Chapter II

Haematological and Biochemical alterations in freshwater fish *Oreochromis mossambicu*s (Peters, 1852) on exposure of Pyrazosulfuron Ethyl– A sub acute study

Introduction

In aquatic ecotoxicology, fish have become the major vertebrate model which represents the largest and most diverse group, a number of characteristics make them outstanding experimental models for basic as well as advance toxicological research, especially for the contaminants which are likely to exert their impact on aquatic systems as they are indicators as well as show greater sensitivity compared to other aquatic organisms due to their high contaminant accumulation power (De la torre *et al.*, 2010, Souza *et al*., 2013, Huang *et al*., 2013; Czédli *et al*., 2014; Dhanakumar *et al*., 2015 and Zhao *et al*., 2015).The contamination of aquatic system by pesticides causes acute and chronic poisoning to fish and results in severe damage to vital organs (Singh, 2012 and 2013). Exposure to contaminants can cause biological changes in organisms. These changes can be measured and used as indicators of exposure to or effects of environmental pollutants, which are called biomarkers. These biomarkers enable the rapid assessment of the health of organisms and warn about possible environmental risks (Van der Oost *et al*., 2003; Parma *et al*., 2007).

Among biological changes, haematological parameters are considered potential biomarkers of exposure to chemical agents, since they can induce an increase or decrease in the various haematological components. Haematological parameters have been widely employed as pathophysiological indicators to diagnose the structural and functional status of fishes

exposed to a variety of toxicants (Kori-Siakpere *et al*., 2005; Saravanan *et al*., 2011; Seriani and Ranzani-paiva, 2012). Haematological parameters are used as an index to detect physiological changes in a number of fish species and to assess general health status and is advocated to provide early signs of critical modifications during stress conditions as they constitute a non-destructive and simple method (Suvetha *et al*., 2010 França *et al*., 2007; Seriani and Ranzani-paiva, 2012) and also provide early warning tools in monitoring environment quality(Pimpao *et al*., 2007; Osman and Kloas.,2010).

Haematological tests provide the information about the state of erythropoiesis. Previous haematological studies of pollutants (Rehulka, 2002b) brought knowledge that erythrocytes are a major and reliable indicator of various sources of stress. Erythrocytes reflect the state of the organism over a prolonged period of time. The blood cell indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) seem to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with values of RBC count, haemoglobin concentration and packed cell volume.

Biochemical parameters in fish are also sensitive for detecting potential adverse effects of pesticides. The activities of various enzymes are considered to be sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water for the presence of toxicants (Gul *et al*., 2004; Muralidharan 2012 and Pereira *et al*., 2013).The utility of biochemical approaches in environmental pollution monitoring and characterization on exposure to stressor for the use in environmental risk assessment is based on the assumption that low concentrations of a toxicant can cause biochemical responses within individual organisms before these effects are observed at higher levels of biological organization (Sarkar *et al*., 2006). Such biochemical responses are considered to be rapidly

responding endpoints (Adams, 2002), and thus most biochemical biomarkers in the laboratory studies are assessed after acute exposure to pesticides. Changes in the biochemical profile indicate alterations in metabolism of the organism resulting from the effect of the pesticide and they make it possible to study the mechanisms of the effects of these pesticides (Luskova *et al*., 2002).

Several ecotoxicological characteristics of Tilapia such as wide distribution in the freshwater environment, availability throughout the year, easy acclimatization to laboratory conditions and commercial importance make this species an excellent test species for toxicity and biochemical studies (Pandey *et al*., 2005; Nwani *et al*., 2010). Tilapia is also considered to be future of aquaculture, and is known as ‘‘aquatic chicken’’ due to its ability to grow quickly with poor-quality inputs. It is a good biological model for toxicological and immunotoxicity studies due to diverse characteristics, namely their high growth rates, efficiency in adopting to diverse diets, great resistance to diseases and handling practices, easy reproduction in captivity at prolific rate and finally, good tolerance to a wide range of environmental conditions (Neeraj, 2010). There have been studies on toxicities of various groups of herbicides however, there is a lacunae as far as sulfonylurea-based herbicides such as PE is concerned. Determination of the toxicity is essential for determining sensitivity of the animals to the toxicants and also useful for evaluating the degree of damage to the target organs and the consequent physiological, biochemical disorders. Thus, to supplement risk assessment studies of these herbicides, it is important to obtain information on their toxicity and effects on some local species like *O. mossambicus.* ***In the view of paucity of information available on PE toxicity, the present work was under taken on fresh water teleosts, O.mossambicus, so as to have an insight regarding its haematological and biochemical alterations.***

MATERIALS AND METHODS:

Experimental design:

Live and healthy male and female adult *O. mossambicus* was procured from the pure brooders of length 12±3cm and weight 25±3g. Fishes (5 males and 5 females) were kept in a clean glass aquaria for an acclimatisation period of 12-15 days in de-chlorinated water at 27 ± 4ºC, pH 7.4 ± 0.05, dissolved oxygen 8 ± 0.3 mg/L and total hardness 188 mg/L $CaCO_3$ with a 12:12 light: dark photoperiod. They were fed with the commercial available healthy food during the period of study. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. Animal maintenance and experimental procedures were following the guideline of A.P.H.A., A.W.W.A. and W.P.C.F. (1998).

Experimental Procedure

On basis of LC_{50} value sub-acute study dose $1/20^{th}LC_{50}$ (Low dose), $1/10^{th}$ LC_{50} (Medium dose), $1/5^{th}$ LC_{50} (High dose) were chosen for haematological and biochemical studies. The experimental regime was maintained in the laboratory for14 days for exposure group and for 28 days for recovery group. A control group was maintained. The experiment was performed semi statically with a group of 10 fish in triplicate, one control and one test aquaria. Haematological and biochemical examinations of the experimental as well as the control fish were carried out at $7^{th}$and 14^{th}days of exposure and on 28^{th} days for recovery. All the groups were kept under continuous observation during the experimental period. Commercially food pallets were given to fish once in day during the experiment *ad libitum*. Test chemical and test media were changed every 24 hour to maintain the toxicant strength and the level of dissolved oxygen as well as to minimize the level of ammonia during experiment.

• Group 1 served as control without any treatment of PE.

• Group 2 were treated with low dose of PE ($1/20^{th}$ LC_{50})

• Group 3 were treated with medium dose of PE ($1/10^{th}LC_{50}$)

• Group 4 were treated with high dose of PE ($1/5^{th}LC_{50}$)

From all the groups after 14 days of exposure the remaining fish were shifted to herbicide free fresh water and blood samples were analysed at 28 days of recovery. Parameters of fish after 28 days of exposure were taken as the initial level (Day 0) for the recovery period. The rate of recovery of the chosen parameters was calculated by dividing the difference between Days 0 and 28 of the recovery period by the actual recovery period. The number of days taken for complete recovery of a chosen parameter from herbicide exposure was calculated by dividing the difference between Days 0 and 28 of exposure period by the rate of recovery (James *et al.,* 1996). Pesticide concentration in the media and fish could not be estimated due to lack of facility.

Haematological and biochemical estimation of fish:

After the completion of the exposure, fish were caught very gently using a small dip net, one at a time with least disturbance. They were slowly released in the tough containing 1% clove oil to make it immobile, and then after each fish was held and wrapped with a clean, dry towel and the posterior half of its body were blotted with a clean coarse filter paper. The total body weight was noted. The caudal peduncle of the fish was severed with a single stroke from a heavy, sharp scissor. After discarding the first drop of blood, the freely oozing blood was collected using separate heparinized disposable syringe. The blood was then transferred to the eppendorf containing anticoagulant, thoroughly mixed using a thin, blunt glass rod, during the process of collection itself. The blood was stored in -4°C prior to haematological

and biochemical estimations. Haemoglobin estimation (HB), Pack Cell Volume (PCV), blood glucose level and total serum protein were analyzed by NIHON KOHDEN Automated Haematology Analyzer (Celtics α, Japan). Red blood cell count (RBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) was determined using the formulas given below.

Blood Cell Count:

The red blood corpuscles (RBC) and White blood corpuscles (WBC) were counted using haemocytometer crystalline chamber using "Hayem's" and "Turch's" diluting fluid, respectively.

Haemoglobin Estimation (HB) and Pack Cell Volume (PCV):

They were analyzed in NIHON KOHDEN Automated HematologyAnalyzer (Celtics α, Japan).

Mean Cell Haemoglobin Concentration (MCHC):

This refers to the percentage of haemoglobin in 100 ml of red blood cell. This was calculated by dividing the haemoglobin content in g/dL by the PCV % of red blood according to the formulae:

MCHC = HB/PCV*1000 g/dL

Mean Corpuscular Volume (MCV):

The value of the corpuscular volume was calculated from the haematocrit value (PCV %) and the erythrocyte count (10^6/ µL) using the formulae:

MCV =PCV*1000/ RBCs fL

Mean Corpuscular Haemoglobin (MCH):

Mean corpuscular Haemoglobin concentration expresses the concentration of haemoglobin in unit volume of erythrocyte. It was calculated from the haemogobin value (HB) and from the erythrocyte count according to the following formulae

MCH = HB/RBCs pg

Statistical Analysis:

Statistical analyses were performed using SPSS (version 16) software. Data are presented as mean ± SE. Data were analysed by one-way of variance analysis (ANOVA). The significant means were compared by Turkey's test and a $p < 0.05$ was considered statistically significant. For recovery experiment, regression analysis based on the least squares method following Zar (1974) was carried out to predict the pre exposure levels of hematological and biochemical parameters.

Result:

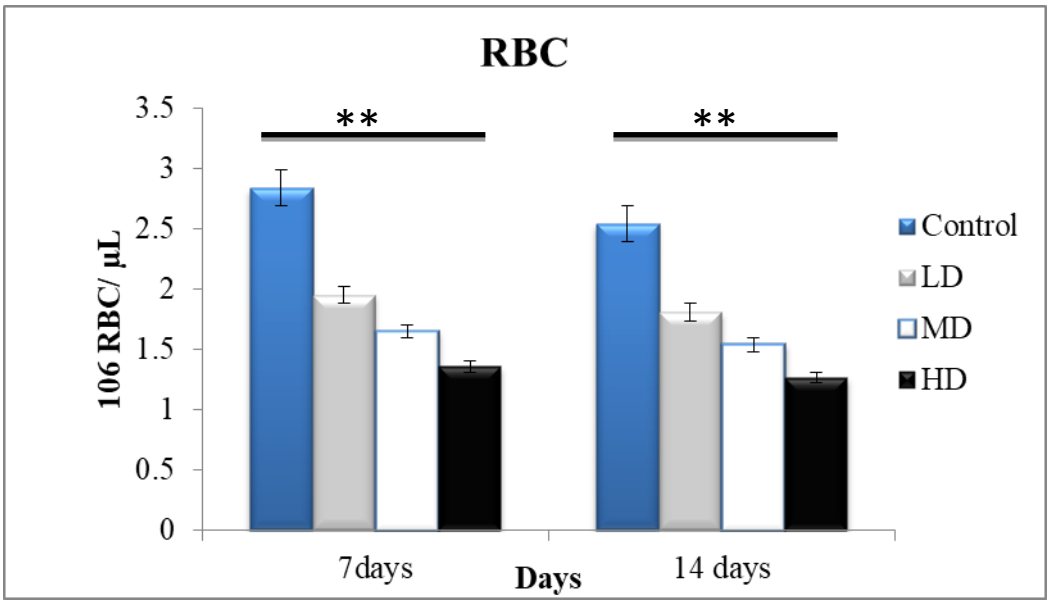
Alterations in the haematological and biochemical parameters of the control group and PE exposed experimental group for the period of 7and 14days on *O. mossambicus* has been tabulated (Tables 2.1 and 2.2) and plotted in Fig: 2.1 to 2.8.

