SUMMARY

Aquatic toxicology is concerned with the adverse effects of various toxicants. The pesticides are mostly used for the control of insects and weeds to improve crop yield in agriculture in the area where this study was carried out. The pesticides are finally find their way into the aquatic environment through water run off. These pesticides have been reported to negative consequence on biota and the environment at large scale. The pesticides have deleterious effects not only on target organisms but also on non target organisms like fishes. The healthy fishes are much affected by these pesticides because they are highly sensitive to the pesticides. Bundelkhand has rich sources of water reservoir as Betwa river, Cane river, Matatila dam and Pahunj river. These reservoirs have become rich sources of fish farming and valuable source of fish food reaching to different areas of our country. The \textit{Clarias batrachus}, \textit{Labeo rohita}, \textit{Heteropneustes fossilis} and \textit{Channa punctatus} species are some fishes mainly used for food. These fishes are continuously challenged by toxicants used in this area. Keeping this in view four toxicants one Glyphosate (herbicide) and three organophosphorus pesticides viz Phosphamidon, Metasystox and Imidacloprid were selected to evaluate data on their toxicity in fresh water fishes \textit{Channa punctatus} of Bundelkhand region. Studies have been shown that when the water quality is affected by toxicants, any physiological changes will be reflected by the haematological and biochemical parameters in fishes. So it is necessary to investigate that how much concentration is required to cause adverse effects of fishes, what blood components are affected by the chemical pesticides and what blood functions are
impaired. Therefore, the aim of this work is the determination of LC<sub>50</sub> of different toxicants (Glyphosate, Phosphamidon, Metasystox and Imidacloprid) and their effects on blood parameters of *Channa punctatus*. The teleost fish *Channa punctatus* was selected in this study because of its wide availability and edibility in India. The literature survey also confers that very little work has been conducted on these toxicants. The toxicological studies in fresh water fishes *Channa punctatus* following exposure of glyphosate and imidacloprid are conducted for the first time in the present thesis.

Chlorine free tap water was used through out the course of the experiment. The physiological characters of water sample like the temperature of the test medium, dissolve oxygen, alkinity, hardness and specific conductivity were tested in the Zonal laboratory U.P. Jal Nigam Babina, Jhansi. The physiological characters of water during all three seasons rainy, summer and winter respectively were pH 7.4, 6.8 and 7.2, dissolve oxygen 7.6, 6.2 and 8.9 mg/liter, alkinity 326, 320 and 308 mg/liter as CaCO<sub>3</sub>, hardness 120, 11.7 and 128.7 mg/liter as Ca, specific conductivity 792, 765 and 782 micro mho.

The fishes (*Channa punctatus*) were collected from different water bodies of Bundelkhand region with the help of professional fisher man. Live and healthy fishes were used in all the toxicological investigations. The selected fishes were checked against injury or infection by keeping 0.2% of potassium permanganate solution for 1-2 minute. They were kept in glass aquaria having a capacity of more than 40 liters. All over the experiment water was changed daily. The fishes were acclimatized in laboratory condition for 6-10 days. During acclimatization the fishes were fed egg albumin, earth worms and
small insects.

Under acute toxicity study LC\textsubscript{50} values after 24h, 48h, 72h and 96h were determined by direct interpolation method, which includes two exploratory and a definitive test. The mortality was recorded after a period of 24, 48, 72 and 96 hrs and dead fishes were removed when observed. In first exploratory test (two concentrations lower and higher) were employed in jar containing five fishes each. Then four and five concentrations were taken to find out narrow range of concentrations in second exploratory test. On the basis of second range finding test 7-9 concentrations were selected for definitive test. The concentrations from the definitive test were employed to determine the LC\textsubscript{50} values by plotting a dose response curve between percent mortality and concentrations of toxicants. A line was drawn between the point represent the % mortality and concentrations. The concentrations at which this line crosses for the 50% lethality line was the actual lethal concentration of toxicant. Therefore the LC\textsubscript{50} values of all the selected toxicants (Glyphosate, Phosphamidon, Metasystox and Imidacloprid) were determined:

- The LC\textsubscript{50} values of Glyphosate in \textit{Channa punctatus} conducted to be as 0.018, 0.015, 0.012 and 0.009 ml/liter at 24, 48, 72 and 96 hours.

- The LC\textsubscript{50} values of Phosphamidon in \textit{Channa punctatus} were 0.023, 0.019, 0.015 and 0.011 ml/liter at 24, 48, 72 and 96 hours.

- The LC\textsubscript{50} values of Metasystox in \textit{Channa punctatus} were found to be as 0.034, 0.030, 0.026 and 0.022 ml/liter at 24, 48,
72 and 96 hours.

The LC$_{50}$ values of Imidacloprid were 0.058, 0.050, 0.042 and 0.034 ml/liter at 24, 48, 72 and 96 hours in *Channa punctatus*.

After calculating the LC$_{50}$ values 40 fishes were collected, acclimatized and divided into 5 groups (A, B, C, D and E) for acute toxicity bioassay. LC$_{50}$ concentrations of selected toxicants were added to the groups A, B, C and D respectively. The fifth group was running without toxicant served as control. The blood was collected after 24, 48, 72 and 96 hours respectively from each group, serum was separated and haematological and biochemical parameters were tested.

For chronic toxicity test 60 fishes were collected from market and washed with 0.2% KMnO$_4$ to avoid any dermal infection and acclimatized for at least 10 days in laboratory condition. The fishes were divided into two groups of 30 each. Group one was exposed to sub lethal concentration (1/10 of 96 hours LC$_{50}$) of toxicants. The second group was kept as untreated control. After 15, 30 and 45 days 10 fishes from each group were sacrificed, blood was collected and serum was separated for the testing of blood parameters. The whole technique of acute and chronic toxicity bioassay were applied for all the toxicants. All the acute and chronic (blood parameters examinations) studies were also conducted in different seasons viz winter, summer and rainy season.

