



Effect of heavy metals cadmium, lead and copper on the blood characteristics of fresh water catfish *Clarias batrachus* (Linn.)

Muneesh Kumar*¹, Dharvinder Kumar², Rajesh Kumar³

¹Department of Zoology, Govt. Degree College Doda, University of Jammu, J&K, India.

²Department of Zoology, G.G.M. Science College, Jammu, University of Jammu, J&K, India

³Department of Zoology, Govt. Banazir Science and Commerce College, Bhopal, Barkatullah University, Bhopal, (M.P.), India.

Corresponding Author: **Dr. Muneesh Kumar**, Department of Zoology, Govt. Degree College Doda, University of Jammu, J&K, India. E-Mail: drmuneesh.ju@gmail.com.

Abstract

Heavy metals are a major problem because they are toxic and tend to accumulate in living organisms. This study was carried out with the aims of studying the effects of sublethal concentrations of CdCl₂, PbNO₃ and CuSO₄ as heavy metals on some haematological parameters of *Clarias batrachus* were exposed to three sublethal concentrations. Sublethal concentrations used were 7.5 ppm for Cd, 3.6 ppm for Pb and 0.5 ppm for Cu. The results obtained revealed that there was significant difference in hematocrit. Exposure of *Clarias batrachus* to sublethal concentration of Cd, Pb resulted in significant decrease ($P < 0.05$) in erythrocyte count and haemoglobin content, while there was a slight increase in case of copper. The microscopical observations showed that exposure of *Clarias batrachus* to sublethal concentration of heavy metals 7.5 ppm for Cd, 3.6 ppm for Pb and 0.5 ppm for Cu resulted in some changes in the morphology of the RBC's which tended to lose its ellipsoidal shape acquiring different shapes (poikilocytosis or anisocytosis).

Keywords: Heavy metals, Hematological parameters, *Clarias batrachus*.

Introduction

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vindodhini and Narayanan, 2008)¹. Increased human activities especially with rapid development of agriculture and industry has resulted in a considerable increase in levels of pollutant such as heavy metals which is the main anthropogenic pollution causing serious and long lasting damage to all living organisms (Sastry and Sukla, 1993; Murugan *et al.*, 2008)^{2,3}. Blood alterations in fish or damage of hemopoietic tissue organs may be associated either with changes in environmental conditions or with water born pollutants (McCay, 1929, Dawson, 1935 &

Gradner and Yevich 1969b). Most of the previous studies on the effect of intoxication with pollutants on blood characteristics were based on differential counts of W.B.C. & R.B.C., as well as the relative concentration of haemoglobin content and hematocrit values (Khadre, 1990, Amin, 1992, Houston *et al* 1993, Zia & McDonald 1994, Adak, 1995 & Abdel Magid, 1997). In present study morphological characteristics of red blood cells were studied beside the other haematological parameters on *Clarias batrachus* exposed to sublethal concentrations of some heavy metals (Cu, Pb & Cd).

Materials and Methods

Adult and live fish *C. batrachus* were collected from the fish farm Patra and Bhabhada Bhopal (M.P.) and were brought to the laboratory, cleaned by using 0.1% KMnO_4 to avoid dermal infection. Only healthy fishes (Length: 12-15cm, Weight: 50-60g) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h. They were put in glass aquaria (60 liters capacity) for about two weeks for acclimatization. Then the tested media were prepared. Experimental fish after this acclimatization were transferred one hour after preparation of the media, into the experimental aquaria. Experimental glass aquaria were cleaned carefully and washed with water and acetone then with running tap water several times and finally.

Pollutants:

1000 ppm stock solutions of the three metals were prepared. Cadmium as cadmium chloride ($\text{Cd Cl}_2 \cdot \text{H}_2\text{O}$), lead as lead nitrate ($\text{Pb}(\text{NO}_3)_2$) and copper as copper sulphate ($\text{Cu SO}_4 \cdot 5\text{H}_2\text{O}$). Standard solutions were stored in clean bottles and diluted to desired concentration just before the beginning of the experiment. Sublethal concentrations used were 7.5 ppm for cadmium, 3.6 ppm for Pb & 0.5 ppm for Cu (Abdel Barr 1997).

