General Considerations

Pesticides, including herbicides, insecticides and fungicides, are used extensively to improve crop yields and as a result, they accumulate in the environment. More than 2.5 million tons of pesticides are applied every year to agricultural crops worldwide (Dimitrov *et al.,* 2006). Agricultural use of pesticides is a subset of the larger spectrum of industrial chemicals used in modern society. Modern agricultural practices reveal an increase of pesticides to meet the food demand of increasing population which results in contamination of the environment leading to the surfaced controversy of use and abuse of pesticide. The rampant use of these chemicals, under the saying "If little is good, a lot more will be better" has played destruction with human and other life forms (EEA, 2013, Agarwal *et al.*, 2015). According to FAO, the average application rates of pesticides per hectare of arable land have been computed and the highest average values have been found to be 6.5–60 kg/ha in Asia and in some countries of South America. In North America and West Europe, the use of herbicides is intensively applied in agriculture and in urban areas. However in the last decades; in Asia, the use of herbicides has remained low in contrast to the use of insecticides in other countries (Carvalho, 2017). The American Chemical Society database indicates that there were some 13 million chemicals identified in 2000 with some 25000 new compounds being added annually (Widener, 2017).

Pesticides pollute water sources through direct application in aquatic systems or through indirect sources such as by erosion from agricultural lands and agricultural wastewater infiltration, which eventually enter in the aquatic ecosystem. According to EPA report (2015), more than 50% of water pollution of streams and rivers occurs due to leaching and mixing of pesticides from agriculture practices (Agrawal *et al.,* 2010). Aquatic environment is not the actual target for pesticide, but the widespread use of them has led to serious problems including toxic residues in grass and toxicity of non-target organisms such as mammals, birds and fish (Saeed *et al.,* 2012; Shankar *et al.,* 2013). Agriculture disrupts all freshwater systems hugely from their pristine states. Both agricultural and freshwater systems are complex and the relationships between them make a mesh of many dimensions. Pesticide residues in drinking water have become a major challenge over the last few years. One of the terrifying effects of pesticide contamination of groundwater came to light when pesticide residues were found in packaged natural mineral water and popular cold drinks (CSE, 2002 and Gupta *et al.,* 2009). Most of the Indian rivers and their tributaries viz., Ganges, Yamuna, Godavari, Krishna, Sone, Cauvery Damodar and the Brahmaputra are reported to be grossly polluted due to the discharge of untreated sewage disposal and industrial effluents directly into the rivers. These wastes usually contain a wide variety of organic and inorganic pollutants including solvents, oils, grease, plastics, plasticizers, phenols, heavy metals, pesticides and suspended solids which are not easily susceptible to degradation. The indiscriminate dumping and release of wastes containing hazardous substances into rivers become a potential source of stress to biotic community.

Bio-accumulation of pesticides in aquatic species is increasing alarmingly, posing a threat to aquatic life. As reported by Singh and Singh (2008) runoff from the agricultural gets accumulated in the fish. The pesticides become parts of the water column and the fish ingest the pesticides, usually through their gills and sometimes through their scales. The pesticides then enter into their organs and fat tissue which are sequestered and gets stored in them. These accumulated pesticides gets biomagnified up the food chain as big fish eat small fish and eventually as humans eat the fish as it is one of the major sources of essential proteins for human beings. Pesticides have been found to be highly toxic not only to fishes but also to fish food organisms, thus threatening the life of the fish (Satayavani *et al.,* 2011, Murthy *et al.,* 2013, Smalling *et al.*, 2013).

Herbicide is a type of pesticide which are widely used in agriculture, industry, and urban areas to control weeds. They are considered to be plant poisons and are relatively easy to apply and they are the only available practical method to control weed in terrestrial as well as aquatic habitat. They have made a large contribution in reducing the workload of crop production. While herbicides with various modes of action are currently used; sulfonylurea herbicides which were developed in late 1970s, are used extensively worldwide, and they hold the central position in weed control globally as paddy rice herbicides. Numerous commercial formulations containing different herbicides (glyphosate, paraquat, sulfonylurea etc) have become popular around the world due to their effective action and low toxicity to mammals. However; they have proven to be harmful to the environment, particularly weed-infested waters with herbicides have proved to be toxic to aquatic life (Rzymski *et al.,* 2013).

