Chapter I

Acute toxicity, Behavioural, Haematological and Biochemical studies of fresh water fish *Oreochromis mossambicus*(peters, 1852) on exposure of Pyrazosulfuron Ethyl

Environmental pollution by various toxicants mainly pesticides has become one of the major problems in the world (Chandran *et al*. 2005). Contamination caused by pesticide and heavy metal of aquatic system has attracted the attention of researchers around the world. It has increased in the last decades due to extensive use of them in agricultural, chemical, and industrial processes that are becoming threats to living organisms (Dutta and Dalal., 2008). Pesticides are among the most hazardous chemicals to living being**.** These chemicals reach to various water bodies as lakes and rivers through rains and wind, affecting many organisms. Only 0.1% reaches the specific target that is pest (Aguiar, 2002).The effects of these chemicals to aquatic environments are innumerable. The substantial increase of chemical emissions in the water resources has led to harmful effects not only to aquatic organisms (Livingstone, 2001; Matsumoto *et al*., 2006) but these persistent molecules also pose a threat to human population consuming the affected aquatic animals (Khalili *et al*., 2012).Unfortunately, artificial ponds are mostly located close to agricultural areas, or are fed with water springs that run through cultivated soil that affect the culture organisms.

Herbicides are the chemical are used for agricultural purposes for controlling the weed and are an integral part of agricultural. However a side effect of usage of these chemicals results in unfortunate consequences to nontarget organisms. The most obvious effect of these chemicals is when they enter the water sources through drift, runoff, soil erosion, leaching, and occasionally accidental or deliberate release (Fishel and Ferrell, 2013 and Sani and Idris, 2016).The toxic effects of herbicides are mainly based on solubility of herbicides in water or any other suitable vehicle. According to Hogan (2014), herbicides concentration biomagnifies into the food chain and food web up to trophic

level of energy pyramid. Each herbicide has a unique mode of action and has proved to be toxic to fish even at a very minimal concentration. The adverse effect of herbicides is reported to aquatic organisms viz., primary producer green algae, primary consumer daphnia as well as fish (Hogan, 2014).

Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. The application of ecotoxicology studies is rapidly expanding, for aquatic systems, and the potentiality of application of the findings from such studies have helped us in understanding the environmental health issues posed by toxic chemicals which have a direct impact on human health and thus has made fish a more attractive modal organism in toxicology research (Pandey, 2009).

Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety (Ayoola, 2008). Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa *et al*., 2003). Acute toxicity of a pesticide refers to the compound capability to cause injury to an animal from a single exposure of the particular concentration generally by short duration. The acute toxicity tests of pesticides to fish has been widely used to acquire rapid estimates of the concentrations that cause direct, irreversible harm to test organisms (Srivastava *et al*., 2007). Measurements of acute toxicity such as LC_{50} are useful to determine the potency of a chemical. Equally useful are the estimations of lowest effect concentrations (LOECs) based on observations of chronic exposure, although they are less accurate and reliable (Kostal *et al*., 2015).

Acute toxicity test usually provide estimates at the exposure concentration causing 50% mortality (LC_{50}) to test organisms during a specified period of the time. The application of LC_{50} has gained acceptance among toxicologists and is generally the most highly rated test for assessing potential adverse effects of toxic substance to the life of organisms. LC_{50} is routine to represent the lethality of a toxicant to a test species in terms of lethal concentration (for aquatic animals) and lethal dose (for

terrestrial animal). It is always expressed in terms of g or mg/kg body weight of the animal and lethal concentrations (LC) in terms of Parts/million (ppm) or parts/billion (ppb) or milligram/liter (mg/L). LC_{50} values are also determined for fish and aquatic organisms based on the concentration of test chemical in water for exposure periods of 24 to 96 hour. There is a vigorous documentation of the use of acute toxicity tests for assessing the potential hazard of chemical contaminants to aquatic organisms (Boyd, 1957, Henderson *et al*., 1960, Sanders and Cope, 1966, Macek and McAllister, 1970, Brack *et al*., 2002, Diez *et al*., 2002). The median lethal dose/$LC_{50}$is analyses using probit analysis in general the data from bioassay give S- shape curve. In order to the curves linear, the proposition is transfer to probit and doses to log10 and the LC_{50} value are estimated using regression analysis. In other words, probit analysis is a type of regression used to evaluate binomial response variables (Finney, 1971).

Along with acute toxicity studies, behavioural responses of fish have been used for decades as methods for environmental monitoring, alterations in the chemical composition of the natural aquatic environment usually affect behavioural and physiological systems of inhabitants, particularly fish (Van der Schalie *et al*., 2001, Khan and Law, 2005, Melvin and Wilson 2013, Denoël *et al*., 2013 and Bradley *et al*., 2013).With the wide range of environmental contaminants finding their way into aquatic environments there is a growing need for monitoring tools that are fast and sensitive to a wide range of compounds, but also indicative of potential effects on survival, growth and fitness. Behavioural analyses show promise for satisfying these requirements, and are often hailed for their rapidity (Gerhardt, 2007; Maradona *et al*., 2012) and sensitivity (Hellou 2008 and 2011; Robinson 2009 and Painter *et al*., 2009) compared to traditional toxicological methods assessing developmental and reproductive effects. Although few would argue that behavioural responses offer comparatively fast and sensitive assessment of environmental perturbations (Gerhardt, 2007 and Amiard-Triquet, 2009).

The exposure of fish to chemical agents induce changes in several haematological variables (Pechiammal and Vasanthi, 2017), and are recurrently used to evaluate fish health (Martinez and

Souza, 2002). The study of blood parameters in fishes has been extensively used for the detection of physiological alterations in different conditions of stress (Pathak *et al*., 2013 and Parikh *et al*., 2014) Haematological parameters such as haematocrit, haemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Adedeji *et al*., 2000). Moreover, haematological and biochemical parameters are used as health indicators to detect the structural and functional status of fish under stress condition (Ramesh and Saravanan, 2010). In recent years, biochemical variables are used more to determine the effects of external stressors and toxic substances. Therefore, the biochemical evaluations are gradually becoming a routine practice for determining the health status in fish (Padma *et al*., 2012).