R.B.Cs count:

R.B.Cs counts were done according to the method of Hesse (1960) & Wintrob (1967) using improved Neubauer haemocytometer. Haemoglobin content was determined according to the calorimetric method of Sahli (Hesser 1960).

Results and Discussion

Normal red blood cells:

The erythrocytes of normal fish are ellipsoidal in shape (the short axis nearly two thirds of the long axis). They are nucleated with a centrally located nucleus, which is ellipsoidal as well. The measurement of the cells varies between $11 \times 5 \mu\text{m}$ and $7 \times 6 \mu\text{m}$ with a mean of $8.6 \pm 1.6 \times 4.7 \pm 0.7 \mu\text{m}$ for long and short axis. The nuclei are dark purple in color and measure between $4 \times 3.6 \mu\text{m}$ and $5 \times 2 \mu\text{m}$ with a mean of $4.2 \pm 0.5 \mu\text{m} \times 3.7 \pm 0.5 \mu\text{m}$ or the long and short axis respectively. R.B.Cs count in normal fish

ranged from 420000 to 720000 cell / mm^3 with a mean value 445000 ± 250000 cell/ mm^3).

Observations:

Hemoglobin content was found to vary between 1.38 & 1.54/ 100 ml blood for normal fish of *Clarias batrachus*.

(1) Effect of sublethal concentration of Cadmium (7.5 ppm):

In the blood film of fish exposed to sublethal concentration of Cd (7.5 ppm) for 4 days it appears that most of the red blood cells lose their normal oval shape. Acanthocytes and crenated cells were apparent beside lysis of some red blood cells. Sticking of the red blood cells and fragmentation of red blood cells were also observed. Upon further exposure (8 days) the phenomena of sticking of the cells is further apparent. Acanthocytes as well as Crenated cells become common. Tear drop cells and sickle cells were apparent. After 12 days of exposure sticking of the cells become more apparent. The stacked cells lose their original shape with appearance of sickle shaped cells. The nuclei of the red blood cells show some variation in their shape. By 16 days of exposure cells acquired different shapes with obvious disintegration of cell wall of some cells and acanthocytes were frequent.

(2) Effect of sublethal concentration of Lead (3.6 ppm):

Lysis of red blood cells is quite clear upon examination of the blood films after 4 days of exposure to 3.6 ppm Pb (cell wall of erythrocyte disintegrated and the nuclear material were clumped). On the other hand diameter of the red blood cells showed a non significant decrease ($7.9 \pm 1.3 \mu\text{m}$ in treated fish and $8.9 \pm 1.5 \mu\text{m}$ in control, $t = -2.0$ & $P < 0.5$). After 8 days of exposure fragmentation of some red blood cells was clear with appearance of tear drop like cells and acanthocytes. After 12 and 16 days of exposure the red blood cells lose their normal oval shape so that they become nearly rounded. Crenated cells acanthocytes and tear drop like cells were frequent. Beside lysis of some red cells, some cells appear to be stacked together.

(3) Effect of exposure to sublethal concentration of copper (0.5 ppm):

