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Environmental Contamination and Toxicology

Bioassay of Distillery Effluent on Common Guppy, *Lebistes reticulatus* (Peter)

S. Kumar, S. S. Sahay, M. K. Sinha

University Department of Botany, Environmental Biology Laboratory, Bhagalpur University, Bhagalpur 812007, India

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Discharge of industrial effluents such as distillery, tannery, pulp and paper, textile mill effluents etc. have been of major concern in water pollution abatement (Johnson 1977; Haniffa and Selvan 1991). Among these, distilleries and sugar mill effluents are highly injurious for plant and animal life (Bilgrami 1991). These effluents without proper treatment are discharged into the nearby aguatic bodies in large quantities, causing massive destruction of aguatic flora and fauna by means of suspended solids (Verma and Dalela 1975), immediate depletion in the oxygen content (Blinski and Jones 1973; Johnson 1977), undesirable test and foul odor creating substances (Thomas 1973; Manivasakam 1977) and by interfering the respiratory metabolism of the animals (David and Ray 1966). Mass death of different varieties of fishes and other aquatic animals were also repoted in river Ganga and its tributaries during Dec.-Jan., 1992 due to discharge of distillery effluents of M/S Shiv Shankar Chemical Industries Limited, Raipura, Distt. Banka, Bihar (India). This industry prepares ethyl alcohol from molasses and discharges its effluents in Ganga near Ghogha (between Bhagalpur and Kahalgaon) through a nullah. Intensity of toxicity can be measured by means of bioassay studies and the extent of necessary dilution could be assessed. Our present knowledge of acute toxicity of industrial effluents are still limited to few (Stephen Maria Antony Raj et al. 1987). The present study was thus undertaken to evaluate the toxic effect of distillery effluents on common guppy, Lebistes reticulatus under laboratory conditions.

MATERIALS AND METHODS :

The samples of distillery effluent (both untreated and treated) were collected on June 4th, 1992 from the discharging tanks of M/S Shiv Shankar Chemical Industries Limited. The physcio-chemical and biological

Correspondence to: S. Kumar

characteristics of the collected samples were determined following the recommended procedures (APHA, 1989) For determining the toxicity of treated distillery effluents, live specimens of common guppy, Lebistes reticulatus (Peter) were procured from fish tank of University Department of Botany, Bhagalpur. These species were selected as test fish because of their easy availability and high survival rate under the laboratory conditions. The specimens were acclimatized to laboratory conditions in an aquarium of 10L capacity at 16-20°C for 10 days. The specimens were fed with plankton each day during acclimatization. Feeding was stopped 48-hr before the commencement of the experiment. Considering the original treated effluent as 100%, different concentrations of effluent such as 16%, 12%, 9%, 6.75%, 5.1%, 3.8% and 2.05% with distilled water were prepared (V/V). Bioassays to determine 24-hr, 48-hr, 72-hr and 96-hr LC_{50} were conducted (in experimental beaker, 500 ml capacity) employing the technique as described by American Public Health Association (1989). A group of 10 specimens of guppy each weighing (70 mg ± 6.2) and body size (2 cm ± 0.5) were introduced into individual experimental beaker containing 400 ml of test solution of treated effluents of different concentrations. Control was treated under identical conditions in distilled water with the same number of fish. The dead specimens were removed immediately when observed. No change in the medium was done during the run.

The behaviour of the guppy was studied during the experimental period and abnormal behaviours were recorded. The mortality of fishes during the different intervals was also recorded. The LC_{50} values were determined by probit analysis (Finney 1971), through natural graph and through the graph on log-probit scale. An average of LC_{50} value was considered. 95% confidence limits were also analysed (Finney 1971).

RESULTS AND DISCUSSION :

Physico-chemical and biological characteristics of untreated and treated distillery effluents are depicted in Table-1. Biological analysis revealed that phytoplankton and zooplankton density was nil in both the untreated and treated effluents. Highly acidic condition and high turbidity of untreated effluent might have caused the absence of phytoplankton and zooplankton. However, concentration of pathogenic bacteria was very high in both the effluents which may be attributed to high nutritional condition particularly glucose and fructose in the distillery effluent (Manivasakam 1987).

Table - 1 Analysis of Distillery Effluents of M/S Shiv Shankar Chemical Industries Limited, Raipura, Banka (India).

