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Toxicity testing of industrial effluents through freshwater fish *Lebistes reticulates* (Peters)

Suresh B. Zade[1], Shashikant R. Sitre[2], Shanta Satyanarayan[3] and Sanjeev S. Gandhewar[4]

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Abstract

Toxic effects of industrial effluents of an herbal pharmaceutical company to freshwater fish *Lebistes reticulatus* (Peters) were investigated during 96 hours static bioassay tests. LC_{50} values for raw, neutralized and physico-chemically treated industrial effluents of an herbal pharmaceutical manufacturing company were found out under standard laboratory conditions. The behavior of the fish is also recorded during experiments. It is evident from the toxicological studies that raw industrial effluent was much more toxic as compared to neutralized and physico- chemically treated effluent and toxicity was reduced by more than 30% with physico-chemical treatment alone. In order to further know the reduction in toxic effect the-physico-chemically treated industrial effluent was subjected to biological treatment by activated sludge system. The effluent after biological treatment revealed no toxic effect to *Lebistes reticulatus* for about a month pointing out that toxicity was fully reduced after biological treatment and the wastewater can be discharged into inland surface waters without harming the aquatic biota.

Keywords: *Acute toxicity* | *Lebistes reticulates* | *industrial effluent* | *Activated sludge*

For Correspondence:

[1]P.G. Department of Zoology, RTM Nagpur University, Nagpur, Maharashtra

[2]N.S. Science and Arts College, Bhadrawati, District – Chandrapur, Maharashtra

[3]National environmental engineering research Institute (NEERI), Nagpur, Maharashtra

[4]J.B. College of Science, Wardha, Maharashtra

Introduction

Today, herbal medicines are popular in India and some of the south East Asian Countries due to very low side effects, cultural acceptability and low cost (Rajashekharan, 2002). The herbal medicines are manufactured from plant materials like roots, stems, barks, gums, resins and certain chemicals like sugars, gums, organic solvents, gelatin, lactose, salts, special minerals, various heavy metals etc.

Herbal medicines generate a lot of waste water (effluent) during manufacturing processes which include washings of medicinal plants to

remove dust, dirt and microbial contaminants. Apart from general washing the wastewater is generated from different processes like crushing, mixing, extraction, distillation, fermentation, decoction and utensil washing based on market demand (Vanerkar et al., 2002). The organic, inorganic and toxic components present in the effluent have direct impact on aquatic organisms and it is very difficult to correlate the observed effect to specific pollutants as these effluents are complex in nature having highly fluctuating characteristics. Due to fluctuating market demands these medicines are prepared in batches and so the characteristics of effluent are continuously changing. These wastewaters have high Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and very high Suspended Solids (S.S.) with large amount of heavy metals, phenolics etc.

Materials and Methods

The static bioassay tests were performed at room temperature using *Lebistes reticulates* as test organism. The required fishes for experimentation purpose were procured from a local fish supplier of Nagpur City (Maharshtra State). The length of fish ranged between 1.5 to 2.0 cm. having a weight of 0.20 to 0.30 gms. approximately. Healthy fish were selected for toxicity testing experiments.

The industrial effluents were collected from a local herbal pharmaceutical manufacturing company from Nagpur and composite sample were collected and utilized for toxicity testing. The raw, neutralized and physico-chemically treated effluents were characterized as per Standard Methods (APHA, 1998) and their characteristics are presented in Table 1. For experimentation standard dilution water was prepared and used, Methods for measuring the toxicity of industrial effluents were followed as per standard protocols (Doudorof, 1951, Sprague, 1969, Rao *et al*.,1982),

The bioassay studies were carried out in glass aquaria of 10 Lit capacity using ten fishes in each container. Similar control was run parallel with dilution water only. Suitable concentrations were prepared and a range finding test and final confirmatory test were performed and readings on fish mortality were recorded at every 24 hours interval.

LC_{50} values were calculated as per Litchfield and Wilcoxon (1949). The results were subjected to statistical analysis. Based on LC_{50} values, 95% confidence limits, slope and regression (R^2) are calculated and presented in Table 2. Fish behaviour was also studied during the experiment of 96 hours duration.

Results and Discussion

The toxicity studies revealed that raw and neutralized wastewaters were much more toxic to *Lebistes* as compared to physico-chemically treated effluents. The fishes came to surface frequently due to distress in raw wastewater, with quick opercular movements and moving erratically. The distress to fishes is due to reduction in dissolved oxygen by raw wastewater because of high Biochemical Oxygen Demand (BOD) values and its acidic nature. Somewhat lessened effect was observed in other two wastewaters. Loss of balance of the fish was observed at higher concentration of wastewater. The LC_{50} values (Table 2) clearly show that raw wastewater was much more toxic and neutralized effluent toxicity was reduced somewhat due to marginal reduction of BOD, COD and Suspended Solids with slight removal of heavy metals.