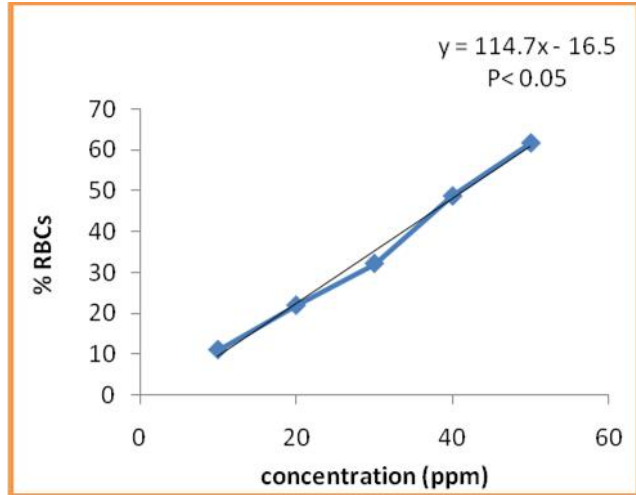
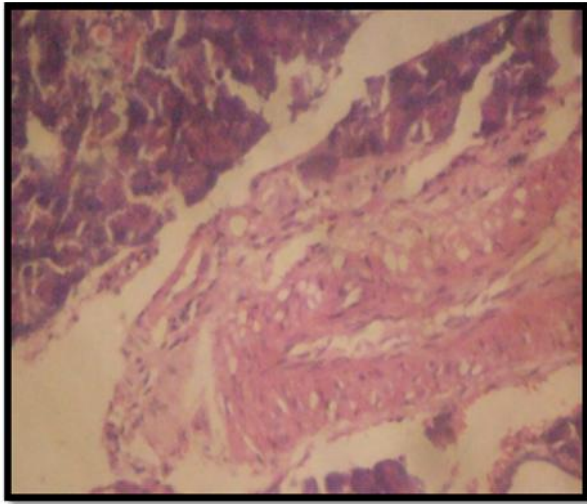
The first observed change in the red blood cell morphology after treatment with Cu was the rouleaux appearance that appeared in the first 4 days of treatment. Also cells lose their normal shape acquiring different shapes or become rounded. After 8 days of exposure the red blood cells started to be stacked with each other with nuclei losing some amount of central dye showing a sign of karyolysis and significantly increase in size ($t = 3.5$ & $P < 0.5$). Sickle cells were apparent and most of erythrocyte cells lose their normal shape. Upon 12 days of exposure most of the

red blood cells lose their normal oval shape and become polygonal or irregular in shape. Sickle cells were apparent. Also spherocyte and tear drop like cells were observed.

R.B.Cs count

(1) Cadmium:

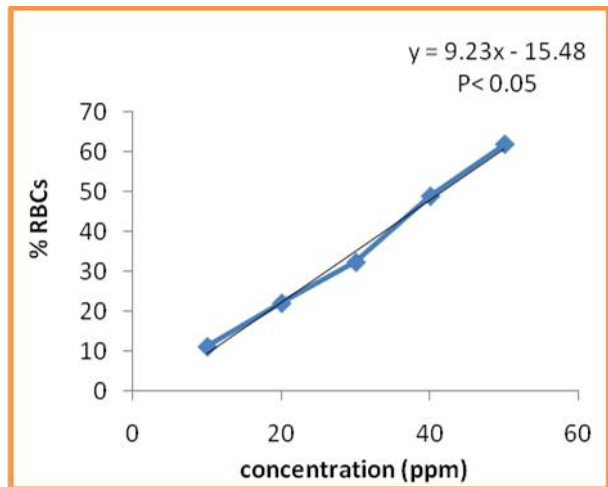
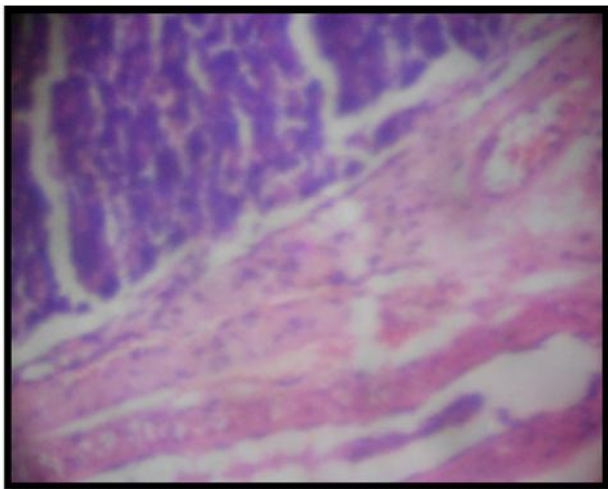
Significant decrease in RBCs count was noticed ($t = -3.9$ (4 d), -14 (8 d) & -3.6 (12 d) $P < 0.05$). A significant decrease in haemoglobin content of fish also occurred ($t = -14.7$ (4 d), -23.1 (8 d) and -16.5 (12 d) $P < 0.05$). (Fig. 1).



