

Haematological Profile of *Clarias batrachus* (Linn.) Exposed to Sub- Lethal Doses of Lead Nitrate

S Mastan, G Indu Priya, E Babu

Citation

S Mastan, G Indu Priya, E Babu. *Haematological Profile of Clarias batrachus* (Linn.) *Exposed to Sub- Lethal Doses of Lead Nitrate*. The Internet Journal of Hematology. 2008 Volume 6 Number 1.

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)

A survey of literature on heavy metals toxicity clearly shows that heavy metals cause several haematological and biochemical disorders both in laboratory animals as well as on aquatic organisms. The toxicity of lead, copper and other metals has been studied since mid 1920, (Qasim 1923, Dilling et al., 1926, Carpenter 1927, Cardift 1937, Ellis 1937 Laurie et al., 1938 Newton 1944, Jones 1947, Davidson

1949, Fry 1957, Cairns And Scheir 1958, Imanishi et al 1959, Hynes 1960, Lloyd 1961, Zavon 1964 ,and Ray et al., 1964) Sprangue (1970) Van Vuren (1986), Lebedeva et al;(1998), Vosy liene (1999), Olifia et al.,(2002) Andhrabi et al; (2006), Shah,(2006), Adeyemo,(2008). The present paper reports the sub-lethal effect of lead nitrate on haematological profile of *Clarias batrachus* (Linn.).

MATERIALS AND METHODS

Alive, healthy and disease free fishes (*Clarias batrachus* 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% KMnO₄ 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 Schmitt 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 (100 mg/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 5.15 ± 0.0085 , 4.85 ± 0.0083 and 4.30 ± 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

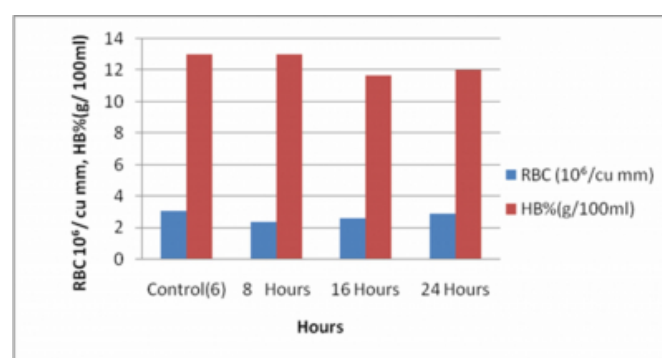


Figure 2

Figure 2: showing RBC count and Haemoglobin percentage of lead nitrate Exposed in (Linn) with (50 mg/l) for 8, 16, 24 Hour

