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Tannery effluent effect on the haematological parameters of freshwater fish, *Channa punctatus*

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Abstract: The present investigation was undertaken to evaluate the tannery effluent toxicity stress symptoms in fish blood during a long term of exposure period. The effect of tannery effluent on various haematological parameters were evaluated exposing fresh water fish, *C. punctatus* to different concentration i.e., [Control, 5% Tannery effluent (TE), 10% TE and 20% TE] of tannery effluent. Exposed of fish to tannery effluent showed a significant decrease in the haemoglobin (Hb) content (9.16± 0.08), red blood cells (3.32 ± 0.12), packed cell volume (34.66 ± 0.33) and mean corpuscular haemoglobin (MCH) values, whereas significant increase in the white blood cells (WBC), erythrocyte sedimentation rate (ESR) and clotting time was recorded with increase in exposure periods as compared to control respectively. Hb, RBC and MCHC values showed fluctuating results. The haematological parameters were decreases from 15th days of exposure periods to 45th days of exposure period. The decrease in haematological parameters clearly indicates that the exposed fishes have become anemic due to tannery effluent exposure.

Keywords: Blood, *Channa punctatus,* Haemoglobin, Tannery effluent, White blood cells

INTRODUCTION

Rivers are the natural source of freshwater. Water pollution has become a global problem; it carries a load of dissolved and particulate matters from both natural and anthropogenic sources along with other contents. These substances move downstream and may cause chemical and biological changes in the quality of water. Thus, the water chemistry of a river is affected by the lithology of the reservoir, atmospheric, and anthropogenic inputs. Industrialization has become an important factor to the development of a country's economy, through the establishment of plants and factories. However, the waste or by-products discharged from them are severely disastrous to the environment. These waste products are various kind of contaminant which contaminates surface water, ground water and soil. The discharges from these industries constitute biohazard to man and other living organisms in the environment because they contain toxic substances detrimental to health (Adebisi *et al*., 2007; Adriano, 2001; Bakare *et al*., 2003). Recently, there has been an alarming and worrisome increase in organic pollutants (Nadal *et al*., 2004). Since many effluents are not treated properly, these products are discharged on the ground or in the water bodies and most of these discharges to water bodies accumulate in the system through the food chain (Odiete, 1999). The waste water discharge form industries are the major source of pollution and affect the ecosystem (Morrison *et al.*, 2001). The degradation of the environment was due to the adverse effect of industrial waste on living organism and agriculture (Anikwe and Nwobodo, 2006).

Kanpur is also known as Industrial city in the world which is mainly famous for tanneries, footwear and leather industries. In the global scenario, Kanpur is the top and famous for tannery industries. The main reason of the pollution in Kanpur is due to the tannery. Only about 20% of chemicals used in the tannery process are absorbed by leather, rest of these were released as waste which absorbed by bioaccumulation process by flora and fauna (Sahu *et al*., 2008). The tannery industry is one of the oldest industries, which is unevenly scattered in the country. There are more than 2,500 tanneries located in different urban centers of India processing about 500,000 tones of hides and 314 kg skins per annum which generates about 100,000 m^3 of wastewater per day (Sugasisni and Rajgopala, 2015).

The discharge of tannery waste water increases from January to May because of higher production rate in tanning industry diurnal this time water quality

decreases. Aquatic system is an ultimate sink of heavy metal pollutants and since aquatic animals tend to accumulate heavy metals from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol, and discharges of waste water (Goodwin *et al*., 2003) they provide the insights of toxicity mechanisms induced by these heavy metals.

Fish is an important component of human nutrition and those from contaminated sited present potential risk to human health. Abnormal physico-chemical characteristics of industrial effluents are responsible for mortality of fishes (Mishra *et al*., 1988; Pawar, 1988). Metals can accumulate in the aquatic organism including fish. When fish are exposed to elevated levels of metals in a polluted aquatic ecosystem they tend to take these metals up from the environment (Adakole, 2012). Haematological tests in fish are important diagnostic tools and valuable indicator of diseases or stress due to environment pollution (Kori-Siakere and Ubogu, 2008). Hence keeping in view the importance the present investigation was carried out to determine the effect of tannery effluent on haematological parameters of fresh water fish *Channa punctatus.*

MATERIALS AND METHODS

The study on tannery effluent toxicity on freshwater fish's *C. punctatus* was conducted to explore the tolerance and toxic limits against different level of diluting tannery effluents. The experiment on aquatic fish *C. punctatus* was conducted at Laboratory in Department of Environmental Science, I.B.S.B.T., C.S.J.M. University, Kanpur.