Toxicity data for variety of herbicide such as paraquat, glyphosate, 2,4 D, atrazine, glufosinate ammonium have been reported for number of fish species by various workers (S. Deivasigamani, 2015, Soloman *et al*., 2008, Rohr and Mccoy, 2010, Gammon *et al*., 2005, Wribisky-hershberger *et al*., 2017., Wribisky and Freeman, 2015 and 2017, Langiano and Martinez., 2007 , Barr *et al*., 2007, DeSesso *et al*., 2014, Peixoto, 2005, Tsui and Chu, 2003, Vigáario *et al*., 2014,Farah *et al*., 2004, Zodrow *et al*., 2004, Santos *et al*., 2010).However there is gap as far as herbicide PE toxicity studies are concerned, hence in the present study an attempt is made to determine the 96hr LC_{50} value, along with the behavioural response and haematological and biochemical alteration on freshwater fish *Oreochromis mossambicus*on exposure of PE.

Materials and Methods

Animal Model:

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 aquarium for an acclimation period of 12-15 days in de-chlorinated water at 27 ± 4ºC, pH 7.4 ± 0.05, dissolved oxygen 8 ± 0.3 mg/Land 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 in accordance with the guideline of A.P.H.A., A.W.W.A. and W.P.C.F. (1998).

Preparation of the Herbicide PE:

The herbicide PE known by the trade name "Saathi" (10% WP) was selected for the present study and was procured from local Pesticide market from Vadodara. The IUPAC name of PE is Ethyl 5-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-1-methylpyrazole-4-carboxylate, available in WP that is wettable powder. Stock solution of PE was prepared by directly dissolving it in water and was kept at room temperature. The experiment was divided into two parts Ia and Ib.

(Ia)Experimental Procedure for LC_{50}determination and Behavioural Studies:

Acute 96-h static bioassay was conducted in the laboratory following the methods of Sprague (1975), APHA (1985) following OECD guidelines. After the acclimation period the fishes were divided into 11 groups having 5 females and 5 males (n=10) and 3 replicates were performed for each group. They were exposed to various concentration of PE for 24, 48, 72 and 96 hour along with one control group. The experiments were conducted in a series of glass aquariums filled with 40 litters dechlorinated tap water. Concentrations of the test compounds used in short term definitive tests were between the lowest concentration for PE (50 mg/L) at which there was no mortality, and the highest concentration for PE (1000 mg/L) at which there was 100% mortality in the range finding tests. Then after, the

mortality rate was recorded for each concentration for 24, 48, 72 and 96 hour. The collected data was computed according to probit analysis method of Finney 1971.Simultaneously behavioural changes of the fish was also observed and recorded during the exposure period.

(Ib)Experimental Protocol for Haematological and Biochemical estimation:

On completion of 96 hour acute toxicity Test, fish was collected, from 50mg, 100mg, 200mg and 400mg/L were the mortality was recorded 0, 10, 20, and 40% respectively. About 3 - 4ml of Blood from the caudal peduncle using heparinised disposable syringes was collected from the live fish from each aquarium for haematology parameters and blood was stored in -4°C prior to estimation. Haemoglobin estimation (HB), Pack Cell Volume (PCV), blood glucose level and total serum protein were analyzed by NIHON KOHDEN Automated Haematology Analyzer (Celtac alpha, 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 Haematology Analyzer (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 haemoglobin value (HB) and from the erythrocyte count according to the following formulae

MCH = HB/RBCs pg

Statistical analysis:

Ia: Probit analysis (Finney, 1971) was used to calculate the median lethal concentration and time with their upper and lower confident limits. Data of Behavioural changes for OBF and TBF were subjected to analysis of variance (ANOVA) for difference between means of both the groups using statistical programme (Biostat 2009 Professional 5.8.1 and Graphpad Prism 5). Other abnormal behaviours were noted and the extent of mucus production on the skin and gills of exposed fish was assessed by feeling with the fingers. A fish was considered dead when it failed to respond to simple prodding with a glass rod. Death was defined as complete immobility with no flexion of the abdomen upon forced extensions (Lockwood, 1976).

Ib: Statistical analysis was performed using Graph pad prism 5 software. The data was analyzed using two-way ANOVA test followed by multiple comparison test (Tukey's). Results were presented as mean ± SEM. The level of significance was set as $P<0.05$, $P<0.01$ and $P<0.001$.

Result

Ia:

Table 1.1 shows the relation between concentration of PE and mortality rate of fish. No mortality was observed in control group. The LC_{50} values according to Probit regression curves was found to be 501.65 mg/l and the Lower Confidence Limit (LCL) value and Upper confidence limit (ULC) were 407.83 mg/l and 595.47mg/l respectively (Fig 1.1, 1.2 and Table 1.2). After 96 h exposure of PE mortality of fishes at 1000 mg/L hundred percent was observed.

Behavioural changes was also observed, were there was no much alteration in the control group. However the exposure of PE resulted into abnormal swimming behaviour which was reported by jerky movement, Agitate swimming and lose of equilibrium, increase fin and tail movement. Overall the exposure of PE resulted into hyperactivity and restlessness. Time and dose dependent increase in the activity was observed. In addition there was a mucous secretion in treated group. The colours of the fish were observed to get lighter with an increase secretion of mucous (Table 1.3 and Fig. 1. 4, 1.5 and 1.6.). Surfacing frequency and gulping of air surface water was also found to increased concentrations.

Ib:

The Haematological alterations as presented in Table-1.4 showed a significant ($p<0.01$) increase in the values of RBC count, Haemoglobin and PCV, when compared to control groups. On the other hand MCV was found to be increased while MCHC decreased but there was no significant change in MCH (Table 4). Moreover WBC count exhibited a significant increase ($p<0.05$) (Table-1.4, Fig.1.6), and blood glucose ($p<0.01$) as well as protein ($p<0.05$) also showed a significant increase in a dose dependant manner in the experimental groups when compared to the control groups (Table-1.4. Fig.1.7).

Table 1.1: The relation between concentration of PE and mortality rate of *O. mossambicus*

Concentration	Actual percent%	Log10 conc.	Total no.	No. dead	Probit
Control	-----	-	10	0	-
50	0.025	1.699	10	0	3.0396
100	0.1	2.0	10	1	3.3183
200	0.2	2.301	10	2	4.1585
300	0.3	2.4771	10	3	4.476
400	0.4	2.6021	10	4	4.7471
500	0.5	2.699	10	5	5.0
600	0.6	2.7782	10	6	5.2529
700	0.7	2.8451	10	7	5.524
800	0.8	2.9031	10	8	5.8415
900	0.9	2.9542	10	9	6.2817
1000	0.975	3.0	10	10	6.9604

Table 1.2: LC_{50} values (mg/L) with their fiducial limits used in acute toxicity tests and for *O. mossambicus*

Agrochemical	Application	Duration	LC50	LCL	UCL
Pyrazosulfuron Ethyl	Herbicide	96 h	501.65	407.83	595.47

Fig 1.1: Plot of adjusted probits and predicted regression line of PE to *O.mossambicus*