(2) Lead:

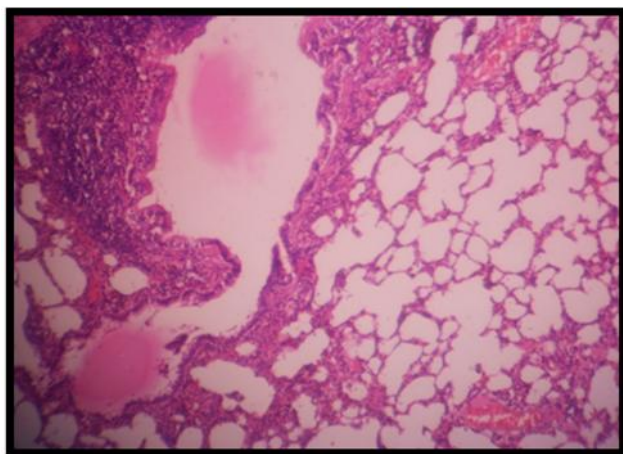
R.B.Cs count significantly decreased than that of control fish after 4, 8 and 12 days of exposure to sublethal concentration of lead (3.6 ppm) ($Pb = -5.7$ (4

d), -24.5 (8 d) & -13.4 (12 d) $P < 0.05$). A significant decrease in haemoglobin content as compared to control fish also occurred ($t = -9.23$ (4 d), -10.55 (8 d) and -15.48 (12 d) $p < 0.05$). (Fig. 2).

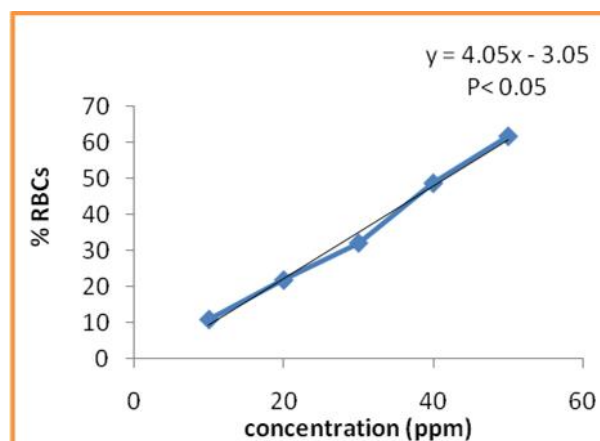


(3) Copper:

A slight increase in RBCs of the fish that exposed to 0.5 ppm Cu after 4, 8 & 12 day was observed ($t = -4.05$ (4 d), -4.3 (8 d) & -3.05 (12 d) $P < 0.05$).



Significant increase in haemoglobin content was also noticed ($t = 8.3$ $P < 0.05$) after 4 days of exposure. However this rise did not continue, either after 8 days or 12 days. (Fig. 3).

**Discussion**

The effect of pollution on the blood picture of fishes made the subject of study of various workers (Osman et al 1993, Zia & McDonald 1994 & Adak, 1995). Comparison of hematological characteristics of *S. rivulatus*, with those of other fishes are given according to the present study exposure of *Siganus rivulatus* to sublethal concentrations of heavy metals Cd (7.5 ppm) Pb (3.6 ppm), and Cu (0.5 ppm) resulted in some changes in the morphology of the R.B.Cs which tended to lose its ellipsoidal shape acquiring different shapes. Among the abnormal cells were the tear drop like cells, acanthocytes, crenated cells and sickle cells. Another effects of metals observed in the present study, are the sticking of the cells together, decrease (in case of Pb) or increase (in case of Cu) in size and appearance of spherocytes. In case of lead and cadmium cell wall of the erythrocytes may disintegrate leaving the nuclei free in the blood film, as well as cell fragments. Few studies are concerned with study of the morphological changes in red blood cells of fishes upon exposure to heavy metals or pollutants as a whole according to available literature. Smith (1968) recorded poikilocytosis and anisocytosis of red blood cells of coho salmon *Onchorhynchus kistuch* fed on folic acid deficient diet. He claimed that these results are characteristic feature of megaloblastic, normoblastic and macroblastic anaemia. Sarivastava and Mishra (1978) found that exposure of *Colisa fasciatus* to sublethal concentration of lead resulted in lysis of the erythrocytes. Shabana