Collection of effluent sample: Tannery effluent is collected from the tannery located at Jajmau, Kanpur in 100 Liter of plastic container and transported to the laboratory.

Collection and acclimatization of fish sample: The small size freshwater fish, *C. punctatus*, weighing 20±2 g and measuring11±2 cm, were collected with the help of local fisherman from water bodies located in the sub region of Kanpur. The fish was properly washed in tap water and treated with 0.02% KMNO4 and 0.004% formalin solution to remove the external infection of fungi, algae, etc. Prior to the experimentation the normal uninfected healthy fish were selected for the experiment. The fish were acclimatized to laboratory conditions for 15 days before taken for experimentation. The animals were fed TOKYO made in Japan on a day at 8 pm every day.

Experimental set up: The bioassay study was conducted using tap water (University water supply) as dilution medium was estimated by the method of APHA, (2005). The fish were divided into 4 equal groups consisting of 10 each and each group was transferred separately to glass aquaria of 100L volume. While the group I fish were maintained as the control without any treatment, the group II, III and IV fish were exposed to different concentration (5% TE, 10% TE and 20%TE) of tannery effluent for 15 and 45 days. The waste products were removed from aquaria water by using good quality of aquaria water filter. The changes in the Temperature, TDS mgL^{-1}, pH, EC dSm^{-1}, DO mgL^{-1}, BOD mgL^{-1}, COD mgL^{-1}, TH mgL^{-1}, TA mgL^{-1}, Chloride mgL^{-1} of the experimental water were recorded throughout the experimental period.

Haematological study: The blood from the caudal vein of control and treated fish was collected for haematological investigation. Hb, RBC, WBC, ESR and PCV were examined following the procedures of Wintrobe, (1957) and Sood, (1996). The anticoagulant heparin liquid power (5,000 I.U) was used for estimation of ESR and PCV.

Statistical analysis: The data observed in the experiment were statistically analyzed for the calculation of standard error (S.E.) and students' 't' test was administered for testing the hypothesis with the help of computer software SPSS program. The data shown are the averages of three replicates ± S.E.

RESULTS AND DISCUSSION

In the present study, exposure to various concentrations i.e., 5%, 10% and 20% of tannery effluent to fish, *C. punctatus* for 15^{th} and 45^{th} days of exposure periods caused significant alterations in haematological parameters. The alteration observed in various physicochemical parameters of tannery effluent along with tap water, such as, Temperature, TDS mgL^{-1}, pH, EC dSm^{-1}, DO mgL^{-1}, BOD mgL^{-1}, COD mgL^{-1}, TH mgL^{-1}, TA mgL^{-1}, Chloride mgL^{-1} and control of experimental system were indicated in Table.1. The Hb% (11.20 ± 0.11) , RBC (5.55 ± 0.13), ESR and PCV (35.33 ± 0.33) increased significantly ($p<0.05$) for 5% TE at 15^{th} day exposure periods, but increase in 10% TE was found after at 15^{th} day, and decreases Hb%, RBC, ESR and PCV was recorded after at 45^{th} day of exposure period. Hb% decreased significantly in 20% TE at all exposure period. The maximum decrease in Hb% was observed after longest exposure of 45^{th} days. The total red blood cells (RBCs) count also registered an increasing 5% of tannery effluent in comparison to controls for 15^{th} and 45^{th} day of exposure periods. Whereas the decrease in total RBC count was significant ($p<0.05$) for 10% and 20% TE in 45^{th} day of the exposure periods. A significant increase in total white blood cells (WBC) count over and above controls was observed for all (5% TE, 10% TE and 20% TE) three sets of exposure periods. The erythrocyte sedimentary rate (ESR) decrease at 15^{th} day exposed fish for 5% TE, and also increased significantly under stress condition for 45^{th} days of exposure periods respectively in comparison with control. All the values of ESR are significant at $p<0.05$. The pact cell volume

Table 1. Effect of Tannery effluent on physico- chemical profile of tap water.

