Haematological Profile of Clarias batrachus (Linn.) Exposed to Sub- Lethal Doses of Lead Nitrate
S Mastan, G Indu Priya, E Babu

Abstract
In the present study, the sub - lethal effect of lead on haematological profile of Clarias batrachus were studied. Lead nitrate was used to prepare stock solution from which different standard concentration were prepared. A total of 64 specimens of Clarias batrachus (weight 80-100 gms and 18-20 cm, respectively) were used in the study. They were divided into four groups and each group has 16 fishes. They were then exposed to various concentrations of (10 mg/l, 50 mg/l, and 100 mg/l) of lead nitrate for acute and chronic studies. In exposed fishes various haematological changes were noticed. The RBC counts, haemoglobin percentage and serum protein levels were decreased significantly in comparison to control groups.

INTRODUCTION
The effect of heavy metals on aquatic organism is currently attracting wide spread attention particularly in studies related to industrial pollution. High toxicity of industrial pollutions have been known since long time, but their hazardous nature as pollution of aquatic environment has been matter of concern only after a large number of deaths of fishes occurring in different areas due to different metals.

Fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflect in their blood component (Wilson and Taylor,1993).In fish , exposure to chemical pollutants can induce either increase or decrease in haematological levels. Early diagnosis is also possible when evaluating haematological data, particularly blood parameters (Folmar,1993;Golovina,1996;Luskova,1997).Furthermore, should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Lebedeva et al.,1998;Vosyliene,1999a and 1999a)


MATERIALS AND METHODS
Alive, healthy and disease free fishes (Clarias batarclus weight 80-100gm and length 18-22cm) were collected from local fish market, Bhimavaram and bought to the laboratory. The fishes were kept in the glass aquarium to observe any visible pathological symptoms. Before introducing into the aquarium fishes were treated with 0.1% KMno solution to obviate any dermal infection. Then the fishes were acclimatized in laboratory condition for a period of one week. No mortality was recorded during this period. The fishes were fed chopped meat daily during acclimatization.

Lead nitrate ( Pb (No3 )2 ) were used for the preparation of various concentration (stock solution) by adopting the dilution techniques. Adequate quantity of distilled water was used to get the required concentration. Sub-lethal level of the above metal was determined on the above said species by the prohibit analysis method (Finney, 1971). Sixteen fishes were exposed to sub- lethal concentration for 8, 16 and 24 hours under acute studies. While chronic studies were also conducted for 45 days. Haematological studies were made after 15, 30, 45 days of exposure. Blood was drawn from
posterior caudal vein according to Schnitt et al., (1999). In acute and chronic studies feeding was stopped one day before the experiment and under chronic studies refeeding was done after one day of exposure.

Large size glass aquaria were chosen to avoid space problem to fish. Various water quality parameters such as water temperature, hydrogen ion – concentration (PH), dissolved oxygen, carbon dioxide, total alkalinity, calcium hardness and total hardness were analyzed before sacrifice of fish. Water quality parameters were analyzed by following the procedure of APHA (2000).

Fish behavior was observed and recorded accordingly. Control groups were maintained for all the above experiments, after exposure the fish was sacrificed for haematological examination. Each experiment was done in triplicates.

STATISTICAL ANALYSIS
The data so obtained was analysed by applying analysis of variance (ANOVA) to test the level of significance.

RESULTS
ACUTE STUDIES
In the present study, attempts have been made to investigate the effect of sub-lethal concentrations of lead nitrate on various haematological and biochemical parameters of Clarias batrachus on comparative approach from 8-24 hours.