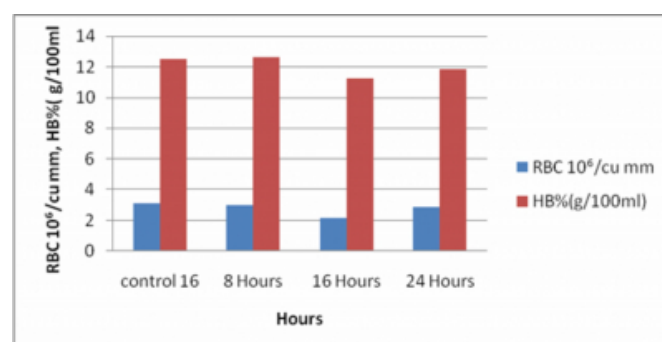


Figure 3

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

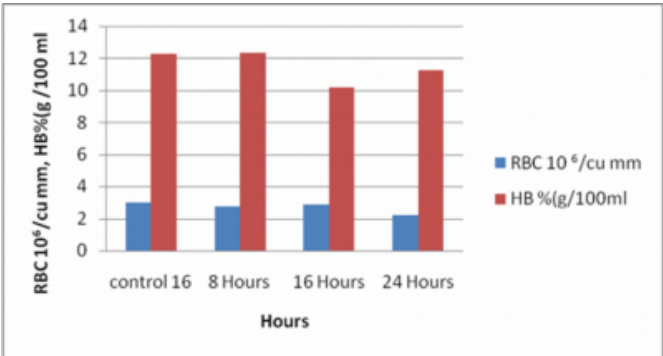


Figure 4

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

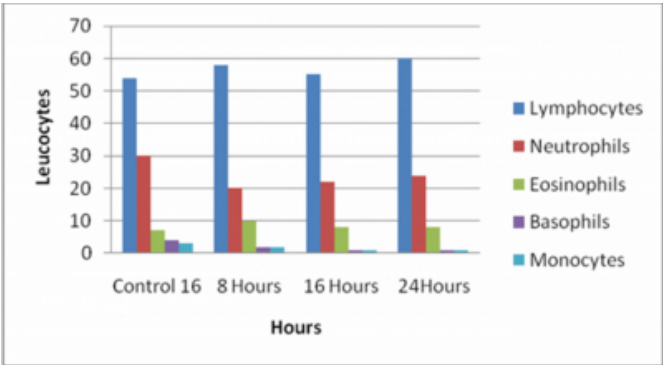


Figure 5

Figure showing Leucocyte differential count of lead nitrate Exposed in (Linn) with (50 mg/l) for 8, 16, 24 Hour

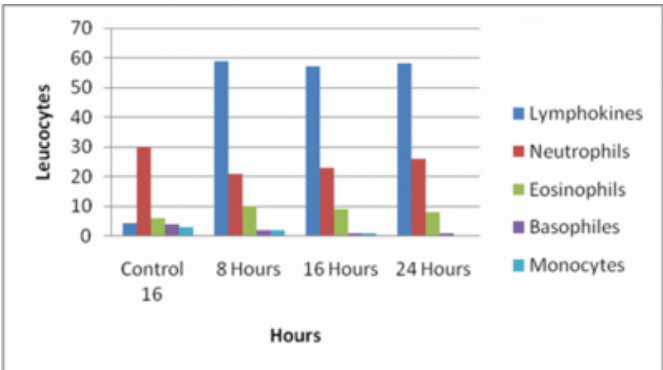


Figure 6

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

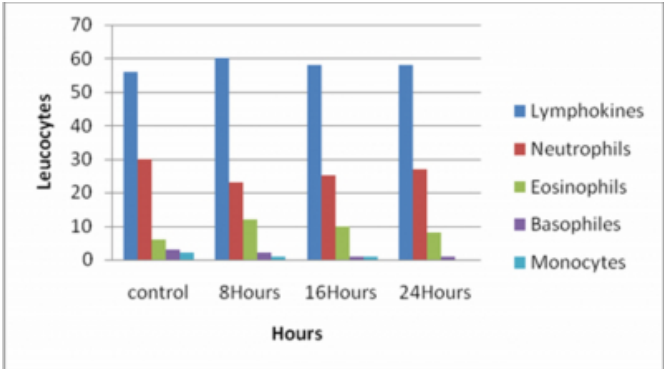


Figure 7

Figure 6 showing Albumin, Globulin and Total serum protein of lead nitrate Exposed in (Linn) with (10 mg/l) for 8, 16, 24 Hours

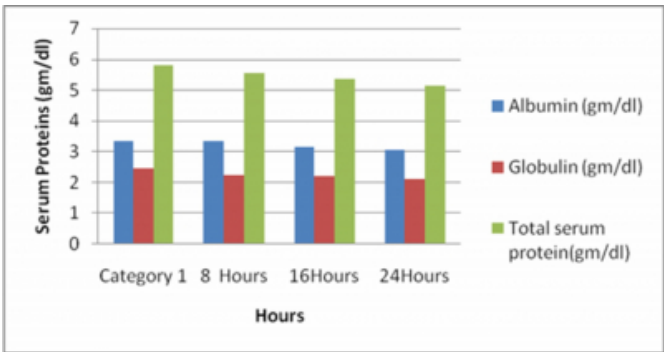


Figure 8

Figure 7 showing Albumin, Globulin and Total serum protein of lead nitrate Exposed in (Linn) with (50 mg/l) for 8, 16, 24 Hours

