Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 6, Issue, 02, pp. 6963-6967, February, 2016

Full Length Research Article

EFFECTS OF NICKEL ON HAEMATOLOGICAL PARAMETERS IN THE ESTUARINE FISH *LIZA PARSIA*

[1]Rani Lalitha, P., [2]Sukumaran, M. and [3,*]Muthukumaravel, K.

[1,2]P. G. and Research Department of Zoology, Rajah Serfoji Government College, Thanjavur – 614 005, Tamil Nadu, India
[3]P. G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701,Tamil Nadu, India

ARTICLE INFO

Article History:
Received 20th November, 2015
Received in revised form
16th December, 2015
Accepted 24th January, 2016
Published online 29th February, 2016

Key Words:
Liza parsia,
Haematology,
Nickel.

ABSTRACT

Effect of 10 and 30 % sublethal concentration (12.4 ppm; 96 h LC_{50}) of nickel on the haematology of Liza parsia after the exposure period of 30 days was studied. The RBC count (-35.82%) and Hb content (-63.28%) decreased significantly with an increase in WBC count (+28.95%). The light microscopic studies revealed many morphological changes in the RBC of fish such as wrinkling of cell membrane, vacuolization and rupture of erythrocytic membrane.

INTRODUCTION

Heavy metals are usually non-biodegradable. Intrusion of heavy metals and their salts in aquatic environment and their toxicity to aquatic system were studied by various investigators. They were reported to cause massive fish kills and other aquatic life (Sastry *et al*., 1972 and Jhingran, 1991). Heavy metal nickel is employed in the industries of pulp, paper mills, paperboard, building paper and board mills, fertilizer, petroleum refining, basic steel works foundries and electroplating industries (Dean *et al*., 1972 and Klein *et al*., 1974). Haematological tests are important diagnostic tools and recent findings have suggested that they may be equally valuable as indicators of disease or stress due to pollutants and environmental fluctuations in fishes (Bhatkar and Dhande 2000). The blood plays an integrated and inevitable part in all immune systems. Haematological parameters are related to responses of the organisms to the changing environmental conditions and therefore can be used to screen the healthy state of fish experimented to the exposed toxicant. Further, haematological observations have greater contribution to the pathological changes obtained during toxicological studies.

***Corresponding author: Muthukumaravel, K.,**
P. G. and Research Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701,Tamil Nadu, India.

Studies on the effect of different toxicants in the haematology of fishes have been made by many workers (Dalela *et al.,* 1981; Sastry and Sharma, 1981; Dutta *et al.,* 1992; Shekar and Christy 1996; Ramaswamy *et al.,* 1996; Anandkumar *et al.,*, 2001; Joshi *et al.,* 2002; Bhatia *et al.,* 2002; Johal and Grewal, 2004 and Gauram and Suneelkumar, 2008). Haematology may be utilized as a means of monitoring the environmental stress in aquatic animals. In view this physiological importance, *Liza parsia* was subjected to toxicological studies upon exposure to different sublethal concentrations of heavy metal nickel.

MATERIALS AND METHODS

Freshwater fish, *Liza parsia* fingerlings (Wt.8 ± 0.5 g; Length 7 ± 1cm) were collected from the Muthupet estuary, Tamil Nadu and acclimated to laboratory conditions for a period of 15 days in large glass aquaria, previously washed with 1% potassium permanganate to free the walls from microbial infection, if any. The fish were maintained in de-chlorinated tap water of the quality used in the test and renewed three times a week, whose physico-chemical characteristics were analyzed following the methods mentioned in (APHA 1976) and found as follows: Temperature: 24 ± 2°C, pH: 8.9. ± 0.3 and Dissolved oxygen: 6.1 ± 0.7 mg/l. The fish were well reared and fed with rice bran and groundnut oil cake to keep the test animals in a normal metabolic state. An acute toxicity

