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Changes in hematological parameters of a freshwater fish, *Cyprinus carpio* (*communis*) exposed under pulp and paper mill effluent

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Abstract
Effect of different concentrations (0 % control, 3, 4, 5, 6 and 7%) of pulp and paper mill effluent selected on the basis of 96 hours LC_{50} test (8%), on certain blood parameters in *Cyprinus carpio* was evaluated after exposure for 7, 15 & 30 days. The hematological parameters studied include hemoglobin concentration, total erythrocytes count, total leucocytes count, packed cell volume, erythrocyte sedimentation rate, mean corpuscles volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Physico-chemical parameters i.e. pH, temperature, conductivity, total dissolved solids and dissolved Oxygen in diluted effluent were also recorded. Decreasing trend was observed in hemoglobin concentration, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration after an initial increase. Decreasing trend in total erythrocytes count and increasing trend in total leucocytes count, packed cell volume, erythrocyte sedimentation rate and mean corpuscles volume were recorded as concentration of effluent and duration of exposure increased. Minute fluctuations were observed in pH and temperature, and conductivity, total dissolved solids and dissolved oxygen of diluted effluent used during the study.

Keywords: *Cyprinus carpio*, conductivity, hematological parameters, LC_{50}, Pulp and paper mill effluent, total dissolved solids etc.

Introduction
The pulp and paper industry is one of the oldest industries from which the hazardous pollutants are continuously released in the water bodies. The release of waste water into the water bodies cause water pollution and is hazardous for aquatic flora and fauna (Balagopalan and Rajalekshmy, 1998; Rastogi, 2007) [4, 29]. Hematological parameters have been extensively used to determine effects of different pollutants and as effective means for the clinical diagnosis of fish physiology (Wepener, 1997; Bhagwant and Bhikajee, 2000) [44, 6]. Blood is considered as a suitable biomarker for detecting the damage caused by close interaction between circulatory system of the fish and the external environment (Singh *et al*., 2008) [36]. Hence, the study of the hematological parameters of the fishes in response to pollution stress has gained recognition as an useful tool for monitoring the level of pollutants in the environment (Sony *et al*., 2006) [38].
Hematological values are also widely used to determine systemic relationship and physiological adaptations including assessment of general health condition of fish (Sastry and Sachdeva, 1994) [31]. The present study was carried out to evaluate the effects of the effluents generated by pulp and paper industry on hematological parameters of a freshwater fish, *Cyprinus carpio* (Common carp), which is widely used in composite carp culture in the country and is also an important species for the fish culture system in hills of Uttarakhand.

Material and methods
Collection of Paper mill effluent
Waste water from the pulp and paper mill Lalkuan is discharged into a small rivulet called Gola Nala which after about 20 km merges into the Gola river. The waste water samples of pulp and paper mill effluent as above were collected from the rivulet, about ½ Km away from the source of discharge located at Ghanshyam Dham, Lalkuan (Nainital). On the basis of LC_{50} value i.e. 8%, six different concentrations (0% control, 3%, 4%, 5%, 6% and 7%) of effluent

were used to evaluate its effect on hematological parameters in the the yearlings of *C. carpio*.

Collection of experimental fish and design of experiment

The yearlings of *C. carpio* (15.10-20.60 cm in length and 132-153 gm in weight) used in this experiment were procured from Instructional Fish Farm, College of Fisheries, Pantnagar. Fish were acclimatized in 1500 litre capacity cemented tank, having clean and sterilized water (temperature- 26.5 ^{0}C±0.5, pH- 8.2±0.04, dissolved oxygen 6.8±0.5mg/l) for one month before transfer of fish to the experimental fiber tanks. During this period the fish were fed with conventional fish feed (rice polish and soya cake in1:1 ratio) at the rate of 4% of body weight.

The yearlings of *C. carpio*, after proper acclimation in the fiber tanks with daily feeding and replenishment of fresh water for one week, were exposed to six different concentrations i.e. 0% (control) and (3%, 4%, 5%, 6% and 7%) of the effluent in duplicate, for 30 days. Observations were made after 7, 15 and 30 days exposure to above concentration of effluent.