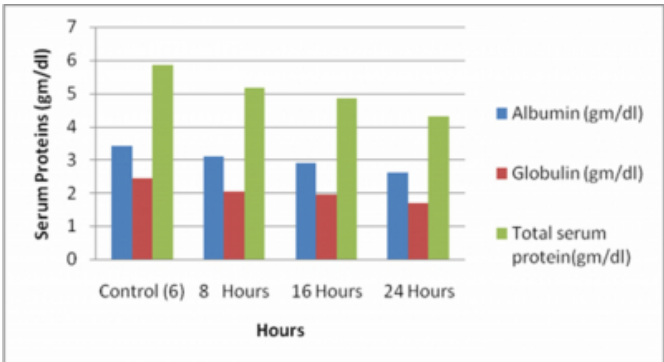


Figure 9

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

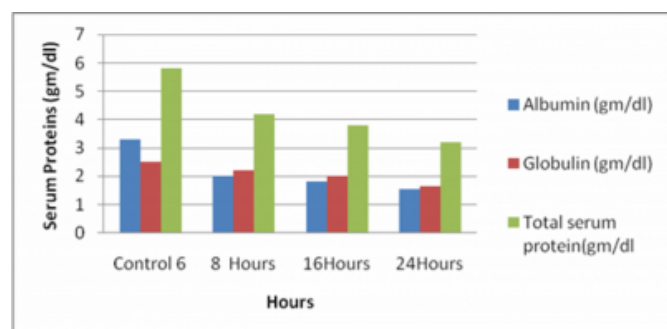


Figure 12

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

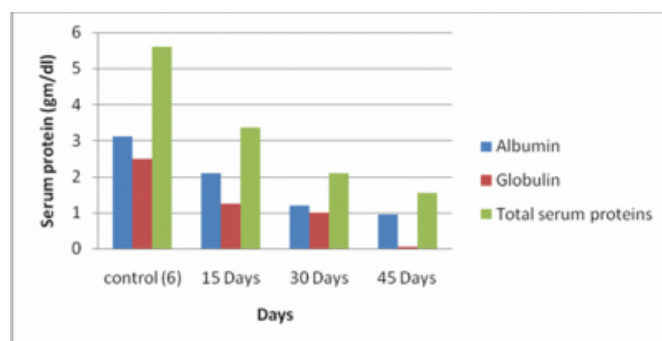


Figure 10

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

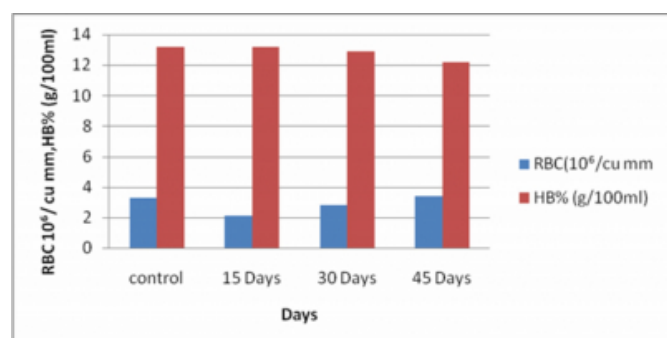
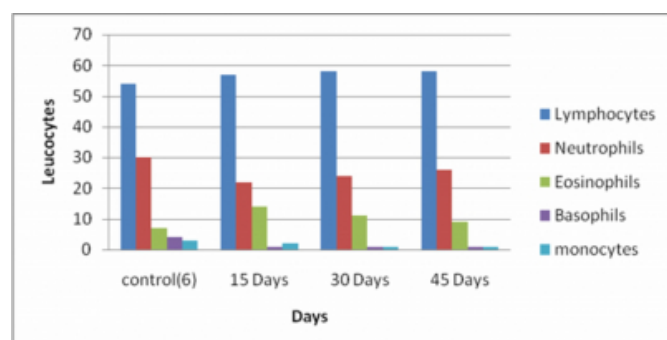


Figure 13

S.No	Parameters	Mean ±S.D
1	Water Temperature (C°)	26.30 ± 1.52
2	Hydrogen Ion – Conc (PH)	7.86 ± 0.40
3	Dissolved oxygen (mg/dl)	6.82 ± 1.20
4	Free Carbon Dioxide (FCO2)	2.86 ± 0.58
5	Total Alkalinity (mg/ml)	10.5 ± 0.86
6	Calcium Hardness	98 ± 0.59
7	Total Hardness	288 ± 2.36

Figure 11

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



References

- r-0. Cairns, J., Jr. and Schcir, A. (1985): Pr. 12 Indus waste cont. Purdue univ. Engin. Ext.Ser. 94,165.
- r-1. Cardiff, I.D. (1937) : Observation with reference to as on food stuffs. Proc. Wesh. Stole. Horticulture Assn. 33: 153- 168.
- r-2. Carpenter, K.E. (1927) : the lethal action of soluble metallic salts on fishes.Brit. J. Expt. Biopl. 4:378.
- r-3. Chandravaty, V. Mary and Reddy, S.L.N. (1996) Lead Nitrate Exposure Changes In Carbohydrates Metabolism Of Fresh Water Fish, J. Environ. Biol. 17 (1), 75- 79.
- r-4. Davidson, J.N (1949) : Nucleoprotein nuclic acidsand derived substances. Ann. Rev. biochem. 18, 155.
- r-5. Dilling, W.J., Henley, c.w. AND Smith, W.L. (1926) : An Appl.Biol. 13: 168.
- r-6. Ellis, M.M. (1937); Detection and measurement of pollution U.S.Bur. Fish. Bull. 48, 365- 437.
- r-7. Fry, E.E.J. (1957) ; The aquatic respiration of Fish. The physiology of fishes (Ed. By M.E.Brown) vol. 6 Academic Press, New York.
- r-8. Hynes, H.B.N.(1960) : The biology of polluted waters in relation to lead toxicity, liver pool, univ, press 262p.
- r-9. Imanishi, N et.al; (1959); studies on the inorganic chemical constituents of sea fishes. Records oceanogr. Works. Japan 3: 135-139.
- r-10. IPCS (1989): Environmental health criteria 85: Lead, Geneva, world health organization, 106 pp.