(LC_{50}) test by the static renewal bioassay method was conducted to determine the toxicity of nickel in *Liza parsia* which were exposed to various concentrations of nickel for 96 h. After 96 h of exposure the data obtained was subjected to Finney's probit analysis method (Finney, 1971) to determine LC50 value. The concentration at which 50% survival/mortality occurred in glyphosate treated fishes was taken as the median lethal concentration (LC_{50}) for 96 h, which was 12.4 ppm. One tenth (1/10) and one thirtieth (1/30) of the LC_{50} value (1.24 & 3.72 ppm) was taken for the sub lethal studies according to Sprague (1973).

concentrations. A marked decrease of 5.2, 3.6 and 6.0% were recorded in the number of RBCs in 10% sublethal concentration at 10, 20 and 30 days .The values were 14.6, 20.7 and 35.8% for 10, 20 and 30 days at 30% sublethal concentration (Table 1). The WBC count of *Liza parsia* was increased with the increasing concentrations of nickel (Table 1). The increase in the number of WBCs was found to be 18.3, 19.6 and 29% at 10, 20 and 30 days exposure at 30% sublethal concentrations, respectively. The haemoglobin content of *L. parsia* in 10 and 30% sublethal concentrations showed

Table 1. Haematological parameters of *Liza parsia* under sublethal concentrations of Nickel

Parameters	Treatment	Exposure periods (days)		
		10 Days	20 Days	30 Days
	control	3.49±0.18	3.34±0.21	3.55±0.08
Total RBC ($\times 10^6$/mm^3)	10% SLC	3.31±0.29	3.22±0.32	3.15±0.29
	%Variation	-5.16	-3.59	-5.98
	30% SLC	2.98±0.51	2.65±0.36	2.15±0.20
	%Variation	-14.61	-20.66	-35.82
	control	10.75±0.31	11.69±0.49	11.47±0.87
Total WBC ($\times 10^6$/mm^3)	10% SLC	12.37±0.16	13.69±0.24	14.21±0.29
	%Variation	+15.07	+17.11	+23.89
	30% SLC	12.72±0.43	13.98±0.64	14.79±0.31
	%Variation	+18.32	+19.59	+28.95
	control	9.67±0.11	8.90±0.19	9.26±0.47
Hb (%)	10% SLC	8.27±0.41	6.85±0.26	5.63±0.67
	%Variation	-14.48	-23.03	-39.20
	30% SLC	7.08±0.37	5.30±0.13	3.40±0.29
	%Variation	-26.79	-40.45	-63.28

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control

After the recovery period, all such treated fishes were separated from the experimental containers and blood samples were collected from three experimental individuals from each group at each time with an interval of 24 h during the experiment. Fish was collected and gently wiped with a dry cloth to remove water.

Caudal peduncle was cut with a sharp blade and the blood was collected in an Eppendorf tubes containing EDTA anticoagulant. The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the Red Blood Corpuscle Count (RBC), Total Leucocyte Count (TLC)and Haemoglobin content (Hb). The haematological parameters were determined the method of Dacie and Lewis (1984). Blood smear slides were prepared from fresh unheparinished blood, air dried, fixed in methanol and stained with Leishman's stain. Blood corpuscles were examined by phase contrast microscopy and photographed.

Fig. 1. Morphology of RBC in control fish (X 450)

RESULTS

The toxic effects of nickel on the blood parameters of *Labeo rohita* such as number of red blood corpuscles (RBC), white blood corpuscles (WBC) and haemoglobin content (Hb)are depicted in Table -1. The observations were made at the end of exposure periods (10, 20 & 30 days) to calculate the percentage of increase and decrease of different blood parameters. Total RBC count of *Liza parsia* showed a significant decreasing tendency at 10 and 30% sublethal

decreasing trend with a significant reduction of 14.5, 23.0, 39.2 and 26.8, 40.5, 63.3% at 10, 20 and 30 days of exposure respectively (Table 1).