Parameters	Exposure periods							
	15th day treatment				45th day treatment			
	Control	5% TE	10% TE	20% TE	Control	5% TE	10% TE	20% TE
Temp °C	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00
TDS mg L^{-1}	125.79±7.16	295.53*±3.76	434.73*±10.58	488.53*±9.74	126.14±7.18	348.36*±9.30	471.96*±8.10	567.03*±10.28
pH	7.93±0.06	8.20±0.30	8.50*±0.05	8.50*±0.05	7.54±0.08	8.13*±0.06	7.76±0.03	7.46*±0.20
EC dSm^{-1}	1.052±0.03	1.797±0.05	2.748*±0.05	3.085*±0.02	1.082±0.03	4.140*±0.11	5.438*±0.20	7.311*±0.13
DO mg L^{-1}	6.36±0.22	6.24±0.11	5.83*±0.03	5.19*±0.25	6.36±0.22	5.46*±0.00	5.27*±0.10	5.18*±0.02
BOD mg L^{-1}	3.13±0.17	4.20*±0.34	6.93*±0.13	8.36*±0.23	3.18±0.17	6.26*±0.24	10.66*±0.26	14.93*±0.29
COD mg L^{-1}	17.66±0.88	125.66*±1.20	204.33*±2.02	253.66*±2.96	18.00±1.15	212.36*±2.96	337.33*±4.05	532.66*±6.56
TH mg L^{-1}	147.33±4.66	160.66*±2.40	209.66*±3.28	303.33*±3.52	148.38±4.66	176.66*±4.80	272*±6.42	354*±8.08
TA mg L^{-1}	571.66±4.06	489.33±4.26	435.33*±3.66	414.00*±2.62	390.66±1.46	300.33*±5.06	310*±4.26	303.66*±2.42
Chloride mg L^{-1}	234.45±5.91	1386.58±11.08	2304.32*±27.77	2665.50* ±13.82	236.25±5.94	1590.52*±17.14	2456.86*±30.55	3629.89*±26.60

TE=Tannery effluent, Temp.= Temperature, TDS = Total dissolved solids, pH= potentia of hydrogenai, EC = Electrical conductivity, DO = Dissolve oxygen, BOD = Biological oxygen demand, COD = Chemical oxygen demand, TH = Total hardness, TA = Total alkalinity. Value are mean of three replicate SE and (*) significance of $p<0.05$.

Table 2. Effect of tannery effluent on haematological parameters in freshwater fish, *C. punctatus.*

Treatments	Exposure periods									
	15th days treatment					45th days treatment				
	Hb	RBC	WBC	ESR	PCV	Hb	RBC	WBC	ESR	PCV
Control	10.33±0.06	2.95±0.04	62.88±0.27	8.00± 0.57	42.33±1.20	10.33±1.20	2.95±0.04	62.88±0.27	8.00±0.57	42.33±1.20
5% TE	10.73±0.06	3.39*± 0.12	82.30*±0.15	5.00*± 0.57	45.00*±1.73	11.20*± 0.11	5.55*± 0.13	83.50*± 0.55	10.33± 0.88	35.33*±0.33
10% TE	10.96±0.08	4.63*± 0.19	87.63*± 0.45	13.00*±0.57	39.33± 0.33	8.40*± 0.11	2.62*± 0.02	136.00*±3.34	20.33*±1.85	31.00*±0.57
20% TE	9.16*±0.08	3.32*± 0.12	89.13*± 1.33	17.66*±0.88	34.66*±0.33	7.26*± 0.29	2.13*± 0.18	191.68*±1.87	24.00*±0.57	27.00*±1.01

TE= Tannery effluent, haemoglobin (Hb%), Red blood cells (RBC $x10^6/mm^3$), white blood cells, (WBC $x10^3/mm^3$), erythrocyte sedimentary rate (ESR mm/hr), pact cell volume (PCV%). Value are mean of three replicate SE and (*) significance of $p<0.05$.

Table 3. Effect of tannery effluent on blood indices in freshwater fish, *C. punctatus.*

Treatments	Exposure periods							
	15th day				45th days			
	CT/sec	MCV cμ	MCHC g %	MCH g %	CT/sec	MCV cμ	MCHC g %	MCH g %
Control	50.52± 0.03	143.68± 6.05	24.45± 0.82	35.04± 0.61	50.51± 0.01	143.68± 6.05	24.45± 0.82	35.04± 0.61
5% TE	52.53± 0.01	132.32*± 3.22	23.91± 0.79	31.66*± 0.95	52.55± 0.01	63.69*± 2.62	31.73*± 0.73	20.17*± 0.36
10% TE	56.35*± 0.04	78.56*± 4.05	27.26*± 0.18	23.22*± 0.85	57.23*± 0.06	79.16*± 2.60	27.10*± 0.13	32.01*± 0.27
20% TE	57.70*± 0.15	104.43*± 3.02	26.44*± 0.39	27.63*± 1.12	59.20*± 0.31	127.94*± 7.59	26.98*± 1.37	34.42± 2.10

TE= Tannery effluent, CT= clotting time, MCV= mean corpuscular volume, MCHC= mean corpuscular haemoglobin concentration, MCH= mean corpuscular haemoglobin . Value are mean of three replicate SE and (*) significance of $p<0.05$.