Hematological studies

Blood sample was collected in a microtube with EDTA from the caudal vein of the fish by cutting the peduncle after 7, 15 and 30 days of exposure to the effluent and hematological analyses were done by using standard methods.

Statistical analysis

One way analysis of variance (ANOVA) test using the statistical package and Least Significant Difference (LSD) test was used to compare the observations.

Results and Discussion

The water quality parameters like pH, temperature, conductivity, total dissolved solids and dissolved oxygen in the water containing different concentration of pulp and paper mill effluent in the experimental tanks recorded at 7,15 and 30 days interval are given in the table-1. Minute fluctuation in pH and temperature, an increase in conductivity in all tanks with different effluent concentrations and in total dissolved solids at 3, 4 and 7% effluent concentrations upto 15 days were recorded. The value of dissolved oxygen showed a decreasing trend with the increase in concentration of effluent and duration of exposure. Temperature and pH recorded during the experiment was within the optimum limit for the carps. Carps thrive well in water with a temperature range of 18.3 to 25 ºC and pH range of 7.5-8.5 (Jhingran, 1982) [17]. The high value of conductivity might have been because of high concentration of salts which decreased progressively with dilution, reducing the effective concentration of these ions in the water. Similar findings were also reported by Senthil *et al.*, (2001) [32] in sugar mill effluent. Increasing trend was recorded in the TDS of water in the experimental tanks. High amount of TDS might increase BOD and COD in the effluent (Chakarvarthi *et al.*, 1996) [10] due to which the dissolved oxygen may decrease with the increase of effluent concentration.

The hemoglobin percentage showed insignificant changes in the specimens exposed to 3 and 4% concentrations of effluent but significant changes were recorded in specimens treated with 5, 6 and 7% effluent concentrations (Fig. 1).

The values are statistically significant ($p< 0.05$) at 5, 6 and 7% effluent concentrations after 7, 15 and 30 days exposure. The maximum reduction was recorded at the 7% concentration after 30 days exposure. The concentration of hemoglobin decreased significantly in the blood of fish exposed to effluent with the increase in concentration and duration of exposure. Similar observations were reported by Witeska and Kosciuk, (2003) [43], which indicated that heavy metals such as Hg, Cd, Cr, Cu, Zn, As, Ni and Pb present in the effluent might alter the amount of hemoglobin by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more fragile and permeable and resulting in cell swelling, deformation and damage.

The changes in TEC after exposure to different concentrations of effluent for 7, 15 and 30 days are shown in fig-2. The variations in TEC are insignificant ($p > 0.05$) after 7 days exposure but are statistically significant ($p< 0.05$) after 15 and 30 days of exposure. The amount of reduction in TEC was higher in specimens treated with 7% effluent concentration for 30 days while lowest reduction was recorded at 3% effluent concentration after 7 days exposure. The results are in good agreement with earlier works that reported a significant decrease in RBC's (TEC), hemoglobin and packed cell volume of fresh water fish exposed to heavy metals (Vutkuru, 2005; Shalaby, 2001) [40, 34]. The same trend were also reported by Shakoori et al. (1994) [33] in grass carp, Hilmy *et al*., (1980) [15]; Beena and Viswaranjan, (1987) [5] in *C. carpio* exposed to HgCl2. Prolonged reductions in RBC's also lead to blood dyscrasis and degeneration of the erythrocytes which is described as a pathological condition in fishes exposed to toxicants (Buckley *et al*., 1976) [8].

The total leucocyte count (TLC) showed insignificant changes (Fig-3) in the specimens treated with 3 and 4% effluent concentrations of effluent but significant changes were recorded in specimens treated with 5, 6 and 7% effluent concentrations. The variations were significant in all treatments after 15 and 30 days except 7 days exposure at 3% effluent concentration. Increasing trend in TLC had been observed after exposure to effluent for (7, 15 and 30 days) in all treatments. The change in leucocyte count could be due to immunological reactions to produce antibody to cope up with the stress induced by pollutants. The increase in WBCs in this study could be correlated with the increase in antibody production to help in survival and recovery of fish exposed to sublethal concentration of toxicants present in test media. The similar observations were also reported in *Heteropneustes fossilis* due to manganese poisoning (Garg *et al.*, 1989) and copper sulphate and potassium dichromate induced toxicity in *Channa punctatus* (Singh, 1995) [35].

