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Impact of Distillery Effluent on Physiological Consequences in the Freshwater Teleost *Channa punctatus*

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In the present scenario there are 246 distilleries operational in India. An average size distillery with a capacity of 30,000 liters/day generates 15-16 liters of waste water for every liter of alcohol produced (Handa and Seth, 1990). This pollution load is equivalent to the daily domestic sewage generated in a town with one million population. Distillery waste is one of major pollutant of ecological concern. It constitutes one of the most complex industrial effluent, having high BOD and COD. Apart from that, large quantities of industrial effluents are discharged into the nearby water bodies. Fish and other aquatic organisms are seriously affected by prolonged exposure (Haniffa and Sundaravadhanam, 1984).

Discharge of effluents into freshwater systems deplete the dissolved oxygen content and, by interfering with respiratory metabolism cause heavy fish mortality (Quasim and Siddique, 1960; David and Ray, 1966; Venkataraman, 1966; Hingoroni *et al.* 1979). After fermentation the waste effluents are released in the environment without proper treatment in most cases and thus cause environmental pollution including changes in soil characteristics (Sahai *et al.* 1979). Pollution of aquatic environment by domestic waste and untreated or partially treated industrial effluents greatly contribute to massive kills of fish and other important aquatic biota (Kumari and Ramkumar, 1997). In the present study, attempts have been made to evaluate the toxicity of distillery effluent on the freshwater catfish Channa punctatus.

MATERIALS AND METHODS

Samples of distillery effluent were collected from M.M. Distillery Ltd., Lucknow and stored under refrigeration to minimize bacterial degradation. Sublethal concentrations viz. 25%, 50% and 100% were prepared using water from the same source. Physico-chemical characteristics of the distillery effluent (Table 1), and water used were analysed pH (6.9 ± 0.02), temperature (23.0 ± 2 ^{0}C), electrical conductivity (268.24 ± 10.59 μmho/cm), dissolved oxygen (8.8 ± 2.5 mg/L), alkalinity (90 ± 10 mg/L) and hardness (118 ± 12 mg/L) as $CaCO_3$ for control experiment using method (APHA,1998). Forty healthy fish (20 ± 2 g live weight) were recruited from the stock and divided into four groups, each group

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consisting of ten individuals. The first group were kept in water and served as control. The second, third and fourth group were also separately introduced in 25%, 50% and 100% effluent respectively. After the stipulated periods of treatment, the fish were dissected and tissues viz. liver, brain, kidney, muscle and blood), were isolated in ice-cold condition for further studies. The tissues were thoroughly washed in normal saline and homogenised (10%, w/v) for one min in triple distilled water (TDW) with Potter-Elvehjam homogeniser using a teflon-coated pestle under ice-cold condition. Lactic acid was estimated according to the method (Barker & Summerson 1967), protein (Lowry *et al.* 1951) using bovine serum albumin (BSA) as standard , glycogen (Montgomery 1957), and glucose (Nelson & Somoggi 1944).The blood samples collected by fish caudal puncture and diluted (1:200) with formal citrate solution for haematology (Dacie & Lewis 1977).

RESULTS AND DISCUSSION

The physico-chemical characteristics of distillery effluent exhibited high BOD, COD, total dissolved solids (TDS) and total suspended solids (Table 1).

Table 1. Physico-chemical characteristics of distillery effluent.

Factors	Values
Color	Dark Brown
Odour	Alcoholic
Temperature	34 ^{0}C
PH	6.4
Dissolved oxygen (DO)	2-3 mg/L
BOD	570 mg/L
COD	960 mg/L
Total suspended solids (TSS)	980 mg/L
Total dissolved solids (TDS)	700 mg/L
Total Nitrogen	40.2 mg/L
Phosphate	13.8 mg/L
Chloride	77 mg/L
Sulfate	34 mg/L
Total hardness	386 mg/L

The opercular activity of the fish was found to be increased when effluent concentrations increased beyond 50%. The fish showed erratic and rapid movement. Similar, changes of exposed fish to altered environment have been reported (Srinivaschar *et al.* 1972; Nagendran and Katre 1979). Hypoxic conditions in fishes are known to cause increase in opercular activity in order to compensate the decreased pO_2 level of the blood (Singh and Singh 1979). The surfacing activity increased with increasing concentrations of the effluent upto 50% and decreased there after. Excess mucous secretion on the body to prevent from the toxicant was noticed. Protein levels in different organs, viz. liver, brain, kidney and muscle, were found to be decreased in treated fish (Table 2). This is due to the degradation of the protein into free amino acid, which is used for different metabolic activities during stress condition (Anees, 1974). Hyperglycemia were noted in the exposed fish at 50% and 100% effluent concentrations (Table 3).