Characteristics	Untreated effluent	Treated effluent	Maximum recommended concentration	Authority
PHYSICO-CHEMICAL				
Colour	Dark brown	Dark brown	No colour	IS:2490
Odour	Odour of molasses (highly disagreeable)	Odour of molasses (highly disagreeable)	No odour	do
Atm. Temp. (°C)	41	41	-	-
Effluent Temp. (°C)	81	48 (discharging point)	40	do
Conductivity (u mho/cm)	5600	4500	-	-
Turbidity (NTU)	60000	50000	-	-
pH	5.0	8.0	5.5 - 9.0	do
TSS (mg/l)	61000	54600	600	IS:3306
TDS (mg/l)	38000	29000	2100	IS:2490
Dissolved oxygen (mg/l)	Nil	Nil	-	-
BOD_5 20°C (mg/l)	52000	30000	30	IS:2490
Chlorides (mg/l)	6700	4700	1000	IS:2490
Potassium (mg/l)	15725	13875	-	-
Sodium (mg/l)	227.5	225	60	IS:2296
BIOLOGICAL				
Phytoplankton	Nil	Nil	-	-
Zooplankton	Nil	Nil	-	-
TBD (x 10^7/l)	23.17	35.73	-	-
TC (x 10^6/100 ml)	0.017	2.4	-	-
E. coli (x10^6/100 ml)	1.6	> 2.4	-	-
C. perfringens (x10^6/100 ml)	0.92	> 2.4	-	-
Faecal coliform	Present	Present	-	-
Streptococci	do	do	-	-
Salmonella	do	do	-	-

- = Values not available; TSS = Total Suspended Solids;
TDS = Total Dissolved Solids; TC = Total Coliform;
TBD = Total Bacterial Density; *E. coli* = *Escherichia coli*
C. perfringens = *Clostridium perfringens*

Table-2 depicts the percentage concentration of effluents, number of test organisms and percentage mortality of test organisms at different time intervals. The table also presents the LC_{50} values, 95% confidence limits and slope of the probit line at different exposure periods. The graphs (Fig. 1 & 2) show the LC_{50} values at different exposure periods.

Effluent intoxication induced certain remarkable behavioural changes in the specimens of guppy. Just after introducing the test organisms into different concentrations of test solution, they showed fast erratic and jerky movement. The tendency of jerky movement was comparatively higher in higher concentrations of effluents (6.75% to 16% progressively). The specimens had the tendency to escape out from the test solutions due to unfavourable conditions created by the effluent. In 2.85% to 5.1% lower concentrations of test solutions, after initial increased swimming activity the specimens attained their normal activities like that of control. A few hours prior to death, the swimming activities ceased, operculum movement slowed and finally they lost their balance and settled to the bottom and died with their belly upwards, open mouth and curved bodies. Transparency and mucus were also noted in the dead bodies of specimens. The gills and kidneys of the specimens were also found to be swollen and damaged. The specimens in the control exhibited no symptom of stress.

The minimum death (10%) was recorded at minimum concentration 3.8% (at 96 - hr.). The minimum concentration which caused 100% mortality was recorded to be 16% at 24-hr, 12% at 72-hr. and 9% at 96-hr. intoxication period. No mortality was recorded in control even after 96-hr. Average LC_{50} values were calculated to be 5.319% at 96-hr., 5.902% at 72-hr., 8.111% at 48-hr and 8.974% at 24-hr exposure of test organisms into test solutions. 95% confidence limits were analysed to be 4.049 and 6.491% at 96-hr, 4.351 and 7.347% at 72-hr, 6.648 and 9.32% at 48-hr and 7.516 and 10.246% at 24-hr.

The present findings reveal that the specimens of guppy are adversely affected by the distillery effluents. The gill and kidney as important organs mainly concerned with the removal of waste material, are easily affected by the toxicants. Autopsy results also indicated the acute toxicity of distillery of distillery effluents on gills and other vital organs of guppy (Personal communication). In view of this, distillery effluents might prove to be an

Table - 2 Effect of Distillery Effluent on Small Fish Guppy, *Lebistes reticulatus* (Peter)