- r-11. Jones, J.R.E. (1947): The oxygen, consumption of *Gasterostues aculeatus* in toxic solution. *J. Exp.Biol.*23, 298-311.
- r-12. Laurie, R.D. and Jones, J.R.D. (1938): The faunistic recovery of lead polluted river in north cardiganshire wales. *Animal Ecol. (Britain)*, 7: 272- 286.
- r-13. Lloyd, R. and Harbest,D.W.M. (1961) : The effect of environment on the toxicity of poisons to fish *J.Inst. Public. Health. Engg.*61: 132-145.
- r-14. Lebedeva, N.E., Vosyliene, V.Z. and Golovkina, T.V.1998. Haematological– biochemical responses of fish to biogenous and anthropogenic chemical stimuli. *Ichthyohaematology. Proceedings of the 4th Ichthyohaematology conference, Hluboka/ Vlt.,Czech:* 85-87.
- r-15. Newton.L. (1944): Pollution of the rivers of waste water by lead and zinc mine effluents. *Ann. Appl. Biol. Brit.* 31: 1-11.
- r-16. Olaife. F.E. Olaifa A.K. and Onwude (2002): Lethal and sub lethal effect of copper to African Cat fish (*Clarias gariepinus*). Dept. of wild life and fisheries management, university of Ibadan (2002) Report – 23- 30.
- r-17. Qasim, S.Z. and Meria, R. Meneger (1923) : determination of acute toxicitity levels of mercury to the fish *Tilapia mossambica*. *Proc. India Acad. Sci.(Anim. Sci.)* vol92. No. 5: 234-237.
- r-18. Ray, P. et. al; (1964) : A case of fish mortality caused by precepitation of ferric iron in the river Daha at Siwan (N.Bihar). *Ind. J. Fish* 9(1) : 117- 122.
- r-19. Rogers, J.T., Richards, j.g. and Wood. C.M. 2003. Ionoregulatory disruption as the acute toxic mechanism for lead in the rainbow trout. *Aquatic toxicol.*, 64(2) : 215-34
- r-20. sShah, S.L.2006 haematological perametars iu tench, *Tinica tinica* after short term
- r-21. exposure to lead. *Journal of applied toxicology*, 26(3) : 223-228.
- r-22. Sprangue J.B. 1971. Management of pollutants toxicity fish. III. Sub- lethal effect and safe concentrations. *Water res.*,5: 245-266.
- r-23. Van Vuren , J.H.J. 1986. The effect of toxicants on the haematology of *Lebio umbratus* (Teleostei; cyprinidae). *Comparative Biochemical and physiological* 83C: 155-159.
- r-24. Vosyliene, M.Z. 1999a. The effect of heavy metal mixture on Haematological parameters of rainbow trout. In D.A. Lovejoy (Ed), *Heavy metals in environment. An integrated approach.*, Institute of Geology. Metalecology Society. Vilnius: 295- 298.
- r-25. Vosyliene, M.Z. 1999b. The effect of heavsy metal on Haematological indices of fish. *Acta Zoologica Lituanica*, 9(2): 76-82.
- r-26. Zavon, Mitchell, L.R. (1964); *Coll. Med. Univ. Cincinnati Ohio, U.S.A. problems in symposium on lead, Cininnati 1963- Arch Environ. Hlth.* (812): 262- 275.

Author Information

S.A. Mastan, Ph.D.

Post - Graduate Department of Biotechnology, D.N.R College, P.G. Courses and Research Centre

G. Indu Priya, M.Sc,(M.Phil)

Post - Graduate Department of Biotechnology, D.N.R College, P.G. Courses and Research Centre

Eswar Ganesh Babu, M.Sc, M.Phil

Post - Graduate Department of Biotechnology, D.N.R College, P.G. Courses and Research Centre