Light microscopic observation of RBC: The blood smear showed number of fragility, vacuolization, wrinkling of membrane, haemolysis of erythrocyte cells were observed in nickel exposed fishes. A distinct changes in the size and irregular shape of red blood cells was also observed (Figs. 1-4).

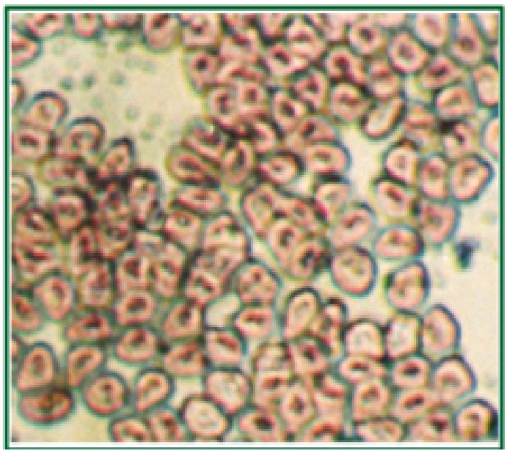

Fig. 2. Morphology of RBC in 30% SLC of nickel treated fish after 10 days

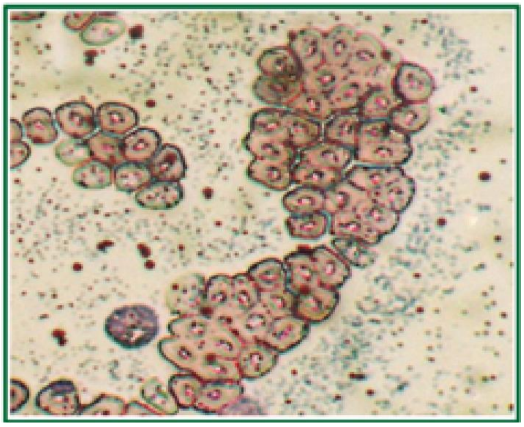

Fig. 3. Morphology of RBC in 30% SLC of nickel treated fish after 20 days

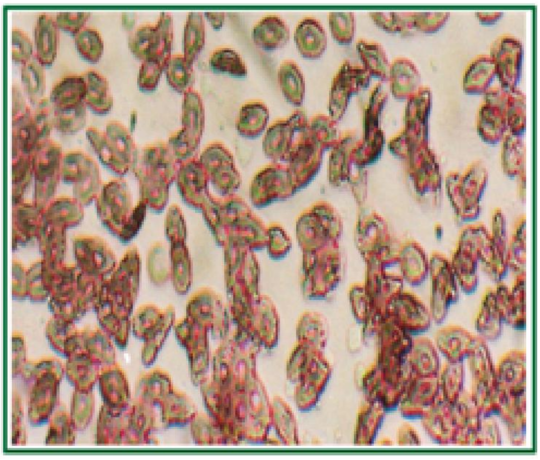

Fig. 4. Morphology of RBC in 30% SLC of nickel treated fish after 30 days

DISCUSSION

Heavy metals and pesticides are the most potentially harmful chemicals released into the environment in an unplanned manner. Though they have contributed considerably to the welfare of humans, their adverse effects on non-target organisms are enormous. The major sources of environmental contamination by these chemicals are from agricultural practices, usage in public health programmes and industrial discharges (Hazarika & Dass 1998). The total red blood cell (RBC) and haemoglobin are reduced in *Liza parsia* on exposure to different sublethal concentrations of nickel. On the other hand WBC values were increased in nickel treated *Liza parsia* when exposed for 10, 20 and 30 days. Similar observations are made available in fishes treated with sublethal concentrations of cypermethrin (Parma *et al.,* 2007). The results recorded in the present investigation corroborate the previous findings in fishes by Nath (1966) and Reddy and Bashamohideen (1989) after exposure to fenvalerate. Agarwal and Chaturvedi (1995) and Chauhan *et al.,* (1994) have made observations with decreased values of above blood parameters in fishes when exposed to rogor. The reduced values of the total red blood cell and haemoglobin content in the species studied also, agree with the investigations made by many workers in fishes exposed to phosphamidon and endosulfan (Shekar and Christy, 1996; Anandkumar *et al*., 2001 and Abidi and Srivastava, 1980). A significant decrease in RBC, haemoglobin and an increase of white blood corpuscles in the fresh water fish *Channa punctatus* due to the toxicity of pesticide endosulfan (Abidi and Srivastava, 1980). Kabeer Ahamed *et al.* (1981) have reported the Malathion toxicity which caused a gradual and highly significant decrease in haemoglobin concentration and the total RBC count in the teleost, *Tilapia mossambica*. They have reported that the decrease in the value of haemoglobin and total RBC counts might be due to anaemia or disturbance in the haemopoietic organs or because of iron deficiency under toxic stress. Verma *et al.* (1982) have reported that a decrease in red blood corpuscles in *Mystus vittatus* might be due to the toxic action of pesticides on peripheral red cells as a result of which the viability of the cell is affected.