(1983) found by microscopical examination of R.B.Cs of poisoned *Clarias lazera* with lead that cells were deteriorated, as clumping of chromatin material and lysis of their cellular and nuclear membrane were noticed. Ahmed and Munshi (1992) found that exposure of Indian carp *Catla catla* to copper resulted in shrinkage and crenation of the configuration of the red blood cells with slight anisocytosis and with a tendency to overlap. Poikilocytosis of red blood cells resulted in association with various types of anaemia e.g. megaloblastic anaemia, iron deficiency anaemia, thalassemia, myelosclerosis or from damage to circulating red blood cells as in microangiopathic hemolytic anaemia (Dacie & Lewis, 1975). This suggests that fish exposed to heavy metals suffer from anaemia.

Acanthocytosis is seen in the liver diseases (Copper, 1980) or in association with abnormal phospholipids metabolism (Dacie and Lewis, 1975). This suggests that the fishes exposed to heavy metals at sublethal doses suffer from liver disease. Tear drop cells may result from the Pitting action of the spleen which removes autophagocytic vacuoles or inclusions. They are also seen in beta thalassemia, Heinz body anaemia, megaloblastic processes and disorders or leukemia and are caused by the removal of precipitated haemoglobin inclusions by the spleen (Bike, 1993). Sickle cells are present in homozygous Hb-S disease in films of freshly withdrawn blood and subjected to anoxia (Dacie and Lewis, 1975).

Exposure of *Siganus rivulatus* to sublethal concentrations of Pb and Cd resulted in significant decrease ($p < 0.05$) in erythrocyte count and haemoglobin content. This case was parallel to the observation of Smith *et al.* (1971) on chinook salmon *Onchorhynchus tshawytscha* subsequent to treatment with phenylehydrazin; in coho salmon *Onchorhynchus kistuch* following exposure to residual chlorine (Buckly *et al.*, 1976) and generally in teleosts exposed to pulp mill effluent (Warner, 1967 and McLeay, 1975) and to cadmium (Larsson *et al.*, 1976). The same observation was also recorded on *Colisa fasciatus* following exposure to lead (Sarivastava and Mishra, 1979), in *Clarias lazera* after acute exposure to lead (Shabana, 1983) on *Anguilla vulgaris* after exposure to mercury (Hilmy *et al.*, 1987), on *Clarias lazera* exposed to hexavalent chromium (Hilmy *et al.*, 1988) on *Oreochromis niloticus* exposed to cadmium (AL-Akel *et al.*, 1988), on *Crassius carassius* exposed to lead (Fantin *et al.*, 1989), on *Clarius lazera* exposed to mercury and gallant (Abd-Allah *et al.*, 1991) on European silver eel exposed to lead (Amin, 1992) and on *Oreochromis mossambicus* exposed to arsenic and zinc (Adak, 1995).

Abdel-Maguid (1997) found that starvation resulted in decrease of both R.B.Cs count and haemoglobin content of *Gobius niger*. On the other hand upon exposure of *Siganus rivulatus* to sublethal concentration of copper significant increase in erythrocyte count and haemoglobin content was observed. Ghazaly and Said (1995) found similar results in *Tilapia nilotica* after exposure to sublethal concentration of copper. Some other metals were found to induce the same effect of copper on fish blood. Thus Frovola (1960) recorded an increase in erythrocyte count and haemoglobin content in carp *Cyprinus carpio* after exposure to cobalt. Also the same results were found in rainbow trout *Salmo gairdneri* (Schiffman and Forman, 1959) and in *Colisa fasciatus* (Srivastava *et al.*, 1979) following exposure to chromium.