Almost 30 commercial herbicides have been identified from sulfonylureas. Five families of ALS inhibitors i.e. Imidazolinone, Pyrimidinylthiobenzoate, Sulfonylaminocarbonyltriazolinone, Triazolopyrimidine and Sulfonylureahese have been commercialized (Vencill 2002 ; Mallory-Smith and Retzinger 2003). Each class of herbicide is unique as they bind to ALS at different sites which are overlapping. It has been postulated that ALS specific herbicides bind to the enzyme at the entry site for ALS substrate or substrate access channel (Pang et al. 2003). This inhibits the activity of the enzyme and leads to the deficiency of branched chain amino acids.

Finally, there is death of susceptible species of plant due to starvation for branched chain amino acids (Tan *et al.*, 2005).

Most of the studies till date have focused on various different insecticides such as Neonicotinoids, which has been well explored by (Tomizawa and Casida 2005, Tennekes, 2010, Jeschke *et al.*, 2011, Parikh *et al.*, 2013, Gibbons *et al.*, 2015, Bayo *et al.*, 2016 and Tyor & Harkrishan, 2016,), fungicides (Juerrgensen *et al.*, 2000, Åkerblom, 2004, Knauer *et al.*, 2007, Mellish, 2013, Parikh *et al.*, 2014,) and fertilizers/plant nutrient (Vivierica and Bowman, 2001; Palanivelu *et al.*, 2005; Yaro *et al.*, 2005; Bobmanuel *et al.*, 2006; 2008, Ofojekwu *et al.*, 2008 a & b; Ufodike and Onusiriuks 2008; Biello,Helfrich, 2009, Kapkin *et al.*, 2010; Parikh *et al.*, 2015 and Auquo *et al.*, 2016). Many herbicides have undergone several toxicity testing to evaluate effects on a nontarget organism such as fish (Masser *et al.*, 2013, Nwani *et al.*, 2010). The herbicide that is toxic to the early life stages of fish has been reported in *Clarias gariepinus* and scientists have opined that herbicide toxicity can lead to a decline in their populations (Ayoola, 2008 a and b, Ayanda and Egbamuno 2012). Acute and chronic exposure to the sublethal concentration of paraquat commercial known by the name roundup of Nile Tilapia (*Oreochromis niloticus*) has been well studied by Jiraungkoorskul *et al.*, 2002, 2003 and Lushchak *et al.*, 2009. Atrazine herbicide toxicity has been well explored on *Clarias punctaatus* (Nwani *et al.*, 2010); on Nile Tilapia (*Oreochromis niloticus)* (Peebua *et al.*, 2007 and De Ventura *et al.*, 2008); on Zebrafish embryo (Weigand *et al.*, (2001) as well as on blue gill sun fish (*Lepomismac rochirus*) by Elia *et al.*, 2002.

Sulfonylurea herbicides are an important class of herbicides used worldwide for controlling weeds in all major agronomic crops. They were discovered by Levitt with DuPont and have strong points such as (i) low toxicity for untargeted organisms (ii) lower application rate than conventional herbicides, (iii) broad herbicidal spectrum ranging from broadleaf weeds to grasses, and (iv) safety to various crops that can be impacted/ imparted by structural modifications. It has come to hold a foremost position among selective herbicides. Among sulfonylurea products, Pyrazosulfuron-ethyl (PE) herbicide is widely used for selective post-emergence control of annual and perennial grasses and broadleaved weeds in cereals. The IUPAC name of PE is Ethyl 5-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-1-methylpyrazole-4-carboxylate. The occurrence of sulfonylurea herbicides in aquatic environments is receiving public attention (Battaglin *et al.,* 2000). Residues of sulfonylureas have been detected in surface water and groundwater due to runoff and leaching after their application (Okamoto *et al.,* 2001). Approaches to the selective action of pyrazosulfuron-ethyl, a herbicide in physiological and biochemical aspects have been little studied, as evidenced by the foregoing literature and thus there is an overall lacunae in the information regarding the toxic effect of herbicides in general and pyrazonsulfuron ethyl in particular to our knowledge. Thus, we hypothesize that the herbicide upon leaching to nearby freshwater ecosystem can possibly enter into the food chain which can result into decrease in the dynamicity of the ecosystem by affecting non target organisms like fishes.

