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CHANGES IN SOME HEMATOLOGICAL AND BIOCHEMICAL INDICES IN FRESHWATER FISH *RASBORA DANICONIUS* EXPOSED TO TRIBUTYLTIN OXIDE

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ABSTRACT
The present study was undertaken to examine hematological and biochemical alteration induced from the exposure of a fish *Rasbora daniconius* to sub-lethal concentration 0.03ppm (1/10 96hr LC_{50}) of tributyltin oxide for a period of 7, 14 and 21 days. The statistically important differences were observed between measured RBC counts, hemoglobin (Hb),packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) from different exposure period. The observed hematological indices of sub-lethal concentration of tributyltin oxide caused a decrease in hemoglobin content from 9.7 to 6.2%, coupled with a decrease in red blood cell count from 2.09 to $1.42\times10^{6}mm^{-3}$,Packed cell volume from 27.06 to19.8% at the end of all exposure period as compared to control. The white blood cells increase from 9.37 to $14.98\times10^{3}mm^{-3}$. The mean corpuscular volume from 130.47 to 141.16fl, mean corpuscular hemoglobin from 47.55 to 50.91pg/cell and mean corpuscular hemoglobin concentration from 37.49 to 41.91g/dl significantly with increase in exposure period. Blood glucose level showed significant increase from 27.63 to 44.56mg/l ($p<0.01$) whereas serum level of protein showed a significant decrease ($p<0.01$) for all exposed treatments.

KEYWORDS: Hematological indices. *Rasbora daniconius*, Tributyltin oxide.

INTRODUCTION

Organotin compounds (OTC) are amongst the most widely used organometallic compounds. They are used for a variety of industrial and agricultural applications including pesticides, fungicides and anti-fouling agents (Golub and Doherty, 2004). These chemicals are hazardous to the natural flora and fauna of the ecosystems and these have increased concerns regarding their toxic effects to living organisms (Rao and Padmaja, 2000, Mitra *et al.*, 2013).

Fish are used as excellent indicator of aquatic pollution due to their high sensitivity to environmental contaminants which may damage certain physiological and biochemical processes when they come in contact with the organs of fishes, Fish hematology is an essential tool for the biologists as a important sensitive indicator of vital physiological and biochemical functions as well as status of nutrition, health, diseases and stress in response to changing environmental conditions (Seth and Saxena, 2003).In ecotoxicology, fish have become the major aquatic vertebrate model, and a number of characteristics make them excellent experimental models for toxicological research, especially for the contaminants which are likely to exert their impact on aquatic systems. Toxicant effects can be studied at different levels of biological organization and consider that the different changes in many biochemical and morphological parameters of fish may be used as successful biomarkers for toxic effects of xenobiotics(Law, 2003; Vinodhini *et al.*, 2009; Torre *et al.*, 2010; Janardana *et al.*, 2011;Murthy *et al.*, 2013; Dhankumar *et al.*, 2015).Although there are some studies on the effects of organotins toxicity in aquatic organisms and mammals, there is lack of data specifically on hematological and biochemical aspects in freshwater fish species of India. Thus we studied the responses of tributyltin oxide at sub-lethal concentration on hematological and biochemical indices in a freshwater fish *Rasbora daniconius*.

The study of hematological and biochemical parameters in blood is much important in the physiopathological assessment of aquatic animals. Little is known about hematological and biochemical changes induced by organometallic compounds in freshwater fish. Changes in the hematological profile of the fish exposed to tributyltinchloride have been observed in *Salmo gairdneri* and *Tilapia rendalli* (Chliamovitchand Kuhn, 1977), *Hoplias malabaricus* (Oliveira-Ribeiro *et al.*, 2006) and *Oplegnathus fasciatus* (Hwang *et al.*, 2013).Blood parameters are considered pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari *et al.*, 2004). A number of hematological indices such as packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBCs) and so on are used to assess the functional status of the oxygen carrying capacity of the bloodstream and have been used as indicator of metal pollution in the aquatic

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environment.

