CHAPTER II

Hematological And Biochemical Alterations In Oreochromis mossambicus And Labeo rohitaExposed To Plant Nutrient

INTRODUCTION

The aquatic environment is currently under threat due to an increase in heavy metal contamination by the human activities and causing high risk to non-target organisms (Kumar et al., 2010; NEPC, 1980). The cry of pollution is heard from all the nooks and corners on global level. It has become a major challenge and threat to the very existence of mankind on the earth. Moreover, an increase in agricultural practices inorder to overcome the needs of increasing population the degradation of aquatic system is a worldwide phenomenon. Heavy metals are major cause of concern for aquatic environment because of their toxicity, persistent, and tendency to accumulate in the organisms. Such an aquatic contamination cause ecological (biological integrity, biodiversity, ecological processes) as well as economic (aquaculture, production of potable water, fishing, bathing, recreation) effects. Fresh water environments compared to seas and oceans, are more vulnerable to pollution stress inasmuch as they are smaller systems and have more limited numbers and kinds of organisms. Pollution of lakes and rivers pose alarming dangers to aquatic life; especially fishes (Caring, 1992). Biomarkers in fishes have been used within environmental monitoring programs to estimate the degradation of aquatic ecosystems (Seriani et al., 2009).

Fish serves as bio-indicator of water quality and the impact of the toxicant can be well understood by analyzing either blood or serum of the fish, because blood is a pathophysiological reflector of whole body (Sharma and Singh, 2004; 2006). Hematological study is important in toxicological research because a hematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound (Kori-Siakpere et al., 2006). Fish blood is known to exhibit pathological changes before the onset of any externalsymptoms of toxicity thus reflecting the physical and chemical changes occurring due to heavy metal accumulation in body of fish. Hematological changes in fish may be used for assessing the effects of contaminants, because blood parameters respond to low doses of pollutants (Ranzani-Paivaet al., 2000; Affonsoet al., 2002; Adhikari et al., 2004; França et al., 2007; Serianiet al., 2009; Seriani et al.,
Thus, fish blood is being studied increasingly in toxicological research and environmental monitoring investigations (Mulcahy, 1975; Bansal et al., 1980). Fishes exposed to metals, pesticides and effluents exhibit hematological changes, not only after laboratory exposure, but also when the exposure occurs in the field (Ranzani-Paiva et al., 1997; Oliveira-Ribeiro et al., 2006; Adhokari, 2004; Shah, 2006; França et al., 2007, Seriani et al., 2010). A thin epithelial membrane separates fish blood from the water and any unfavorable change in the water body is reflected in the blood (Kori-Siakpere et al., 2008). It is a pathophysiological indicator of the whole body function and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant. A number of haematological indices such as haemoglobin (Hb), hematocrit (Hct), red blood cells (RBCs), white blood cells (WBCs) and so on, have been used as an indicator of metal pollution in the aquatic environment. Furthermore, it should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Dordevic et al., 2000; Gagnon et al., 2006; Akinrotimi et al., 2012). Previous haematological studies of pollutants brought to the knowledge that erythrocytes are the major and reliable indicators of various sources of stress (O’neal and Weirich, 2001).

Blood parameters are considered good physiological indicators of the whole body conditions and therefore can be used in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari et al., 2004; França et al., 2007; Seriani et al., 2009). They have been increasingly employed in environmental monitoring programs to indicate physiological changes due to toxicants (França et al., 2007; Seriani et al., 2009; Zutshi et al., 2009). However, the knowledge on the fish hematology still needs to be expanded, to provide data for different species (Affonso et al., 2002, França et al., 2007; Ranzani-Paiva et al., 2000; 2008; Zutshi et al., 2009; Seriani et al., 2010) and its exposure to the different toxicants. Such a study would be helpful as the exposure of fish to several different types of chemical agents may induce differential changes in hematological variables (Shahi and Singh, 2011). The values of hematocrit, hemoglobin, and number of erythrocytes are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Barcellos et al., 2003; Ahmad et al., 2004; Miron et al., 2005; Ferrari et al., 2007). In addition, the determination of the packed cell volume (PCV), and obtaining total erythrocyte counts and red blood cell indices, such as mean cell volume, mean corpuscular hemoglobin concentration, and mean corpuscular...
hemoglobin, all can be useful in diagnosing disease. PCV varies within and between species and seems to correlate with the normal activity level of the fish. Hematological abnormalities have also been studied in various toxicants exposed fish: *Channa punctatus* to lead (Hymavathi and Rao, 2000); *C. punctatus* to cadmium (Karuppasamy *et al.*, 2005); and *O.mossambicus* to fungicide curzate (Desai and Parikh, 2012). 

