Insecticides. Asian J. Agric. Food Sci. 3, 40–60.

Saravanan, M., Prabhu Kumar, K., Ramesh, M., 2011. Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. Pestic. Biochem. Physiol. 100, 206–211. https://doi.org/10.1016/j.pestbp.2011.04.002.

Shaikh, I.A., Gautum, R.K., 2014. Effect of Organophosphate Pesticide, Nuvan on Serum Biochemical Parameters of Fresh Water Catfish *Heteropneustes fossilis* (Bloch.). Int. Res. J. Environ. Sci. 3, 1–6.

Shirdel, I., Kalbassi, M.R., Esmaeilbeigi, M., Tinoush, B., 2020. Disruptive effects of nonylphenol on reproductive hormones, antioxidant enzymes, and histology of liver, kidney and gonads in Caspian trout smolts. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 232, 108756. https://doi.org/10.1016/j.cbpc.2020.108756.

Singh, S., Bhutia, D., Sarkar, S., Rai, B.K., Pal, J., Bhattacharjee, S., Bahadur, M., 2015. Analyses of pesticide residues in water, sediment and fish tissue from river Deomoni flowing through the tea gardens of Terai Region of West Bengal, India. Int. J. Fish. Aquat. Stud. 3, 17–23.

Sun, F., Chen, H.-S., 2008. Monitoring of pesticide chlorpyrifos residue in farmed fish: Investigation of possible sources. Chemosphere 71, 1866–1869. https://doi.org/10.1016/j.chemosphere.2008.01.034.

Sun, K.-F., Xu, X.-R., Duan, S.-S., Wang, Y.-S., Cheng, H., Zhang, Z.-W., Zhou, G.-J., Hong, Y.-G., 2015. Ecotoxicity of two organophosphate pesticides chlorpyrifos and dichlorvos on

non-targeting cyanobacteria *Microcystis wesenbergii*. Ecotoxicology. https://doi.org/10.1007/s10646-015-1458-0.

Sunanda, M., Rao, J. Chandra, S., Neelima, P., Simhachalam, G., 2016. Toxicity and effects of chlorpyrifos in a non-target organism (Fish) – A review. J. Atoms Mol. 6, 966–976.

Swar, D.B., 2002. The status of cold water fish and fisheries in Nepal and prospects of their utilization for poverty reduction, in: Peter, T., Swar, D.B. (Ed.), Cold Water Fisheries in the Trans-Himalayan Countries. FAO Fisheries Technical Paper No.431. Food and Agriculture Organization of the United Nations, Rome, pp. 79–96.

Tuck, M.K., Chan, D.W., Chia, D., Godwin, A.K., Grizzle, W.E., Krueger, K.E., Rom, W., Sanda, M., Sorbara, L., Stass, S., Wang, W., Brenner, D.E., 2009. Standard operating procedures for serum and plasma collection: Early detection research network consensus statement standard operating procedure integration working group. J. Proteome Res. 8, 113–117. https://doi.org/10.1021/pr800545q.

Ullah, R., Zuberi, A., Naeem, M., Ullah, S., 2015. Toxicity to hematology and morphology of liver, brain and gills during acute exposure of mahseer (*Tor putitora*) to cypermethrin. Int. J. Agric. Biol. 17, 199–204.

Ural, M.-Ş., Çalta, M., 2005. Acute toxicity of dichlorvos (DDVP) to fingerling mirror carp, *Cyprinus carpio* L. Bull. Environ. Contam. Toxicol. 75, 368–373. https://doi.org/10.1007/s00128-005-0763-3.

Varó, I., Navarro, J.C., Nunes, B., Guilhermino, L., 2007. Effects of dichlorvos aquaculture treatments on selected biomarkers of gilthead sea bream (*Sparus aurata* L.) fingerlings. Aquaculture 266, 87–96. https://doi.org/10.1016/j.aquaculture.2007.02.045.

Velisek, J., Wlasow, T., Gomulka, P., Svobodova, Z., Dobsikova, R., Novotny, L., Dudzik, M., 2006. Effects of cypermethrin on rainbow trout ( *Oncorhynchus mykiss* ). Vet. Med. (Praha). 51, 469–476.