The selected fish *Rasbora daniconius* is a very hardy fish, which is found in, lakes, ponds, swamps and inland water bodies of India. Several studies have been carried out on the effect of heavy metals on the hematology of different fish species. A very few literature available on the effect of tributyl compound on hematology of freshwater fishes therefore the freshwater fish *Rasbora daniconius* was selected as biological indicators of ecotoxicological studies for the assessment of hematological and biochemical parameters induced by sub-lethal concentration of tributyltin oxide.

MATERIALS AND METHODS

The medium size freshwater fish *Rasbora daniconius*, 6 to 7 cm in length and 8 to 9 gm in weight irrespective of sex were collected with the help of local fisherman from Godavari river at the Kayagaon village of Aurangabad. The fish was properly washed in tap water and treated with 0.01% KMNO solution to remove external infection of fungi, algae, etc. The normal healthy and non-infected fish were selected for experiment. The fish were acclimatized to laboratory conditions for 15 days before commencement of experiment. All the precautions laid down by APHA *et al.,(*1998) are followed, for maintaining the fish in laboratory. The animals were fed twice in daily with commercially available fish food. The feeding was stopped 24hr before taken for experimentation. The 96hrLC_{50} value of tributyltin oxide was found to be 0.3ppm exposed to *Rasbora daniconius* using the probit analysis method Finney (1971).

The fish were divided into four groups consisting of 10 each and was transferred to glass aquaria of 100 L volume separately. The group I contained control fish without exposure to toxicant and the group II, III and IV fish were experimental exposed to sub-lethal concentration (0.03ppm) 1/10th of 96hr LC_{50} of tributyltin oxide for 7, 14 and 21 days. The blood samples were taken from the control and experimental fish. The blood from the caudal vein of control and treated fish was collected for hematological and biochemical analysis RBC, WBC, Hb and PCV were examined following the Wintrobe (1967). Erythrocyte indices such as mean corpuscular volume (MCV), mean hemoglobin content (MCH) and mean hemoglobin concentration in the cell (MCHC) were analyzed using standard formulae (Dacie and Lewis, 2001). Total serum protein and glucose concentration were determined by Lowry *et al.,* (1951) and Somogyi (1952) method respectively.

The mean values of the different hematological parameters of the fish were analyzed for statistical significance using the student's t-test at 0.01 and 0.05 levels.

RESULTS AND DISCUSSION

The hematological alteration in *R.daniconius* obtained from exposure to sub-lethal concentration of tributyltin oxide for 7, 14 and 21 days showed significant reduction in RBC count, hemoglobin percentage and packed cell volume whereas WBC count increased significantly as compared to control The RBC, Hb and PCV were reduced in fish exposed to tributyltin oxide in all periods as well as being in each case lower than in the control ($p<0.05$). The erythrocyte count of control showed a mean value of $2.09\times10^{6}mm^{-3}$. The fishes exposed to sub-lethal concentrations of tributyltin oxide showed mean values of RBC's were $1.97\times10^{6}mm^{-3}$, $1.76\times10^{6}mm^{-3}$ and $1.42\times10^{6}mm^{-3}$ for 7, 14 and 21days treatments respectively (Table 1 Fig. 1). The values obtained for white blood cells of the control fish showed a mean value of$9.37\times10^{3}mm^{3}$(Table1 Fig. 2). In control, the Hb level was recorded to be 9.7%. The Hb levels were observed as 8.9%, 7.9%and 6.2% at 7, 14 and 21days respectively (Table 1 Fig.3). The Hb levels were in decreasing trend with increase in the exposure period of tributyltin oxide. The overall decline was observed throughout the exposure period. The PCV showed a significant difference ($p<0.05$) between exposed fish and the control.27.06%, 25.8%, 22.6% and 19.8% for control, 7, 14 and 21days respectively (Table 1 Fig.4). The fishes exposed to sub-lethal concentrations showed the mean values of WBC as $10.21\times10^{3}mm^{-3}$, $12.76\times10^{3}mm^{-3}$ and $14.98\times10^{3}mm^{-3}$for 7, 14 and 21 days of tributyltin oxide treatments respectively(Table 1 Fig.2). The values observed showed a significant increase when compared to the control ($P<0.01$).