The pattern of changes in packed cell volume (PCV) after exposure to different concentrations of effluent for 7, 15 and 30 days are shown in fig-4. An increase in PCV was recorded with the increase in effluent concentration from 3 - 4% effluent but continuously decreasing trend was found as concentration and dilution increased. The reduction in mean of (PCV) values was reported due to decrease in circulating RBC number and also shrinkage of the cell (Ahmad *et al.*, 1995) [2]. The reduction in PCV may be correlated with reduced cell counts and hemoglobin concentration. The reduction in PCV has also been observed in *Colisa fasciatus* due to exposure to zinc sulphate (Mishra and Srivastava,1980) [21]; *H. fossilis* due to manganese (Garg and Tyagi, 1989) [13], *C. punctatus* caused by copper sulphate and potassium dichromate poisoning (Singh, 1995) [35], *H. fossilis* exposed to washing effluents (Gupta *et al*., 2001) [14] and *Oreochromis mossambicus* exposed to copper and zinc mixture (Nussy *et* *al*., 2002) [22].

Significant changes were found in the in erythrocyte sedimentation rate (Fig-5) of the specimens exposed to higher concentrations of effluent in comparison to control. Except decreasing trend in specimens treated with 5% effluent concentration for 15 days a continuous increasing trend in ESR value was recorded in all other groups. Increasing value of ESR showed an increase in the fibrinogen or serum globulins may also cause an increase in the ESR. Similar observations were reported in *Oreochromis mossambicus* after copper and zinc toxicity (Sampath *et al*., 1998) [30] and to copper induced toxicity in *Labeo rohita* (Sinha *et al*., 2000) [37]. After exposure to different concentrations of effluent for 7, 15 and 30 days MCV showed an increasing trend as concentration of effluent and duration of exposure increased (Fig-6). Mean Corpuscular Volume (MCV) increased with increasing concentrations of the effluent. The changes may be due to reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood. The MCV gives an indication of the status of the red blood cells that reflects an abnormal or normal cell division during erythropoiesis (Hodson *et al*., 1978). The increase in MCV indicates decrease in RBC count but increased erythrocyte size and a low percentage of immature red blood cells in the circulation after long-term exposure to the effluent. This different erythrocyte status related parameters are suggested about less release of microcytic cells in the circulation of the fish due to effluent toxicity.

Significant changes were also recorded in the mean corpuscular hemoglobin (MCH) after exposure to the effluent (Fig-7). After an initial increase in MCH of specimens exposed to 3, 4 and 5% effluent concentrations for 7, 15 and 30 days exposure and in specimens exposed to 6% effluent concentration for 15 days a decreasing trend were observed in specimens of remaining groups.

A decreasing trend was found in Mean corpuscular hemoglobin concentration MCHC of all groups in comparison to control (Fig-8). Hematological indices like hemoglobin, hematocrit and red blood cell count have been reported to indicate secondary responses of an organism to irritants (Rogers et al., 2003). Mean cell hemoglobin concentration was used to assess the amount of red cell swelling by decreased MCHC or shrinkage by increased MCHC (Milligan & Wood, 1982) [20]. The present study revealed that exposure of fish to effluent induced marked red cell shrinkage as reflected by increased MCHC. The fluctuation (up and down) in MCH clearly indicated that the concentration of hemoglobin in the red blood cells were lower in the exposed fish than in the control fish causing anaemic condition. The result of the toxicological studies carried out in the present work was in good agreement with Wade *et al*., 2002 [41], who reported the toxicity of cassava effluent on the Nile tilapia following 96 hrs exposure. In view of the above findings it is therefore recommended that hematological alterations may be effectively used as a potential biomarker of pulp and paper mill effluent toxicity especially at their lowest concentrations, at which either mortality was nil or very low to the freshwater fish in the field of environmental biomonitoring.