Velmurugan, B., Selvanayagam, M., Cengiz, E.I., Unlu, E., 2007. Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. Environ. Toxicol. Pharmacol. 24, 286–291. https://doi.org/10.1016/j.etap.2007.07.001.

Wang, X., Xing, H., Jiang, Y., Wu, H., Sun, G., Xu, Q., Xu, S., 2013. Accumulation, histopathological effects and response of biochemical markers in the spleens and head kidneys of common carp exposed to atrazine and chlorpyrifos. Food Chem. Toxicol. 62,

148–158.

Yousafzai, A.M., Khan, A.R., Shakoori, A.R., 2008. Hematological and biochemical responses of blood of an endangered South Asian fresh water fish, *Tor putitora* against aquatic pollution. Pak. J. Zool. 40, 123–134.

Zahran, E., Risha, E., Awadin, W., Palić, D., 2018. Acute exposure to chlorpyrifos induces reversible changes in health parameters of Nile tilapia (*Oreochromis niloticus*). Aquat. Toxicol. 197, 47–59. https://doi.org/10.1016/j.aquatox.2018.02.001.

## Table

Table 1

Blood parameters of golden mahseer at different sampling intervals

| | 24 h | | 96 h | | 1 WR | |
|---|---|---|---|---|---|---|
| **Parameters** | Control | Exposed | Control | Exposed | Control | Exposed |
| Glucose (mg/dl) | 83.08±12.44 | 129.18±43.20$^{*}$ | 69.04±9.42 | 79.89±24.40$^{\circ\circ}$ | 84.86±13.11 | 84.78±13.40 $^{\circ\circ}$ |
| Protein (g/dl) | 4.94±0.54 | 4.50±1.13 | 3.96±0.59 | 3.73±1.01 | 4.14±0.73 | 4.66±0.78 |
| Albumin (g/dl) | 1.53±0.16 | 1.15±0.28 | 1.14±0.17 | 0.98±0.28 | 1.16±0.37 | 1.13±0.21 |
| Globulin (g/dl) | 3.42±0.38 | 3.35±0.93 | 2.81±0.55 | 2.75±0.79 | 2.99±0.57 | 3.53±0.82 |
| TG (mg/dl) | 178.26±15.18 | 129.05±32.75 | 156.97±53.93 | 94.53±56.49 $^{*}$ | 144.14±34.50 | 112.22±40.30 |
| Urea (mg/dl) | 8.12±1.25 | 9.61±2.71 | 7.64±1.88 | 10.03±3.27 | 8.85±0.98 | 9.07±1.88 |
| Creatinine (mg/dl) | 0.43±0.10 | 0.58±0.17 | 0.39±0.08 | 0.52±0.11 | 0.48±0.11 | 0.45±0.10 |
| ALT (IU/L) | 14.60±4.16 | 30.88±11.59 | 17.40±5.73 | 41.60±15.85 $^{**}$ | 15.60±3.51 | 21.60±7.08 $^{\bullet\bullet\bullet}$ |
| AST (IU/L) | 191.40±40.24 | 480.00±226.97 | 267.20±73.85 | 577.40±363.72 | 220.40±53.51 | 281.33±84.47 $^{\bullet\bullet}$ |
| ALP (IU/L) | 26.30±5.92 | 45.00±20.93 | 28.30±6.67 | 70.30±32.23$^{**,\circ}$ | 33.10±10.14 | 27.13±8.36 $^{\bullet\bullet\bullet}$ |

Values are mean ± SD. Asterisk denotes significant difference between control and pesticide treated groups at same sampling intervals (*P< 0.05; **P< 0.01); white circle denotes significant difference between same groups of 96 h and 1 WR compared to 24 h ($^{\circ}P$< 0.5) and dark circle denotes significant difference in same groups between 96 h and 1 WR ($^{\bullet\bullet}P$< 0.01; $^{\bullet\bullet\bullet}P$< 0.001).