Along with alterations in the haematological profile the fishes also exhibit alterations in the metabolism and biochemical processes (Luskova *et al.*, 2002). For example, several studies indicate that after exposure to a toxicant, fish may exhibit an increase or decrease in levels of plasma glucose, serum protein, creatinine and urea. However the exact changes can vary depending on the toxicant type, species of fish, water quality and length of exposure (Monteiro *et al.*, 2005; Jee *et al.*, 2005). Microelements such as Zn, Mn, Fe, Cu and Mn play key roles in living processes and either an excess or deficit can disturb biochemical functions in both humans and animals (Przybyl and Koligot, 1997). In this study, the effect of the micronutrient mixture on the haematological and biochemical profile of freshwater teleosts, *Oreochromismossambicus* and *Labeorohita* were studied. Till now there is lack of knowledge about the toxic potential of a micronutrient mixture having EDTA chelated chemistry in trace amounts. Such a study is vital as it not only assesses the health of fish subjected to changing environmental conditions but also for the deteriorating water quality.
MATERIALS AND METHODS

Experimental design:

The specimens of freshwater fishes, *O. mossambicus* (12 ± 2 cm, 25 ± 1.9 g) and *L. rohita* (20 ± 2 cm, 125 ± 5 g) of similar size in length and weight were brought to the laboratory from a local pond of Baroda district, stocked in well aerated tanks containing chlorine free water and acclimated for 10 days. Temperature, pH, and dissolved oxygen of the water were maintained at 27 ± 2° C, 7.1 ± 0.5, and 3.9 ± 0.02 mg/L, respectively. If in any batch, mortality exceeded 5% during acclimatization, that entire batch of fish was discarded. They were fed with commercial fish pallets. 30% Water was renewed every alternate day to provide freshwater, rich in oxygen. Ten well-acclimatized fish were transferred from the stock to each experimental tank containing 40 L of water exposed to the concentration of 500 mg/L in *O. mossambicus* and 600 mg/L in *L. rohita* for the period of 45 days. A control group was also maintained in the same condition for the basic test. After the study period the fishes were sacrificed. The LC50 values in the respective time intervals were calculated using software by transforming mortalities (percentage values) into a probit scale (Finney, 1971).

Experimental Procedure

On basis of LC50 value sub acute study dose LC50/20 was chosen for hematological and biochemical studies. The experimental regime was maintained in the laboratory for 45 days. A control group was also maintained. The experiment was performed semi statically with a group of 10 fishes in experimental aquaria. Hematological and biochemical examinations of the experimental as well as the control fish were carried out at 15th, 30th, and 45th days of exposure. All the groups were kept under continuous observation during the experimental period. Commercially food pallets were given to fishes once in day during the experimental period *ad libitum*. 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, blotted dry and blood was collected by tail ablation.
**Haematological and biochemical estimation of fish:**

The tail ablation was done using a single stroke from a heavy, sharp seizure. The caudal peduncle of the fish was severed and first drop of blood was discarded. Afterwards 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 hematological and biochemical estimations. An alterations in the hematology and biochemical profile was recorded using NIHON KOHDEN Automated Hematology Analyzer (Celtics α, Japan). The difference between the control and the Librel exposed fishes was determined by One-Way ANOVA. If there was significant difference, Dunnett t-tests were employed to recognize difference in the alterations found in between the control and the exposed groups. The significant level of the tests was set at 5% (p<0.05).
RESULTS

The alterations in the haematological profiles of the exposed and control fishes of both the fishes are shown in the graphs (Table I and Fig. I-XII). There was a species specific change in the parameters. There was significant (p<.05) increase in the HB, RBCs ad PCV in *O.mossambicus* in contrast to *L.rohita* which have shown a significant increase in the exposed fishes compared to control. The level of significance varied with time, showing most significant (p<.001) alteration at the 30th day compared to the 15th and the 45th day in both the fishes. MCH, MCV and MCHC values significantly (p<.05) increased at 15th day with a sudden insignificant decrease at 30th day and at increase at 45th day in both fishes.