The red cell indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were significant at($P<0.01$) when compared to the control. Mean corpuscular volume (MCV) of experimental fishes increased from130.47fl in control fishes to 134.67fl, 139.47fl and 141.16fl after 7, 14 and 21 days of exposure (Table 1, Fig.5). Following exposure the MCH values fluctuated in narrow ranges at different exposure period (Table1, Fig.6). The mean corpuscular hemoglobin concentration (MCHC) of experimental fish also decreased from the control value 37.49g/dl to 33.68g/dl at 7 days exposure and then steadily

increased to 38.56g/dl after 14 days showed not significant, again significantly increased to 41.91g/dl of 21 day of exposure (Table 1 Fig.7).

The glucose levels were observed 27.63mg/l in control and 32.11mg/l, 39.63mg/l and 44.56mg/l at 7, 14 and 21 days respectively. Whereas reverse trend was observed in serum protein level. Serum protein level decreased significantly throughout the exposure period of tributyltin oxide compared to control from 4.97mg/l to 3.17mg/l, 2.96mg/l and 2.55mg/l at 7, 14 and 21 days(Table 1, Fig.8 and Fig.9).

Hematological indices have been used regularly to examine the oxygen carrying capacity of the blood as well as an indicator of metal pollution in aquatic environment (Shah and Altindag, 2004). A steady decrease in the RBCs might be one of the main causes of anemia. According to Gill and Epple (1993) the reasons for anemia might be impaired erythropoiesis caused by the direct effect of metal on hematopoietic centers. Hematological indices like hemoglobin (Hb) content, red blood cell count (RBC), white blood cell (WBC), packed cell volume (PCV),mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), may be altered in fish after exposure to sub-lethal concentration of tributyltin oxide. Agrawal *et al.,* (1988) reported that metals are known to induce anemia in fish. Anemia might be one of the earliest indications of metal toxicity. Decline in Hemoglobin and haematocrit was observed in *Channa punctatus* exposed to Mercury (Sastry and Sharma, 1980).The steady decrease in PCV values were observed for all exposure period and this might be due to the decreased number of RBC count. The metallic compound mercuric chloride also caused significant decrease of PCV value in fish *Clarias batrachus* (Maheswaran *et al.* ,2008). Oliveira Ribeiro *et al.,* (2006) reported no significant changes in MCH and MCHC but differences were found in erythrocytes, hemoglobin, PCV, MCV, and white blood cells counts in fish *Hoplias malabaricus* exposed to sub chronic tributyltin chloride.

In another study of Chliamovitch and Kuhn (1977) the packed cell volume, the hemoglobin concentration and erythrocyte count increased after exposure of tributyltin oxide to fish *Salmo gairdneri* and *Tilapia rendalli*. There results indicate that tributyltin oxide interfered with the process of respiration. Exposure to of TBT even at lower concentration resulted in significant decrease in hematological parameters in fish (Hwang *et al.,* 2013).They reported significant decline in the red blood cell count, hematocrit value and hemoglobin concentration in the fish exposed to tributyltin compounds can cause significant changes in growth and hematological parameters of rock bream *Oplegnathus fasciatuso*. Nasser *et al.,* (2015) reported significant decrease in erythrocytes, hemoglobin and PCV content in blood of fish *Oreochromis niloticus* exposed to various concentrations of cadmium chloride.