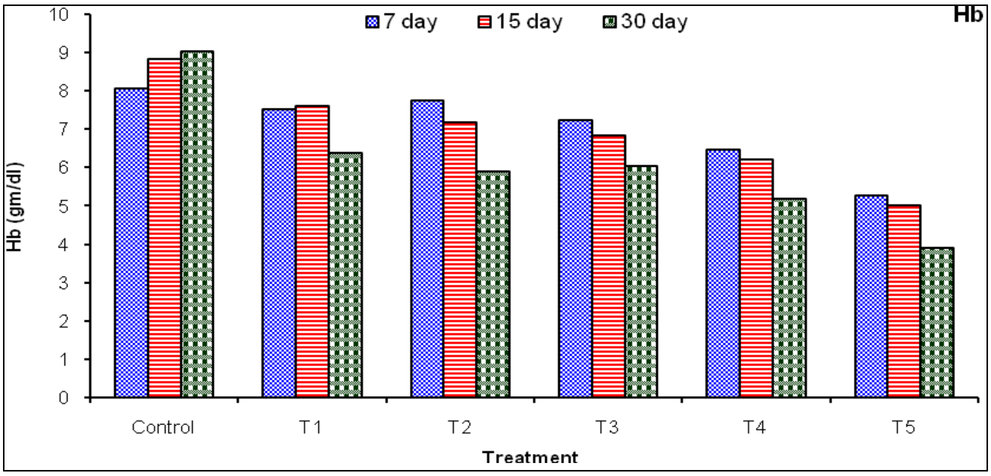

Fig 1: Changes in hemoglobin content

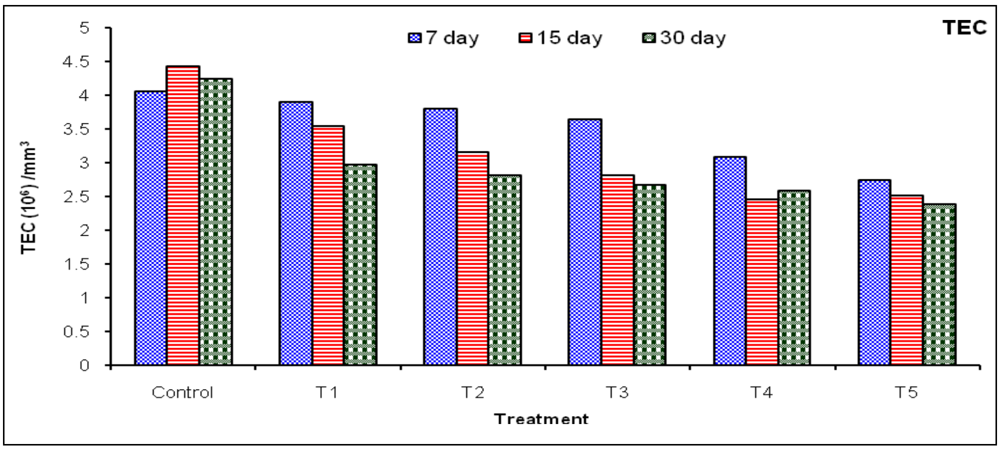

Fig 2: Changes in total erythrocyte count

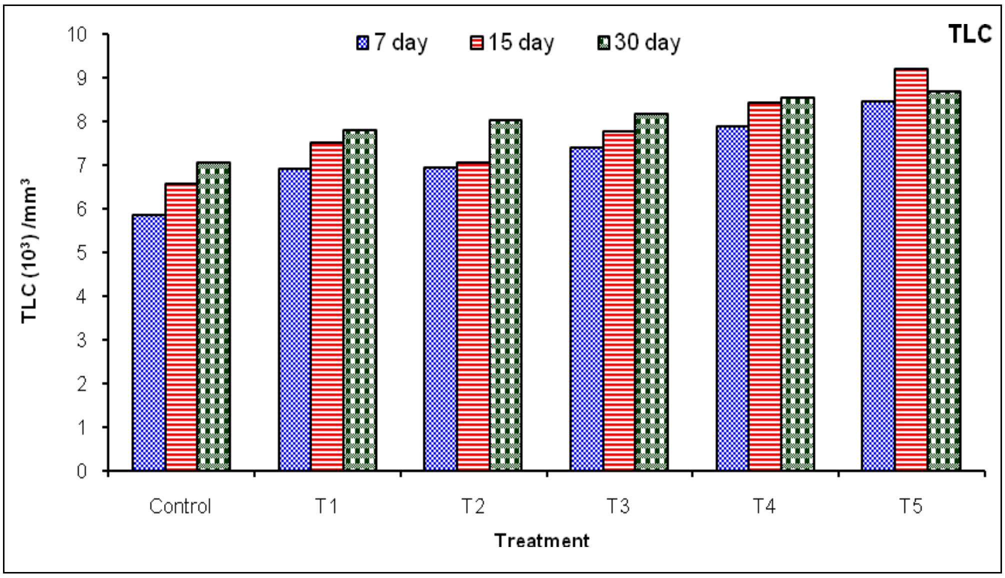

Fig 3: Changes in total leucocyte count

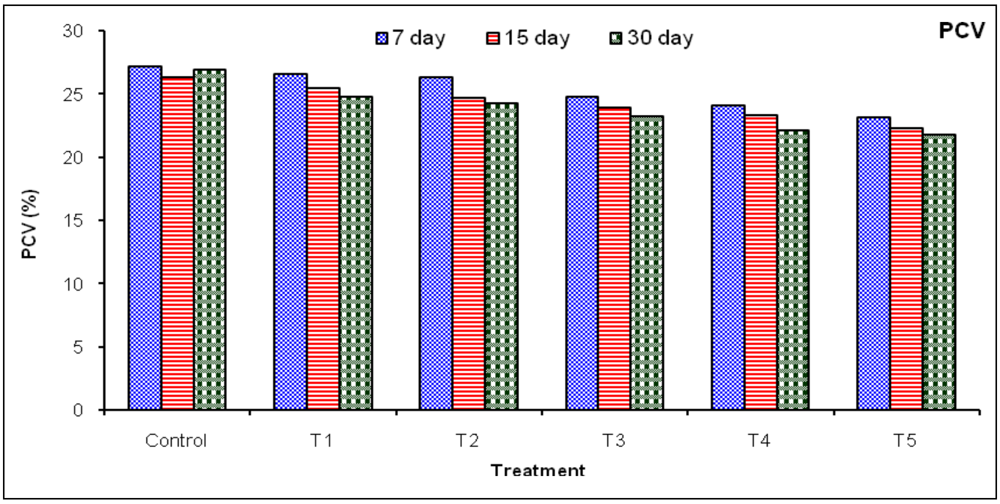

Fig 4: Changes in packed cell volume

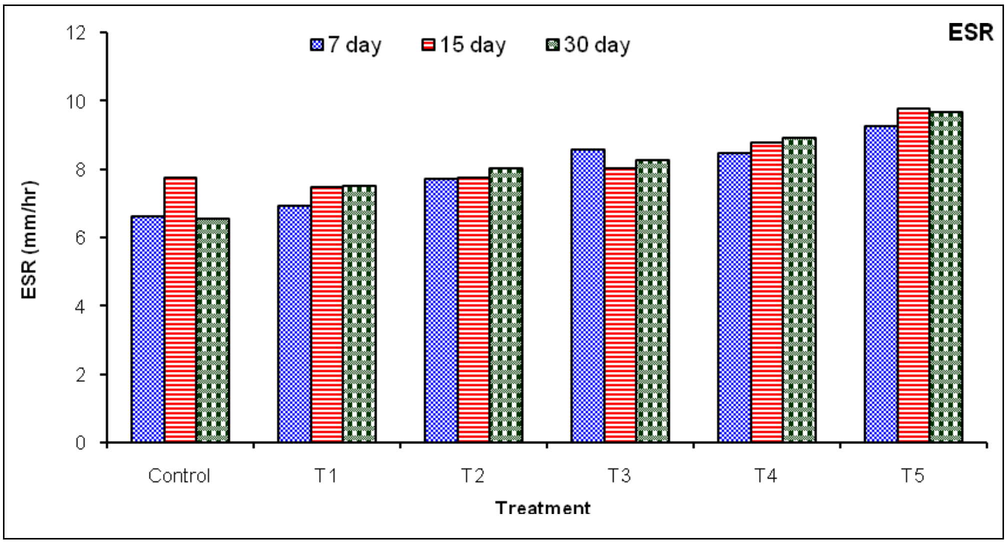

Fig 5: Changes in erythrocyte sedimentation rate

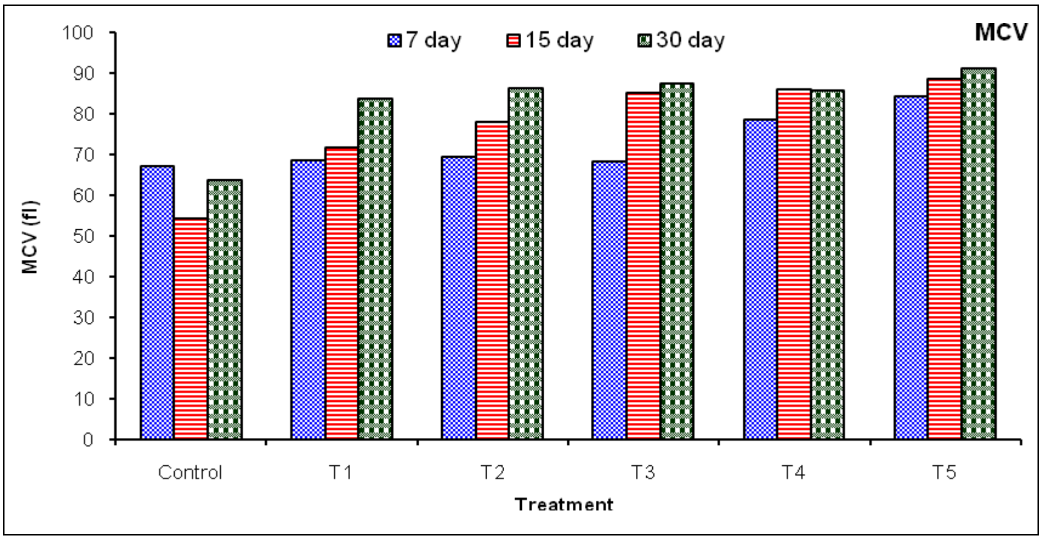

Fig 6: Changes in mean concentration volume

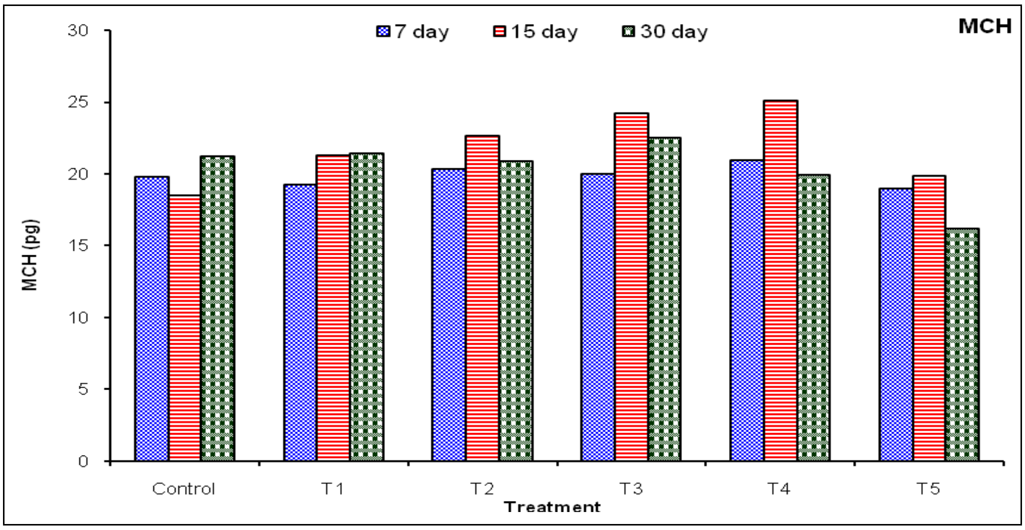