From the study it is obvious that the total number of RBCs in Tilapia (Table 2.1) significantly ($p<0.001$) decreased from 2.84 x 10^6/µL ± 0.01 to 1.95 x 10^6/µL ± 0.01 at low dose, 1.65 x 10^6/µL ± 0.01 at medium dose and 1.36 x 10^6/µL ± 0.01 at high dose for 7th day exposure period while at 14th day exposure period 2.44 x 10^6/µL ± 0.01 to 1.81 x 10^6/µL ± 0.01 at low dose, 1.54 x 10^6/µL ± 0.01 at medium dose and 1.27 x 10^6/µL ± 0.01 at high dose when exposed to PE. A significant decrease ($p<0.001$) in Hb and PCV were observed at low, medium and high dose compared to the control for both the duration i.e at 7th and 14th day (Fig 2.1 and 2.2). There was a significant ($p<0.001$) time and dose dependent alterations in MCV, MCH and MCHC in the fish *O. mossambicus* when exposed to PE. The total number of WBC in *O. mossambicus* increased from 15.21 x 10^3/µL ± 37.39 to 16.24x 10^3/µL ± 7.36 at low dose, 18.39x 10^3/µL ± 22.64 at medium dose and to 20.91 ± 19.06 x 10^3/µL ± 19.06 at high dose when exposed to PE for 7th day while it 15.18 x 10^3/µL ± 6.64 to 17.35x 10^3/µL ± 22.54 at low dose, 20.47x 10^3/µL ± 17.9 at medium dose and to 22.14x 10^3/µL ± 21.93 for 14 day exposed to PE.

As far as biochemical parameters are concerned, PE exposure resulted into a significant increase in Glucose, Urea, BUN and Creatinine ($P<0.01$) in a time and dose dependent manner. On the other hand, protein, Albumin and Globulin depicted a significant decrease ($p<0.01$) in the entire PE exposed group compared to control.

When fish were shifted to pesticide free fresh water after 28 days of exposure, RBC gradually increased during the recovery period Table 2.1 and figure 2.9 there was significant and positive correlation between RBC count and recovery period at all concentrations. The rate of recovery and extrapolated time between day 0 and 28 of the recovery period indicated that completed recovery would occur on days 31, 32 and 32 in fish exposed to low, medium and high dose of PE (Fig. 2.9). Similarly the WBC gradually declined towards the pre-exposure level. The correlation coefficient obtained for the relationship between WBC counts was significant and negative at all tested concentrations. (r= 1.0). The rate of recovery and extrapolated time between days 0 and 28 of recovery period revealed that WBC recorded completely 38, 41 and 43 in fish exposure to low, medium and high dose of PE (Fig.2.15). Dose and time dependent decrease in Hb and PCV, MCV, MCH and MCHC gradually improved at all concentration (Table 2.1 and Fig 2.10 to 2.14).

As far as the biochemical parameters are concerned, the rate of recovery and extrapolated time between Days 0 and 28 of the recovery period indicated that complete recovery would occur on Days 28 in fish exposed to LD, MD and HD of PE for Glucose, protein , Albumin and Globulin respectively. However, for Urea, BUN and Creatinine the rate of recovery and extrapolated time between Days 0 and 28 of the recovery period indicated that complete recovery would occur on Days 28, 29 and 32 in fish exposed to LD, MD and HD of PE **(**Table 2.2 and Fig. 2.15 to 2.21).



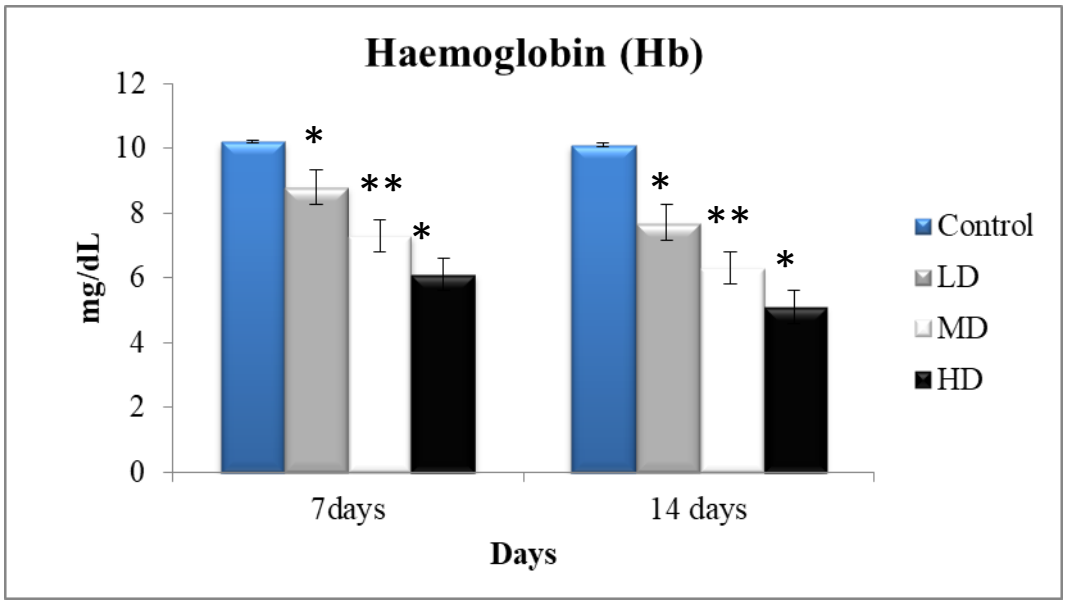
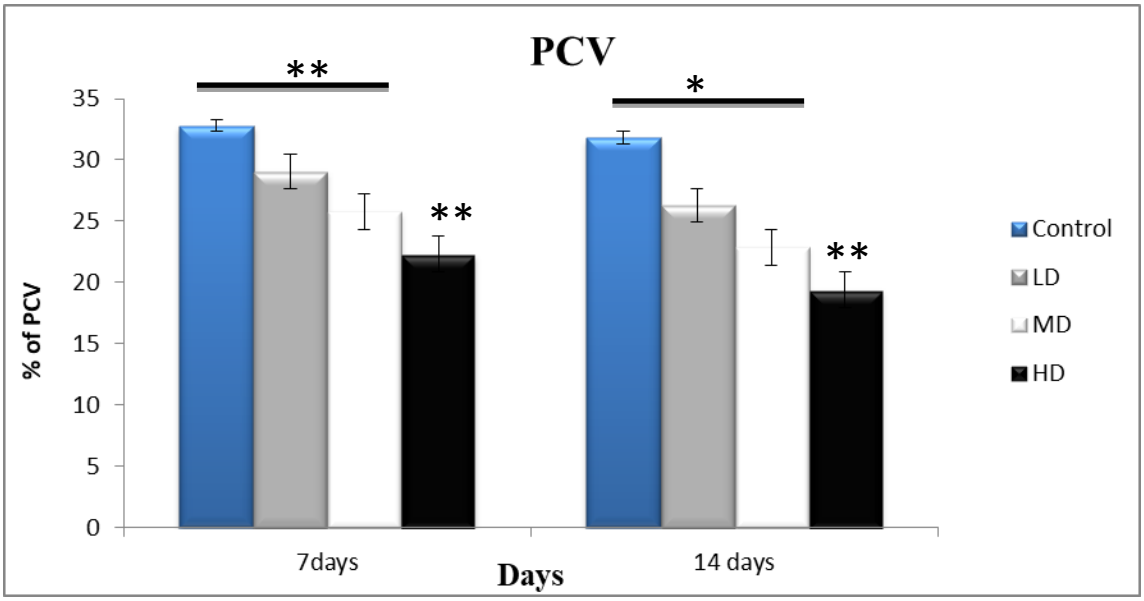


Fig 2.1: Changes in Haematological Parameter: RBC and Hb of *O.mossambicus* on exposure of PE



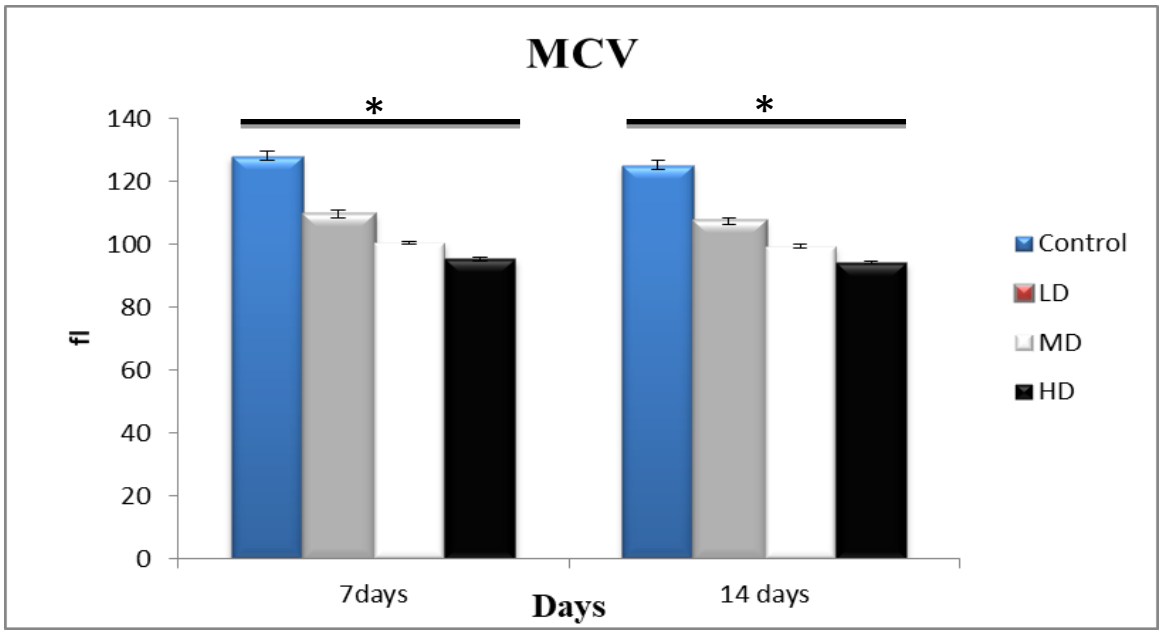
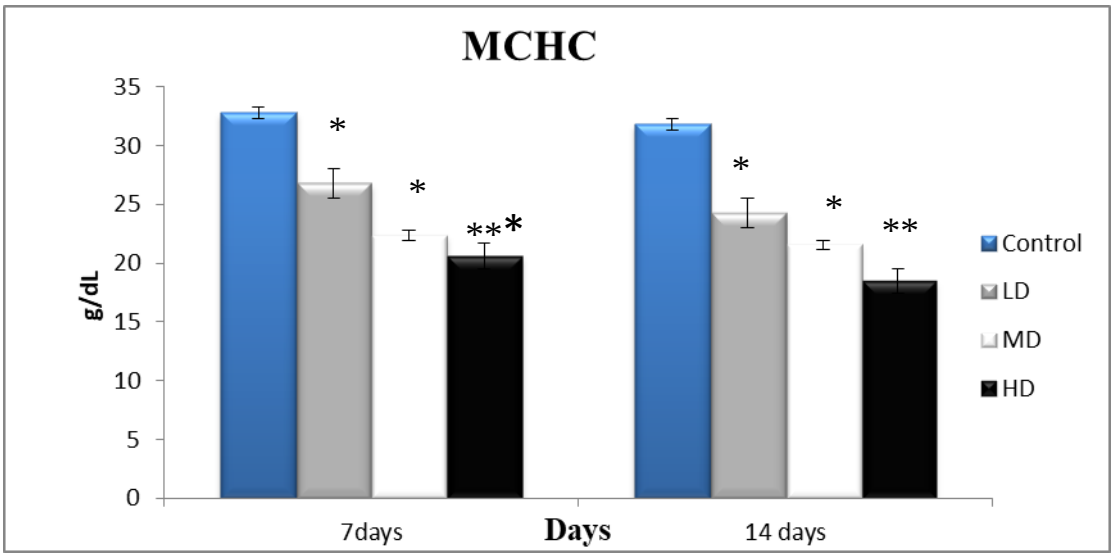


Fig 2.2: Changes in Haematological Parameter: PCV and MCV of *O.mossambicus* on exposure of PE