In the present study increase of WBC observed as the exposure period increases. White blood cells or leucocytes are the cells of immune system which defend the body against infectious as well as foreign materials. Presently our results are in accordance with workers who have observed an increase in total WBC count of fish on exposure to different xenobiotics (Torres *et al.,* 1984; Garg *et al.,* 1989; Sinha *et al.,* 2000; Oliveira *et al.,* 2006; Devi and Banerjee 2007). Also Saroch *et al.,* (2012) reported a significant increase in the total count of WBC in a time dependent manner after exposure to Hg. Total WBC count increased in *Tinca tinca* exposed to lethal and sub-lethal dose to Hg in a time dependent manner. This increase in total WBC can be attributed to an activation of the immune system in response to stress caused by sub-lethal concentration of tributiltin oxide.

The blood of fish showed significant increase ($p < 0.01$) in glucose in all exposure period compared to control. Blood glucose is an important indicator of pollutants causing environmental stress in fish. Our results are in agreement with data obtained by Shalaby (2007) showed that elevated blood glucose levels in Nile tilapia exposed to cadmium chloride 4.64 mg/l (25% of 96hr LC_{50}) for 15 and 30 days because of induced hyperglycemia with an associated decrease in liver glycogen in catfish *Heteropneustes fossilis*. The decrease in serum protein level observed in the present work is in accordance with the findings of Mekkawy *et al.,* (2011). Similar results were showed in fish *Cyprinus carpio* exposed to calcium chloride (Bedii and Kenan 2005). The decrease of serum protein might be due to the destruction of protein-synthesizing sub cellular structures and inhibition of the hepatic synthesis of blood protein (Fontana et al., 1998).

In conclusion decrease of RBC, Hb and PCV were observed in fish exposed to sub-lethal concentration of tributyltin oxide. In contrast, white blood cells showed elevation as an immunogenic response. Increase or decrease of hematological indices like MCV, MCH, MCHC depends upon the levels of RBC and Hb since they are derived from them. On exposure to tributyltin oxide, glucose level showed increase to cope up energy demand might be due to

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glycogenesis and gluconeogenesis while decrease in serum protein because of inhibition in blood protein synthesis. Therefore the assessment of different hematological indices and biochemical were serving as useful biomarkers to assess the fish health.

Table 1: Variation in Hematological and Biochemical parameters of *Rasbora daniconius* exposed to sub-lethal concentration of 96hr LC_{50}tributyltin oxide

Sr. No.	Parameters	Control	7 Days	14 Days	21 Days
1)	RBC count ($\times 10^6 mm^{-3}$)	2.09±0.11	1.97±0.025*	1.76±0.063*	1.42±0.29*
2)	WBC count ($\times 10^3 mm^{-3}$)	9.37±0.041	10.21±0.044**	12.76±0.046**	14.98±0.21**
3)	Haemoglobin%	9.7±0.82	8.9±0.44*	7.9±0.66*	6.2±0.13*
4)	PCV %	27.06±0.48	25.8±0.89*	22.6±1.67*	19.8±0.15**
5)	MCV (fl)	130.47±1.23	134.67±1.88*	139.47±2.95**	141.16±1.72**
6)	MCH (pg/cell)	47.55±1.82	43.59±1.72*	$46.79±1.30^{NS}$	50.91±1.93**
7)	MCHC (g/dl)	37.49±2.43	33.68±1.78**	$38.56±2.78^{NS}$	41.91±1.38**
8)	Blood glucose (mg/l)	27.63±0.98	32.11±1.95**	39.63±1.46**	44.56±1.16**
9)	Serum protein(mg/l)	4.97±0.61	3.17±1.83**	2.96±1.17**	2.55±0.98**

Values are the mean of 5 observations and S.D. indicated as (±)
* indicates significant ($p<0.05$) and ** indicates significant ($p<0.01$).