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 the fiat adjective of the present study was to determine the 96hr LC50 value along with the behavioural response and haematological and biochemical alterations on freshwater fish Oreochromis mossambicus on exposure of PE.

Acute toxicity (LC- 50) was investigated in static test during a 24, 48, and 96- hours exposition of PE. The LC50 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. The exposure of PE resulted into abnormal swimming behaviour which was reported by jerky movement, Agitate swimming and loses of equilibrium, increase fin and tail movement. Overall the exposure of PE resulted into hyperactivity and restlessness. 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). The kinds of behavioural 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 behaviours (Kasumyan, 2004). Hence, any impairment of these organs would produce behavioural faults in the fishes. Therefore, the behavioural changes, particularly those concerned with respiratory insufficiency, observed in *O. mossambicus*, might be contributing to the mortality in these stressed fishes. With regards to the

Haematological alterations are concerned, there occurred a significant increase in the values of RBC count, Haemoglobin and PCV, when compared to control groups. 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. Increased WBC count indicates the hypersensitivity of immune cell which in turn stimulates the immunological reactions to produce antibodies so as to cope up with the stress induced by PE (Ramesh and Saravanan, 2008). In the present study the significant increase in glucose was a manifestation of stress induced by herbicide PE. 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. The alterations of the haematological and biochemical parameters thud has provided the early sign for the determination of acute toxic level of herbicide and their effects on aquatic medium and also give a better understanding of toxicological endpoint of aquatic pollutants and safer level of these herbicides in the aquatic environment and protection of aquatic habitats.

After establishing the LC_{50} of PE, to supplement risk assessment studies and in the view of paucity of information available on PE toxicity at a sub-acute level the next objective was to have an insight regarding the biochemical and haematological alterations on fresh water

teleost, O. mossambicus on exposure of PE at sub-acute level. Studies on the elimination of pesticides from fish are most important from the point of view of human health and there is a paucity of information on the recovery of blood cells after exposure to pesticides (Sampath et al., 1993; Adhikari et al., 3004). Knowledge of the prediction or extrapolation of recovery time is important. The time of complete recovery of fish to pre-exposure levels would be of direct help to fish health and indirect help to the human health. Therefore, in the present study an attempt was also made to evaluate the sublethal effects of PE) on certain hematological and biochemical parameters of a freshwater fish, O. mossambicus, and to assess the duration for complete recovery from PE intoxication (Chapter II)

Sub-acute toxicity was investigated in a semi-static experiment by exposing fish to different PE concentrations (1/20th of LC50 as low dose (LD; 1/10thLC50 as Medium dose (MD) and 1/5thLC50 as high dose (HD) for 7 and 14 days. From all the groups after 14 days of exposure the remaining live fish were shifted to herbicide free fresh water and blood samples were analyzed at 28 days of recovery. A dose and time dependent significant decrease in all the hematological parameters with a significant alteration in MCV, MCH and MCHC in the fish *O. mossambicus* was observed exposed to PE. In addition to the hematological alterations, biochemical parameters also evoked a significant decrease in protein content associated with a significant increase in Glucose, BUN, Urea and Creatinine parameters on PE exposure. However, these parameters gradually improved during the recovery period at all the concentrations tested for PE. The improvement in blood and biochemical parameters of the test fish when transferred to herbicide-free freshwater suggests that herbicides entering into the system are slowly eliminated and hence the blood parameters recover from PE toxicity.

The condition factor is an organism – level response, to factors such as nutritional status, pathogen effects and toxic chemical exposure, causing greater – than normal and less – than – normal weights (Azmat *et al.,* 2007). The condition factors are used as indicator of the well being of individual organism, because it integrates many levels of the organizational processes. For example, a decrease in condition factor is considered a reflection of depletion in energy reserves because these indices are positively related to muscle and livers energy content (Lizama *et al.,* 2002; Hasan and Seces, 2003). Condition factor has been used as an indicator of health since the beginning if the 20^{th} century as it provides the information on the variation of the fish physiological status and may be used for its overall health status. Organo-somatic indices are yet another simple biomarker of toxicity, described as the ratios of organs to body weight (Das and Gupta 2014) and can be directly linked to toxic effects of chemical on target organ (Maxwell and Dutta, 2005 and Giullo and Hinton, 2008). ***Hence, keeping in mind the above facts, the present study is aimed to look into the alterations in the oraganosomatic index (HSI, GSI, KSI, SSI and CSI) as well as the condition factor (K) of freshwater teleost fish, Oreochromis mossambicus on exposure of the herbicide PE.***