Fig 7: Changes in mean concentrated hemoglobin

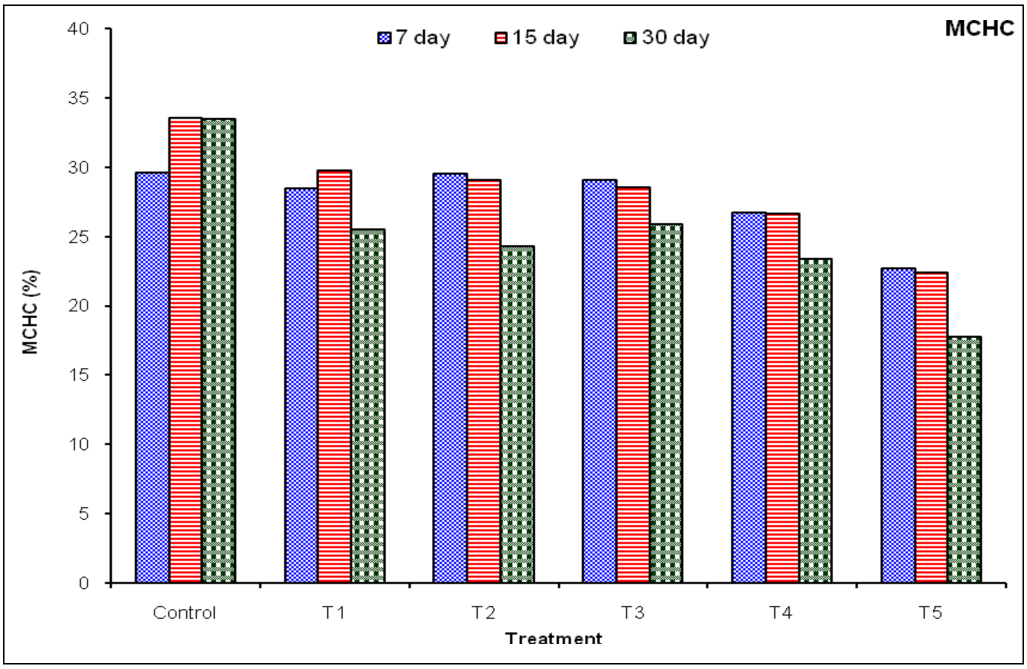

Fig 8: Changes in mean concentrated hemoglobin concentration

Table 1: Physicochemical parameters of water having different effluent concentrations in the experimental tanks

Parameters/ Concentrations (Mean ± SD)	Days	pH	Temperature (^{0}C)	Conductivity (µs/cm)	Total dissolved solids (mg/l)	DO (mg/l)
Control	7	7.56±0.84	32.06±0.37	142.16±0.20	15.49±0.25	5.75±0.29
	15	7.47±0.35	32.43±0.85	156.28±0.96	13.66±0.28	6.78±0.10