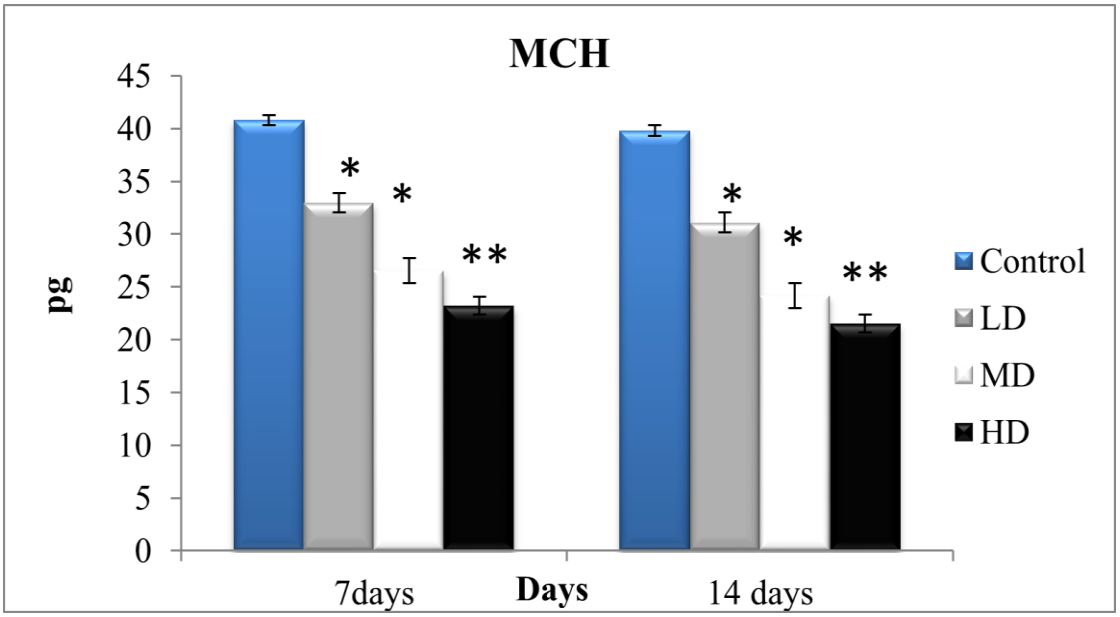


Fig 2.3: Changes in Haematological Parameter: MCHC and MCH of *O.mossambicus* on exposure of PE

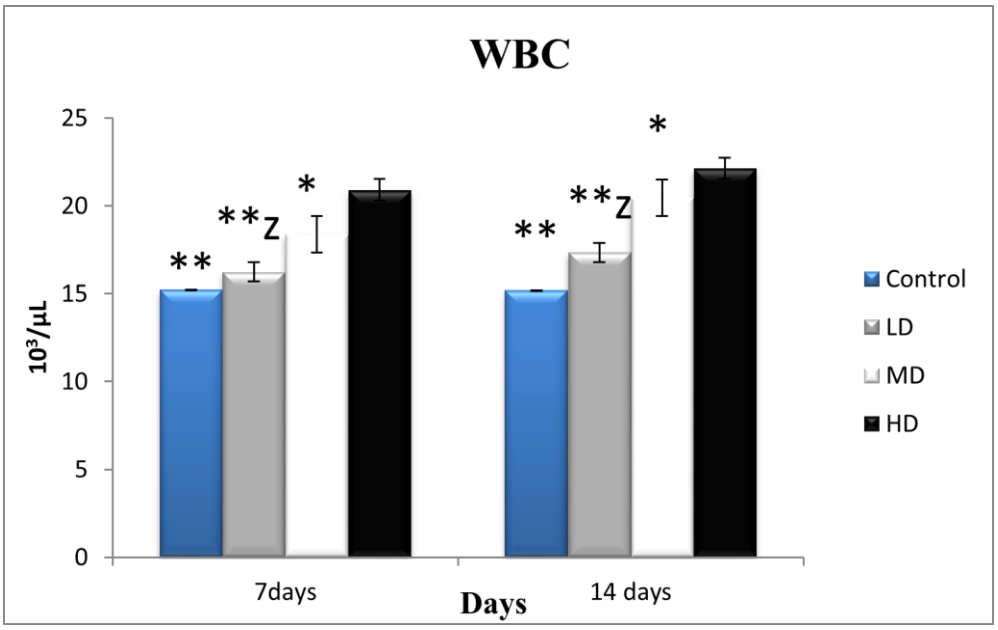
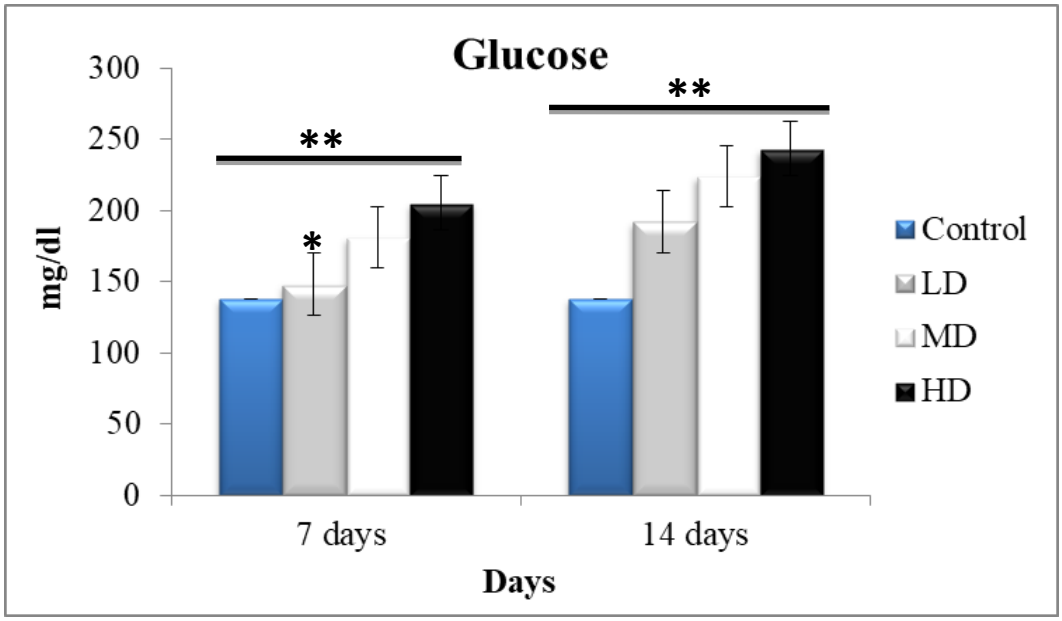


Fig 2.4: Changes in Haematological Parameter: WBCs of *O.mossambicus* on exposure of PE



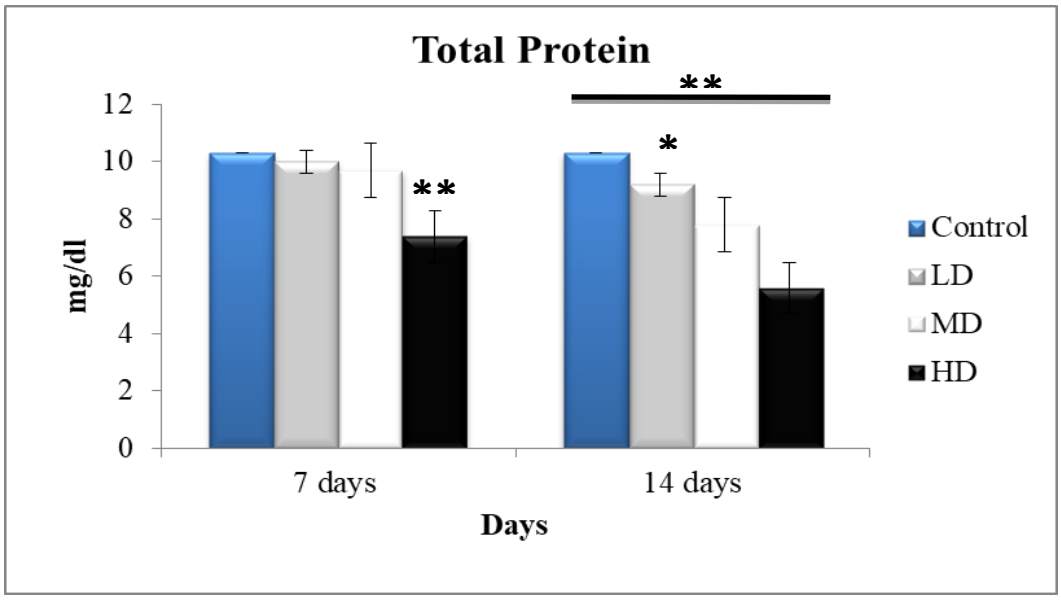
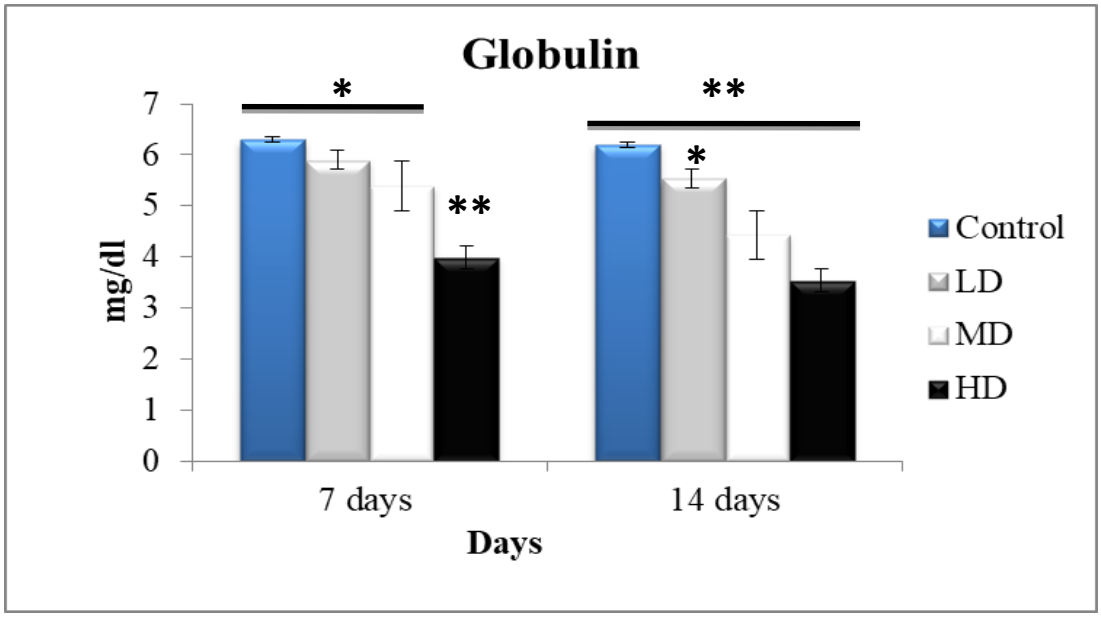


Fig 2.5: Changes in Biochemical Parameter: Glucose and Total Protein of *O.mossambicus* on exposure of PE