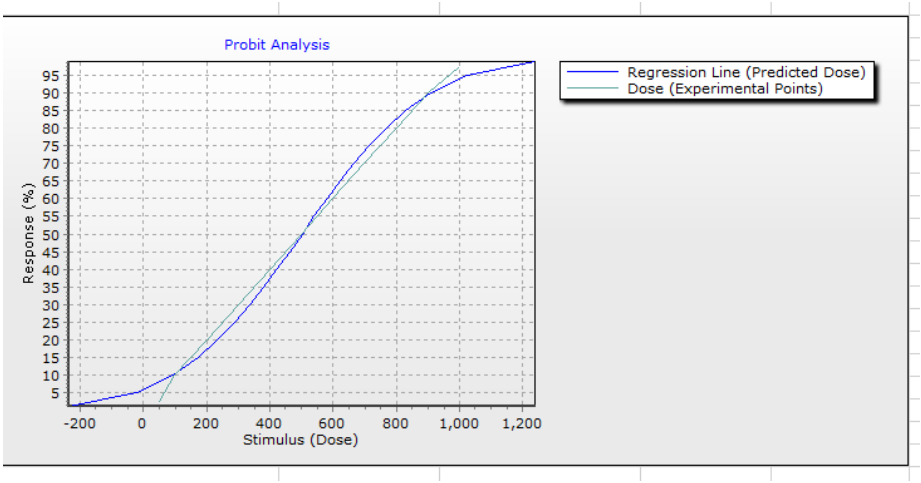


Fig 1.2: Plot of adjusted probits and predicted regression line of PE to *O.mossambicus* in log10base

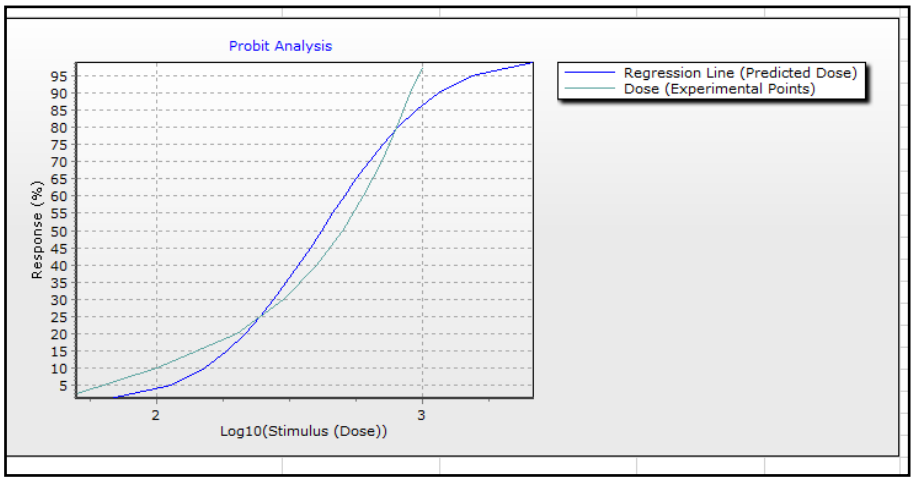


Table 1.3: Behavioural response of *O. mossambicus* in different concentration of PE up to 96 h.

Exposure time	24 h							48 h							72 h							96 h						
Conc. (mg/L)	0	50	100	200	300	400	500	0	50	100	200	300	400	500	0	50	100	200	300	400	500	0	50	100	200	300	400	500
Jerky movement	-	+	+	+	++	++	+++	-	+	+	+	++	++	+++	-	+	+	+	++	++	++	-	+	+	+	++	++	++
Hyperactivity	-	+	+	+	++	++	+++	-	+	+	+	++	++	+++	-	+	+	+	+	++	++	-	+	+	+	+	++	++
Restlessness	-	+	+	+	+	++	+++	-	+	+	+	+	++	+++	-	+	+	+	+	++	++	-	+	+	+	+	+	++
Loss of Equilibrium	-	+	+	+	++	++	+++	-	+	+	+	++	++	+++	-	+	+	+	++	++	++	-	+	+	+	+	++	++
Fin Movement	-	++	++	++	+++	+++	+++	-	++	++	++	++	+++	+++	-	++	++	++	++	++	+++	-	++	++	++	++	++	+++
Mucus Secretion	-	+	+	+	++	+++	+++	-	+	+	+	++	+++	+++	-	+	+	++	++	+++	+++	-	+	++	++	++	+++	+++
Opercular Beat Frequency	-	+	++	++	++	+++	+++	-	+	+	++	++	++	++	-	+	+	+	++	++	++	-	-	+	+	++	++	++
Tail Beat Frequency	-	++	++	++	+++	+++	+++	-	++	++	++	++	++	+++	-	++	++	++	++	++	+++	-	+	+	+	++	++	+++

Note: Normal → (-), (+) → mild, (++) → moderate, and (+++) → maximum behaviour sign of minus(-, - -, - - -) denoting decreased behaviour, as compared to control.

Table 1.4: Haematological Parameter, Blood Glucose and Total Protein in *O. mossambicus* affected by acute exposure of PE:

Parameters	Concentration mg/l of PE (Mean ±SD)				
	Control	**50mg/l**	**100mg/l**	**200mg/l**	**400mg/l**
RBCs10^6/µL	1.04± 0.025	1.90±0.027	1.69 ±0.023	1.40±0.028	1.28±0.022
HB g/dL	5.3±0.154	9.7±0.159	8.6±0.152	6±0.157	5.80±0.162
PCV(Htc) %	14.6±0.555	29.6±0.551	32.6±0.558	23.4±0.557	19±0.554
MCV fL	140.38±3.52	155.79±3.56	192.9± 3.54	144.44±3.57	142.42±3.51
MCHC g/dL	36.3±1.03	32.77±1.03	26.38±1.03	34.62±1.03	31.3±1.03
MCH pg	50.96± 1.66	51.96± 1.68	50.89± 1.64	50± 1.65	53.54± 1.69
Total WBC /µL	45,000± 655	151,600±653	109,500±653.64	79,600±657.89	76,800±652.23
Glucose	138±3.162	215± 3.113	322±3.15	133±3.19	222±3.14
Protein	10.3± 0.462	11.9± 0.471	12.7± 0.469	14.3± 0.465	16.5±0.473
Albumin	5.64± 0.48	3.4± 0.49	2.40±0.48	4.80±0.47	5.70±0.48
Globulin	6.3±0.354	6.9±0.355	5.50±0.357	8.00±0.360	10.8±0.359

Fig 1.3 Graphs showing alterations in Jerky Movement, Hyperactivity and Restlessness of *O. mossambicus* on exposure of PE