Conclusion

Exposure of *C. batrachus* to sublethal concentrations of Zn and Cd resulted in significant decrease ($p < 0.05$) in erythrocyte count and hemoglobin content, while there was slight increase in case of copper. The microscopical observations showed that exposure of *C. batrachus* to sublethal concentrations of heavy metals Cd (7.5 ppm), Pb (3.6 ppm) and Cu (0.5 ppm) resulted in some changes in the morphology of the R.B.Cs which tended to lose its ellipsoidal shape

acquiring different shapes (poikilocytosis or anisocytosis). Among the abnormal cells were the tear drop like cells, acanthocytes, crenated cells and sickle cells. Another effects of metals observed in the present study, are the sticking of the cells together, decrease (in case of Pb) or increase (in case of Cu) in size and appearance of spherocytes. In case of copper and cadmium cell wall of the erythrocytes may disintegrate leaving the nuclei free in the blood film, and appearance of cell fragments.

References

- Abd-Allah, G.A., Ibrahim, M.S., Bahnasawy, M.H. and T.F. Abdel-Baky (1991).** Toxic effects of some water pollutants (Gallant and mercury) on blood parameters of cat fish *Clarias Lazera*. J.Egypt.Ger.Soc.Cool.6 (A), 201-209.
- Abdel-Barr M., (1997).** Toxicological studies of heavy metals on *Signus rivulatus*. M. Sc. thesis, Fac. Sci. Alex. Univ. (188 pp).
- Abdel-Maguid, S.A. (1997).** Biological and haematological studies on marine coastal fish, family, Gobiidae in Alexandria, Egypt. Ph.D. Thesis, Fac. Sci. Alex. Univ. (206 pp).
- Adak. S.K. (1995).** Hematological studies on fresh water telost, *Oreochrmis (Tilapia) mosambicus* (Peters) in the normal conditions and under the stress of zinc and Arsinc. Ph.D. Thesis, zoology, Sambalpur Univ.
- Ahmed, I.M. and S.Munshi (1992).** Scanning electron microscopic evaluation of changes on the morphology of blood cells of an Indian major carp *Catla catla* (Halm). Following exposure to copper. J. Environ. Biol. 13 (14): 297-301.
- Al-Akel, A.S., Shami, M.J. Al-Kalem, H.F., Ghaudhary, M.A. aand Z.Ahmed (1988).** Effects of cadmium on the cichlid fish *Oreochrmis nilotictus* behavioural and physiological responses. J. Univ. Kuwait, Sci., 5(2): 341-345.
- Amin, E.M. (1992).** Haematological changes and light microscopic study of peripheral blood cells in European eel exposed to lead concentrations. Rapp. Comm. nt. Mer. Medit. 33:165.
- Bike, R.L. (1993).** Haematology: Clinical and Laboratory practice 1:12 Morphology of erythron, St. Louis, Missouri.
- Buckley, J.A., Whitenore, C.M. and R.I.Matsuda (1967).** Changes in blood chemistry and blood cell morphology in Coho-Salmon *Oncorhynchus Kistuch* following exposure to sublethal levels of total residual chlorine in municipal waste water. J. Fish. Res. Can. 33: 776-782.