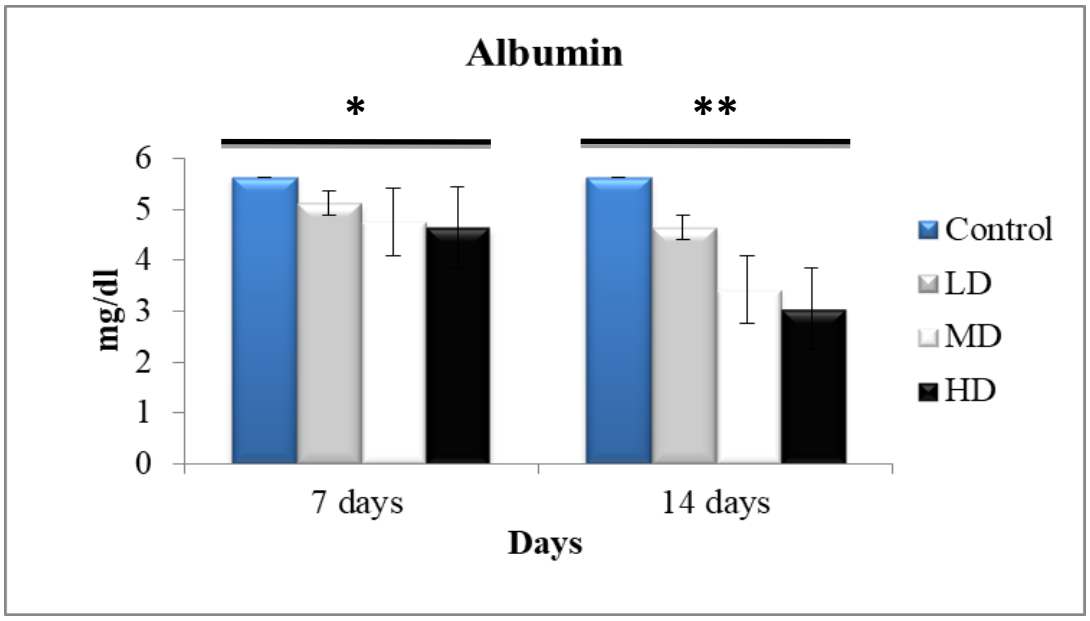
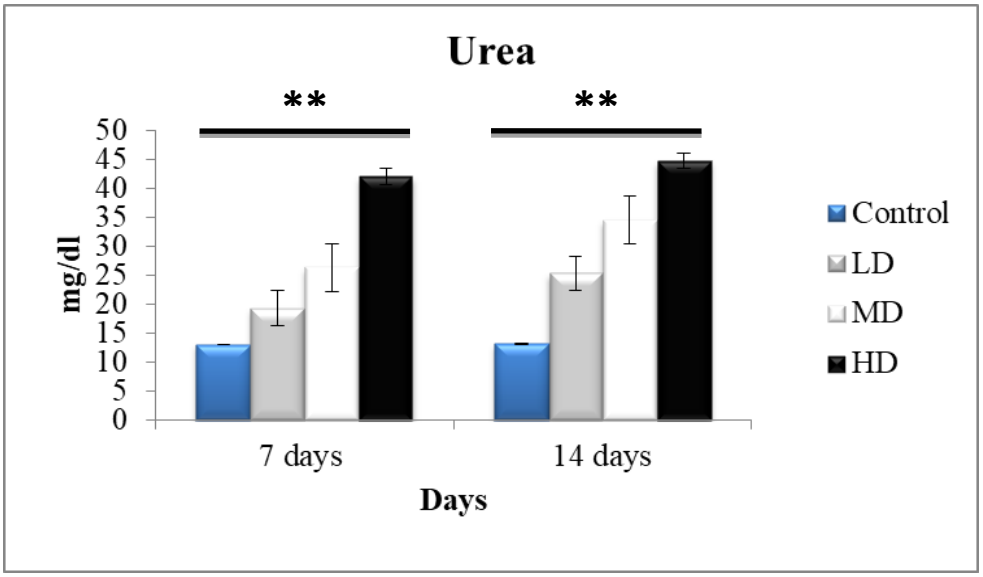


Fig 2.6: Changes in Biochemical Parameter: Globuline and Albumin of *O.mossambicus* on exposure of PE



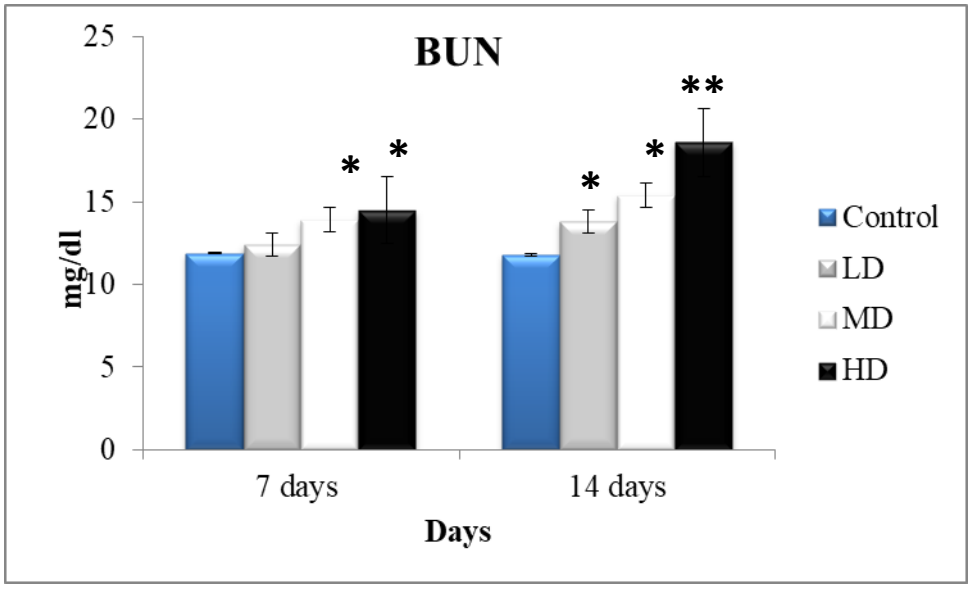


Fig 2.7: Changes in Biochemical Parameter: Urea and BUN of *O.mossambicus* on exposure of PE

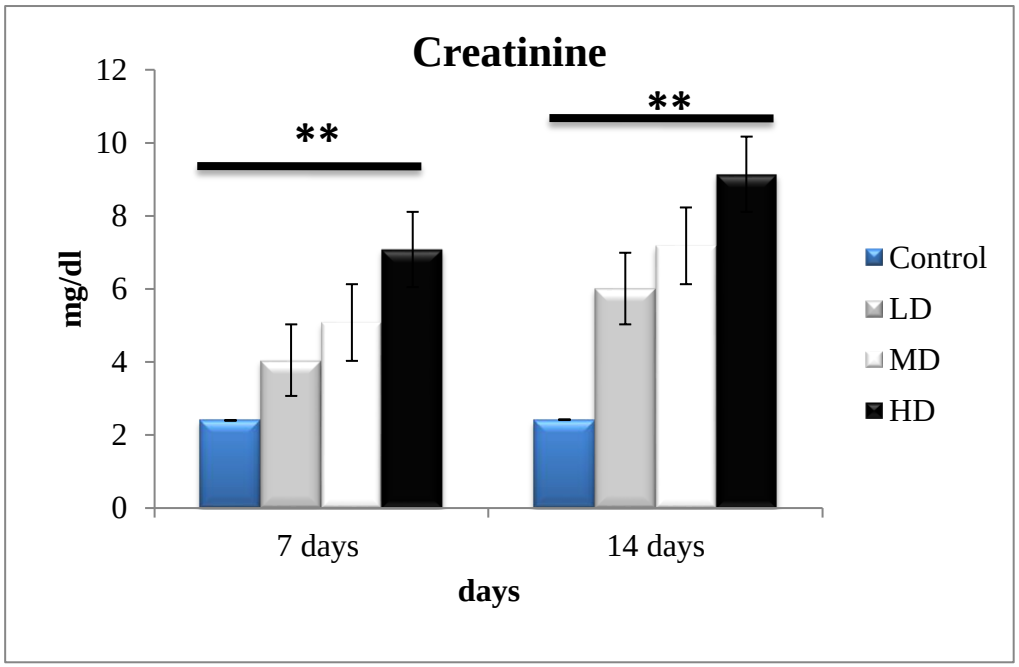


Fig 2.8 Changes in Biochemical Parameter: Creatinine of *O.mossambicus* on exposure of PE

Table 2.1: Changes in Haematological parameters during exposure and recovery treatment in *O.mossambicus* as a function of sublethal levels of PE

Concentration	Exposure period (Days)		Recovery period (Days)	Rate of Recovery	Complete Recovery
	7th	**14th**	**28th**		
Total erythrocyte count (10^6 mm3)- RBCs 10^6 /ml					
Control	2.84 ± 0.01	2.54 ± 0.01	2.68 ± 0.04	-	-
1/20th LC50 (LD)	1.95 ± 0.01***	1.81 ± 0.01***	2.64 ± 0.05	0.029	31
1/10th LC50 (MD)	1.65 ± 0.01***	1.54 ± 0.01***	2.6 ± 0.07	0.037	32
1/5th LC50 (HD)	1.36 ± 0.01***	1.27 ± 0.01***	2.59± 0.02	0.047	32
Hemoglobin (g%) HB g/dL					
Control	10.2 ± 0.01	10.1 ± 0.09	10.2 ± 0.08	-	-
1/20th LC50 (LD)	8.8 ± 0.03***	7.7 ± 0.09***	10.1± 0.05	0.085	28
1/10th LC50 (MD)	7.3 ± 0.06***	6.3 ± 0.06***	9.9± 0.09	0.128	30
1/5th LC50 (HD)	6.1 ± 0.03***	5.1 ± 0.06***	9.7± 0.01	0.164	31
Pack Cell Volume -PCV(Htc)%					
Control	32.8 ± 0.03	31.8 ± 0.09	32.7 ± 0.09	-	-
1/20th LC50 (LD)	29.1 ± 0.06***	26.3 ± 0.06***	32.1± 0.04	0.207	27
1/10th LC50 (MD)	25.8 ± 0.03***	22.9 ± 0.06***	31.7± 0.09	0.314	28
1/5th LC50 (HD)	22.3 ± 0.06***	19.4 ± 0.09***	31.5± 0.01	0.432	29
MCV fL					
Control	128.2 ± 0.03	125.2 ± 0.06	128.4± 0.03	-	-
1/20th LC50 (LD)	109.6 ± 0.1***	107.4 ± 0.12***	127.5± 0.09	0.717	25
1/10th LC50 (MD)	100.5 ± 0.06***	99.3 ± 0.06***	127.1± 0.05	0.992	26
1/5th LC50 (HD)	95.4 ± 0.21***	94.2 ± 0.2***	126.7± 0.02	1.16	27
MCHC g/dL					
Control	32.8 ± 0.07	31.8 ± 0.09	32.7± 0.01	-	-
1/20th LC50 (LD)	26.77 ± 0.01***	24.25 ± 0.0***	32.4± 0.04	0.291	26

1/10th LC50 (MD)	22.38 ± 0.01***	21.54 ± 0.02***	31.8± 0.08	0.366	28
1/5th LC50 (HD)	20.62 ± 0.02***	18.47 ± 0.01***	31.2± 0.04	0.454	29
MCH pg					
Control	40.80 ± 0.03	39.80 ± 0.09	40.7± 0.02	-	-
1/20th LC50 (LD)	32.96 ± 0.01***	31.10 ± 0.09	40.6± 0.03	0.339	26
1/10th LC50 (MD)	26.54 ± 0.0***	24.16 ± 0.01	39.4± 0.07	0.544	29
1/5th LC50 (HD)	23.22 ± 0.01	21.54 ± 0.01	39.1± 0.02	0.627	29
Total WBC $10^3/\mu L$(Increase)					
Control	15.21 ± 37.39	15.18 ± 6.64	15.2± 8.78	-	-
1/20th LC50 (LD)	16.24 ± 7.36***	17.35 ± 22.54***	15.9± 9.26	0.048	38
1/10th LC50 (MD)	18.39 ± 22.64***	20.47 ± 17.9***	15.7± 17.37	0.087	41
1/5th LC50 (HD)	20.91 ± 19.06***	22.14 ± 21.93***	16.1±20.32	0.209	

Table 2.2: Changes in Biochemical parameters during exposure and recovery treatment in *O.mossambicus* as a function of sublethal levels of PE

Concentration	Exposure period (Days)		Recovery period (Days)	Rate of Recovery	Complete Recovery
	7th	14th	28th		
Glucose					
Control	138 ± 2.31	138 ± 2.03	139± 2.03	-	-
1/20th LC50 (LD)	148 ± 1.86***	192 ± 2.33***	140± 2.43	1.857	28
1/10th LC50 (MD)	181 ± 1.76***	224 ± 1.76***	142± 2.02	2.928	28
1/5th LC50 (HD)	205 ± 2.31***	243 ± 2.03***	145± 2.08	3.5	28
Protein					
Control	10.3 ± 0.22	10.3 ± 0.32	10.2± 0.31	-	-
1/20th LC50 (LD)	10.0 ± 0.12*	9.2 ± 0.09*	10.1± 0.05	0.032	28
1/10th LC50 (MD)	9.7 ± 0.09***	7.8 ± 0.12***	10± 0.14	0.078	28
1/5th LC50 (HD)	7.4 ± 0.06***	5.6 ± 0.17***	9.9± 0.08	0.153	28
Albumin					
Control	5.64 ± 0.04	5.64 ± 0.01	5.7± 0.03	-	-
1/20th LC50 (LD)	5.12 ± 0.04***	4.64 ±0.02***	5.5± 0.05	0.03	28
1/10th LC50 (MD)	4.75 ± 0.01***	3.42 ±0.03***	5.5± 0.08	0.07	28
1/5th LC50 (HD)	4.64 ± 0.07***	3.05 ±0.02***	5.3± 0.02	0.08	28
Globulin					
Control	6.3 ± 0.38	6.2 ± 0.17	6.1± 0.17	-	-
1/20th LC50 (LD)	5.9 ± 0.07	5.54 ± 0.03**	5.9 ± 0.19	0.012	28
1/10th LC50 (MD)	5.4 ± 0.15 **	4.43 ± 0.03***	5.9 ± 0.21	0.052	28
1/5th LC50 (HD)	4.0 ± 0.26**	3.54 ± 0.19***	5.7± 0.12	0.077	28
Urea					
Control	13.1 ± 0.25	13.2 ± 0.29	13.3± 0.31	-	-