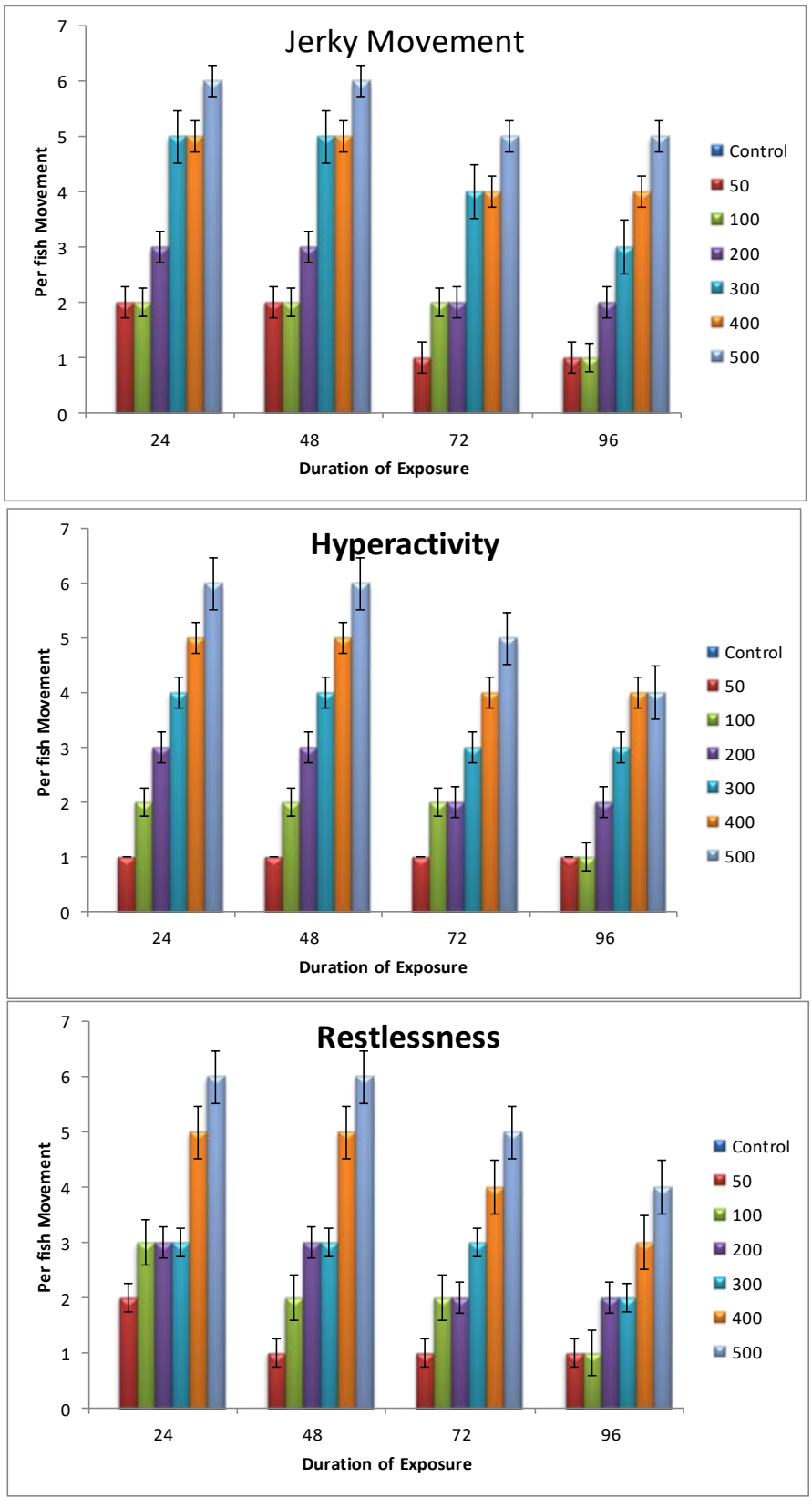


Fig.1. 4 Graphs showing alterations in Loss of Equilibrium, Fin Movement and Mucus Secretion of *Oreochromis mossambicus* on exposure of PE