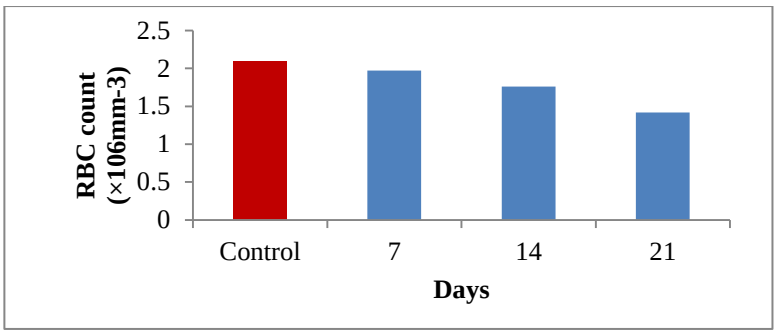

Figure 1: Variation in RBC count of *R. daniconius* exposed to sub-lethal concentration of tributyltin oxide

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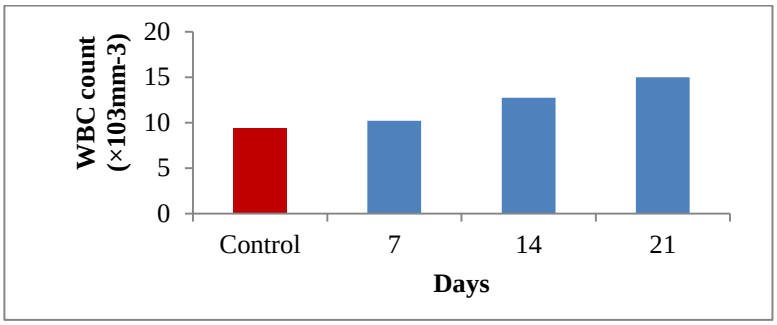

Figure 2: Variation in WBC count of *R. daniconius* exposed to sub-lethal concentration of tributyltin oxide

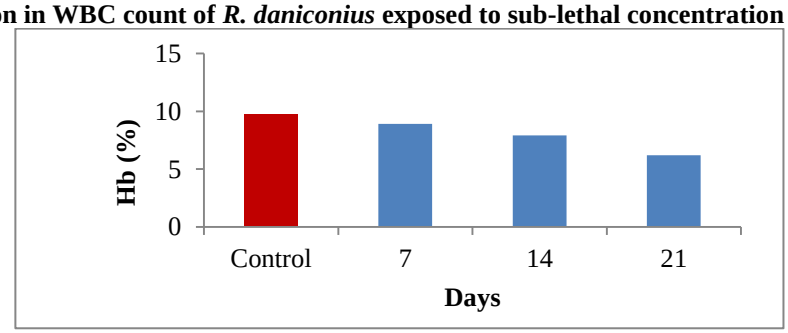

Figure 3: Variation in Hemoglobin of *R. daniconius* exposed to sub-lethal concentration of tributyltin oxide

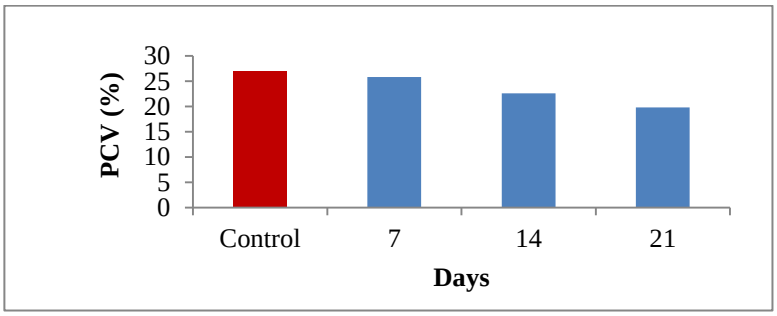

Figure 4: Variation in Packed cell volume of *R. daniconius* exposed to sub-lethal concentration of tributyltin Oxide

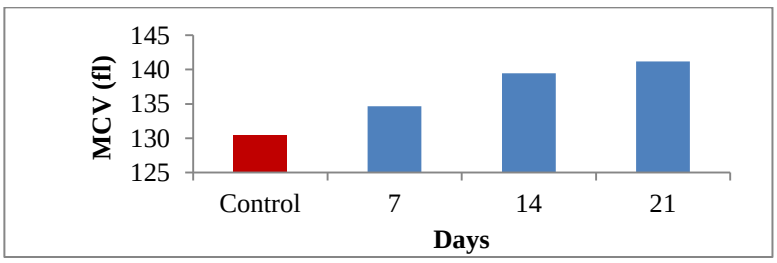

Figure 5: Variation in MCV of *R. daniconius* exposed to sub-lethal concentration of tributyltin oxide