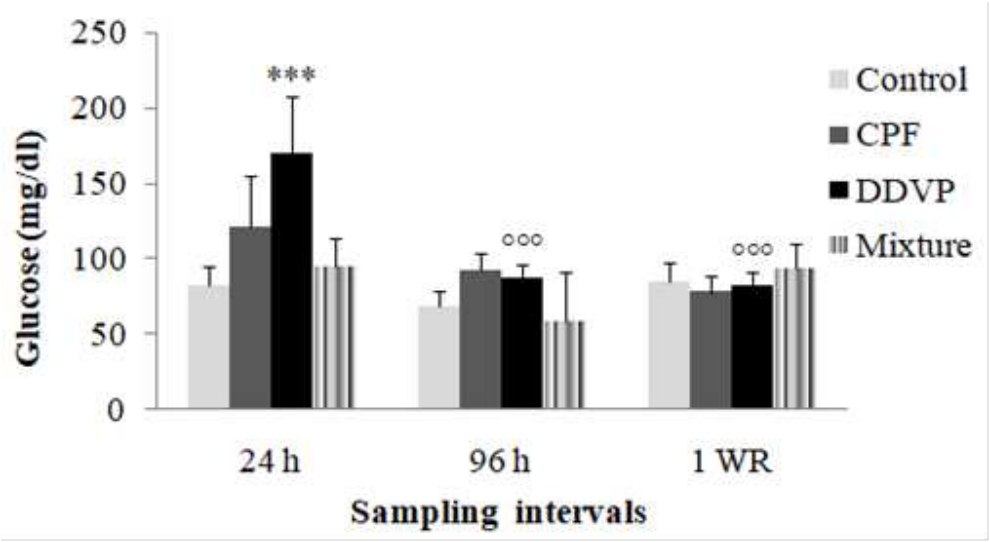


**Figure 1.** Blood glucose (mg/dl) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). Asterisk denotes significant difference between control and pesticide treated groups at same sampling intervals ($^{***}P < 0.001$) and white circle denotes significant difference between same groups of 96 h and 1 week recovery (1 WR) compared to 24 h ($^{\circ\circ\circ}P < 0.001$). No significant difference was observed in same groups between 96 h and 1 WR.

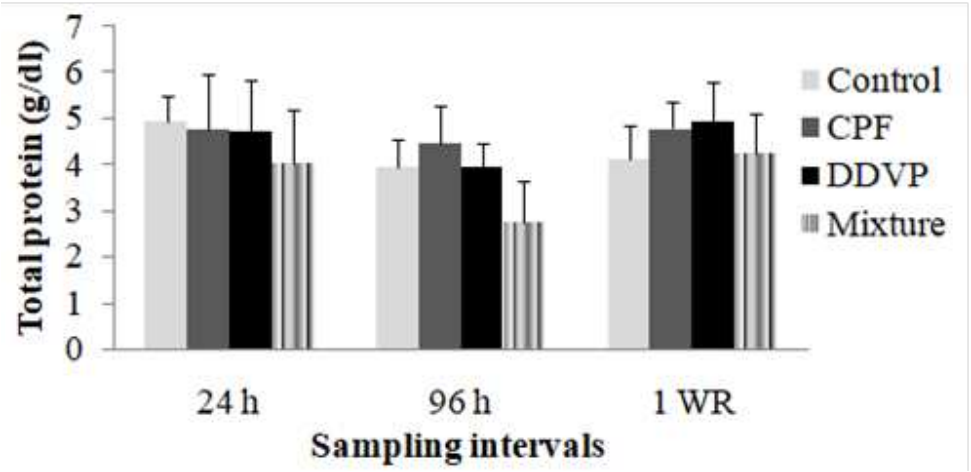


**Figure 2**. Blood total protein (g/dl) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). No significant difference was observed between control and pesticide treated groups at same sampling intervals; between same groups of 96 h and one week recovery (1 WR) compared to 24 h and in same groups between 96 h and 1 WR.