- Cooper, R.A. (1980).** Red cell membrane abnormalities in liver disease. *Semin, Hematol.* 17:103.
- Dacie, J.V. and S.M. Lweis (1975).** Practical hematology. 5th ed. Churchill Livingstone.
- Dawson, A.B. (1953).** The hemopoietic response in the cat fish *Ameiurus nebulosus*, to chronic lead poisoning. *Biol. Bull.* 68: 335-346.
- Fantin, A.M.B., Tervisan, P., Pederzoli, A. and M.Bergoni (1989).** Effect of experimental pollution by lead on some haematological parameters in *Carassius carassius* var *anratus*. *Bollctino Dizooologia* 55(4): 251-256.
- Frovol, L.K. (1960).** The effect of inorganic cobalt on the morphological picture of *Cyprinus carpio*. *Dolel. Akad, Nauk. SSSR.* 131: 933-984.
- Gardner, G.R. and P.P, Yevich (1969b).** Toxicological effects of cadmium on *Fundulud heteroclitus* under various oxygen, Ph., Salinity and temperature regimes. *Amer. Zool. g:* 1096 (Abstr.).
- Ghazaly, K.S. and K.M. Said (1995).** Physiological characteristics of *Tilapia nilotica* under stress of copper. *J. Egypt. Ger. Soc. Zool.*
- Hesser, E.F. (1960).** Methods of routine fish haematology. *Progressive fish culturist*, 22(4): 164-171.
- Hilmy, A.M., Shabaua, M.B., El-Domiati, N.A. and L.E. Yaacout (1987).** Mercury levels and haematological correlation in *Anguilla Vulgaris*. *Bull. Fac. Sci. Alex. Univ.,* 27(4), A: 125-140.
- Hilmy, A.M., El-Domiati, M.A., Moussa, F.I. and A.A El-Masry (1988).** Haematological and pheyical changes induced by Hexavalent chromium in *Clarias Lazera*. *Bull. Inst. Oceangr. & Fish. A.R.E* 14(3): 101-110.
- Houston, H., Korm, M.D., Cohen, Y., and M. Bernhard (1993).** Trace metal content in deep water sharks in eastern Mediterranean Sea Marin. *Biol.* 115: 31-338.
- Khardre, S.M. (1990).** Changes in the gill structure and blood profiles following acute copper toxicity in two fresh water teleosts. *Proc. int. On Biology and culture of Tilapias*, 27-31 October, 1990. Alex., Egypt.
- Larrson, A., Bengtsson, B.E. and A. Svanbery (1976).** Some haematological and biochemical effects of cadmium on fish. *From the effect of pollutants on aquatic organisms.* A.P.M. Lockwood. Cambridge University Press.
- McCay, C.M. (1929).** Studies upon fish blood and its relation to water pollution. A Biological survey for the Erie-Niagra system. New York conservation Dep., N.Y., 140-149.
- McLeay, D.J. (1975).** Sensitivity of blood cell count in juvenile coho salmon *Onchorhynchus Kistuch*. *J. Fish. Res. Bd. Can.,* 32: 2357-2364.
- Osman, M.A., Ghaly, Y.S., Afify, A.M.R., El-Dosoky, G and M.E.F.Mahmoud, (1993).** Lend pollution of water resources and its biochemical effects. *Proc. 3rd. Int. Conf. on Environ. Prot. is a must,* 75-87.
- Sarivastava, A.K. and S.Mishra (1979).** Blood dyscarsia in teleost, *Colisa fasciatus* after acute exposure to sublethal concentration of lead. *J. Fish Biol.,* 14: 199-203.
- Schiffman, R.H. and P.O. Forman (1959).** Chromium induced changes in the blood of rainbow trout *Salmo gairdneri*. *Sewage and industrial wastes* 31: 205-211.
- Shabana, M.B. (1963).** Induced pathological and Biological stress of acute lead poisoning in Egyptian cat fish *Clarieas lazera*. *Bull. Fac. Sci. Alex. Univ.,* 23: 1-141.
- Smith, C., (1968).** Haematological changes in cho salmon fed on folic acid deficient diet. *J. Fish Res. Bd. Can.,* 25(1): 151-156.
- Srivastave, A.K., Agrawal, S.J. and H.S. Chaudhry, (1979).** Effects of chromium on blood of fresh water fish. *Ecotoxicol. Environ. saf.,* 3: 321-324.
- Vinodhini, R. and Narayanan, M., (2008).** Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinius carpio*. *Int. J. Environ. Sci. Tech.,* 2008, 5(2):179-182.
- Warner, R.E., (1967).** Bioassay for micro chemical environments. *Bull. W.H.O.,* s6: 181- 207.
- Wintro, M.M, (1967).** Clinical haematology. London, Henry Kimpton. 448pp.

Access this Article in Online	
	Website: www.ijarbs.com
	Subject: Aquatic Biology
Quick Response Code	
DOI:10.22192/ijarbs.2017.04.01.014	

How to cite this article: Muneesh Kumar, Dharvinder Kumar, Rajesh Kumar. (2017). Effect of heavy metals cadmium, lead and copper on the blood characteristics of fresh water catfish *Clarias batrachus* (Linn.). *Int. J. Adv. Res. Biol. Sci.* 4(1): 129-134. DOI:<http://dx.doi.org/10.22192/ijarbs.2017.04.01.014>