There was time dependent decrease in the values of protein of both the fishes, in case of *L. rohita* the decrease was significant at 45th day (p<.05) while in case of the *O.mossambicus* the values decrease significantly at 30th (p<.05) and 45th (p<.001) day. Albumin showed a contrasting results, in case of *O.mossambicus* it decreased significantly (p<.05) at 30th and 45th day while in *L.rohita* it increased significantly (p<.001) at 30th and 45th day. Globulin showed a significant (p<.001) time dependent decrease in its levels in both fishes. WBC count have shown a differential results, in *L.rohita* it decreased significantly (p<.001) at 15th and 45th day and increased on 30th day in contrast of *O.mossambicus* where an insignificant increase was reported at 15th day and significant (p<.001) decrease in 30th and 45th day. Platelet count have shown the following results, in *L.rohita* it decreased but the decrease was significant (p<.001) at 30th day while in *O.mossambicus* and increase was seen which was significant (p<.001) at 15th day. Urea showed a time dependent significant (p<0.05) increase in both species.
Table I: Time dependent alterations in blood parameters in both species

<table>
<thead>
<tr>
<th>Parameters</th>
<th>O.mossambicus</th>
<th>L.rohita</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>15 days</td>
</tr>
<tr>
<td>Hb</td>
<td>8.24±0.2 9</td>
<td>8.30±0.0</td>
</tr>
<tr>
<td>RBCs</td>
<td>1.84±0.1 4</td>
<td>1.51±0.0</td>
</tr>
<tr>
<td>PCV</td>
<td>27.66±0.66</td>
<td>25.62±0.86*</td>
</tr>
<tr>
<td>MCV</td>
<td>142.95±3.09</td>
<td>166.44±3.27</td>
</tr>
<tr>
<td>MCH</td>
<td>41.23±1.11</td>
<td>53.68±1.55*</td>
</tr>
<tr>
<td>MCHC</td>
<td>30.99±0.55</td>
<td>32.44±1.07</td>
</tr>
<tr>
<td>WBC</td>
<td>110±5 6.55</td>
<td>114.1±1 25</td>
</tr>
<tr>
<td>Platelet</td>
<td>90.66±1 7.00</td>
<td>227±23.64</td>
</tr>
<tr>
<td>Glucose</td>
<td>65.22±0.95</td>
<td>47.44±0.50</td>
</tr>
<tr>
<td>Protein</td>
<td>7.22±0.6 7</td>
<td>6.18±0.1 1</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.42±0.0 5</td>
<td>4.11±0.1 0*</td>
</tr>
<tr>
<td>Globulin</td>
<td>2.73±0.2 3</td>
<td>1.92±0.2 4</td>
</tr>
<tr>
<td>Urea</td>
<td>32.3±0.6 0</td>
<td>34.3±0.8 5</td>
</tr>
</tbody>
</table>

*The mean difference is significant at the 0.05 level.
**The mean difference is significant at the 0.01 level.
Fig. I: Alterations in the Hemoglobin (Hb) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

Fig. II: Alterations in the Red blood cells (RBCs) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.
Fig. III: Alterations in the Packed Cell Volume (PCV) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

![Graph showing alterations in the Packed Cell Volume (PCV) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.](image)

Error Bars: +/- 2 SE

Fig. IV: Alterations in the Mean Corpuscular volume (MCV) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

![Graph showing alterations in the Mean Corpuscular volume (MCV) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.](image)

Error Bars: +/- 2 SE
Fig. V: Alterations in the Mean corpuscular hemoglobin (MCH) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

![Graph showing changes in MCH](image)

Groups

Error Bars: +/- 2 SE

Fig. VI: Alterations in the Mean corpuscular hemoglobin concentration (MCHC) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

![Graph showing changes in MCHC](image)

Groups

Error Bars: +/- 2 SE
Fig. VII: Alterations in the Glucose in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

![Graph showing alterations in glucose levels.](image)

Fig. VIII: Alterations in the Protein in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

![Graph showing alterations in protein levels.](image)
Fig. XI: Alterations in the Albumin in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

Fig. X: Alterations in the Globulin in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.
Fig. XI: Alterations in the Hemoglobin (Hb) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.