The damaging effects of erthrocytes might be due to the action of the toxicant on the erythropoietic tissues which has led to a failure in the production of red blood cells. Anaemia is due to the low rate of production or to an increase in the destruction of red blood cells. Reduction of RBC count of fish blood after exposure to pesticide in *Saccobrachus fossilis* was reported by Verma *et al.* (1979). In the present study, similar pattern of reduction in RBC and Hb content indicated anaemic stage of experimental fish caused due to decreased erythopoietic activity or increased destruction of blood cells. The reduction in red blood cell count and haemoglobin percentage indicated the occurrence of acute anaemia (Nanda, 1997). White blood cells play a major role in the defence mechanism of fish and they consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies (Ellis and Robert, 1978; Wedemeyer and McLeay, 1981). In the present investigation, white blood corpuscles concentrations of *Liza parsia* showed increase and quite different pattern of changes with the effect of nickel when compared with the erythrocyte levels of the control group. Blood of all experimental groups contained higher concentrations of leucocytes than those of controls. An increase in leucocyte numbers may be the compensatory response of lympoid tissues to the destruction of circulating lympocytes (Shah and Altindag, 2005). Pooja Gupta and Kumar Saxena (2006) observed increased WBC counts in *Channa punctatus* after cyhalothrin and permethrin. They have suggested that significant increase in WBC in the fish could be due to lympocytopoeisis and enhanced release of lympocytes from lympoid tissues such as kidney, spleen and thymus. The increase in WBC observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by pesticides. Karuppasamy *et al.* (2005) have reported that the stimulation of the immune system causes an increase in lymphocytes by an injury or tissue damage. Gautam and Suneelkumar (2008) have also reported an increase in the leucocyte counts in fish exposed to an organophosphate, Nuvan. The leucocytosis during metabolic stress has been considered to be of adaptive values to overcome the stressful conditions of the animal. Maheswaran *et al.* (2008) have reported the decrease in RBC, Hb and increase in WBC in the blood of *Clarias batrachus* exposed to mercuric chloride. Sastry *et al.* (1982) have observed significant increase in WBC count in *Channa punctatus* exposed to quinolphos. They have reported that the increase in WBC count may be due to lecocytosis, which is adaptation to meet stressful condition of the fish.