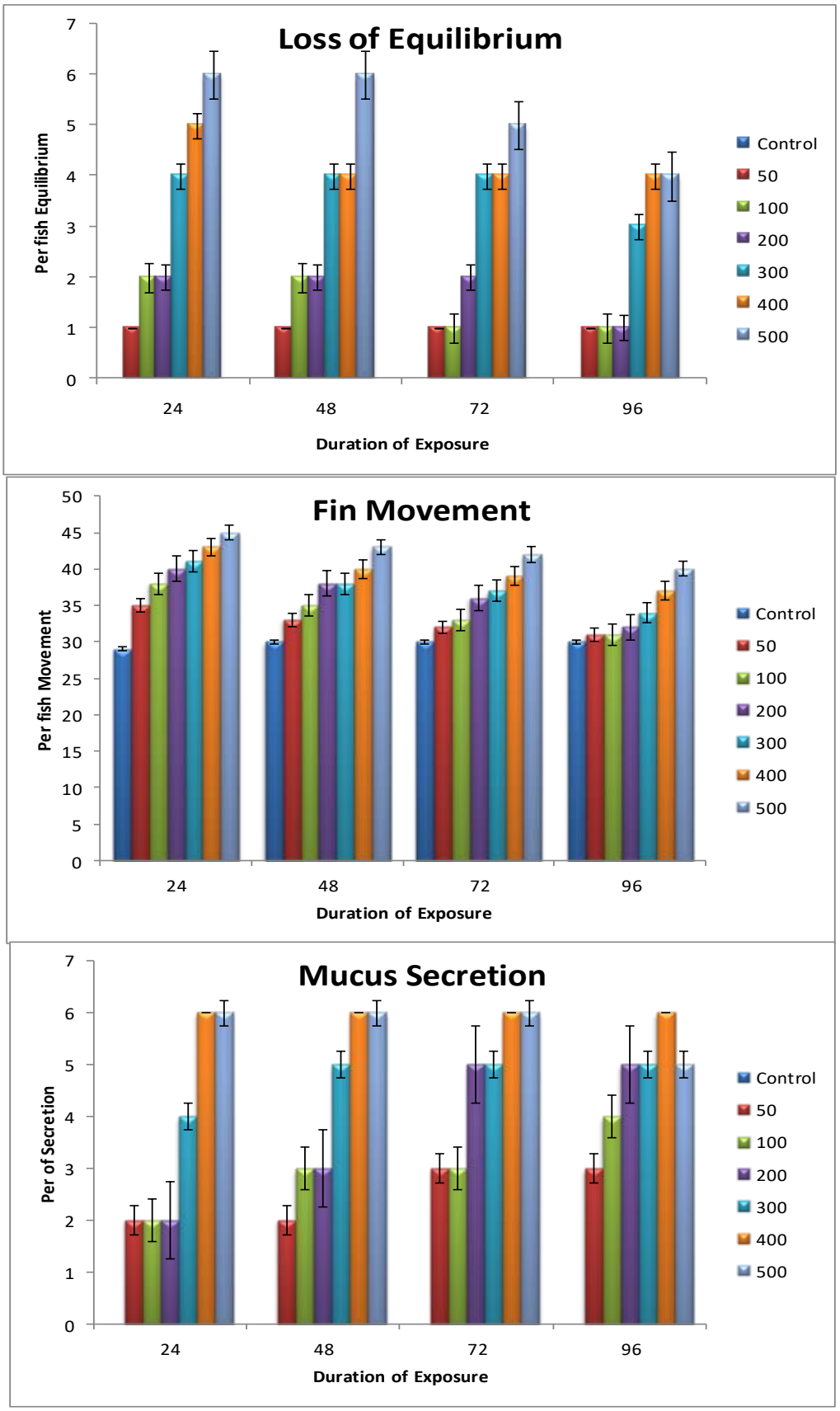
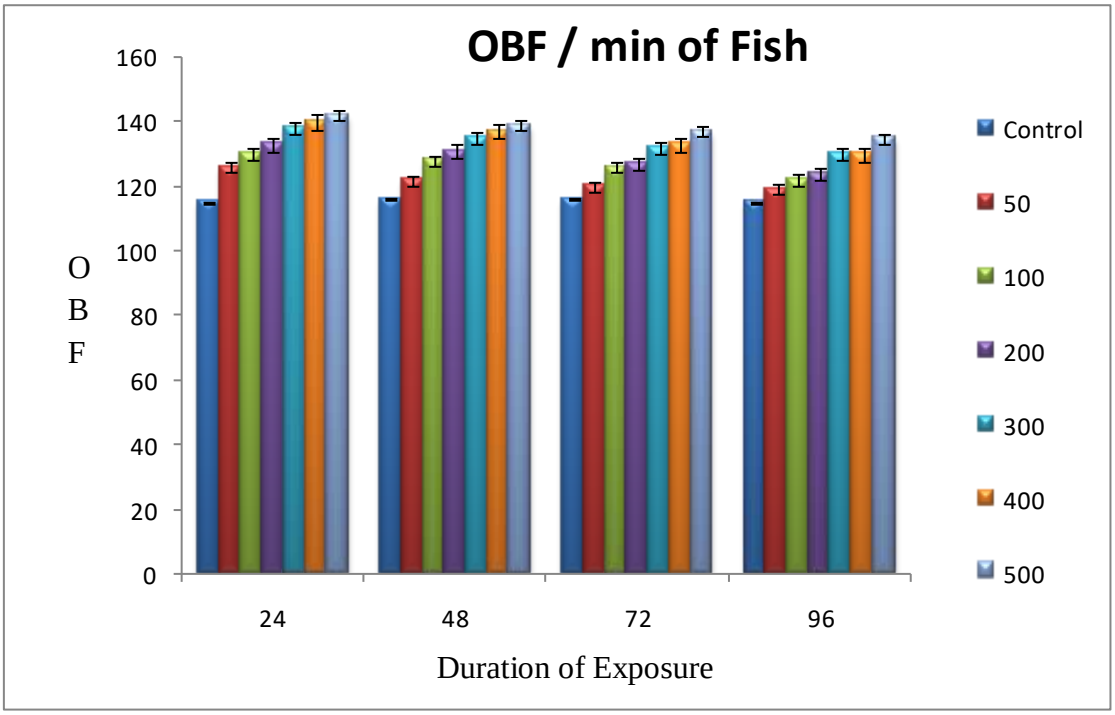


Fig. 1.5 Graphs showing alterations in OBF and TBF of *O. mossambicus* on exposure of PE



