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Distillery effluent induced alterations in the haematological profile of fingerlings of *Colisa fasciatus*

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Abstract

Present study deals with the impact of Gorakhpur distillery effluent at various concentration levels (5, 10 and 20%) on the blood profile of fingerling of, *Colisa fasciatus* after 30 days of exposure. Observations revealed that 5% of effluent concentration produced no significant alterations in various haematological parameters except for clotting time and immature erythrocytes. However, 10% of effluent concentration brought significant alterations in hematocrit as well as clotting time and 20% of effluent concentration produced highly significant (P<0.001) alteration in most of the haematological parameters except for hemoglobin percentage.

Key words

Colisa fasciatus, Distillery effluent, Erythrocytosis, Fingerlings, Haematology, Leucopenia

Introduction

India is the major producer of sugar in the world having about 579 sugar mills and 319 distilleries (Patil and Ghole, 2010). Apart from sugar and alcohol, sugar industries generate many by-products and waste materials (Raju and Manickan, 1997). Industrial effluents in developing countries are indiscriminately discharged into aquatic ecosystem and even into nearby fields without any pretreatment (Srivastava *et al*., 2007; Shukla and Shukla, 2012 a, b). Industrial effluents contain a variety of pollutants such as heavy metals, pesticides, detergents, organic and inorganic salts *etc.,* which create serious problem to the non-target fauna, especially fishes (Ramakrishnan *et al.,* 1999; Ramakritinan *et al.,* 2005). Haematological indices have different sensitivities to various environmental factors and chemicals. In fish, changes in haematological parameters and their peculiarities depend upon the concentration of pollutants and duration of exposure (Venkatramreddy *et al*., 2009).

The major effective parameters in distillery effluent are dissolved solids, chlorides, sulphates, less amount of highly toxic sulphides and a high percentage of dissolved organic as well as inorganic matters (Joshi, 1999: Ramakritinan *et al.,* 2005). In addition, high biological oxygen demand causes depletion of dissolved oxygen and proves deleterious to aquatic fauna. The distillery effluent is a potential water pollutant in two ways, first, its highly colorful nature may block sunlight and hence becomes detrimental to aquatic life and second, it has a high pollution load that results in eutrophication of water (Joshi, 1999; Ramakritinan *et al*., 2005). Thus, the untreated effluents pose a toxic impact on fish and aquatic fauna (Krishna and Prakash, 2010; Shukla and Shukla 2012 a, b). Hence, it becomes essential to reduce the toxic level of various pollutants in the distillery effluent before discharging it into adjacent water course or land. Various studies have been carried out on the toxicity of industrial effluent on various biochemical parameters in the fishes. (Kumar *et al.,* 1995; Pant and Adholeya, 2007),

however, deleterious impact of various concentrations of distillery effluent particularly on hematological profile is very scarce. Hence, present study was undertaken to observe the alterations in the hematological parameters of fingerling of *C. fasciatus,* exposed to distillery effluent.

Materials and Methods

Fingerling of *C. fasciatus* (weight 3.82 ± 0.32 gm) were procured from local lake for the study and were brought to the laboratory in an oxygen pack. They were acclimatized for 7 days under natural photoperiod in glass aquaria containing laboratory tap water having temperature 21.30 ± 1.64°C; pH 7.28 ± 0.22 ; hardness as $CaCO_3$ 128.30±6.24 mg l^{-1} and EC 128.30 µmhos cm^{-1}. They were fed with dried shrimp powder daily but feeding was allowed only after 5 days, during the experimental period.

The physico-chemical properties of distillery effluent was analyzed following standard methods of APHA (2005) (Table 1). Three groups each consisting of 25 fingerling fish were exposed to 5, 10 and 20% distillery effluent for 30 days. One group of 25 fingerling kept in tap water served as control.

Experimental media was aerated 2 to 3 hrs daily with stone diffusers. Twenty five fingerling were kept in each aquarium tank (90"×45" ×45") along with control. Distillery effluent was changed after 5 days during long term experimentation comprising of 30 days. Each fingerling was anesthethized with MS 222 (99.5%, pure Tricaine Methanesulfonate) blotted dry with absorbent paper. Fresh heparinised blood sample were collected from the caudal artery by transaction of caudal penduncle of the fingerling. The hematological assay was carried out following the methods outlined by Blaxhall and Daisley (1973) and Dacie and Levis (1984). Erythrocyte and leucocyte counts were calculated with Newbar haemocytometer. Haemoglobin was estimated using Haldane's haemoglobinometer. The blood samples were centrifuged at 6000 rpm for 10 min in haematocrit tubes for the estimation of haematocrit value. Mature as well as immature RBC's and small and large lymphocytes were examined following the methods outlined by Ghai (1986). Significant student 't' test was applied to data using the formula outlined by Fisher (1983).

Results and Discussion

The total erythrocytes count (6.04±0.22) and heamatocrit value (48.32±2.20%) were significantly elevated from control values in 10 and 20% effluent concentration after 30 days of exposure (Table 2).

The number of thrombocytes per thousand cells were also significantly depressed in 10% and 20% effluent concentration. Significant reduction was noticed in leucocytes count in 20% effluent concentration.