In (10mg/l) lead nitrate exposed fishes, the number of RBC was observed to be 2.4 ± 0.0816, 2.6 ± 0.0815, 2.9 ± 0.0816 for 8, 16, and 24 hours exposure, respectively. The haemoglobin content was found to be 13.02 ± 0.0085, 11.70 ± 0.0083, 11.02 ± 0.0084 for 8, 16, and 24 hours exposure, respectively. (Fig-1)

In (50 mg/l) lead nitrate exposed fishes, the number of RBC exposed to be 3.00 ± 0.0084, 2.15 ± 0.0081, and 2.85 ± 0.0085 for 8, 16, and 24 hours exposure respectively. The haemoglobin content was found to be 12.60 ± 0.0083, 11.25 ±0.0082 and 11.85 ± 0.0084 exposed fishes, respectively, while in (100mg/l) exposed fishes, the number of RBC was recorded to be 12.30 ± 0.0083, 10.20 ± 0.0084 and 11.25 ± 0.0085 for 8, 16 and 24 hours exposure, respectively. (Fig-22)

In all cases, the differential leukocyte count was deviating significantly from normal values. The increase was observed in the number of lymphocytes and eosinophils while decrease was noticed in the number of monocytes and neutrophils. (Fig 4,5,6).

SERUM PROTEIN PROFILE
The serum protein profile of healthy and exposed fish was carried out. The total serum value is highest in healthy fish i.e. 5.90 ± 0.0081. In (10mg/l) lead nitrate exposed fishes the total the serum protein was recorded to be 5.56 ±0.0085, 5.36 ± 0.0082 and 5.15 ± 0.0081 for 8, 16 and 24 hours exposure, respectively. In (50 mg/l) lead nitrate exposed fishes the total serum protein was recorded to be 4.20 ± 0.0083, 3.80 ± 0.0084 and 3.20 ± 0.0082 for 8, 16 and 24 hours exposure, respectively. (Fig-7,8,9)

While in (100mg/l) lead exposed fishes the total serum protein was recorded to be 4.20 ± 0.0083, 3.80 ± 0.0084 and 3.20 ± 0.0082 for 8, 16 and 24 hours exposure, respectively. (Fig-5)

In all the cases, the total serum protein was decreased as the period of intoxication was increased.

CHRONIC STUDIES
In (10mg/l) lead nitrate exposed fishes, the number of RBC was observed to be 2.1 ± 0.0085, 2.80 ± 0.0083, and 3.4 ± 0.0082 for 15, 30 and 45 days exposure, respectively. The haemoglobin content was found to be 13.2 ± 0.0081, 12.89 ± 0.0084 and 12.20 ± 0.0085 for 15, 30 and 45 days exposure, respectively. (Fig-10)

In all cases, the differential leukocyte count was deviating significantly from normal values. The increase was observed in the number of lymphocytes and eosinophils while decrease was noticed in the number of monocytes and neutrophils. (Fig-11)

SERUM PROTEIN PROFILE
In (10mg/l) lead nitrate exposed fishes the total the serum protein was recorded to be 3.35 ± 0.0081, 2.10 ± 0.0084 and 1.45 ± 0.005 for 15, 30 and 45 days exposure, respectively. The total serum protein was decreased as the period of intoxication was increased. The results of various physicochemical parameters of experimental water are present in graph. (Fig-12).

PHYSICO-CHEMICAL PARAMETERS
The result of Physico-chemical parameters of experiment water are given in the table -1
DISCUSSION

Aquatic environment is constantly polluted from a variety of sources and presently it has assumed a dangerous proportion for aquatic life and fish species are no exception. In reality, heavy metals intoxication cause deleterious effect such as enzyme inactivation, reduction in RBC’s life span and haemoglobin surface area, mitochondrial dysfunction, breakage of genetic material, interference with immunology, alterations in haematological and biochemical organization of different fish species.

Among the heavy metals, lead is one of the metal known to man since medieval times. It is a non-essential element being released into the media either terrestrial or aquatic and is causing several toxicological problems to aquatic animals and man. The natural water is continuously being contaminated by lead due to increase anthropogenic activity and industrial exploitation of metal (Chandravathi and Reddy, 1996). The harmful effects caused by lead include haematological, biochemical and physiological alterations in several aquatic species (Chandravathi and Reddy, 1996).