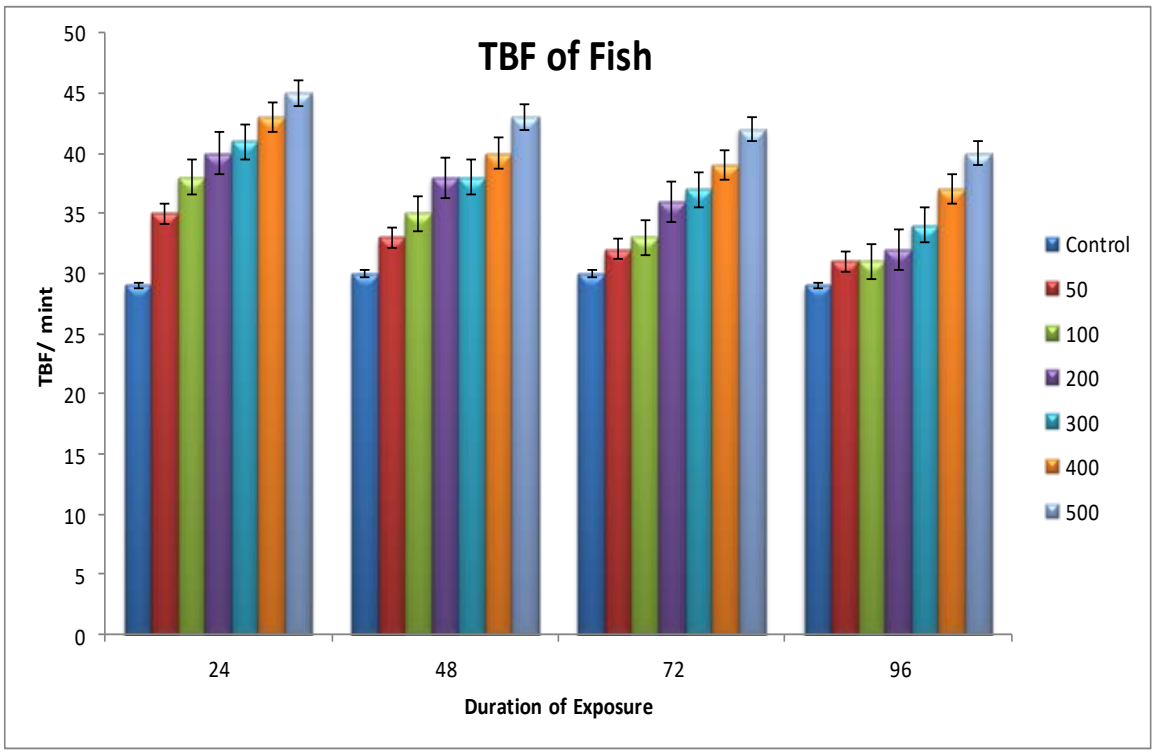
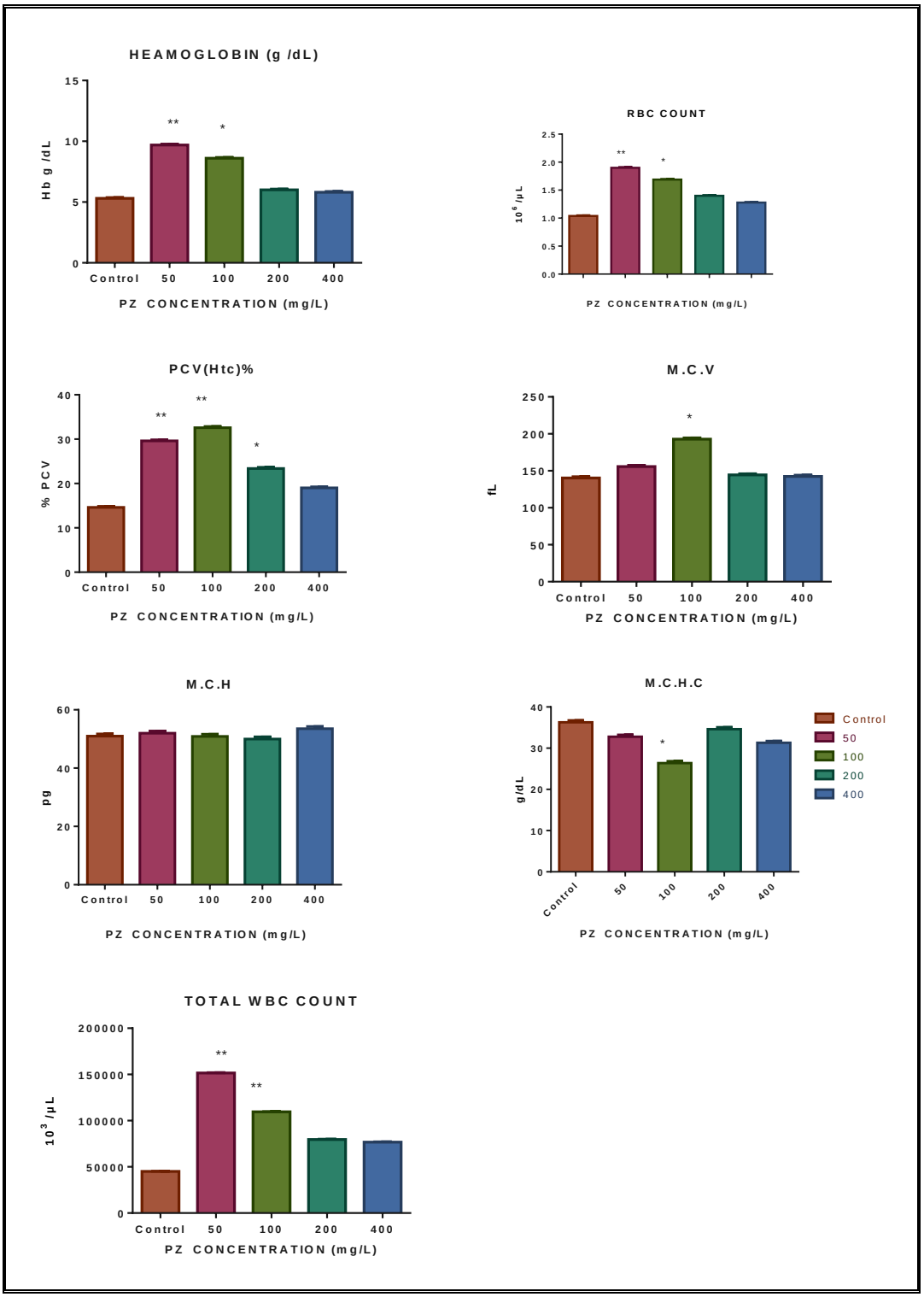
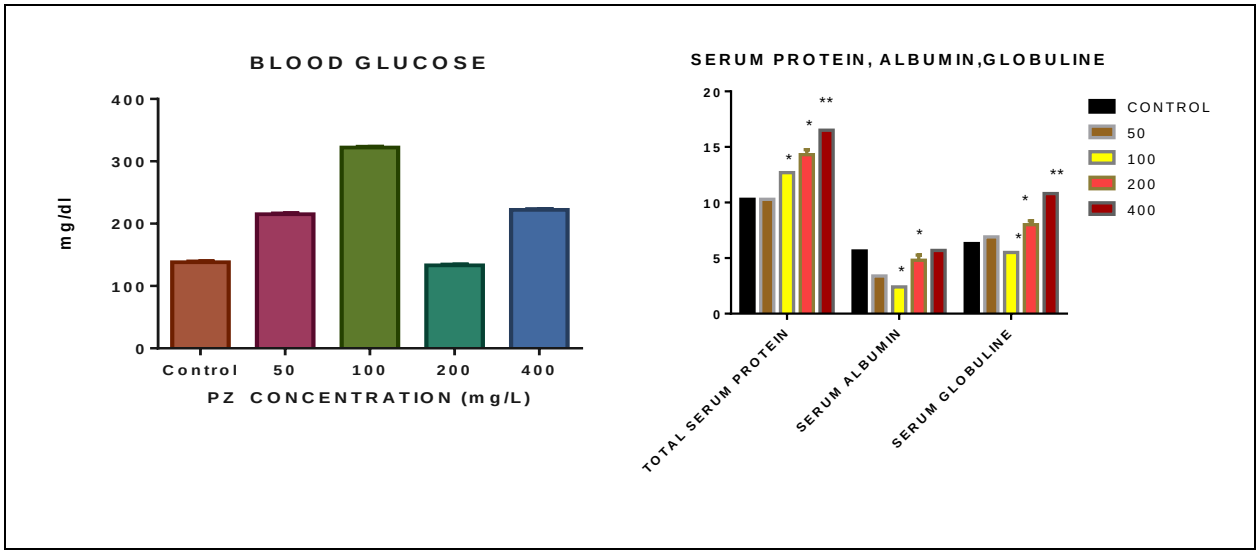


Fig 1.6: Changes in Haematological Parameter of *O. mossambicus* on exposure of PE



Values are mean ± SEof five individual observations, Values are significant at (*) p<0.05, (**) p<0.01