1/20th LC50 (LD)	19.4 ± 0.21***	25.4 ± 0.32***	13.9± 0.23	0.410	29
1/10th LC50 (MD)	26.4 ± 0.07***	34.6 ± 0.24***	14± 0.27	0.735	29
1/5th LC50 (HD)	42.1 ± 0.29***	44.8 ± 0.06***	14.2± 0.23	1.092	32
BUN					
Control	11.9 ± 0.17	11.8 ± 0.10	11.8± 0.10	-	-
1/20th LC50 (LD)	12.4 ± 0.23*	13.8 ± 0.15 ***	12± 0.12	0.064	28
1/10th LC50 (MD)	13.9 ± 0.12***	15.4 ± 0.13***	12.1± 0.19	0.117	30
1/5th LC50 (HD)	14.5 ± 0.17***	18.6 ± 0.1***	12.3± 0.13	0.225	32
Creatinine					
Control	2.40 ± 0.12***	2.42 ± 0.18	2.46± 0.12	-	-
1/20th LC50 (LD)	4.05 ± 0.02***	6.01 ± 0.33***	2.49± 0.13	0.125	28
1/10th LC50 (MD)	5.08 ± 0.01***	7.18 ± 0.06***	2.51± 0.19	0.166	29
1/5th LC50 (HD)	7.08 ± 0.02***	9.14 ± 0.02***	2.54± 0.04	0.235	29

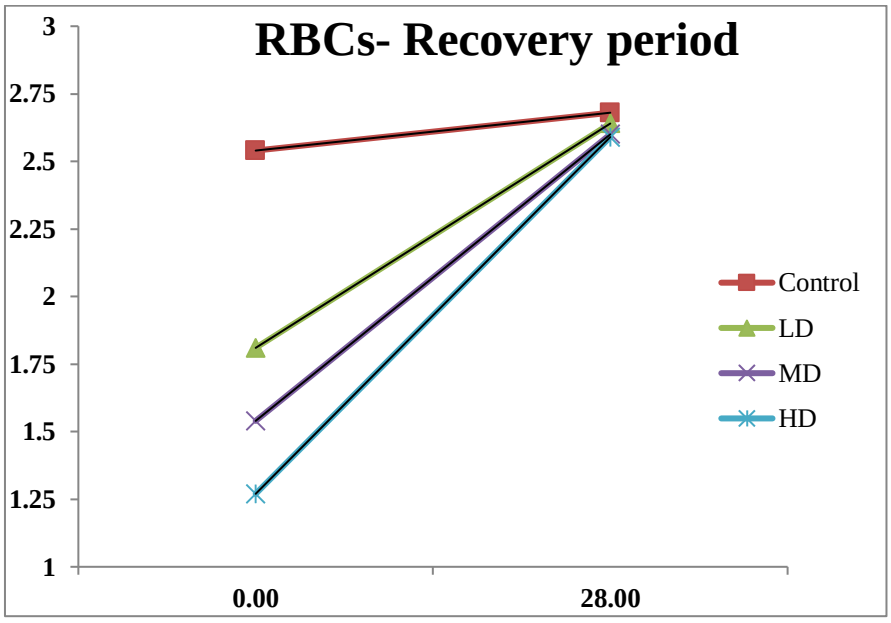


Fig 2.9: Recovery of blood perameters. in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: RBCs: Control, Y = 0.14x + 2.4; LD, Y = 0.83x + 0.98; MD Y= 1.06x + 0.48, HD, Y = 1.32x - 0.05

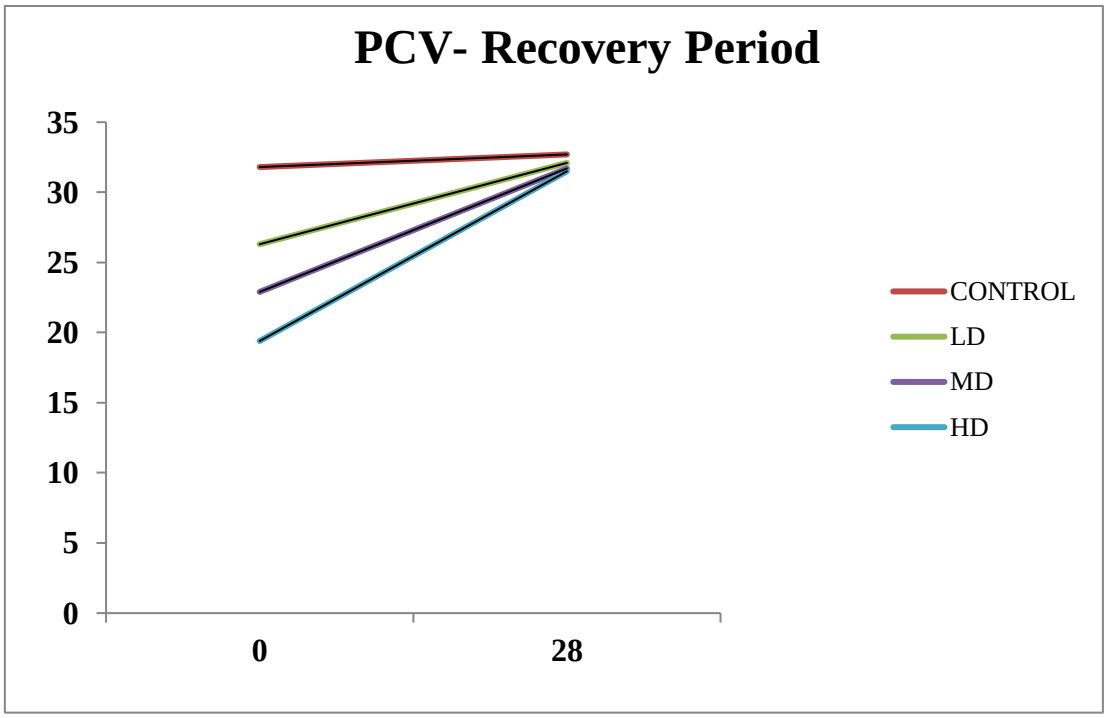


Fig 2.10: Recovery of PCV in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: PCV: Control, Y = 0.9x+30.9; LD, Y = 5.8x+20.5; MD Y= 8.8x+14.1, HD, Y = 12.1x+7.3.

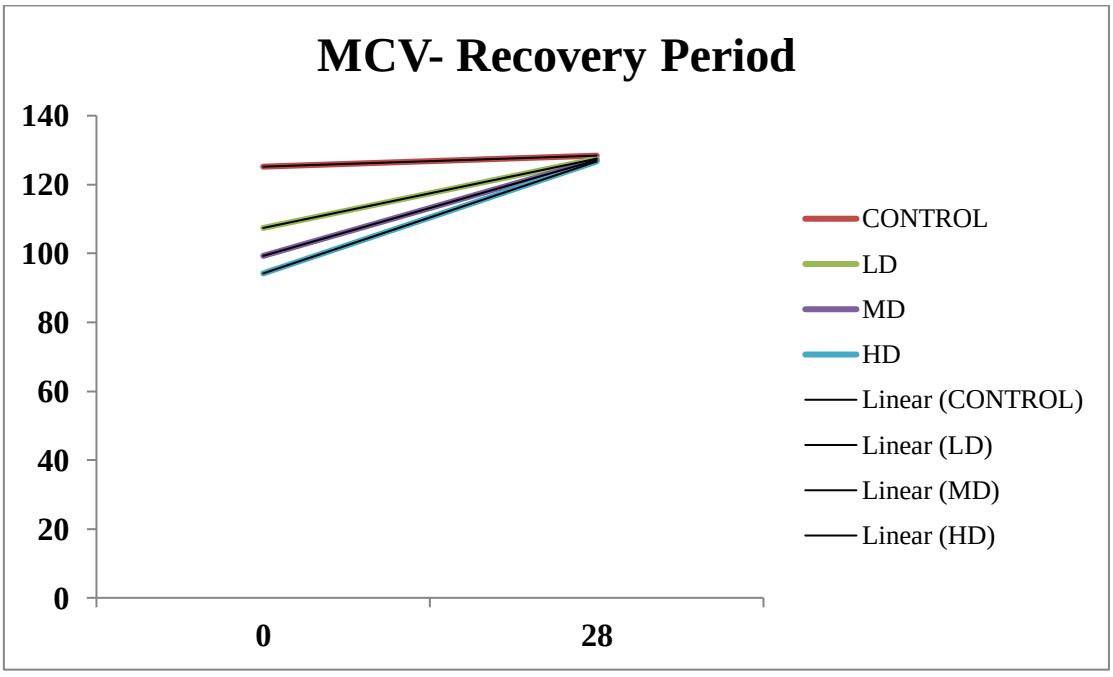


Fig 2.11: Recovery of MCV in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained MCV: Control, Y 3.2x+122; LD, Y = 20.1x+87.3; MD Y= 27.8X+71.5, HD, Y = 32.5x+61.7

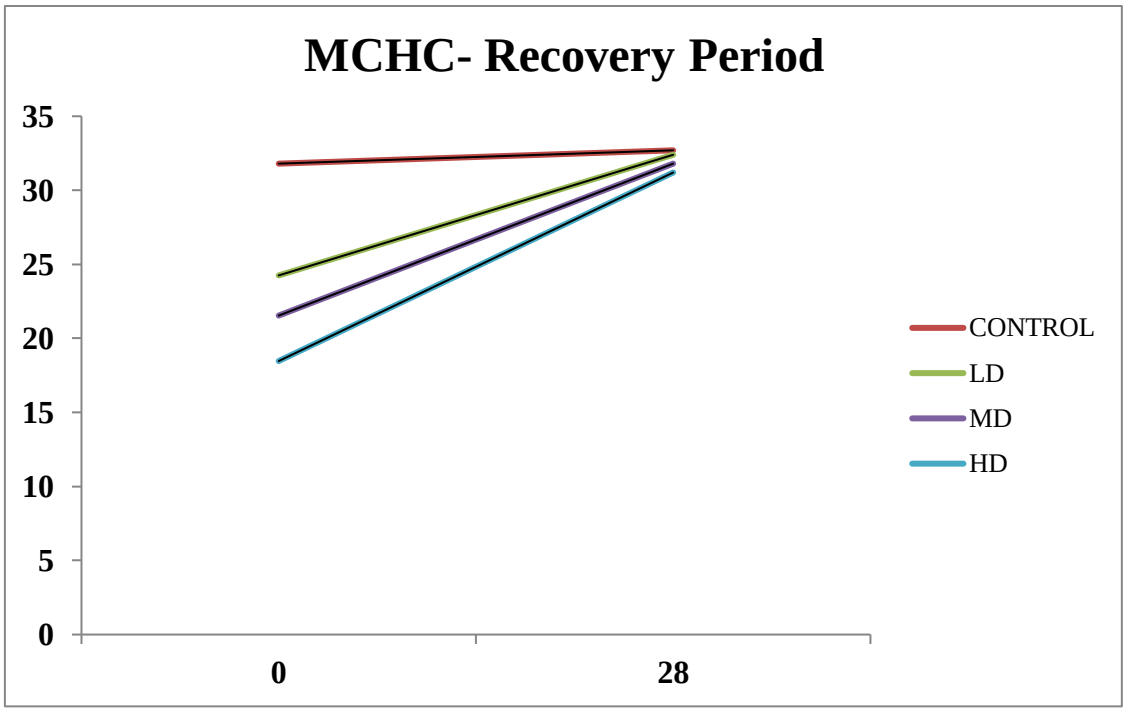


Fig 2.12: Recovery of MCHC in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: MCHCe: Control, Y = 0.9x + 30.9; LD, Y = 8.15x + 16.1; MD Y= 10.26x + 11.28, HD, Y = 12.73x + 5.74