(PCV) was found increased in 5% TE at 15th day and also decreased significantly after 45th day of exposure periods. The PCV was found decreased for 10% TE and 20% TE significantly after 15th and 45thday of exposed fish (Table 2). The clotting time (CT) registered an increasing trend over and above controls for all exposure periods. The mean corpuscular volume (MCV) value, mean corpuscular haemoglobin (MCH) and mean corpuscular haeamoglobin concentration (MCHC) values decreased after 15th day 5% TE exposed fish, and MCHC values increased 45th days of exposure to 5%, 10% and 20% TE exposed fish in comparison with control value (Table 3). Short term exposure to pollutants and low concentration of heavy metals mostly induce an increase in these haematological indices to tolerate the stress condition. Similar results were reported by Singh and Singh, (1982) in fish *Mystus vittatus* exposed to copper and Zinc Sulphate. Haniffa and Porchelvi (1985) and Bhatkar (2010) reported erythrocytosis and increase in haemoglobin after exposure to stress condition. This can be due to impairment of gas exchange. According to Shah (2006) it may be due to the consequent stimulation of erythropoesis or compensatory erythropoesis in fish *Tinca tinca*.

In the present study when the freshwater fish *C. punctatus* was exposed to various concentration of tannery effluent and evaluated haematological toxicity. The Hb%, RBC count and PCV% was significantly increased after 15th day of exposure period, but decreased Hb%, RBC count and PCV% values after 45th day of exposure period leading to anemia. The anemic condition of fish resulting from an unusually low numbers of red blood cells or too little haemoglobin content in the red blood cells Kori-Siakepere *et al.* (2006). Bhagwant and Bhikajee (2000) reported that the prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in Oreochromis hybrid fish.

The fall in RBC count, Hb% and PCV%, in the fish *C. punctatus* upon treatment with both copper and chromium, shows the occurrence of acute anemia in toxic condition Mallesh *et al*. (2015). *Cirrhinus mrigala* exposed to hexavalent chromium. A decrease in the concentration of haemoglobin in the blood, which is usually caused by the effect of toxic metals on gills, as well as a decrease in oxygen also indicates anemia or confirms negative changes occurring in fish. Singh *et al*. (2008) revealed in *Cirrihna mrigala* and *Catla catla* fishes that decreased haemoglobin percent, RBC count and PCV percent values leading to anemia. They also suggested that the anemia might have lead to fall in the red blood cell, haemoglobin concentration and hematocrit values and anemia under metal stress may be due to blood cell injury and disturbed haemoglobin synthesis. Hematocrit is also used to determine the ratio of plasma to corpuscles in the blood (Larsson *et al*., 1985) and hemopoietic functioning of tissues (Kori-Siakepere *et al*., 2006). Ates *et al.* (2008) also found the reduction in erythrocyte, haemoglobin and hematocrit values in fish *Onchorynchus mykiss e*xposed to lead and copper which can be an indicator of anemia with the subsequent result of inhibition of erythropoietin in the hemopoietic organism.

The white blood corpuscles count was significantly increased in the experimental groups at the end of 15th and 45th day of exposure periods. Increased population of white blood corpuscles might indicates a stimulation of immune system to protect the fish against infections under hexavalent chromium toxicity. Singh, (1995) also found similar findings, increase in leucocytes count in *C. punctatus* exposed to chromium and copper, and in *Cyprinus carpio* exposed to hexavalent chromium (Shaheen, 2009). Leucocytes increased following copper exposure and were significantly higher in *Prochilodous scrofa* fish exposed to increased copper concentration (Mazon *et al.*, 2002). Similar trends were reported in the fish exposed to various pollutants in *C. punctatus* exposed to zinc (Tyagi and Srivastava, 2005).

Conclusion

Haematological characteristics are an important tool that can be used to understand as an effective and sensitive index to monitor physiological and pathological changes in fishes. Exposed of fish to tannery effluent showed a significant decrease in the haemoglobin (Hb) content (9.16± 0.08), red blood cells (3.32 ± 0.12), packed cell volume (34.66 ± 0.33) and mean corpuscular haemoglobin (MCH) values, whereas significant increase in the white blood cells (WBC), erythrocyte sedimentation rate (ESR) and clotting time was recorded with increase in exposure periods. Changes in haematological parameters depend on the aquatic biotope, fish species, age, and sexual maturity and health status. Haematological parameter are highly useful as the early warning for the process of xenobiotic and their effects, which make it possible to implement corrective measures before aquatic organisms and their communities suffer irreversible damage.

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