Decrease in haemoglobin is proportional to concentration of the pesticides and duration of exposure. The low levels of Hb indicated anaemic conditions in fish due to stress caused haemolysis (Panigrahi and Misra 1978 and Satyajit, 2010). The lower haemoglobin levels of treated fish in the present study might be due to the distruption of the iron synthesizing machinery (Beena and Viswarajan, 1987). Erythrocytes are fundamentally capable of few stereotypic responses to a variety of environmental perturbations, which are sometimes considered to be vital physiological significance. Furthermore, it is implicated that modifications of the shape and size of the erythrocytes represent the most common morphological abnormalities that occur in pathological conditions (Barnnart, *et al.,* 1983). The fragitity of RBC membrane and the rate of haemolysis were maximum after 30 days of exposure to nickel, which is indicate cumulative response of this toxicant on blood cells. Similar observation was reported by Karuppasamy *et al* (2005) in cadmium treated *Channa punctatus*. Further, observations of the present study are in good agreement with those of Kapila and Ragothaman (1999) in *Boleophthalamus dussumieri* exposed to different heavy metals. Patil (1986) reported vacuolization of RBCs, thickening of cell membrane after treatment of monocrotophos in *Boleophthalamus dussumieri*. Sriwasta and Sriwasta (1980) observed cellular and nuclear hypertrophy, change in shape, agglutination and bursting of erythrocytes in *Cirrhinus mrigala* fingerlings treated with urea. Devi *et al* (2008) also observed in similar findings in fish, *Channa punctatus* treated with pesticide endosulfan. Thus, this study clearly indicates the changes in haematological parameters and morphology of RBC of *Liza parsia* when exposed to sublethal doses of nickel.

REFERENCES

A P H A, 1976. American Public Health association. Water Pollution Control federation. Standarded methods for the examination of water and wastewater 1193.

Abidi, R. and Srivastava, U. S.1980 Effect of endosulfan on certain aspects of haematology of the fish, *Channa punctatus*. *Proc. Natl. Acad. Sci. India*. 58B(1): 55-65.

Agarwal, K. and Chaturvedi, L. D. 1995 Anomalies in blood corpuscles of *Heteropneustes fossilis* induced by alachlor and rogor. *Adv. Bios*., 14: 73-80.

Anandkumar. A., Tripathy, A. P. and Tripathy, N. K. 2001 Effect of dimecron on the blood parameters of *Heteropneustes fossilis*. *J. Environ. Biol*., 22(4): 297-299.

Barnnart, M. I., Walaoe, M. A. and Lusher, L. M. 1983 Red blood cells. In: Biomedical research Applications of Scanning Electron Microscopy .Eds. Hodges G M and Corr K E Vol.3. Academic press, New York.p. 171 – 243.

Beena, S. and Viswarajan, S. 1987 Effect of cadmium and mercury on the haematological parameters of the fish, *Cyprinus carpio*. *Environ. Ecol.,* 5: 726-732.

Bhatia, N.P., Sandhu, G. S. and Johal, M. S. 2002. Endosulfan induced changes in blood chemistry of *Hetropneustes fossilis. Poll. Res.* 21(3): 323-327.

Bhatkar, N. V. and Dhande, R. R . 2000 Furadon induced haematological changes in the fresh water fish *Labeo rohita*. J. Ecotoxicol. Environ. Monit. 10(3): 193-196.

Cavalcanate, D.G., Martinaz, C.B. and Sofia, S. H. 2008. Genotoxic effects Roundup on the fish *Prochilodus lineatus. Mutat. Res*., 655:41-46.

Cavas, T and Konen, S. 2007. Detection of cytogenetic and DNA damage in peripheral erythrocytes of gold fish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and comet assay. Mutagenesis 22:263-268.

Chauhan, R .R .S., Saxena, K. K. and Kumar, S. 1994. Roger induced haemotological alterations in *Cyprinus carpio.* Adv. Bios. 13: 57-62

Dacie, V. and Lewis, S. M., 1984. Practical haematology. Sixth ed. Churchill Livingstone, p.1-6.

Dalela, R..C., Rani, S., Kumar, V. and Verma, S.R. 1981 *In vivo* haematological alterations in a freshwater teleost *Mystus vittatus* following subacute exposure to pesticide and their combinations. *J. Environ. Biol*., 2(2): 76-86.

Deen, J.G., F.L. Bosqui and V.H. Lanouette, 1972. Removing heavy metals from waste water. *Eviron.Sci.Technol*., 6:518-522.