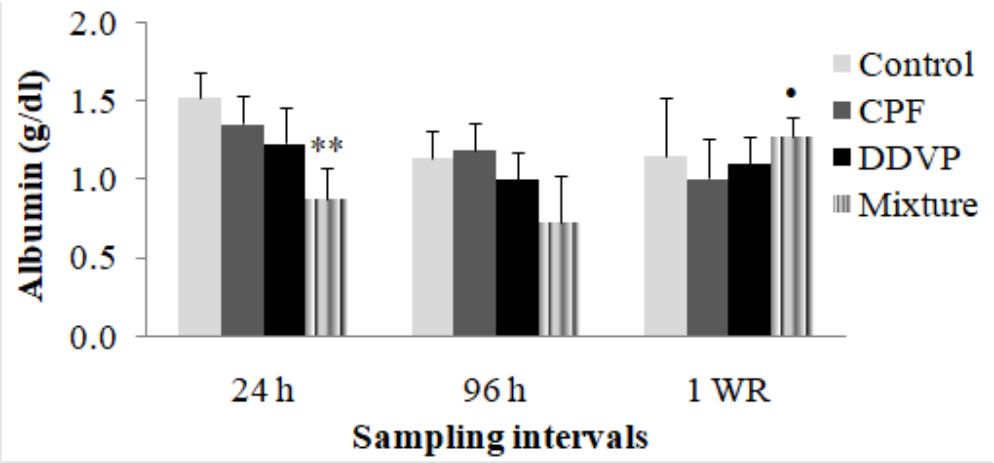


**Figure 3.** Blood albumin (g/dl) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). Asterisk denotes significant difference between control and pesticide treated groups at same sampling intervals ($^{**}P < 0.01$) and dark circle denotes significant difference in same groups between 96 h and one week recovery (1 WR) ($^{\bullet}P < 0.05$). No significant difference was observed between same groups of 96 h and 1 WR compared to 24 h.

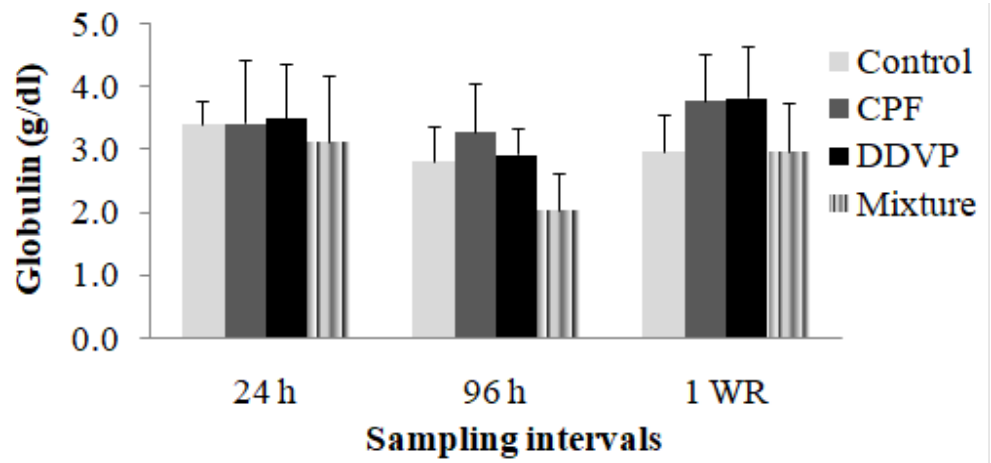


**Figure 4.** Blood globulin (g/dl) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). No significant difference was observed between control and pesticide treated groups at same sampling intervals; between same groups of 96 h and one week recovery (1 WR) compared to 24 h and in same groups between 96 h and 1 WR.