Table 2. Effect of distillery effluent on tissue protein (mg/g wet wt) in C. punctatus exposed for 96 hours.

Tissue	Control	25%	50%	100%
Liver	7.06 ± 2.06	7.02 ± 1.2	6.58 ± 1.47*	4.92 ± 0.96**
Brain	2.6 ± 0.02	2.50 ± 1.2	2.30 ± 0.47	2.00 ± 0.03*
Kidney	2.90 ± 0.05	2.85 ± 0.19	2.70 ± 1.5	2.65 ± 0.01*
Muscle	8.92 ± 1.66	8.91 ± 0.8	7.62 ± 1.47*	5.17 ± 0.55**

Each value represent the mean ± SE of five observations; * = P < 0.05; ** = P < 0.01; (Student's 't' test).

It may be suggested that stress conditions created by effluent stimulates the adrenal glands to liberate epinephrine which in turn would elevate the level of glucose. Similar observation has been established in some fishes under the environmental stress (Silbergeld 1974). It is well established that hyperglycemia induced in fish due to hypoxic condition (Bansal *et al.* 1979; Sastry and Siddique, 1982). Gopal *et al.* (1980) suggested that the disrupted carbohydrate metabolism may be due to the enhanced breakdown of liver and muscle glycogen (glycogenolysis). The lactic acid significantly increased in tissue viz. liver, brain, kidney, and muscle at 50% and 100% effluent concentrations (Table 4).

Table 3. Blood glucose and glycogen level in C. punctatus exposed to distillery effluent for 96 hours.

Tissue	Control	25%	50%	100%
Blood glucose (mg/dL)	71 ± 4.0	86 ± 5.0	108 ± 6.7*	112 ± 6.9**
Muscle (mg/100 mg wet wt.)	1.06 ± 0.27	1.05 ± 0.24	0.87 ± 0.29*	0.78 ± 0.02**
Liver (mg/100 mg wet wt.)	13.10 ± 0.42	13 ± 0.18	11.27 ± 0.29**	10.07 ± 0.34*

Each value represent the mean ± SE of five observations; * = P < 0. 05; ** = P < 0.01 (Student's 't' test).

Lactic acid was found to be accumulated in the tissues due to the toxic effect of effluent. Sharma and Gopal (1995) suggested that the accumulation of lactic acid

Table 4. Alterations in the level of lactic acid in tissues (mg /g wet wt.) of C. punctatus exposed to distillery effluent for 96 hours.

Tissue	Control	25%	50%	100%
Liver	1.25 ± 0.29	1.30 ± 0.23	2.43 ± 0.27***	2.53 ± 0.21***
Brain	0.59 ± 0.02	0.66 ± 0.05	0.73 ± 0.06*	0.79 ± 0.66*
Kidney	0.80 ± 0.08	0.82 ± 0.06	1.01 ± 0.07	1.07 ± 0.10*
Muscle	3.42 ± 0.28	3.70 ± 0.41*	4.86 ± 0.35**	4.92 ± 0.45**

Each value represent the mean ± SE of five observations; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ (Student's 't' test).

in different tissues indicates the inhibition of the Kreb's cycle and a shift from aerobic to anaerobic pathway due to stress.

The RBC and WBC count increased however, haemoglobin percentage decreased in exposed fish (Table 5). It seems that erythropoiesis has been accelerated to avoid anemic state leading to higher production of erythrocytes. Since young RBC of fish contains less haemoglobin, the small reduction of Hb and increase in MCH may be indication of altered turnover of circulatory erythrocytes (Harding 1978; Areechon and Plumb 1990; Lal *et al.* 1986; Kumar *et al.* 1999). The clotting time significantly increased in exposed fish at both the concentrations 50% and 100%. Blood coagulation mechanism was affected due to the toxic effect of effluent. Similar, observations was noticed in the teleost fish (Kumar *et al.* 1999).

Table 5. The effect of distillery effluent on the blood parameters of C. punctatus exposed for 96 hr.

Parameters	Control	25%	50%	100%
RBC (10^6x mm^3)	2.98 ± 0.24	2.90 ± 0.18	2.70 ± 0.23	3.58 ± 0.14**
WBC (10^3x mm^3)	11.5 ± 1.2	11.8 ± 0.8	11.4 ± 0.5	17.0 ± 1.8*
Hb %	23 ± 0.21	22.9 ± 0.68	22.7 ± 0.58	21.1 ± 0.7*
Clotting time (sec)	90.0 ± 0.01	95.7 ± 0.34	95.7 ± 0.44	96.1 ± 0.5**
MCH (10^6 Pg)	7.95 ± 0.13	7.89 ± 0.08	8.40 ± 0.15*	8.17 ± 0.07**

Each value represent the mean ± SE of five observations; * = $P < 0.05$ ** = $P < 0.01$ (Student's 't' test).

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