Haematological indices are very important parameters in the evaluation of physiological status under pollution stress. Alterations in the blood indices depend on the concentration and duration of exposure under stress. In the present study, increase in the number of circulating R.B.C. reveal more or less similar trend as found in certain teleost under metallic stress (Nussey *et al.*,1995). The increase in RBC count probably reflects hypoxic stress exposure as reported by Ramakritinan *et al.,* (2005) resulting in secondary polycythemia. Further, a mechanism by which fish might compensate for poor oxygen uptake during hypoxic condition is via, release of large number mature RBC ingeneral circulation (Alwan *et al.,*2009). The blood haematocrit value significantly increased as compared to control. Similar results were also reported by Polio and Hytterod (2003) in *Salmosalar* exposed to aluminium contaminated water for 3 weeks at slight basic pH. Significant decrease in WBC count in effluent stress may be a consequence of sharp decline in the number of lymphocytes and thrombocytes. Such changes may be due to the enhanced secretion of adrenocorticotrophic hormone secreted by adenohypophysis which results in higher blood-titers of corticosteroids which bring about the lysis of lymphocytes and thrombocytes (Alkahem,1995). Reports are also available on lymphopenia and leucopenia in fishes under various pollutional stress.The diminishing blood clotting time in the fingerlings of *C. fasciatus* after long

Table 1 : Physico-chemical characteristics of distillery effluent of GIDA, Gorakhpur distillery (U.P.)

Parameters	Units	Raw distillery effluent
Temperature	(°C)	32.5±2.2
pH		4.0-5.2
Oxygen	(mg l^{-1})	ND
COD	(mg l^{-1})	8000-12000
BOD	(mg l^{-1})	1500-1800
Total solids	(mg l^{-1})	3600-4200
Suspended solids	(mg l^{-1})	1800-2200
Volatile solids	(mg l^{-1})	6000-8000
total hardness	(mg l^{-1})	ND
Free CO_2	(mg l^{-1})	ND
Organic nitrogen	(mg l^{-1})	ND
Total nitrogen	(%)	0.80-1.20
Total phosphours	(%)	0.034-1.02
Potassium as K_2O	(mg l^{-1})	1.16-1.28
Sulphate as SO_4	(mg l^{-1})	3200-3800
Ferrous	(mg l^{-1})	260-340
Sulphide	(mg l^{-1})	160-240
Calcium as Ca^{++}	(mg l^{-1})	180-260
Chloride as Cl^{-}	(mg l^{-1})	500-680
Salinity	(ppt)	ND

ND = not determined; Values are mean of eight replicates ± SE

Table 2 : Effect of different concentrations of distillery effluent on hematological parameters of *Colisa fingerling*

Parameters	Control	5% effluent	% Change	10% effluent	% Change	20% effluent	% Charge
Erythrocytes ($X10^6$ mm^{-3})	6.04±0.22	6.38±0.24*	+5.62	6.78±0.32*	+12.25	6.98+0.26**	+15.56
Leucocytes ($X10^3$ mm^{-3})	60.62±3.26	58.36±2.22*	-3.72	52.22±3.14*	-13.85	46.72±3.48**	-22.92
Thrombocytes ($X10^3$ mm^{-3})	36.12±4.04	35.33±2.16*	- 2.18	32.48±3.10*	- 10.07	24.46±2.24**	-32.28
Mature erythrocytes	848.62±2.36	856.20±2.68*	+0.893	872.36±2.44**	+2.79	902.12±2.52***	+6.30
Immature erythrocytes	2.24±0.22	2.14±0.26**	- 4.46	1.68±0.18	- 25.00	1.52±0.14**	-32.14
Small lymphocytes	32.46±1.62	30.38±1.58*	-6.40	28.84±2.02*	-11.15	18.24±1.92*	-43.80
Large lymphocytes	1.88±0.32	1.72±0.28*	-8.51	1.54±0.26*	18.08	1.04±0.24***	-44.68
Thrombocytes	3.24±0.12	3.18±0.10*	-1.85	3.00±0.16*	-7.40	2.22±0.18***	-1.48
Haematocrit (%)	48.32±2.20	50.24±1.92*	+3.97	58.43±1.62***	+12.5	59.29±0.36***	+22.70
Hb (gm%)	12.04±1.62	11.98±1.68*	-0.49	11.74±1.24*	-2.49	11.08±102*	-7.97
Clotting time (sec)	28.26±0.08	30.32±0.26***	+7.28	32.16±0.28***	+13.80	36.12±0.34***	+27.81

* = insignificant; **= significant (P<0.05); *** = highly significant (<0.001)

term exposure under distillery effluent may have resulted mainly from a decrease in the number of circulating thrombocytes which regulate the function of blood clotting mechanism. Venkatramreddy *et al.* (2009) reported that clotting rate of blood in teleost is an apparent function of the number of thrombocytes present. The findings of this study confirms the fact that higher the number of thrombocytes, shorter is the blood clotting in teleost. Present observations also reveal that besides leucopenia, erythrocytosis and hypocoagulability of blood of fingerling may be an index of distillery effluent toxicity. Since, the juvenile stages of the fishes are highly sensitive hence, the present study on the blood profile under distillery effluent may be regarded as an index of sensitive toxicity.

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