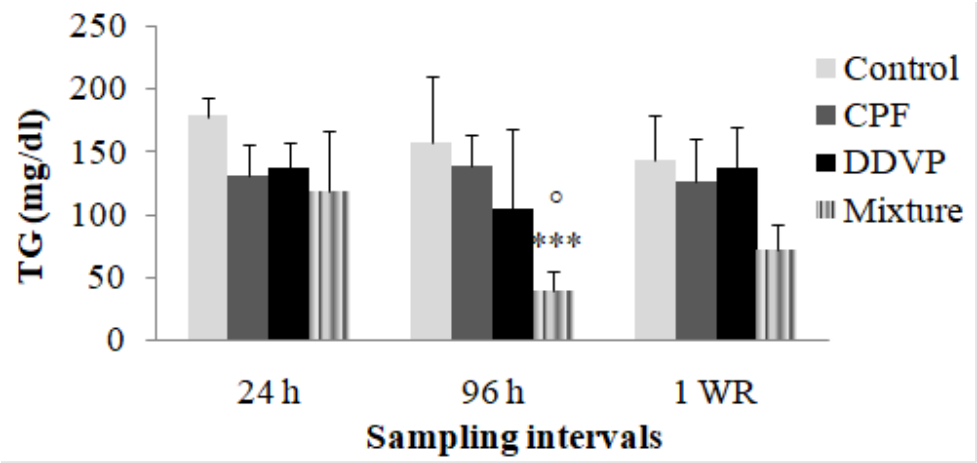


**Figure 5.** Blood triglycerides (mg/dl) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). Asterisk denotes significant difference between control and pesticide treated groups at same sampling intervals (***$P < 0.001$) and white circle denotes significant difference between same groups of 96 h and one week recovery (1 WR) compared to 24 h (°$P < 0.05$). No significant difference was observed in same groups between 96 h and 1 WR.

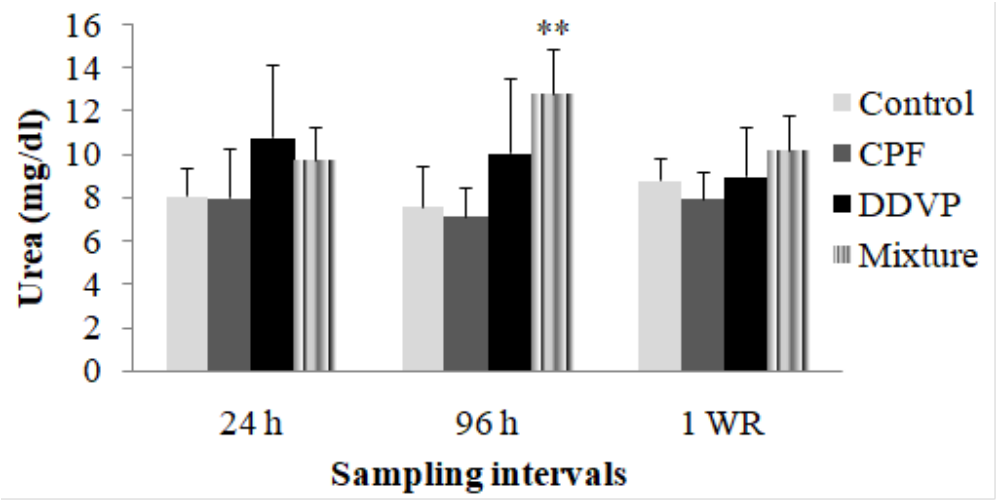


**Figure 6.** Blood urea (mg/dl) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). Asterisk denotes significant difference between control and pesticide treated groups at same sampling intervals (**$P < 0.01$). No significant difference was observed between same groups of 96 h and one week recovery (1 WR) compared to 24 h and in same groups between 96 h and 1 WR.