The mean organosomatic index and condition factor of *O.mossambicus* exposed to different concentrations of PE resulted into a dose dependent and time dependent decrease. Although these parameters are not very sensitive, they may serve as an initial screening bio meter and is a practical tool for biologist and managers to guess the overall health of fish population and a good indicator of fish habitat quality and pollution levels. HSI is most important because it describes stored energy in fish, in the present study significant decrease with time and doses thus suggest the usage of the stores accumulated in the liver for supplying energetic requirements (Akermen *et al.,* 2003; Garcia-Diaz *et al.,* 2006; Kumar *et al.,* 2007; Montaser *et al.,* 2010). Further, a decrease in the weight of liver also suggests a reduction in the production of endoplasmic reticulum for protein systhesis in liver tissue under PE exposure (Bennet and Wolke, 2004), Further, liver reduction could also be as a result of decreased lipid storage. (Gabriel *et al.*, 2010). Our results are in agreement with the earlier reported decreased HSI in (Oreochromis niloticus) juvenile exposed to glyphosate herbicide by Ayoola (2008), Clariasgariepinus exposed to cypermethrin by Ariweriokuma *et al.,* 2011, Channa punctata exposed to butachlor by tilak *et al.*, 2007.

Gonadosomatic index (GSI) indicates the gonadal development and maturity of fish. Although it may be unrealistic to include detailed reproductive data into routine toxicity assessments, accurate measurement of reproductive condition is essential for determining reproductive fitness (Lowerre-Barbieri *et al.,* 2011). In the present study a significant time and dose dependent decrease in male and female GSI was witnessed. HSI is related with GSI because of vitelogenesis process that synthesizes vitelogenin. In fish, vitelogenin is yolk precursor that is synthesized in liver and indiced by estradiol 17β (Babin *et al.,* 2007 and Yaron *et al.,* 2011). Vitelogenin is secreted in blood and transported in oocytes causing the accumulation of yolk this accumulation cause the changes of oocytes size and enhancement of ovary weight. This vitelogenesis activity can increase HSI and in turn GSI (Le men *et al.*, 2007). In the present study as HSI was found to be decreased, so a possible explanation for a decrease in GSI can thus be correlated with the titer of HSI (Intanurfemi *et al.,* 2015). Furthermore the dose dependent decrease in the GSI is also suggestive that GSI is directly proportional to the concentration of PE as proposed by Murthy *et al.*, 2013 for various pesticides. Our results are in agreement with the earlier work of Vasath *et al.,* 2015 who have reported a decrease in GSI and HSI on exposure of herbicide atrazine. Further, SSI, KSI and CSI were also studied, and all the three revealed a dose and time dependent decrease The present study thus suggest that the biological indicator approach is an effective technique to assess the integrative effect of stress on fish, as well as being a tool to obtain biological information in a system and could be used to manage contaminated sites or exposure of the pollutants (Parikh *et al.,* 2013, Sadekarpawar and Parikh, 2013, Parikh and Pandya, 2015).

A variety of approaches have been used to evaluate the effect of stress on the general health of the Fish population. Recently many studies have made use of histopathological assessment of fish in aquatic system systems (McHugh *et al.,* 2011 Smith and Wagenaar, 2012, McHugh *et al.,* 2013, Wagenaar *et al.,* 2012 and Gerber *et al.,* 2017). Histopathological biomarkers are closely related to stress since many pollutants either toxic or non toxic have to undergo metabolic activation in order to be able to culminate cellular change in the affected organism. Histopathological studies have been conducted to help for establishment of casual relationships between contaminant and exposure and other various biological responses. These investigations have also been proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments (Satpal, 2010, Akinsorotan *et al.,* 2013, Mariyadasu, 2014, Adebola and Folorunsho, 2014, Nibamureke *et al.,* 2016, Robson *et al.,* 2017). Such analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads (Mariyadasu and Kumari 2017). Investigation of such nature may therefore prove to be a cost effective tool to determine the nature of fish tissue damage, hence reflecting the well being of an organism.