**Glyphosate:-**

In acute toxicity test Hb % and TEC were decreased significantly (P < 0.001). A significant decrease was also observed in
levels of PCV, MCH & MCV at (P < 0.01). The other parameters like TLC and MCHC levels increased significantly at (P < 0.01). ESR was also increased from the control fishes. The level of glucose was increased significantly (P < 0.01) in treated fishes.

In chronic toxicity bioassay the Hb %, TEC, PCV, MCH & MCV decreased significantly (P < 0.01) after 15 days, 30 days and 45 days exposure of Glyphosate. Significant increased in the level of TLC and MCHC was observed (P < 0.01). Although an increased level of ESR was observed when compared with the unexposed fishes but statistically the difference was not significant. Glucose level were elevated significantly (P < 0.01) when the fishes were subjected to glyphosate treatment.

**Phosphamidon:-**

The Hb % TEC were decreased significantly P < 0.001. Although the values of Hb% and TEC were all the times lesser than untreated fishes but at 72 hours the total erythrocytes counts was insignificant. The values of ESR were insignificantly increased, but at 96 hours it was significant (P < 0.01). The data illustrated that the value of PCV, MCH and MCV were significantly decreased (P < 0.01). TLC and MCHC were found to increase at P < 0.01. The glucose level estimated intreated fishes were markedly increased after all exposure periods.

In chronic toxicity bioassay the levels of Hb % TEC, PCV, MCH & MCV were decreased after 15 days, 30 days and 45 days exposure at 1/10 of 96 hours of phosphamidon LC$_{50}$ concentration. It was observed that the levels of Hb%, TEC, MCH, PCV and MCV
were less than control fishes, but gradually increased with rising exposure periods. The blood parametric levels of TLC, ESR and MCHC were increased significantly. The level of blood glucose was increased significantly at P < 0.01. It was lower during 15 days but higher in 45 days exposure period.

**Metasystox:-**

In acute toxicity test the Hb% and TEC were increased significantly after 24, 48, 72 and 96 hours exposure periods at P < 0.01 and P < 0.001. The values of PCV, MCH and MCV were significantly increased at P < 0.01 and P < 0.001. TLC, ESR and MCHC were decreased significantly. The biochemical parameters like glucose level also increased in comparison to control fishes. The levels of glucose increased significantly at P < 0.01 in treated fishes.

During chronic test the levels of Hb%, TEC, PCV, MCH and MCV were significantly increased at P < 0.01 in treated fishes than untreated ones after the end of 15 days, 30 days and 45 days intoxication of metasystox. A significant decreased level of TLC, ESR and MCHC were observed at P < 0.01 and P < 0.001. The blood glucose level was increased significantly in the insecticidal treated fishes.

**Imidacloprid:-**

In case of imidacloprid same results were observed as glyphosate and phosphamidon i.e. Hb% TLC, PCV, MCH and MCV decreased significantly while TLC, ESR, MCHC and glucose were increased after 24, 48, 72 and 96 hours exposures.
In chronic toxicity bioassay the Hb % TEC, PCV & MCH decreased significantly after 15 days, 30 days and 45 days exposure of imidacloprid (1/10 of 96 hours LC$_{50}$ concentration). The level of TEC was decreased but no significant difference was observed after the end of 15 and 30 days of exposure periods. A statistically insignificant increased was observed in erythrocytes sedimentation rate expect 15 days exposure period which was significant at P < 0.001. The level of blood glucose was increased significantly (P < 0.01) in treated fishes.

Fishes lives in very intimate contact with their environment. They are susceptible to physical and chemical changes which may be reflected in their blood components. The changes in water quality can alter the haematological and biochemical parameters. Water temperature, O$_2$, pH, and other factors also causes disturbance in metabolic rate. Hence this study was also performed to obtain data on seasonal changes in blood parameters of fresh water fishes *Channa punctatus*. If only control fishes were taken into consideration, it was found that Hb%, TEC, TLC, PCV, ESR, MCV, MCH, MCHC and blood glucose level were minimum during summer and maximum during rainy season. When compared to untreated control using Glyphosate, Phosphamidon, Metasystox and Imidacloprid. The activity of Hb%, TEC, PCV, MCH and MCV were decreased while TLC, ESR, MCHC and blood glucose were increased. In case of metasystox treated fishes Hb%, TEC, PCV, MCV and MCH were increased but TLC, ESR and MCHC were decreased. Blood glucose level was also significantly increased. It was found that activity levels of all the parameters were decreased during summer season comparatively.
Toxicological study of pesticides (Glyphosate, Phosphamidon, Metasystox and Imidacloprid) on blood parameters of fresh water fish *Channa punctatus*

Determination of LC50 by direct interpolation method

Acute toxicity bioassay using LC50 concentration

Chronic toxicity test using sub lethal concentration (1/10 of 96 hours LC50)

Blood collection and examination of haematological and biochemical parameters

Blood collection and examination of haematological and biochemical parameters

Control after 15 days

Treated after 15 days

Control after 30 days

Treated after 30 days

Control after 45 days

Treated after 45 days

Blood collection and examination of haematological and biochemical parameters

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*All the acute and chronic toxicity experiments were carried out during winter, summer and rainy seasons. Comparisons of different haematological and bio chemical parameters were presented graphically among different season.*