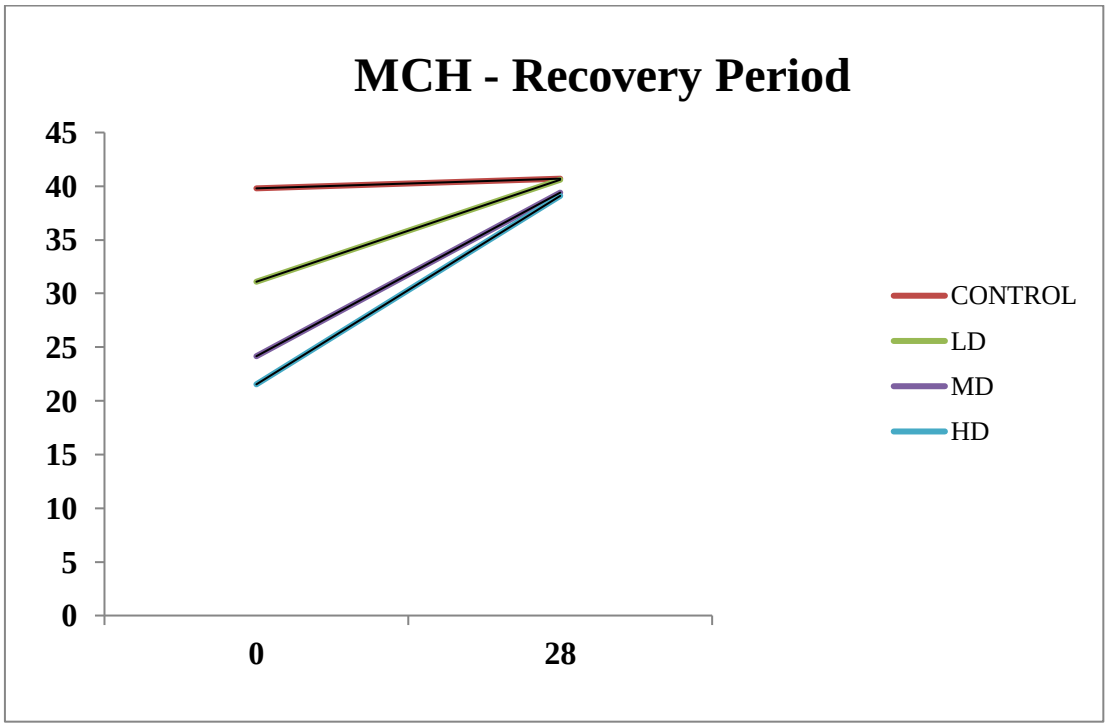


Fig 2.13: Recovery of MCH in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: MCH: Control, Y = 0.9x + 38.9; LD, Y = 9.5x + 21.6; MD Y= 15.24x + 8.92, HD, Y = 17.56x + 3.98

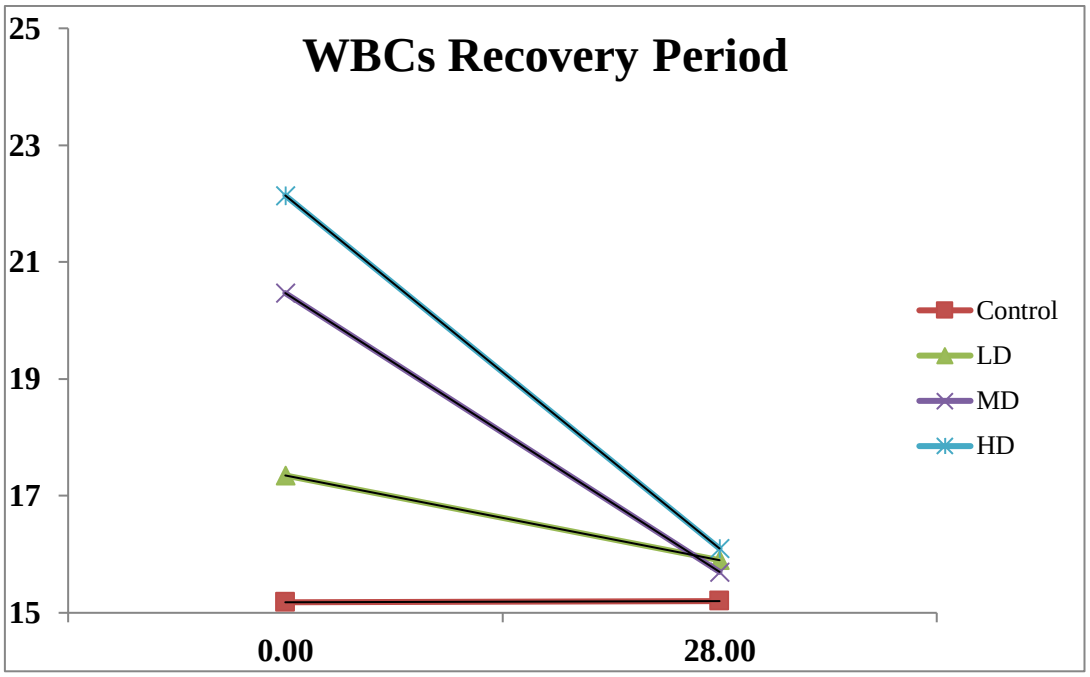


Fig 2.14: Recovery of WBCs in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: WBCs: Control, y = 0.02x + 15.16; LD, Y = -1.45x + 18.8; MD Y= -4.77x + 25.24, HD, Y = -6.04x + 28.18

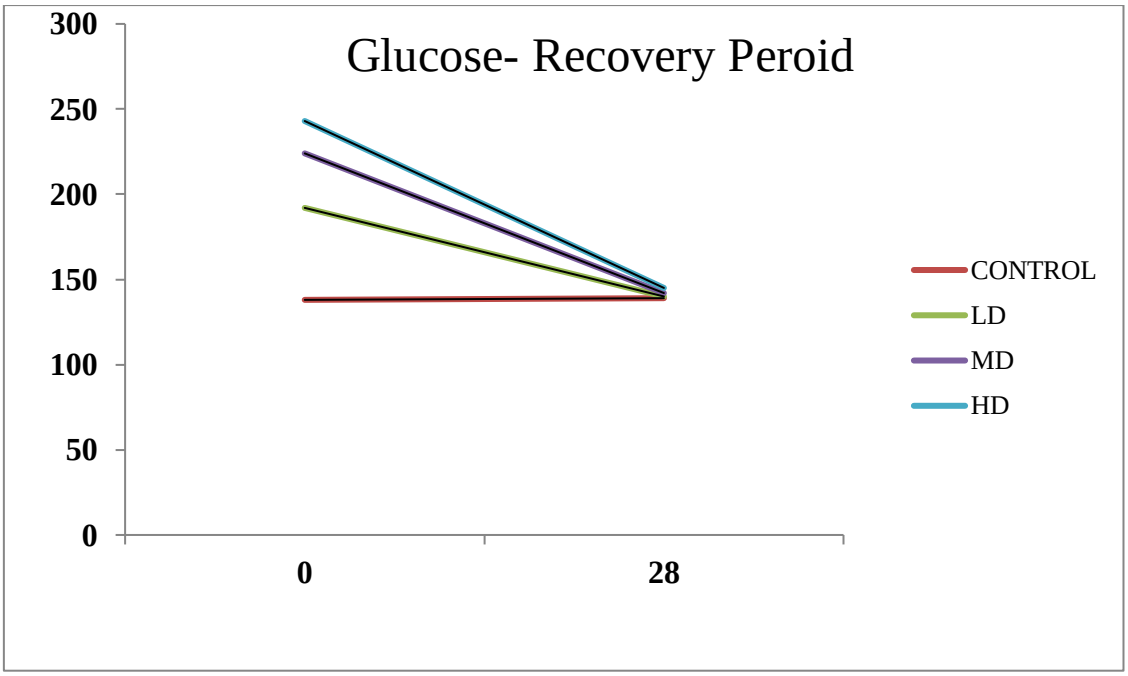


Fig 2.15: Recovery of Glucose in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: Glucose: Control, Y = X +137; LD, Y = -52X +244; MD Y= -82X+306, HD, Y = -98X +341

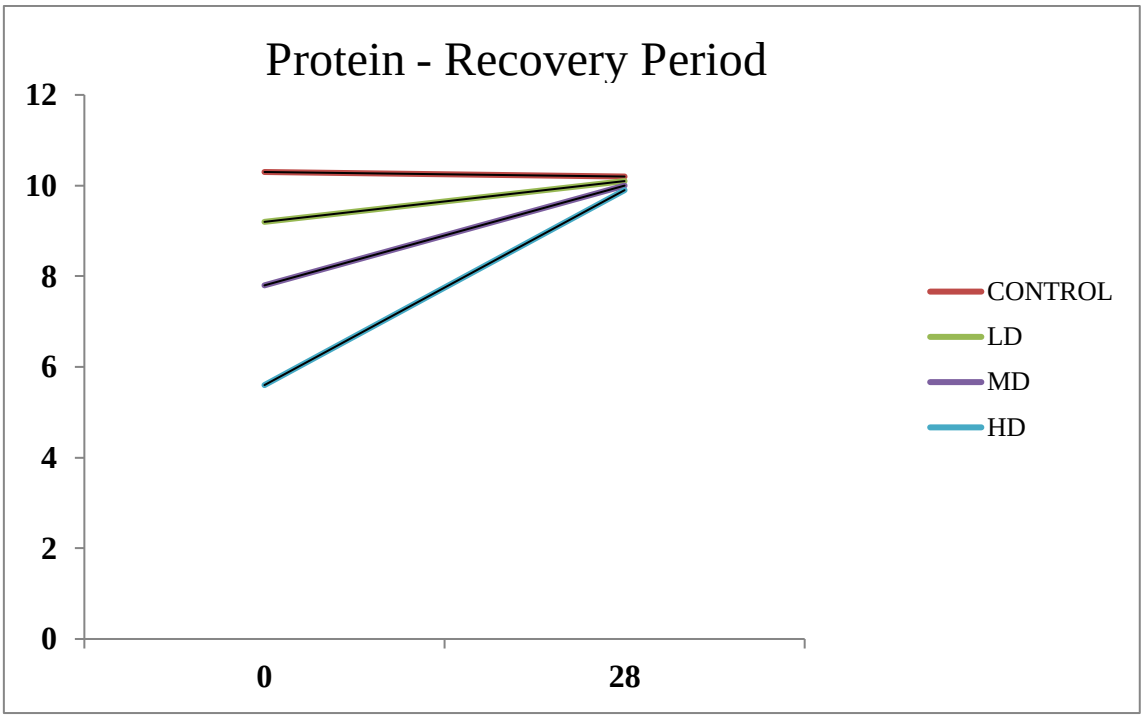


Fig 2.16: Recovery of Protein in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: Protein: Control, Y = -0.1x+10.4; LD, Y = 0.9x+8.3; MD Y= -2.2x+5.6, HD, Y = 4.3x1.3

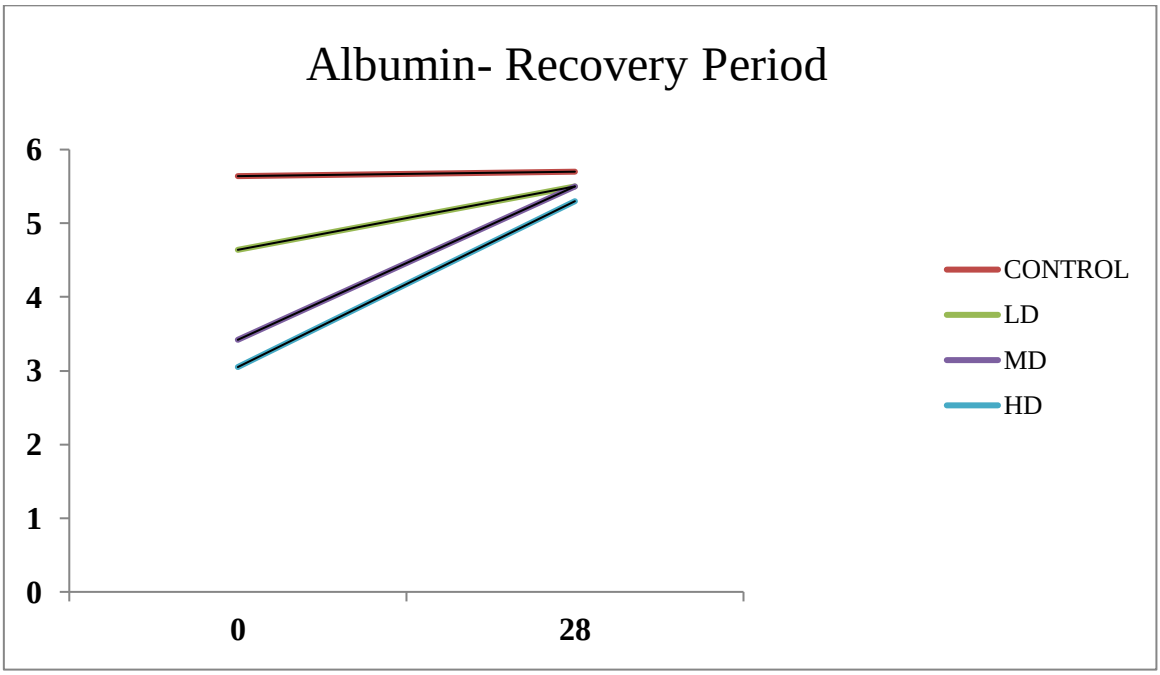


Fig 2.17: Recovery of Albumin in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtain5ed: Albumin: Control, Y = -0.06x+5.58; LD, Y = 0.86x+3.78; MD Y= 2.08x+1.34, HD, Y = 2.2x+0.8