Fishes are particularly sensitive to a wide variety of pesticide chemicals, and toxic concentrations may rise not only from spillage of agricultural practices if their use is excessive but also from several other sources. Apart from causing death either directly or due to starvation by destruction of food organisms, many pesticides have been shown to effect growth rate, reproduction and behavior with the evidence of tissue damage. ***Keeping in mind the above facts an attempt is made to have an insight into the histopathological alteration of Gills, Liver and Kidney with a semi quantitative approach (Chapter IV).***

The reaction index (I org rp) gives an indication of the quality of the lesions in the specific organ, and the organ index (Iorg) gives an indication to the degree of damage. Time and dose dependent alteration in the reaction pattern was obtained for Gills, Liver and Kidney. Histological alterations were confirmed by the semiquantitative analysis, which includes circulatory disturbances, regressive changes and progressive alterations. On the basis of the reaction pattern, the organ index of all the tissues after 7 days of PE exposure exhibited a slight histological alteration which represents class I. However, at low dose; Gills, liver and Kidney exhibited moderate histological alterations which represent class II. At medium dose, all the tissues exhibited severe histological alterations which represent class III, and at High dose, Gills, Liver and Kidney exhibited pronounced histological alterations which represent class IV. In conclusion, the present study indicates that sub acute exposure to PE induces histopathological and ultra structural changes in gills, liver, and kidneys.

The cytopathological lesions in all three tissues recorded less than two different time period and three doses demonstrate the cumulative physiological and biochemical effects of PE exposure. The effects were more pronounced in the kidney compared to liver and gills and at HD in comparison to the MD and LD which ultimately indicated greater disturbance of cellular metabolism as well as serious structural alterations in the kidney. Therefore, these histopathological including ultra structural alterations under different dose and time for fish could be considered as sensitive biomarkers of xenobiotic exposure. The different responses probably are functions of tissue specificity, the time of exposure. The observed cellular damage may be as a result of the ROS generated by PE which may lead to overall cell apoptosis. However, organisms are equipped with a self repairing mechanism to alter the damaged/altered histoarchitecture, which was evident by the recovery test for biochemical and hematological parameters, where the recovery test revealed a time dependent alteration to cope up with the disturbed structure of the tissues, where all the three tissues did show a macromolecules, produced during normal metabolism or due to exposure to xenobiotics.

Different species of aquatic organisms, such as clams, mussels, fish, and amphibians, are used to investigate the genotoxicity of toxicants (Buschini *et al.,* 2004; Lemos *et al.,* 2005; Souza and Fontanelli, 2006). **A**grochemicals are employed worldwide in agriculture to protect crop from pests, weeds, pathogens and parasites. The pesticides enter the aquatic ecosystem through runoff from agricultural fields that lead to the pollution of aquatic environments such as rivers, ponds, lakes etc. The bioaccumulation and persistence of these pollutants in the aquatic environment pose a serious threat to aquatic life and to human beings indirectly through the food chain (Binelli and Provini, 2004). The aquatic ecosystem as a greater part of the natural environment is faced with the threat of a shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Omitoyin *et al.*, 2006). The majority of these hazardous chemicals are mutagenic in nature (Garaj- Vrhovac and Zeljezic, 2002), either linked to the cancers .

Fish are excellent subjects for the study of the mutagenic and carcinogenic potential of contaminants present in water samples since they can metabolize, concentrate and store waterborne pollutants and the micronucleus test (MN) is one of the simplest and quickest tests for biomonitoring the genotoxicity of pesticides. Micronucleus is produced by fragments of chromosomes or whole chromosomes, which remains behind during cell division, the remaining back is due to lack of centromeres, or have damaged or have defects centromeres in cytokines. During cell division, the shape, size and number of chromosomes are the same, and divided equally between the daughter cells. If during cell division, chromosomes are damaged or cut off by the action of various toxic substances, the distribution of genetic material between two daughter cells is not the same, because the chromosomes of all or part of them may remain outside nucleuses cells daughters then the material is rolled membrane forming a micronucleus, which can be clearly seen under the microscope. The MN method using peripheral blood smears of fish is widely used and recommended (Çavas and Ergene Gozukara, 2003; Conen and Cavas, 2006; Zeqiraj *et al.,* 2015). ***In the present study was to verify genotoxic potentisl of herbicide PE in peripheral blood erythrocytes of O.mossambicus, and to prove damage to the genetic material in erythrocytes by MN test, and further to validate whether the damage has led to apoptosis FACS was also carried out. (Chapter V).***