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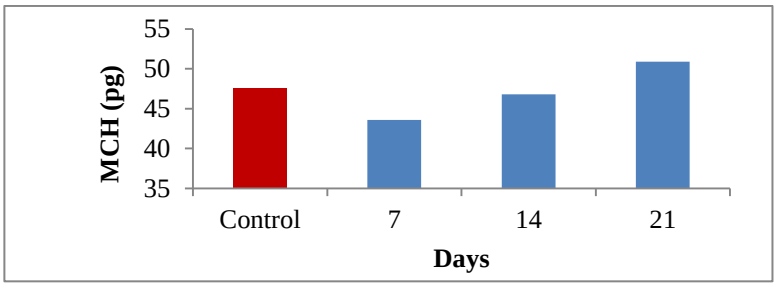

Figure 6: Variation in MCH of *R. daniconius* exposed to sub-lethal concentration of tributyltin oxide

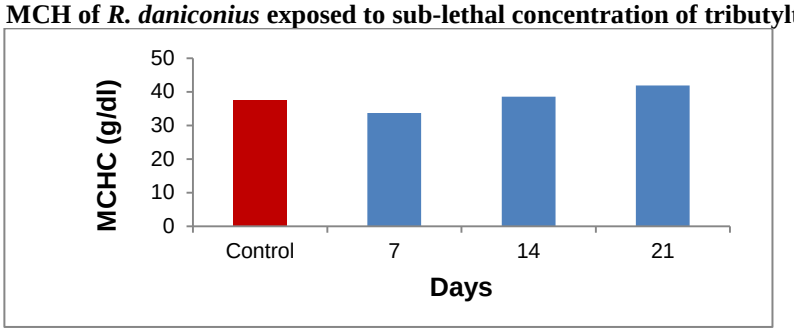

Figure 7: Variation in MCHC of *R. daniconius* exposed to sub-lethal concentration of tributyltin oxide

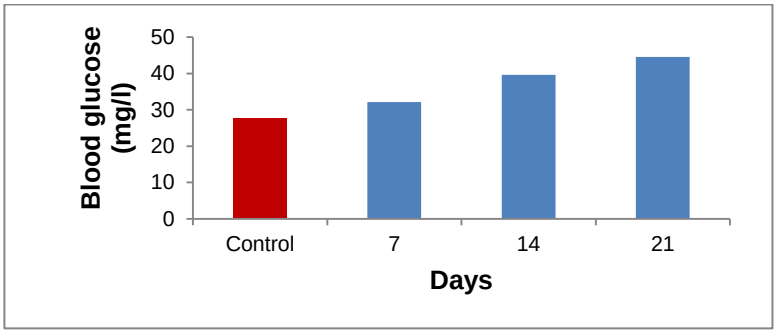

Figure 8: Variation in Blood glucose of *R. daniconius* exposed to sub-lethal concentration of tributyltin oxide

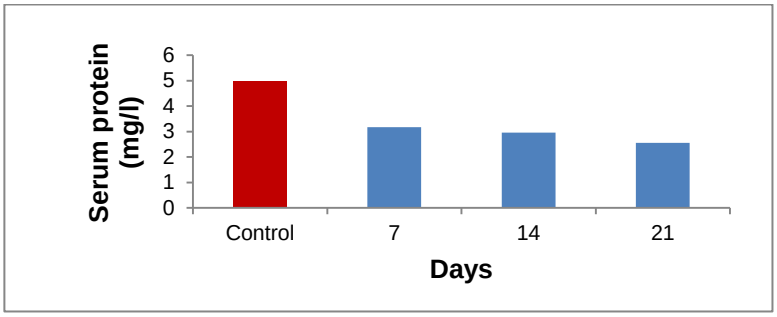

Figure 9: Variation in serum protein of *R. daniconius* exposed to sub-lethal concentration of tributyltin oxide

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REFERENCES

Adhikari S., Sarkar B., Chatterjee, A., Mahapatra C.T. and Ayyappan S. (2004). Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost; *Labeo rohita* (Hamilton). *Ecotoxicol. Environ.Safety*. 58:220–226.