Fig 1.7: Changes in Blood Glucose and Total Serum Protein on *O. mossambicus on* exposure of PE



Values are mean ± SE of five individual observations, Values are significant at (*) p<0.05, (**) p<0.01

Discussion

Fish are often used as sentinel organisms for ecotoxicological studies because they play number of roles in the trophic web, accumulate toxic substances and respond to low concentration of mutagens (Cavas *et al*., 2005) therefore, the use of fish biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems (Van Der Oost *et al*., 2003). The result of the LC_{50} (median lethal concentration) for PE in the present study at 96 h was 501.65mg/·L. The results show that the toxicity of PE belonging to the sulfonylurea group for *O. mossambicus*is both time and concentration dependent. The LC_{50} value obtained for *O. mossambicus* in this study is higher than that reported by PPDB manual for *Onychorhynchus myskiss* (180 mg/ L). This could probably due to the size difference in the fish, as for acute toxicity studies the standard size of the fish for the experiment is always 4-6 cm with a weight of around 5-10 g according to OECD guidelines and the size and weight of the fresh water fish used in the present study was 12-15 cm with the weight around 25-28 g. The LC_{50} of other herbicides belonging to sulfonylurea such as Metsulfuronmethyl has also been reported by other workers (Da Fonseca *et al*. 2008 and Pretto *et al*. 2011 for *leporinuso btusidens*; by dos Santos Miron *et al.* 2005 for silver catfish (*Rhamdia quelen*), 2008; Moraes *et al*. 2007, 2011 for *Leporhinus obtusidens* and *Cyprinus carpio* respectively; Rossi *et al*. 2011.Further, Baghfalaki *et al*., 2012 has reported LC_{50} on three non-target freshwater fish Caspian roach *Rutilusrutilu scaspicus,* Common carp *Cyprinus carpio* and silver carp *Hypopthalmicthys molitrix* were 152.74, 289.08 and 139.45 ppm respectively.

Behaviour arises from the cumulative interaction of a variety of biotic and abiotic factors and represents the animal's response to internal (physiological) and external (environmental and social) factors, relating one organism to another. In short, behaviour is the how the animal "talks" to us about what it knows (Morris 2005).Behaviour is a visible reaction of an organism to a stimulus on the whole-organism organization level. However, being based on biochemical reactions and exerting consequences on the population and biocoenosis levels, behaviour can be regarded as highly integrative (Dell'Omo 2002, and. Gerhardt A, 2007).There has been recent interest in behavioural

responses for aquatic toxicity testing and is considered to be a promising tool in ecotoxicology (Melvin and Wilson, 2013). To study changes in behaviour due to chemical exposure is therefore an essential part of behavioural science, which can be called behavioural ecotoxicology. As chemicals are one type of environmental stressors, behavioural ecotoxicology thus become an integral part of stress ecology and is among the most sensitive indicators of environmental alterations and have been proposed as an index of sublethal toxicity in wildlife (Dell'Omo 2002). Hence, Behavioural analyses has become promising due to its rapidity, sensitivity and noninvasive compared to traditional toxicological methods assessing developmental and reproductive effects (Maradona *et al*., 2012, Amiard-Triquet, 2009).

In the present study the PE treated group fish exhibited irregular, erratic and darting swimming movements. Abnormal swimming and loss of balance may be due to the deficiency in nervous and muscular coordination which can be due accumulation of acetylcholine in synaptic and neuromuscular junctions (Rao *et al*., 2005). Toxicological effects of the herbicide oxyfluorfen on acetylcholinesterase in two fish species: *Oreochromis niloticus* and *Gambusiaaffini* has been reported by Hassanein, 2002 and by 2,4-D herbicide on piava freshwater fish (*Leporinus obtusidens*) by da Fonseca *et al*., 2007 and by yet another herbicide, glyphosate by Modesto, and Martineze on Juveniles of *P. lineatus.* Pesticides are lipophilic and are rapidly absorbed in fish gills which cause respiratory limitations that also attack neural organs resulting in imbalance, spiral swimming and collapse to the bottom of the aquarium. These were very much evident in the fishes of the group exposed to 400 and 500 mg/L concentration of PE. Change in respiration rate is one of the common physiological responses to toxicants (Pandey *et al*., 2009)**.** Gulping air at the surface and swimming at the water surface were also observed along with abnormal swimming pattern, intense opercula movements and loss of coordinated movement. Increased surface breathing and opercula movement in the stressed *O. mossambicus* points to the fact that, PE exposure has resulted into sustained respiratory discomfort. Our perceived behavioral changes are in accordance with previous researchers on methylsulfuron herbicide (Baghfalaki *et al*., 2010) where they have studied acute toxicity of tribenuron-methyl herbicide in Silver Carp (*Hypophthalmict hysmolitrix*), Common Carp (*Cyprinus carpio*) and Caspian

Roach (*Rutilusrutilus caspicus*). Further, the abnormal behavioral responses increased with increasing concentration and exposure time. Similar behavioral responses determined in this study have been observed with the spotted snakehead *Channa punctat*us exposed to various concentrations of atrazine herbicide (Nwani *et al*., 2010).

The kinds of behavioral burdens in orientation and locomotion, as observed in the present study, can be related to the mutilation of sensory organ systems particularly the mechano and chemo-receptor systems. Sensory organs like lateral line, olfactory organs and membranous labyrinths helps the fishes in maintaining harmony with their environments and also control their vital behaviors (Kasumyan 2004). Hence, any impairment of these organs would produce behavioral faults in the fishes. Therefore, the behavioral changes, particularly those concerned with respiratory insufficiency, observed in *O. mossambicus*, might be contributing to the mortality in these stressed fishes.

A thick coat of mucus was observed all over the body of the fish along with the reduced pigmentation of the skin. The secretion of mucus over the body and depigmentation is due to dysfunction of the endocrine gland under toxic stress causing changes in the number and area of mucus glands and chromatophores as reported by Nwami *et al*., (2013) in *Tilapia zilli.* There are several studies considering herbicide toxicity in fish, especially concerning metabolic and oxidative parameters in response to glyphosate exposure (Fonseca *et al*., 2008; Salbego *et al*., 2010; Glusczak *et al*., 2011). The mucus layer is important not only because of to its effective role as a protective barrier, but also because it performs a number of functions, including disease resistance, respiration, ionic and osmotic regulation, locomotion, reproduction, communication, and feeding (Subramanian *et al*., 2008).