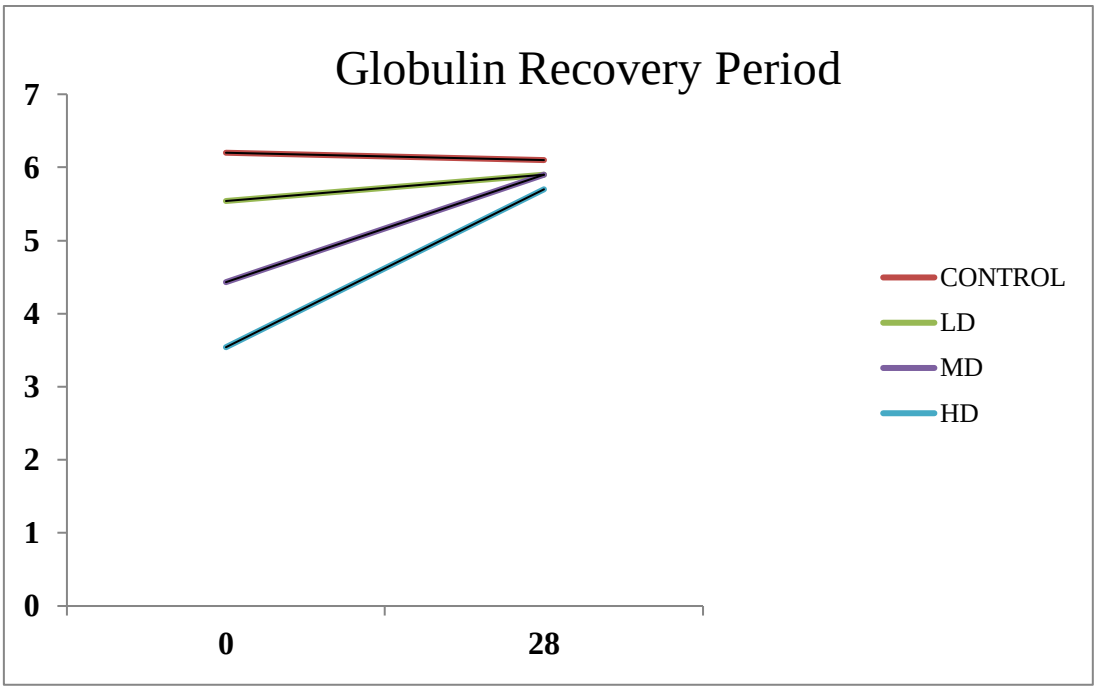


Fig 2.18: Recovery of Globulin in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: Globulin: Control, Y = -0.1x+6.3; LD, Y = 0.36x+5.18; MD Y= 1.47x+2.96, HD, Y = 2.16x+1.38

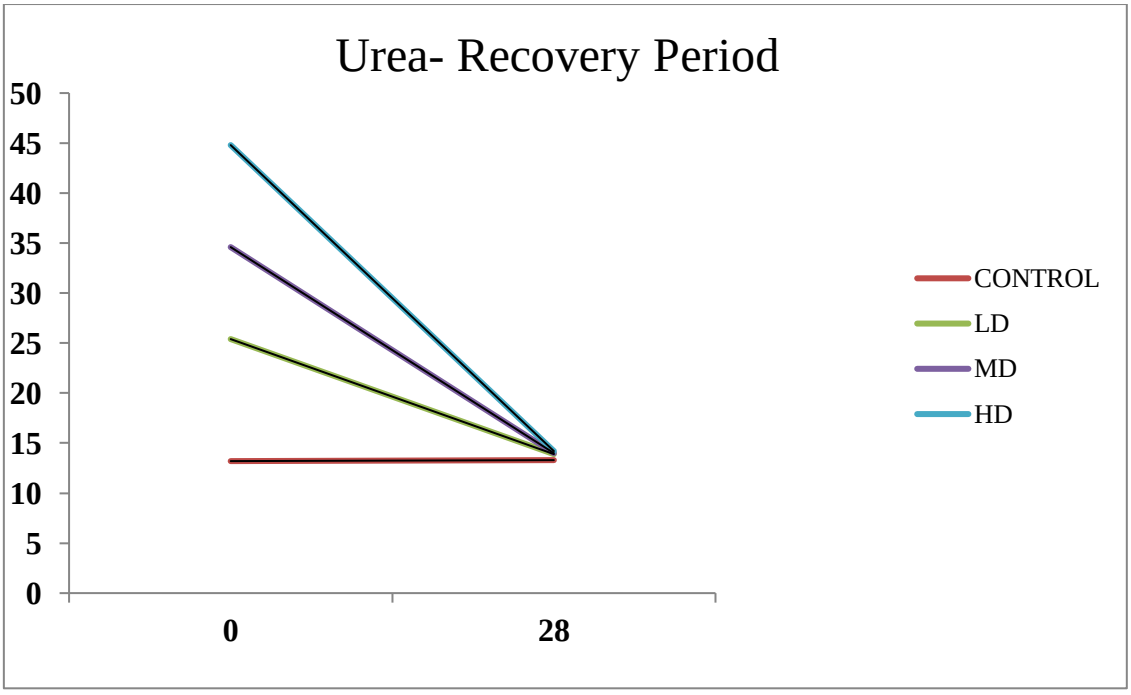


Fig 2.19: Recovery of Urea in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: Urea: Control, Y = -30.6x+75.4; LD, Y = -20.6x+55.2; MD Y= -11.5x+36.9, HD, Y =0.1x13.1

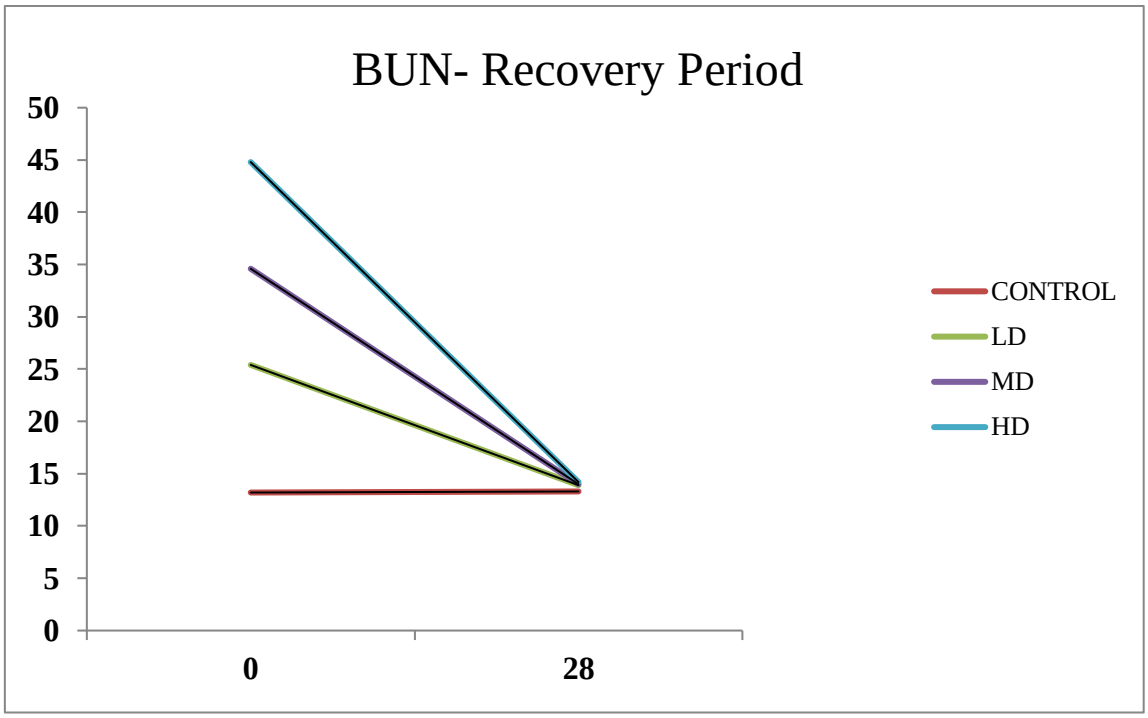


Fig 2.20: Recovery of BUN in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: BUN: Control, Y =0.1x+13.1; LD, Y =11.5x+36.9; MD Y= -20.6x+55.2, HD, Y = -30.6x+75.4

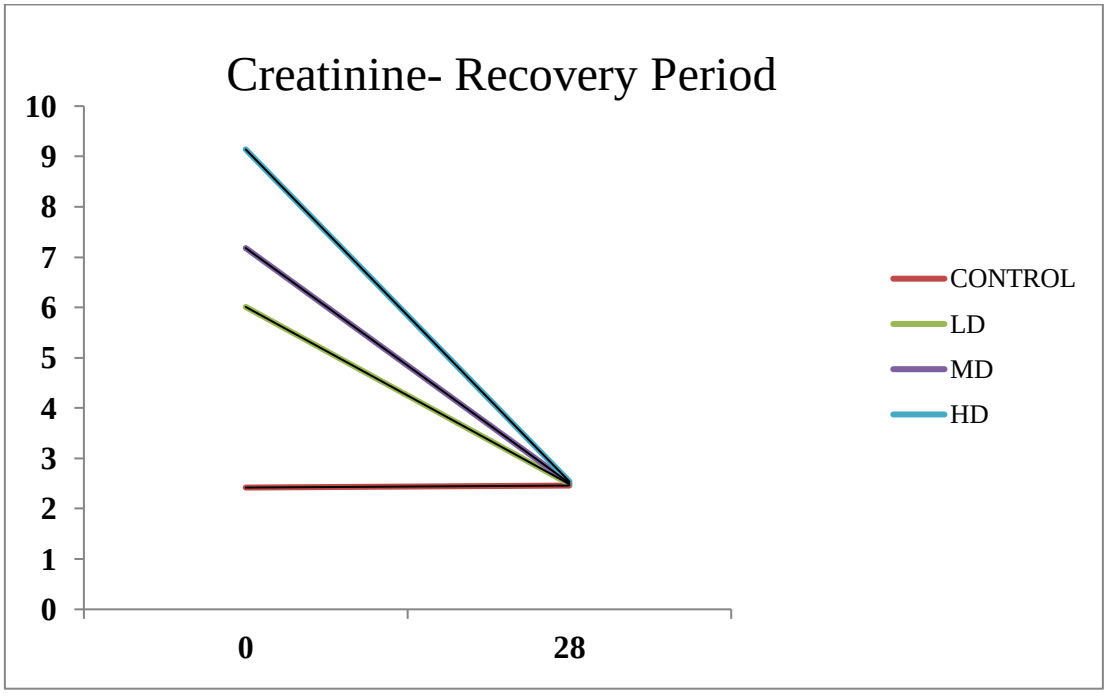


Fig 2.21: Recovery of Creatinine in *O. mossambicus* in herbicide-free water after 28 days of exposure to sublethal concentrations of PE. The following regression equations were obtained: Creatinine: Control, Y = 0.04x+2.38; LD, Y = -3.52x+9.53; MD Y= -4.67x+11.85, HD, Y = -6.6x+15.74