	30	7.40±0.75	30.06±0.56	154.99±0.22	14.49±0.30	5.14±0.82
3%	7	8.05±0.24	30.99±0.17	154.16±0.17	94.16±0.21	3.85±0.46
	15	7.28±0.56	30.48±0.22	242.49±0.68	244.83±0.11	4.60±0.70
	30	7.22±0.28	30.29±0.33	203.00±0.46	191.16±0.11	4.23±0.84
4%	7	7.21±0.16	29.29±0.47	244.33±0.12	239.16±0.44	4.21±0.10
	15	7.81±0.77	31.03±0.17	251.16±0.20	279.66±0.10	3.44±0.34
	30	7.21±0.16	29.18±0.11	226.66±0.57	253.16±0.35	3.56±0.62
5%	7	8.46±0.77	29.06±0.48	247.99±0.23	248.83±0.64	3.39±0.27
	15	7.56±0.40	30.21±0.17	253.33±0.47	230.00±0.32	3.46±0.34
	30	7.23±0.14	29.21±0.14	173.83±0.44	234.33±0.39	3.12±0.13
6%	7	7.69±0.19	28.75±0.21	255.33±0.14	223.49±0.32	3.07±0.50
	15	7.61±0.32	29.25±0.63	257.66±0.37	237.66±0.52	2.92±0.26
	30	7.31±0.28	29.23±0.28	205.83±0.56	239.83±0.45	3.62±0.10
7%	7	8.04±0.28	28.98±0.63	270.33±0.56	231.33±0.35	3.39±0.65
	15	7.72±0.29	30.43±0.14	269.66±0.61	253.83±0.43	3.44±0.51
	30	7.40±0.15	29.46±0.14	220.66±0.66	214.83±0.11	3.27±0.51

Values are Mean ± SD in the experimental tanks (n = 6)

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