Devi, P., Baruah, D., Baruah, B. K. and Borkotoki, A. 2008 Impact of endosulfan on some haematological parameters of *Channa punctatus* (Bloch). *Poll. Res*., 27(3): 485 – 488.

Dutta, H. M., Dogra, J. V. V., Singh, N. K ., Roy, P. K., Nasar, S. S .T., Adhikari , S, Munshi, J. S. D and. Richmonds, C .R. 1992 Malathion induced changes in the serum proteins and haematological parameters of an Indian cat fish *Heteropneustes fossilis* (Bloch.), Bull. Environ. Contam. Toxicol. 49: 91-97.

Ellis, E .A. and Robert, R .J. 1978. *Fish Pathology*. Bailliere Tindall, London, p.318.

Finney, D. J., 1971 *Probit Analysis.* Cambridge Univ. Press, London.

Gautam, R. K. and Suneel Kumar, 2008. Alteration in haematology of *Channa punctatus* (Bloch). J. *Exp. Zool. India*, 11(2): 309-310.

Grisolia, C. K. 2002. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutat. Res*., 518: 145-150.

Hazarika, R. and Das, M. 1998. Toxicological impact of BHC on the ovary of the air-breathing catfish *Heteropneutes fossilis* (Bloch). Bull. Environ. Contam. *Toxicol*., 60(1): 16-21.

Jhingran, V.G. 1991. Fish and Fisheries of India, Hindustan, New Delhi.

Johal, M. S. and Grewal, H. 2004. Toxicological study on the blood of *Channa punctatus* (Bloch) upon exposure to carbaryl. *Poll. Res*., 23(4): 601-606.

Joshi, P., Harish, D. and Bose, M. 2002. Effect of lindane and malathion exposure to certain blood parameters in a freshwater teleost fish *Clarias batrachus*. *Poll. Res.,* 21(2): 55-57.

Kabeer Ahmed, I., Jaganatha Rao, K. S. and Ramana Rao, K.V. 1981. Effect of malathion exposure on some physiological parameters of whole blood and on tissue ions of teleost, *Tilapia mossambica.* J. Biol. Sci., 3: 17-21.

Kapila Manoj and Ragothaman, G. 1999. Effect of mercury, copper and cadmium on the red blood cells of *Boleophthalmus dussumieri* (Cuv). *Poll. Res*. 18 (2): 149 – 152.

Karuppasamy, R. S., Subathra, S. and Puvaneswari, S. 2005. Haematological responses to exposure to sublethal concentration of cadmium in air breathing fish, *Channa punctatus* (Bloch). *J. Environ. Biol*., 26(1): 123-128.

Karuppasamy, R., Subathra, S. and Puvaneswari, S. 2005 Haematological responses to exposure to sublethal concentration of cadmium in air breathing fish, Channa punctatus (Bloch). *J. Environ. Biol.,* 26(1) : 123 – 128.

Klein, L.A., M. Lang, N. Nash and Krischner, S.L. 1974. Sources of metals in New York City waste water. JWPCF, 46:2653-2662.

Lushchak, O. V., Kubrak, O .I., Storey, J .M., Storey, K. B., Lushchak, V. I. 2009. Low toxic herbicide roundup induces mild oxidative stree in gold fish tissues. Chemosphere 76:932-937.

Maheswaran, R., Devapaul, A., Muralidharan, S., Velmurugan, B. and Ignacimuthu, S. 2008. Haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride. *Int. J. Integ. Biol.,* 2(1): 49-54.

Nanda, P. 1997 Haematological changes in the common Indian catfish *Heteropneustes fossilis* under nickel stress. *J. Ecobiol*., 9: 243-246.

Nath, R. 1996 Effect of fenvalerate on blood parameters of fish *Heteropneustes fossilis* (Block). *Environ. Ecol*., 14: 710-712.