Blood is a pathophysiological reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari *et al*; 2004).The toxicity of herbicides on aquatic organisms can be studied by assessing the alterations in the haematological and biochemical parameters and these parameters can be used as health indicators of the aquatic environment and also provide early warning tools in monitoring environment quality (Pimpao *et al*., 2007, Saravanan *et al*., 2011). The aim of the measurement of blood

parameters is that in recent years, this method is used as an easy and useful method to assess the effects of sublethal toxicity of herbicides on fish (Pour *et al*., 2011).

On acute exposure of different concentration of herbicide PE to fishes the haematological parameters red blood cell count, haematocrit (PCV), and haemoglobin have shown a significant increase compared to control. However the maximum change was seen in the fishes which were exposed to the concentration of 50 mg/l. An increase in this parameter may occur in situations of acute stress, when the adrenergic stimulus triggers splenic contraction, releasing large quantities of red blood cells into the bloodstream (Pereira *et al*., 2013). Furthermore, this increase in erythrocyte number and haematocrit value could be due to increased hypoxia. According to Mairbäurl (2013) spleen and liver may reactivate the erythropoiesis to compensate the demand due to the increased oxygen transport to the peripheral tissue. Erythropoiesis is well explained by Sawka *et al*., (2000) as a mechanism whereby the number of red blood cells in the circulation is increased for the compensation for poor oxygen uptake in prevailing hypoxic condition. Moreover, hypoxic conditions also contributed to the increase surfacing and gulping in surface water, which could also have been an attempt by the fish to avoid breathing in the herbicide PE infected water. Hypoxic conditions arise primarily due to damage of gills of fish exposed to herbicide, which hampers oxygen uptake. The increased mucus secretion by the fish after PE exposure is probably an adaptive response to counter the irritating effects of the herbicide on body surface and mucus membrane. The observed abnormal behaviour alterations in PE exposed fish are consistent with previous reports on pesticides such as malathion, profenofos, praziquantel (Pandey *et al*. 2011, Nwani *et al*., 2014) and atrazine (Nwani *et al*,. 2012).Though there is another mechanism via the release of a large number of mature red blood cells in general circulation which is thought to be stimulated by β adrenergic action on the haemopoetic tissue (Christoph *et. al*; 2015). However this mechanism might work well for the compensation for short-term variation in oxygen concentration in blood or water (Nespolo and Rosenmanm, 2002). Thus one can say the increase in Pack cell volume of *O. mossambicus* is likely to be due to either increased metabolic demand or gill damages resulting in impairment of oxygen transport or both (Varadarajan, 2010).

Similar results were found in reports of acute intoxication by dichlorvos on *Clariasbatrachus* (Benarji,1990), by trichlorfon on *Piaractus mesopotamus* (Tavares, 2004) and by quinalphos on *O. mossambicus* (Sampath,1993). It has been shown that the erythrocyte number and haemoglobin level may vary with oxygen requirements (Hubrec *et. al*; 2000, Tavares *et. al*; 2004).Pereira *et al*., (2013) evaluate the possible effects of the clomazone-based herbicide on the fish *Prochilodus lineatus*, juveniles were exposed for 96 h and showed that RBC increased significantly at the lowest concentration.

White blood cells are the smaller number compared with red blood cells, and they have the defensive role in the body of organisms. WBCs act as an immune response of infections and chemical irritants in fish. Joshi and his co-worker (2002) explained that increase in WBC count can be correlated with an increase in antibody production which help in survival and recovery of the fish exposed to lindane and malathion. In the present work, the increased WBC count indicate the hypersensitivity of immune cell which interned stimulate their immunological reactions to produce antibodies to cope up with the stress induced by PE (Ramesh and Saravanan, 2008).

Biochemical analysis can provide valuable information for monitoring the health condition of fishes. Biochemical changes depend on the fish species age, the cycle of sexual maturity and health condition. In present study the analysis of blood glucose, total protein, albumin and globulin level showed useful information in detection and diagnosis of metabolic disturbances in fishes. Analysis of glucose concentration in blood is widely used as indication of stress response. Barton (2002) reported that blood glucose appeared to be a sensitive indicator of environmental stress in fish. The stress – related hyperglycaemia reported in many species of teleosts is mediated mainly by the effects of catecholamines (CAs) on glucose release from the liver, the main carbohydrate store in fish, with epinephrine being more potent than norepinephrine (Jenkins *et al*., 2003). Generally glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma. In many fish species, the blood glucose level has the tendency to increase due to the experimental stress. In the present study the significant increase in glucose which was may be

manifestation of stress induced by herbicide PE. Wedemeyer *et al.,* (1981)stated that high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition such as chemical stresses. The increase of glucose can be interpreted as a consequence of glycogenolytic activity of catecholamines and gluconeogenetic effect of glucocorticoids as an organism reaction to the stress stimuli. Both of these groups of hormone produce hyperglycaemia. It is generally thought that, under conditions of stress, hyperglycaemia may provide additional energy during times of high metabolic need such as flight and fight response. Ramesh and Sarvana (2008) have found the similar result in *Channa punctatus* when exposed to chloropyrifos.

Proteins are mainly involved in the architecture of the cell. During stress conditions, fish need more energy to detoxify the toxicant to overcome stress. Protein is also considered one of the important biochemical parameter which has been used to understand the general status of health and biological mechanism of metabolism under the pollutant stress. In the present study the gradual dose dependent increase in the protein level could be due to the mobilization of this molecule to meet energy demand for the maintenance of increased physiological activity, to detoxify the hepatocellular damage in the liver (Martinez *et al;* 2004) or increased rate in the protein syntheses as general adaptation of stress by an animal (Sweety *et al*; 2008).

Serum proteins composition and levels of their separate components which depend on fish species, age, life cycle and sexual maturity, diet, health and environmental factors. (Kovyrshina *et al*., 2012). Serum protein mainly contains albumin and globulin. Albumin is thought to have three basic functions in fish osmotic regulation of blood volume, easily available protein reserve and transport protein. Also the Albumin in fish involves in plastic metabolism and plays an important role in transport functions of exogenous chemicals and endogenous metabolites (Baker, 2002).Thus albumin determination in fish plasma or serum is considerable diagnostic tool which reflects the health of the animal, liver function, metabolic status and stress conditions. In the present study serum albumin level decreased.

Looking to the value of LC_{50} of PE it can be consider to be moderate toxic. However, PE exposure during the acute treatment induces significant changes in the haematological and biochemical parameters of the freshwater fish *O. mossambicus.* The alterations of the parameters may provide the early sign for the determination of acute toxic level of herbicide and their effects on aquatic medium. The findings of present study also provide a better understanding of toxicological endpoint of aquatic pollutants and safer level of these herbicides in the aquatic environment and protection of aquatic habitats.