Fig. XII: Alterations in the Hemoglobin (Hb) in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.
Fig. XII: Alterations in the Urea in *O.mossambicus* and *L.rohita* exposed to Plant nutrient Librel.
DISCUSSIONS

Hematological indices are very important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in hematological levels (Kori-Siakpere, 2006; Oboh, 2011). The impacts of Librel on the hematological profile of tilapia have been assessed in the present investigation.

Hematological system is the most important vital system which reflects the total health status in any organism as it provides movement for useful and useless components. It is the only transport medium for gases during respiration along with the transport of nutritive materials and other important biomolecules to various parts of body. Any alteration in animal’s body like in liver, kidney, brain, digestive system or any infection is reflected in the haematological system. Blood being a liquid connective tissue connects all tissues to each other. It is through this system that compounds are transported in the other parts of the body. These compounds in turn gradually harm the haematopoietic system to damage RBCs, WBCs and other cells resulting into the disturbed haematological parameters (Pathak et al., 2013). In the present study, reduction in hemoglobin was accompanied by lowest PCV value in O.mossambicus. Moreover an increase in WBC count; reflect the occurrence of leucocytosis in the treated fish samples. This was perhaps, a typical defensive response of the fish against a toxic invasion or probability may be of leukemia (Sudha, 2012). This decrease in the erythrocyte count or in the percent of PCV indicates the worsening of an organism state and developing anemia as they are positively correlated. The anemia could be due to the destruction of RBC triggered by the influx of micronutrient into the erythrocytes and may also be of hemolytic type of RBC. Similar observations were also reported by Tilak et al., (2007), Saravanan et al., (2010) and Saeed et al., (2012). In contrast L.rohita has shown increased levels of RBCs, Hb and PCV values which could be due to an increased loss of scales and haemorrhage. Moreover here the duration of exposure also plays an important role. With the increase in the exposure period L.rohita showed, erratic swimming, air gulping, loss of reflex, loss of scale, haemorrhage and molting. They finally settled at the bottom motionless with slow opercular movement. Such results were also reported by Ayotunde and his coworkers (2004) in O.niloticus when exposed to drumstick.

MCV, MCH and MCHC increased considerably with time compared to control. However the increase in these indices can be attributed to direct or feedback responses of structural damage to RBC membranes resulting in hemolysis and impairment in hemoglobin synthesis, stress related
release of RBCs from hemopoietic organ and hypoxia, induced by micronutrient exposure. Our results are in agreement with earlier reported alterations in these indices of Clarian gariepinus exposed to lead nitrited (Adeyemo,2007; Shah, 2006)

In the present investigation, total leucocyte count was increased aftertreatment of parathion and malathion in all the three fishes *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* as compared to control fishes of the same group (Table-3). The increase is more in *Catla catla* following *Cirrhinusmrigala* and *Labeorohita* and dose dependent i.e. effect is increasing with increased sublethal dose. Both thepesticides were harmful but malathion was more reactive than parathion. The increasewas gradually non-significant to significant with increasing dose. The present result is in accordance to the findings of Agarwal and Srivastava (1980) in fresh water fish *Colisa fasciatus* after manganese poisoning; Goel and Kalpana (1985) in *Heteropneustes fossilis* after zinc treatment; Aruna and Gopal (1987) in fish aftersublethal exposure of mercury; Saravanan and Harikrishnan (1999).