DISCUSSION:

Blood forms a unique medium between external and internal environment and any agent including toxic substances that causes stress can alter blood composition either directly or indirectly and thus is a pathophysiological reflector of the whole body. Thus blood parameters are important in diagnosing the structural and function status of the fish exposed to toxicant (Adhikari *et al*., 2004, Seriani *et al*., 2009). Haematological parameters are used as indicators for evaluating physiological changes in the fish. Age, sex, nutritional status, environmental factors and stress are considered as known factors to cause changes in haematological parameters (Kazemi *et al*., 2011). In recent years the purpose of the measurement of blood parameters that is an easy and useful method to assess the effects of toxicity on fish (Khajeh *et al*., 2011). Red blood cells play an important role in oxygen transportation in the body and an insufficient amount of red blood cells has the negative effect on the body of aquatic animals. The number of red blood cells can have a significant effect on the total energy balance of the body. The results of the present study revealed that PE exposure led to a significant time and concentration dependent decrease in RBC, Hb and PCV. A decrease in the erythrocyte count or in the percent of haematocrit indicates the worsening of an organism state and developing anaemia. In the light of the present study, PE exposure directed the fish to an anaemic condition. Probably PE has altered the O2 carrying capacity of the blood cells, effecting gas transfer from the medium leading to anaemia and retardation of physical activity due to the deficient oxygen as a defensive reaction or due to disturbances of the metabolic and haematopoietic activities leading to reduced erythropoiesis (Lavanya *et al*., 2011 and Hashemi *et al*., 2017). The number of red blood cells can have a significant effect on the total energy balance of the body. When the fish has less metabolic activity, a large number of red blood cells are not needed and their number continues to decline (Silveira-Coffigny *et al*., 2004). Our results are in agreement with earlier reported

work on herbicides Paraquat (Kori-Siakpere *et al*., 2007), Glyphosate (Giesy *et al*., 2000 and Glusczak *et al*., 2006), and for Atrazine (Soorena *et al*., 2011).

The erythrocyte indices like mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) seems to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with values of RBC count, haemoglobin concentration and packed cell volume. A time and dose dependent fall in the blood indices: MCV, MCH and MCHC of *O. mossambicus* exposed to PE compared to the control group. The reduction in size and quantity of haemoglobin of red blood cells is measured by the indices MCV, MCH, MCHC which can be a sign of anaemia in fish, resulting from the stress of PE exposure (Feiz, 20010 and Mikula *et al*., 2008). The presence of a large percentage of immature red blood cells in the bloodstream also may be a reason for reduction of MCV and MCH. On the other side, reduction of MCHC in may be due to decreased production of haemoglobin after exposure to PE (Kazemi *et al*., 2011). Another reason for reduction in MCHC is reduction in cell haemoglobin or hypochromic (Hii *et al*., 2007). During the anaemia, MCHC values reduced because large cells had less haemoglobin concentration (Sarikaya and Yılmaz, 2003). MCHC reduction resulted from increased production and secretion of reticulocytes that had a larger size but less haemoglobin content compared to mature red blood cells (Lermen, 2004).

The WBCs in fish respond to various stressors including infection and chemical irritants (Svobodova *et al.,* 1994). Thus, altered number of WBCs is a normal reaction on the exposure of the toxicant (Kori-Siakpere *et al*., 2006). In the present study a significant increase in WBC was observed with time and dose. An increase in WBCs count has occurred as a pathological response since these WBCs play a great role during infestation by

stimulating the haemopoetic tissues and the immune system by producing antibodies and chemical substances working as defence against infection (Hassen , 2010; Kathya *et al.*, 2010; Pereira *et al.*, 2013). WBCs are important cells in the immune system, because of their main defensive function. The WBCs respond immediately to the change in medium due to toxicant. During toxic exposure period of PE, the WBC counts were enhanced. It indicates that fish has developed a defensive mechanism to overcome the toxic stress. In an attempt to see whether the changes are reversible or not the recovery studies were also conducted. It was observed that there was an improvement in blood parameters of the test fish when transferred to herbicide free fresh water, suggestive of that the herbicide which has entered in to the system are slowly getting eliminated and the blood parameters recover from the herbicide toxicity. The rate of recovery or extrapolation shows that WBC has recovered faster than RBC.

The haematological analysis and biochemical parameter are routinely used as health indicators for fish following different stress conditions (Vutukuru, 2003; El-Sayed *et al*., 2007 and Lavanya *et al*., 2011). They have significant values in toxicological evaluations because of their alterations long before the clinical symptoms produced by toxicants become apparent in organisms (VenkateswaraRao, 2006). Among biochemical profiles plasma glucose extensively used as parameter to study stress and also used as a sensitive indicator of environmental stress in fish (Kavitha *et al*., 2010 and Parikh *et al*., 2013). Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma. In the present study the significant time dependent increase in glucose thus indicate an adaptive manifestation of stress induced by PE herbicide. A variety of stressors stimulate the adrenal tissue resulting in increased level of circulating glucocorticoids and catecholamines. Both of these groups of hormones produce hyperglycemia. It is generally thought that, under conditions of stress, hyperglycemia may

provide additional energy substrates to vital organ to cope with the increased energy demand. (Ramesh and Saravanan., 2008; Saha and Kaviraj, 2009 and Banaee *et al*., 2011). The present result are in accorandace with that of reported by Velisek *et al* (2010) and Zhi-Hua Li on exposure of fungicide propiconazole on a freshwater teleost and Rainbow Trout respectively.

Proteins are the most important and abundant macromolecules in living beings, which play a vital part in architecture and physiology of the cell and in cellular metabolism and they also play an important role in the metabolism and regulation of water balance (Inyang *et al*., 2016). All biological activities are regulated by enzymes and hormones, which are also proteins. Assessment of protein content can be considered as a diagnostic tool to determine the physiological phases of the cells (Khaled and Zakaria, 2000). Total protein is an essential constitutes of cells and tissues which aid in the physiological functions of the cells. Due to the fact that fish has low carbohydrates, protein which is architecture of the cell and main source of nitrogenous metabolism is used to enhance the energy demand (Adamu and Kori-siakpere, 2011 and Adamu *et al.,* 2013). Albumin and globulin make up most of the protein within the body and are measured in the total protein of plasma. Total protein, albumin and globulin test are used to monitor the course of diseases in immune disorder, liver dysfunction and impaired kidney activity (John, 2007; Banaee *et al*., 2008 and 2011). According to the test results, a time and dose dependent decrease in the levels of total protein, albumin and globulin was found in fish exposed to PE. A decline in total protein concentration of other fish, against atrazine herbicide has been observed and our results are in agreement with the results obtained by different researchers; by Crestani *et al.,* 2006 in silver catfish, *Rhamdia quelen*, by Velisek *et al.,* 2008 and 2012 in Rainbow trout, *Oncorhynchus mykiss* and common carp respectively and by Khaled and Zakaria, 2016 on Catfish, *Clarias Gariepinus* by Inyang *et al* (2016) in total protein and albumin in *Parpohiocephalus obscurus.* The reduction in protein could be attributed to adjustment of the fish to its new environmental

conditions as a result of stress response. Unlike mammals, fish consume protein and do not store it in the body tissue for muscle energy when a carbohydrate source is absent; hence the exposed fish meet their extra energy requirements from body proteins, which are used to produce glucose for fish by the process of gluconeogenesis. A decreased protein level may be attributed to stress-mediated immobilization of these compounds to fulfil an increased element for energy by the fish to cope with environmental conditions exposed by the PE which was very much evident by a significant increase in glucose titre. Thus, an overall reduction in protein, albumin, globulin and albumin/ globulin ratio are string indicators of not only for energy immobilization but also an indicator of hepatic dysfunction and immunosuppressive effect of the herbicide as reported by Ahsan Khan *et al*., (2016) on chronic exposure of atrazine on the biochemical parameters total protein and serum albumin in carp *Ctenopharyngo donidella*.

Urea is quantitatively the most important non protein nitrogenous constituent of blood. It is the chief end product of protein metabolism and excreted by kidneys. Hence, its blood concentration is directly related to the protein content and renal excretory capacity. Liver is the important site of urea formation ,in any pathological condition that involves liver, urea formation is upset as evidenced by either increase oe decrease. In teleost fish, the main end product of nitrogen metabolismis ammonia which accounts for 55-80% of total nitrogen excretd , while urea excretion is about 6-8%. The amino acids which are precursors of purines also contribute to urea ouput. Common cause for increase in blood urea is inadquate excretion usually due to kidney damage and urinary obstructions. Renal dysfunctions with severe renal insufficiency or excessive bodily breakdown of protein due to toxic stress may be reponsible for the elevation of urea level, Thus, urea estimation is mainly useful for the diagnosis of renal disese or damage Jothi and Narayan, 2000). Stress results in an increase in

cortisol levels as well as an increase in protein catabolism and ammonia production which gets converted to the less toxic urea, but this is a metabolically expensive process which is found only in terrestrial vertebrates (Srivastava *et al*., 2016). Teleost fishes are primarily amminotelic but their blood contains 6-8% of urea and indeed in some teleosts it may account for 20 % or more of the total nitrogen excreted. Occurrence of uremia has been reported by many workers (Amin and Hashem, 2012; Yousef *et al*., 2006; Parikh *et al.,* 2013; Desai and Parikh 2014; Srivastavaand Singh, 2013; Barad and Kulkarni, 2010). In the present study elevated urea with reduced protein content is suggestive of biochemical transformation of protein nitrogen into the other nitrogenous product. An elevated level of urea implies uraemia which is possibly due to the inability of the toxicated kidney by PE to filter urea in adequate amount (Adham *et al*., 2002).

Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage lead to increased creatinine levels. In the present study serum Creatinine showed insignificant decrease in experimental group in comparison to control suggesting that there occurs an alteration in glomeruli filtration rate. Excretion occurs through a combination of glomerular filtration and tubular secretion (Orun *et al*., 2014; Nagarajun and Venkata, 2013). The alteration in the levels of serum creatinine may, therefore, be due to a combination of these two factors. Our results are in agreement with the results of Gaafar (2010) who has reported a significant increase of creatinine in *Oreochromis niloticus* following chronic exposure to edfenphos.

Blood Urea Nitrogen (BUN) measures the amount of nitrogen, a waste product of protein metabolism in the blood. Urea is formed by the liver and carried by blood to the kidneys for excretion. So this test can be used as a test of renal function. An increase in BUN in various fresh water fish has been reported for different pesticides (Kerem *et al.*, 2007, Zaki *et al*.,

2008; Vijakumar *et al*., 2009; Zhang *et al*., 2011 and Parikh *et al*., 2013 and 2014; Thoker *et al*, 2016). The elevated blood urea nitrogen (BUN) indicates low renal perfusion or renal dysfunction due to PE exposure which suggestive of harmful effects on kidney tissues resulting in its functional impairment. The present data suggest that PE exposed fish adapt glomerular dysfunction rather than tubular insufficiency, In consistent with these explanations of decreased total protein level on PE with a marked increase in blood urea nitrogen could be attributed to impaired excretion of urea through kidney and this explanation is supported by increasing blood creatinine level which is more sensitive and specific indicator of impaired kidney function.

During a subsequent recovery study period of 28 days, the haematological parameters as well as the biochemical titres were found to get normal values which can be interpreted as either a compensatory response by the fish against the toxic conditions, or insufficient recovery time to elicit a complete recovery. The improvement in haematological profile and biochemical parameters of the test fish when transferred to herbicide-free freshwater suggests that PE entering into the system is slowly eliminated and hence the haematological and biochemical parameters recover from the herbicide toxicity. Our results are in agreement with the earlier work of Zhang *et al.*, (2004), Menezes *et al.,* (2011) and Cattaneo *et al.*, 2011 who reported a recovery after roundup herbicide exposure in Carp and have suggested a possible species-specific difference in susceptibility to recovery period.

Based on the present study, the following conclusions can be drawn: Fish exposed to sub-lethal levels of PE undergo significant time- and dose-dependent decreases in total erythrocyte count, haemoglobin content, and haematocrit values, and increases in total leukocyte count. Furthermore, the recovery study showed that RBC recovers faster than the

WBC. Further, the haematological and biochemical parameters of fish exposed to HD of PE took more time to completely recover than fish exposed to MD and LD of PE.