A significant time and dose dependent increase in the frequency of MN in peripheral blood erythrocytes of fish treated with herbicide PE was detected compared with control group of fish. In the present investigation, we have evaluated the genotoxicity of herbicides PE that has measured the induction of structural chromosomal aberrations and micronuclei. Chromosomal aberrations qualitatively and quantitatively detect clastogenic activity, while the micronucleus assay detects both clastogenic effects and damage to the mitotic apparatus, some of which might have aneugenic consequences. Although the MN test is a sensitive assay to evaluate genotoxic compounds in fish under controlled conditions as an index of cumulative exposure (Yin *et al.,* 2009; Bhatnagar *et al.,* 2015), however, it might suffer variations according to tests organisms. Further, as the preexisting mature (and non-dividing) erythrocytes would predominate in the blood, the detection of induced MN in mature blood cells will be at a low frequency in the beginning of the exposure. On the other hand, with the progression of the experiment, a greater number of the dividing cells (polychromatic erythrocytes) would be expected to predominate in the blood and, therefore, a latency period is normally required between treatment and subsequent MN peak (Nwani *et al.,* 2010; Bhatnagar *et al.,* 2015). So, due to above constraint, cell cycle analysis and apoptosis rate was performed with the help of FACS.

The MN studies and FACS results thus postulates that erythrocytes have entered the apoptosis; together these data show that PE exposure is potentially hazardous to *O. mossambicus*, even at sub-lethal levels as evidenced by the cell death study which was found to be significantly increasing in a dose and time dependent manner. At 7 day exposure, there was linearity in the increase in the apoptotic population of erythrocytes i.e. from LD to HD. While, this linearity was also observed for 14 days exposure period. The highest cell death was accounted for HD of both the durations suggesting cells were under stressed which lead them to undergo apoptosis at sub lethal exposure of PE. Hence, the study suggest, in field, even the sub-lethal concentration can cause toxicity to fresh water fish and results in altering the aquatic ecosystem.

The important observations can be summarized:

- 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. Acute studies also resulted into marked behavioral alterations, such as abnormal swimming behavior with jerky movement, Agitate swimming and lose of equilibrium, increase fin and tail movement. Overall the exposure of PE resulted into hyperactivity and restlessness.
- Sub-acute exposure of PE herbicide resulted in significant alterations in hematological and biochemical parameters. The recovery studies revealed the fact that the rate of recovery and extrapolated time was between day 0 and 28 and complete recovery would occur on days 31, 32 and 32
- As general indicators of the overall health and well-being of the fish, an alteration in the indices indicates deleterious effect of the herbicide. From the altered values of CF and indices in the present study it is apparent that PE has resulted in to considerable alteration in the overall health and well-being of the fish.
- Semi Quantify Histopathological observations envisaged the deleterious anatomical and morphological alterations induced in gill, liver, kidney tissues at sub-lethal exposure of the PE. Histological alterations included circulatory disturbances, regressive changes and progressive alterations. On the basis of the reaction pattern, the organ index of all the tissues after 7 days of PE exposure exhibited a slight histological alteration which represents class I. However after 14 days at low dose; Gills, liver and Kidney exhibited moderate histological alterations which represent class II. At medium dose, all the tissues exhibited severe

histological alterations which represent class III, and at High dose, Gills, Liver and Kidney exhibited pronounced histological alterations which represent class IV.

- The cytotoxic and genotoxic potential of PE in *O.mossambicus* revealed increase in the intensity of MN and other nuclear abnormalities. FACS results proved that erythrocytes have entered the apoptosis.

Future Prospects

- Herbicides have proved to alter the hematological and biochemical parameters; however, the mechanism of action at molecular level to source the physiological changes at tissue level will give better understanding to prove the toxic potential of PE.
- Tissue damage which was semi quantified has proved to show some of the regressive changes, pointing to the fact that the tissue might have entered the apoptotic pathway. Hence the exact signaling pathway and cascade involved in cellular genotoxicity can be validated by Immuno-histochemical localization and sequencing of genetic markers.
- To prove whether the toxicity caused is reversible or not, recovery studies will help in comprehending the detoxification mechanism.