Glucose is one of the most important sources of energy for the animals and has been studied as an indicator of stress caused by physical factors in particular pollutants (Manush et al., 2005). An increase in the levels of glucose in fishes in response to micronutrient mixture exposure indicates quantum of stress imposed on fish during subchronic toxicity and its physiological attemptsto overcome it. Glucose is synthesized from hepatic tissue proteins and amino acids due to the intensive glycogenolysis induced due to stress (Almeida et al., 2001). Nakno and Tomlinson et al., observed that all types of stress elevated the secretion of catecholamine which in turn increased the break downof glycogen and elevated blood glucose level. Stressors induce the changes that alter the homeostasis of the animals (Luebke et al., 1997; Bols et al., 2001; Rehulka, 2002). The stressors first activate the chromaffin cell present in the wall of the cardinal veins and in some cases the heart and the kidneys of the teleosts (Mazeaund and Mazeaund, 1981), which in turn releases the adrenalin and small amount of nor-adrenalin that stimulates the conversion of liver glycogen into blood glucose and the utilization of the glucose by muscle. Umingel, (1977) reported that blood sugar has a direct co-relation to metabolism. On the other hand the increase in blood sugar noticed could also be attributed to the differences in the respiration and the activity (Ghosh, 1987). The progressive accumulation of the blood glucose reported in this investigation reveal that both the fishes become hyperglyceamic. Same results were observed by Omoregie et al., (1990) in tilapia exposed to stressed environmental condition
as a result of an incomplete metabolism of the blood glucose due to impaired osmoregulation. According to Coles (1980) increased blood glucose concentration results from an imbalance between the hepatic output of glucose and the peripheral uptake of sugar. Though there are no reports on diabetes in fishes, however stress imposed upon the fish during the toxicity trial might be the possible reason for hyperglycemia. In the present study, exposure of librel at different time period caused an increase in the blood glucose level leading to lethargy.

The proteins are most diverse bio-molecule which are of prime importance in biochemical reactions and cellular structures. Serum proteins have immunological properties in fishes and other animals as well as in human (Kumar and Dahiya, 2013). Proteins are indispensable constituents of the body and their metabolism is almost confined to the liver. Fall in serum protein level may be due to impaired function of kidney or due to reduced protein synthesis owing to liver cirrhosis (Garget et al., 1989; Ravichandran et al., 1994; Kumari and Kumar, 1995). There are reports on the changes induced by pollutants on protein content of serum (Abidi, 1990; Das and Mukherjee, 2001). Serum proteins were found to decrease due to Librel exposure in the present study. This could be attributed to renal excretion or impaired protein synthesis or due to liver disorder (Kori-Siakpere, 1995; 2008). Moreover this could also result from the breakdown of protein into amino acids first and possibly into nitrogen and other elementary molecules. Verma et al., (1979) and Abdel-Tawwab and Wafeek (2008) have observed the same results in fishes. Moreover by Abdel-Tawwab and Wafeek (2008) have opined that such alterations result in the depletion of total protein in the plasma of fish. It is obvious that prolonged exposure offish to most toxicants, interferes with protein metabolism and the present work also supports the observations. Moreover the histopathological damages caused to the kidney offish by these toxicants (Sastry and Sharma, 1981) can lead to significant loss of blood proteins by renal excretion, further augmenting its depletion in the blood (Verma et al., 1979).

Proteins are mainly involved in the architecture of the cell. During chronic period of stress they are also a source of energy. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amounts of carbohydrates so next alternative source of energy is protein to meet the increased energy demand. The depletion of protein fraction in liver may have been due to their degradation and possible utilization of degraded products for metabolic purposes (Singh et al., 2010). Chronic study in Channa punctatus exposed to endosulfan have also shown the decrease in the total serum protein levels.
due to low assimilation of food (Joseph and Raj, 2010). The decrease in serum protein level in fish exposed for longer duration in the present study may be due to the low assimilation of food. Das et al., (2004) stated that reduction of protein content in serum occurs due to shrinkage and lysis of RBCs causing plasma dilution and/or protein catabolism where structural protein converts to energy. According to Radha et al., (2005) the reduction of protein content may be due to increased proteolytic activity and decreased anabolic activity of protein as observed by Jenkins et al., (2003). Further, due to this degradation of protein in liver, the serum protein level has been increased which was released. Reddy et al., (1995) and Singh and Sharma (1998) have also reported decline in protein constituent in different fish tissues exposed to sub-lethal concentration of insecticides in liver and increase in serum. Serum or organ proteins of fish are occasionally studied to estimate the toxic potential of many substances including metals. Results of such investigations are valuable in observing the proteins involved in the metabolism of the toxic substance. The decrease in total protein may be due to the inhibition of RNA synthesis disturbing the protein metabolism. Micronutrient exposure has led to serum total protein depletion probably because of excessive renal excretion or due to the diver disorder (Singh et al., 2011). Decrease in the serum proteins also supports the view of (Abdel-Tawwab, M., and Wafeek, 2010), who reported that the decrease in amino acid incorporation and desegregation of polysomes lead to decrease in protein synthesis. In the present study reduction in the serum oral protein may also be attributes to intensive proteolysis which contributes to the increase in the free amino acids to be fed into TCA cycle as ketoacids. It is, therefore, evident that in case of continous exposure of the micronutrient mixture the deleterious effects of these substances on protein synthesis and kidney function accounts for the progressive reduction in the concentration of total serum protein.