Agrawal V.P. and Tyagi M. (1988). Haematological alterations of *Ophiocephalus punctatus* induced by endosulfan. *Nat. Symp. Threatened habitats. Moradabad:* 24.

APHA. (1998). Standard methods for the examination of water and wastewater. American water works association and water environment federation. 20th Edition. American Public Health Association, Washington DC, USA.

Bedii K. and Kenan N. (2005). The effect of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissue of *Cyprinus carpio*(L, 1758). *Turk. J. Vet. Anim. Sci.* (29): 113-117.

Chliamovitch Y.P. and Kuhn C. (1977). Behavioral, haematological and histological studies of acute toxicity of bis (tri-n-butyltin) oxide on *Salmo gairdneri* Richardson and *Tilapia rendalli* Boulenger. *J. Fish. Biol.* 10:575-585**.**

Dacie J.V. and Lewis S.M. (2001). Practical Haematology, 9th ed., Churchill Livingstone, London, UK, 1986.

De La Torre C., Petochi T., Corsi I., Dinardo M. M., Baroni D., Alcaro L., Focardi S., Tursi A., Marino G., Frigeric A. and Amato E. (2010). DNA damage, severe organ lesions and high muscle levels of As and Hg in two benthic fish species from a chemical warfare agent dumping site in the Mediterranean Sea. *Sci. Total Environ.* 408(9): 2136-2145**.**

Devi R. and Banerjee T.K. (2007). Estimation of the sub-lethal toxicity of lead nitrate in the air breathing fish *Channa striata* employing certain hematological parameters. *Biochem. Cell. Arch.* 7: 185-191.

Dhanakumar S. G., Solaraj R. and Mohanra. (2015). Heavy metal partitioning in sediments and bioaccumulation in commercial fish species of three major reservoirs of river Cauvery delta region, India. – *Ecotoxicol. Environ. Safety.* 113: 145-151**.**

Finney D. J. (1971). Probit Analysis 3rd edition. Cambridge University Press, Cambridge London.

Fontana L., Moreira E., Torres M., Ferna'ndez I., Rios A., Sanchezde Medina, F. and Gil A. (1998). Dietary nucleotides correct plasma and liver microsomal fatty acids alterations in rats with liver cirrhosis induced by oral intake of thioacetamide. *J. Hepatol.* 28: 662-669.

Garg V.K., Garg S.K. and Tyagi S.K. (1989). Manganese induced haematological and biochemical abnormalities in *Heteroneustes fossillis*. *J. Environ. Biol.* 10: 349-353.

Gill T.S. and Epple A. (1993). Stress-related changes in the hematological profile of the American eel (*Anguilla rostrata*).*Ecotoxicol. Environ. Saf.* 25(2):227–235.

Golub Mari S. and Doherty John D. (2004). Triphenyltin as a potential human endocrine disruptor'. *J. Toxicol. Environ. Health Part B.* 7: 281 – 295**.**

Hwang, Un-Gi., Kim, Jun-Hwan. and Kang Ju Chan.(2013). Changes of Growth and Hematological Constituents in the Rock Bream *Oplegnathus fasciatus* Exposed to TBT Journal title: *J. Fish Pathol.* 26 (3) 219-229.

Janardana R. S., Kiran R. T. and Reddy D. C. (2011). Influence of heavy metals on biochemical and metabolic biomarkers of Indian major carp *Labeo rohita*. *Bioscan.* 6: 167-173.