It is thus inferred that this wastewater needs treatment to reduce toxicity and lime neutralization is effective in reducing the toxicity. So the wastewater was subjected to physicochemical treatment using conventional coagulants like ferric chloride, alum, lime and ferrous sulphate. The cationic polymer Oxyfloc-FL 11 gives good results at a dose of 300:0.25 mg/ liter at optimum combination for reduction in toxicity of the effluent. It was found that the toxicity was reduced by more than 30%. Still it is not safe to discharge the effluent as fishes do not survive for a long duration into it. So the effluent was subjected to further biological treatment by aerobic activated sludge system and toxicity tests were conducted.

It was confirmed from the toxicity tests that the effluent was completely safe for discharge after biological treatment as no fish mortality was observed for a period of one month. The completely treated effluent is now non toxic to fish as shown by healthy fish.

The herbal pharmaceuticals though age old and referred in our Vedic scripts did not receive much attention till recently but its importance was understood quite late and they gained popularity due to low side effects. Moreover being herbal in nature their toxicity testing aspect was not seriously considered so far and so they were not fully understood.

Today bioassays provide a safe tool for assessing the toxic effects of industrial wastewaters and chemicals and LC_{50} values play an important role in protecting the fish communities (Basak and Konar 1977). The results obtained from the bioassays will help the industries to take necessary pollution control measures before discharging effluents into natural waters which will help in minimizing the pollution and safeguarding our aquatic organisms.

Parameters	**Raw effluent**	**Neutralized effluent**	**Physic-chemically treated effluent**
Colour	Dark Yellow	Grey	Light Yellow
pH	3.6-4.00	6.9-7.50	6.6
Total acidity	1385	590	192
Total suspended solid	1800	1603	295
Total solids	4169	2536	538
BOD (5 days at 20°C)	6892	4820	1660
COD	12430	9600	3860
Sulphide as S^{-2}	28	20	09
Total Phosphates (PO_4^{-2})	136	98	42
Total Nitrogen	223	132	60
Oil and Grease	82	36	15
Heavy Metals			
Iron	34.40	15.85	8.20
Mercury	0.015	0.006	0.002
Copper	0.5790	0.312	0.222
Manganese	3.540	1.100	0.169
Zinc	0274	0.1624	0.100
Nickel	0.808	0.28	0.142
Lead	1.562	0.92	0.72
Chromium	0.2330	0.1204	0.0721
Selenium	0.211	0.131	0.095
Arsenic	N.D.	N.D.	N.D.

Table 1: Characteristics of Herbal Pharmaceutical Industrial Effluents

Note:

All the values are expressed in mg/Litre except colour and pH. The heavy metals were analyzed on atomic absorption spectrophotometer.

Time in Hrs	Parameter	Raw effluent	Neutralized effluent	Physico- chemically treated effluent
24 hrs	LC_{50} 95% Confidence Limit, Slope R^2	6.50 4.2 - 10.7 y= 0.0613x + 3.60 0.962	7.50 5.22-10.90 y=0.04812x+ 5.166 0.966	38.00 33.2-43.00 Y=0.057x + 35.40 0.989
48 hrs	LC_{50} 95% Confidence Limit, Slope R^2	6.00 3.05-11.50 Y= 0.0623x + 2.90 0.979	7.00 5.60-8.75 Y= 0.0413x + 4.936 0.974	37.50 33.8-42.2 Y= 0.055x + 35.08 0.984
72 hrs	LC_{50} 95% Confidence Limit, Slope R^2	4.90 2.70-8.80 Y= 0.0625x+ 1. 794 0.978	6.70 4.82-9.10 Y= 0.0405x+ 4.612 0.972	37.30 33-41.00 Y= 0.057x+ 34.6 0.2785
96 hrs	LC_{50} 95% Confidence Limit, Slope R^2	2.45-7.80 2.45-7.80 Y= 0.0558x+ 1.597 0.982	6.30 4.25-9.30 Y= 0.041 x+ 4.261 0.983	36.80 33.50-40.20 Y= 0.06Ix+ 33.90 0.975

Table 2: LC_{50} and Other Estimated Values of Acute Toxicity Tests for *Lebistes reticulates* (Peters) Exposed to Industrial Effluents (Herbal Pharmaceutical)

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