Concentration of effluent, % by volume	No. of Test organisms	Percentage Mortality of Test Organisms at 4hr	8hr	12hr	24hr	48hr	72hr	96hr
16	10	10	20	50	100	100	100	100
12	10	0	10	30	80	90	100	100
9	10	0	0	20	60	60	90	100
6.75	10	0	0	10	20	30	70	90
5.1	10	0	0	0	10	10	30	30
3.8	10	0	0	0	0	0	10	10
2.85	10	0	0	0	0	0	0	0
0 (Control)	10	0	0	0	0	0	0	0
LC_{50}, %, estimated from Natural graph					9.0	8.175	5.9	5.38
LC_{50}, %, estimated from graph on Log-probit Scale					9.042	8.175	5.957	5.308
LC_{50}, %, estimated by Probit analysis					8.881	7.984	5.848	5.27
Average LC_{50}, %					8.974	8.111	5.902	5.319
95 % Confidence limits					7.516	6.648	4.351	4.049
					10.246	9.32	7.347	6.491
Slope of probit line					5.667	6.654	6.949	10.258

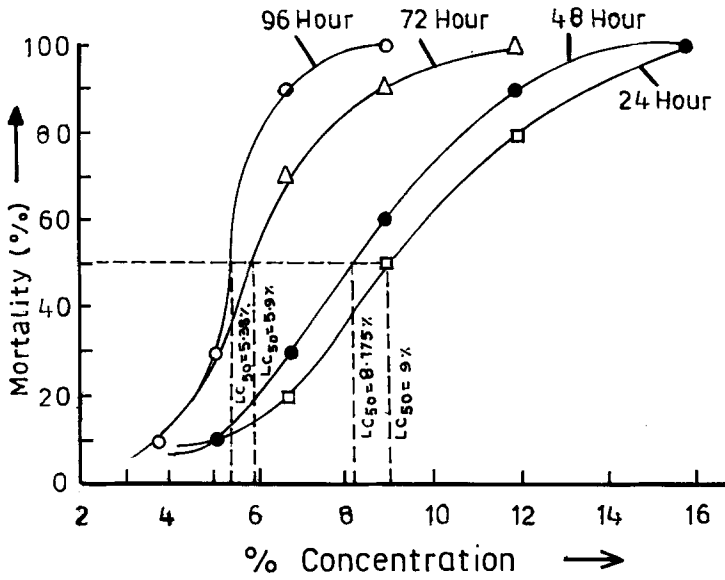

Fig.1. Natural graph showing LC_{50} values.

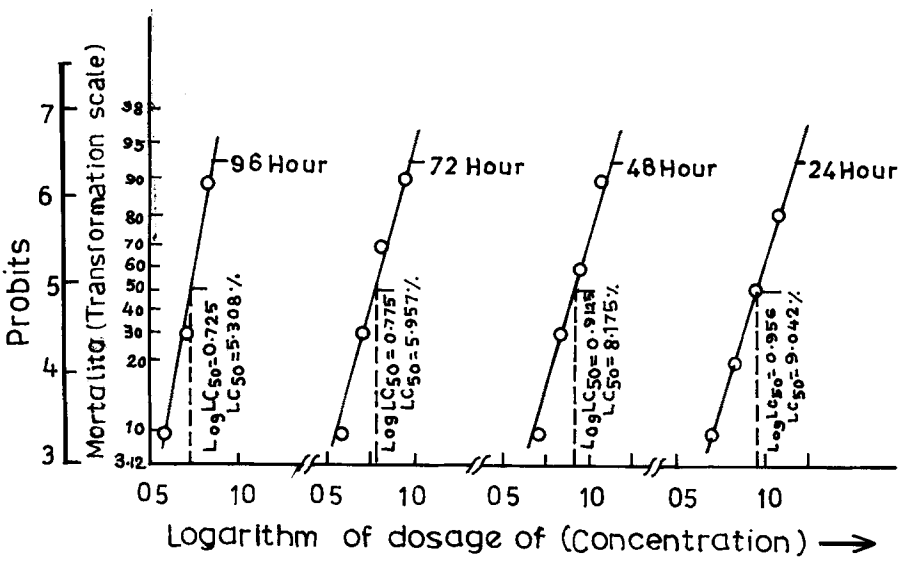

Fig. 2. Graph on log-probit scale showing LC_{50} values.

environmental hazard to the fishes and other aquatic biota. The mixing of such industrial effluents into aquatic system without proper treatment may affect the normal life of aquatic flora and fauna which may result in shrinkage of biodiversity. Bioaccumulation and biomagnification of toxic substances in the fishes may also pose a great health risk to human beings who eat them. Besides these, discharge of effluents through land cause seepage and subsequent ground water pollution. Further the obnoxious odour spreads to a few kilometers and it is a serious public health problem (Manivaskam 1987). A survey of the nearby village also confirms the fact where villagers were suffering from high respiratory problems, high abortion rate of women and cattle, vomiting, diarrohea, tuberculosis, various types of skin diseases etc.

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