The uptake and accumulation of lead by aquatic organisms from water and sediment are influenced by various environmental factors such as temperature, salinity, pH, dissolved oxygen, alkalinity, hardness etc. in sediment, only a minor fraction is dissolved in water. Lead is accumulated mostly in gill, liver, kidney and bone, fish accumulates lead from water as well as sediments, aquatic uptake is influenced by presence of cation and oxygen content of water (IPCS, 1989). Heavy metals probably exact their toxic effect on fish by reacting with the mucous on the surface of the gills causing precipitation, coagulation and thus interfere with the normal exchange of gases (Carpenter 1927, 1930). The toxic effects of heavy metals on fish are multidirectional and manifested by numerous changes in the physiological and chemical process of their body system (Dimitrova et al., 1944). Sub-lethal toxicity of lead to fish produces haematological and neurological effects (Hodson et al., 1984). Literature shows the changes in haematological indices of fishes caused by heavy metals and their mixture are different. They are pre determined both by the concentration of heavy metals in the water and time of exposure, and both this factors can cause reversible and irreversible changes in the homeostatic system of fish. Haemoglobin concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible.

In present study it has been observed that, in the exposed fishes the number of RBCs and haemoglobin percentage decreased significantly from normal values. However, the differential leucocyte counts were deviating significantly from normal values. The increase was observed in the number of lymphocytes and eosinophils while decrease was noticed in the number of monocytes and neutrophils. the total serum protein was also decreased as compared to normal value. This is in the agreement with the work of Shan (2006) and Olanike K. Adeyemo et al (2008).

Figure 1
Figure 1: showing RBC count and Haemoglobin percentage of lead nitrate Exposed in (Linn) with (10 mg/l) for 8, 16, 24 Hours

Figure 2
Figure 2: showing RBC count and Haemoglobin percentage of lead nitrate Exposed in (Linn) with (50 mg/l) for 8, 16, 24 Hour
Haematological Profile of Clarias batrachus (Linn.) Exposed to Sub-Lethal Doses of Lead Nitrate

Figure 3
Figure 3: showing RBC count and Haemoglobin percentage of lead nitrate Exposed in (Linn) with (100 mg/l) for 8, 16, 24 Hours

Figure 4
Figure 4: showing Leucocyte differential count of lead nitrate exposed in (Linn) with (10 mg/l) for 8, 16, 24 Hours

Figure 5
Figure 5 showing Leucocyte differential count of lead nitrate Exposed in (Linn) with (100 mg/l) for 8, 16, 24 Hours

Figure 6
Figure 6 showing Leucocyte differential count of lead nitrate Exposed in (Linn) with (100 mg/l) for 8, 16, 24 Hours

Figure 7
Figure 7 showing Albumin, Globulin and Total serum protein of lead nitrate Exposed in (Linn) with (10 mg/l) for 8, 16, 24 Hours

Figure 8
Figure 7 showing Albumin, Globulin and Total serum protein of lead nitrate Exposed in (Linn) with (50 mg/l) for 8, 16, 24 Hours
Haematological Profile of Clarias batrachus (Linn.) Exposed to Sub-Lethal Doses of Lead Nitrate

Figure 9
Figure 8 showing Albumin, Globulin and Total serum protein of lead nitrate Exposed in (Linn) with (100 mg/l) for 8, 16, 24 Hours

Figure 10
Figure 9 showing RBC count and Haemoglobin percentage of lead nitrate exposed in (Linn) with (10 mg/l) for 15,30,45 days.

Figure 11
Figure 10 showing Leucocyte differential count of lead nitrate exposed in (Linn) with (10 mg/l) for 15,30,45 days.

Figure 12
Figure 11 showing Albumin, Globulin and Total serum protein of lead nitrate exposed in (Linn) with (10 mg/l) for 15,30,45 days.

Figure 13

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<th>S.No</th>
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<tr>
<td>1</td>
<td>Water Temperature (°C)</td>
<td>26.30 ± 1.52</td>
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<td>2</td>
<td>Hydrogen Ion – Cone (pH)</td>
<td>7.86 ± 0.40</td>
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<td>3</td>
<td>Dissolved oxygen (mg/dl)</td>
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<td>Total Alkalinity (mg/ml)</td>
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<tr>
<td>7</td>
<td>Total Hardness</td>
<td>288 ± 2.36</td>
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References


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