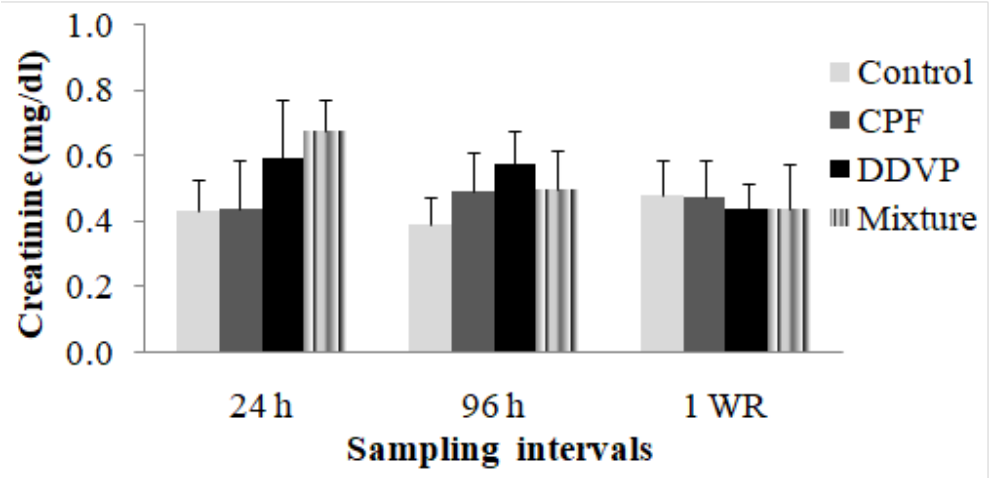


**Figure 7.** Blood creatinine (mg/dl) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). No significant difference was observed between control and pesticide treated groups at same sampling intervals; between same groups of 96 h and one week recovery (1 WR) compared to 24 h and in same groups between 96 h and 1 WR.

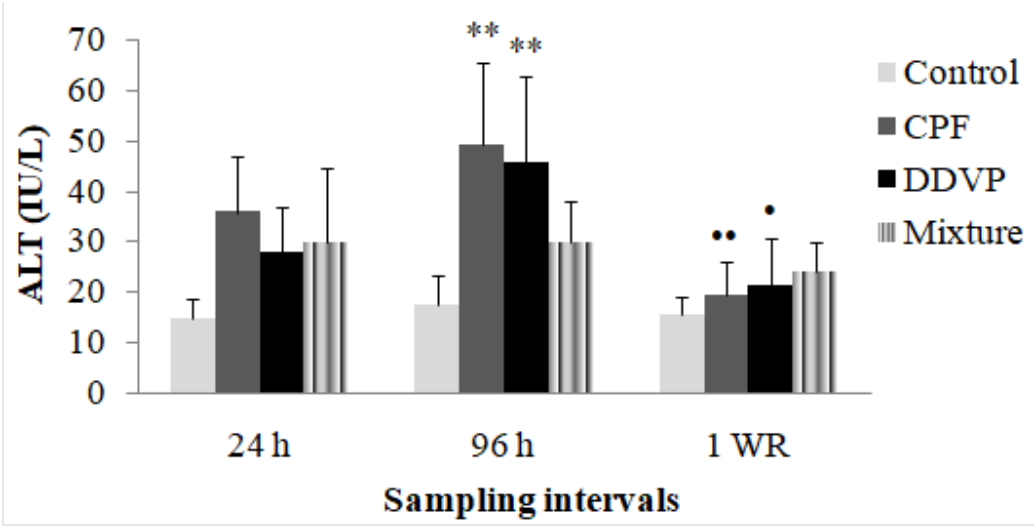


**Figure 8.** Blood ALT (IU/L) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). Asterisk denotes significant difference between control and pesticide treated groups at same sampling intervals ($^{**}P < 0.01$) and dark circle denotes significant difference in same groups between 96 h and one week recovery (1 WR) ($^{\bullet}P < 0.05$; $^{\bullet\bullet}P < 0.01$). No significant difference was observed between same groups of 96 h and 1 WR compared to 24 h.