Albumin, globulin and A/G ratio have found to be decrease with the increase duration of micronutrient exposure. Measurement of albumin, globulin, and total protein in serum or plasma is of considerable diagnostic value in fish, as it relates to general nutritional status (Schaperclaus et al., 1992). The decline in albumin, globulin and A/G ratio correlates with decrease in protein content as these are the integral content of protein itself (Singh et al., 2011). Serum protein, albumin, and globulin were significantly lower in Tilapia and Rohu. These results may be due to the disturbances in the liver protein metabolism due to micronutrient toxicity, as was found to be the case with other contaminants (Dange and Masurekar 1984; Abdel-Tawwab et al., 2007a; b).
On the other hand, Nguyen (1999) reported that a low albumin may result from impaired synthesis, loss through urine or feces, or increased catabolism. Serum albumins are synthesized in liver, and changes in serum proteins are inevitable during the pesticide/metal toxicity. Further, Singh and Agarwal (2006) have reported changes in albumin, globulin and A/G ratio after the exposure of pesticides in *Channa striatus*.

Urea is synthesized from $\text{NH}_4^+$ and $\text{HCO}_3^-$ in the liver via the ornithine-urea cycle (OUC). Urea may also formed by the degradation of uric acid or arginine. Elasmobranchs utilize the ornithine-urea cycle whereas teleost synthesize urea by uricolysis or arginolysis. Few teleostean species synthesize significant amounts of urea in response to environmental conditions that limit ammonia excretion (*O.a. grahami*, Randall *et al.*, 1989; *Opasanus beta*, Walch *et al.*, 1990; *Heteropneustes fossilis*, Saha and Ratha, 1989). These species are unique in expressing the full complement of OUC enzymes but in other adult teleosts some of the genes for OUC enzymes appear to be repressed (Wright, 1993). The OUC is used as a ‘safeguard’ mechanism to prevent ammonia toxicity during particularly sensitive stage of neural development (Wright, 1995) Teleost fishes are primarily aminotelic but their blood contains significant amount of urea and indeed in some teleost it may account for 20% or more of total nitrogen excreted (Joshi, 2002). Renal disorders also elevate serum urea release. Creatinine is another nitrogenous waste product that is eliminated by kidneys when excretion is suppressed in renal insufficiency. The high levels of blood urea and creatinine result either from increase breakdown of tissue or dietary or impaired excretion or increased synthesis or decreased urinary clearance by the kidney or decrease degradation of these compounds (Adham *et al.*, 2002) the present suggest that micronutrient exposed fish adapt glomerular dysfunction rather than tubular insufficiency as blood levels of urea and creatinine depends largely on glomerular function. In consistent with this explanation of decreased total protein level with micronutrient exposure urea is the end product of protein catabolism in mammals but in fish ammonia is the end product of protein, so the marked increase in blood urea nitrogen could be attributed to impaired excretion of urea through kidney which is supported by increase in blood creatinine level, a more sensitive and specific indicator of impaired kidney function (Amin and Hashem, 2012).

Thus from the present study it can be concluded that evaluation of haematological parameters along with biochemical changes of fish exposed to toxicants provide valuable information in the assessment of fish health and in monitoring stress responses. The exposure of *O.mossambicus*
and *L. rohita* to sublethal concentrations of Librel caused marked variations in the blood indices of both the fishes an indication that this micronutrient mixture is highly toxic to fish and its use should be controlled so as to prevent its entrance into water bodies to minimize aquatic pollution consequent of agrochemicals. Furthermore, the results of the present investigation reveal that under experimental condition, blood parameters of tilapia and rohu were sensitive to Librel exposure. These findings permit us to conclude that Librel is highly toxic to fish. Hence, the presence of micronutrient in waterways surrounding the agriculture fields could have adverse impact on the survival of the fish. Therefore it is necessary to monitor, the level of micronutrient content in the surrounding aquatic environments.