Parma, M.J., Loteste, A. Campana, M. and Bacchetta, C. 2007. Changes of hematological parameters, in *Prochilodus lineatus* (Pisces: Prochiloddontidae) exposed to sublethal concentration of cypermethrin. *J. Environ. Biol*., 28(1): 147-149.

Patil, V. 1986. Some toxicological effects of an organophosphorus insecticides, Monocrotophos on the mud skipper, *Boleophthalmus.* M.Phil., dissertation submitted to South Gujarat University, Surat.

Pooja Gupta and Kumar Saxena, 2006 Biochemical and haematological studies in freshwater fish *Channa punctatus* exposed to synthetic pyrethroids. Poll. Res. 25(3): 499-502.

Ramaswamy, M., Thangavel, P., Dhanalakshmi, S., Govindaraj, P. and Karuppiah, D. 1996. Comparative study on the synergistic and individual effects of dimecron and cuman on oxygen uptake and haemotological parameters of a fresh water edible fish, *Sarotherodon mossambicus*. (Peters). Bull. Environ. Contam. *Toxicol*. 56: 796-802.

Reddy, M.P. and Basha Mohideen, M. 1989. Fenvalerate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio*. Acta. Hydrochem. Hydrobiol. 17: 101-107.

Sastry, C.A., C.K. Khare and A.V. Rao, 1972. Water pollution problems in Madhya Pradesh. *Ind. J. Environ. Res*., 16(1-3): 270-278.

Sastry, K.V. and Sharma, S.K. 1978. The effect of endrin on the histopathological changes in the liver of *Channa punctatus* Bull. Environ. Contam. *Toxicol*. 20: 674-677.

Sastry, K.V., Siddique, A. A. and Singh, S.K. 1982. Alterations in some biochemical and enzymological parameters in the snake head fish, *Channa punctatus* exposed clinically to quinalphos. Chemosphere 11: 1211.

Satyajit Hota, Rajesh Behera, Alivapatnaik and Milan Kumar Behera 2010. Arsenic induced changes in some haemato-biochemical parameters of an air breathing fish *Channa punctatus.* J. Ecotoxicol. Environ. Monit. 20(4):313-318.

Shah, S.L. and Altindag, A. 2005. Alterations in the immunological parameters of tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal treatments with mercury, cadmium and lead. *Trunk J. Vet. Anim. Sci*., 29: 1163-1168.

Shekar, P. and Christy, I. 1996. Haematological changes in the fresh water catfish *Mystus vittatus*, exposed to sublethal concentrations of phosphamidon. *J. Ecol*., 8(1): 25-28.

Sprague, J.B. 1973. The ABC's of pollutant bioassays using fish. In: Biological methods for the assessment of water quality*. American Society for Testing and Materials*, 528: 6-30.

Srivastava, D.K. and Srivastva, V.M.S. 1980. Erythrocytic abnormalities under urea stress in *Cirrhinus mrigala* (Hamilton) fingerlings. Curr.Sci. 549(20):799.

Szarek J, Siwicki A, Andrzejewska, A, Terech Majewska E and Banaszkiewicz 2000 Effects of the herbicide Roundup on the ultrastructural pattern of hepatocytes in carp (*Cyprinus carpio*). Mar. Environ.Res. 50:263-266.

Verma, S.R., Rani, S. and Dalela, R. C. 1982. Indicators of stress induced by pesticides in *Mystus vittatus*. Haematological parameters. *Indian J. Environ. Hlth*. 24: 58-64.

Verma, S.R., Tyagi, A.K. and Datta, R. 1979 Effect of distillery waste on some fresh water teleost – *Biochemical studies. Environ.* Poll. 19: 225.

Wedemeyer, G. R. and McLeay, D.J. 1981. Methods of determining the tolerance of fish to environmental stressors. In: Stress and fish. Ed: Pickering AD Academic Press, New York. USA. p 247-275.

******