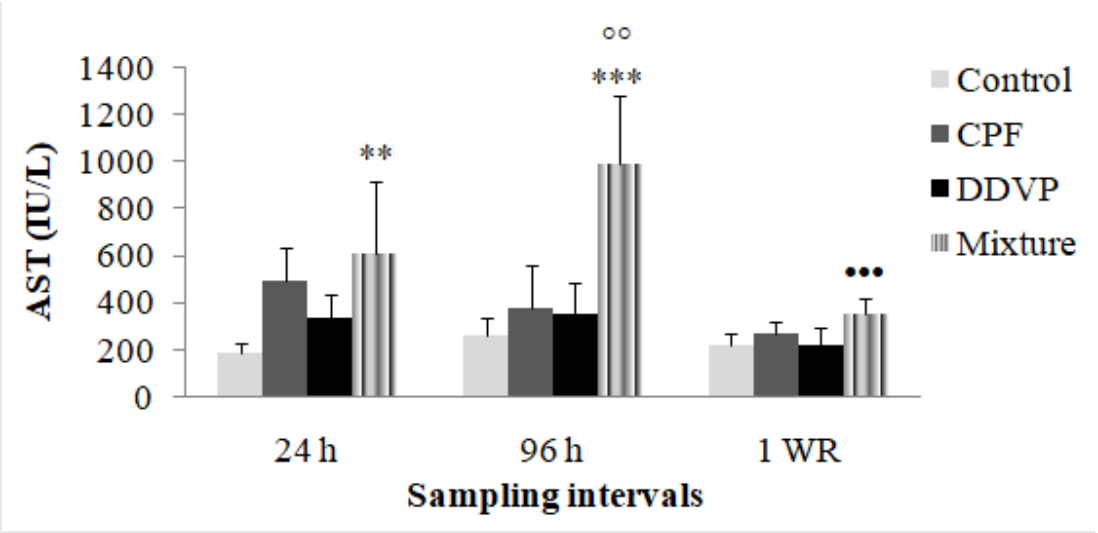


**Figure 9.** Blood AST (IU/L) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values are mean ± SD (n=5-7). Asterisk denotes significant difference between control and pesticide treated groups at same sampling intervals ($^{**}P < 0.01$; $^{***}P < 0.001$); white circle denotes significant difference between same groups of 96 h and one week recovery (1 WR) compared to 24 h ($^{\circ\circ}P < 0.01$) and dark circle denotes significant difference in same groups between 96 h and 1 WR ($^{\bullet\bullet\bullet}P < 0.001$).

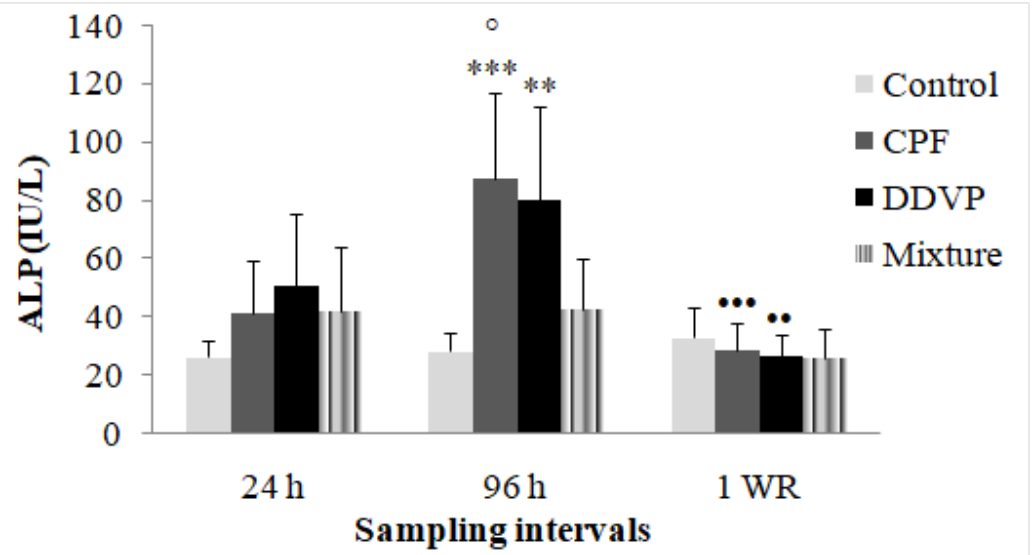


**Figure 10**. Blood ALP (IU/L) of golden mahseer exposed to chlorpyrifos (CPF- 0.075 mg/L), dichlorvos (DDVP- 1.29 mg/L) and mixture (CPF- 0.075 mg/L and DDVP- 1.29 mg/L). Values

are mean ± SD (n=5-7). Asterisk denotes significant difference between control and pesticide treated groups at same sampling intervals ($^{**}P < 0.01$; $^{***}P < 0.001$); white circle denotes significant difference between same groups of 96 h and one week recovery (1 WR) compared to 24 h ($^{\circ}P < 0.5$) and dark circle denotes significant difference in same groups between 96 h and 1 WR ($^{\bullet\bullet}P < 0.01$; $^{\bullet\bullet\bullet}P < 0.001$).