Law J. M. (2003). Issues related to the use of fish models in toxicologic pathology: session introduction. *Toxicol. Pathol*. 31: 49-52**.**

Lowry O.H., Rosebrough M.J., Far A.L. and Randall R.L. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265–275.

Maheswaran R., Devapanl A., Muralidharan S., Velmurugan B. and lgnaeimuthu S. (2008). Hematological studies of fresh water fish, *Clarius batrachus* (L.) exposed to mercuric chloride, *IJIB* 2(1) 49-54.

Mekkawy A.A., Mahmoud U.M., Wassif E.T. and Naguib M. (2011). Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. *Fish Physiol. Biochem.* 37:71–84.

Mitra S., Gera R., Siddiqui W.A. and Khandelwal S. (2013). Tribultyltin induces oxidative damage, inflammation and apoptosis via disturbance in blood – brain barrier and metal homeostasis in cerebral cortex of rat brain: An in vivo and in vitro study. *Toxicol* 310:39–52.

Murthy K. S., Kiran B. R. and Venkateshwarlu M. (2013).A review on toxicity of pesticides in Fish. *Int. J. Open Sci. Res.* 1(1): 15-36.

Nasser A. Al-Asgah, Abdel-Wahab A. Abdel-Warith, El-SayedM.Younis, Hassan Y. Allam. (2015). Haematological and biochemical parameters and tissue accumulations of cadmium in *Oreochromis niloticus* exposed to various concentrations of cadmium chloride. *Saudi J. Biol. Sci.* 22(5): 543–550.

Oliveira Ribeiro C.A., FilipackNeto F., Mela M., Silva P. H., Randi M.A. F. and Costa J. R.A. and Pelletier E. (2006). Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead and tributyltin chloride. *Environ. Res.*101: 74-80.

Rao L.M. and Padmaja G. (2000). Bioaccumulation of heavy metals in M. cyprinoids from the harbor waters of Visakhapatnam. *Bull. Pure Appl. Sci.*19: 77-85**.**

Saroch J.D., Nisar H., Shrivastav R., Qureshi T.A. and Manohar S. (2012). Haematological studies of mercuric chloride affected freshwater catfish *Clarias gariepinus* fed with spirulina. *J. Chem. Bio Physci.* B 2:1862–1869.

Sastry K.V. and Sharma K. (1980). Mercury induced hematological and biochemical anomalies in *Ophiocephalus (Channa) punctatus*. *Toxicol. Lett.* 5: 245-9**.**

Seth N. and Saxena, K. K. (2003). Haematological responses in a fresh water fish *Channa punctatus* due to fenvalerate. *Bull. Environ. Contam.toxicol*. 71:1192-1199.

Shah S.L. and Altindag A. (2004). Hematological parameters on tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sub-lethal mercury treatments, Bull. Environ. *Contam. Toxicol.* 73, 911-918.

Shalaby A.M.E. (2007). Effect of EDTA on toxicity reduction of cadmium in relation to growth some haematological and biochemical profiles of Nile tilapia (*Oreochromius niloticus). J. Fish. Aquatic Sci.* 2(2), 100-109.

Sinha A.K., Sinha M. J. and Adhikari S. (2000). Effect of copper toxicity on haematological profile on indian major carp *Labeo rohita*. Hand Book Ind. Environ. *Pollut.* 166-172.

Somogyi M.(1952). Notes on sugar determination. *J. Biol. Chem.* 195(1): 19-23**.**

Torres P., Tort L., Planas J. and Flos R. (1984). Effects of confinement stress and additional zinc treatment on some blood parameters in the dogdish (*Scyliorhinus canicula*). *Comp. Biochem. Physiol*. 83:89-92**.**

Vinodhini R. and Narayanan, M. (2009). The impact of toxic heavy metals on the hematological parameters in common carp (*Cyprinus carpio L.*). *Iran J. Environ. Health Sci. Eng.* 6: 23-28.

Wintrobe M.M. (6th Eds). (1967). Clinical hematology Lea and Febiger, Philadelphia, Library of Congress, Print USA.

