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Toxicity of rubber wood processing effluent exposed to fresh water fish *Poecilia reticulata*

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Abstract
Acute toxicity tests were performed over a period of 120 hours using different concentrations of effluent of treated rubber wood processing wastewater on freshwater fish, *Poecilia reticulata*. The LC_{50} values of the effluent exposed to *P. reticulata* for 24, 48, 72, 96 and 120 hours were 19.77, 19.55, 19.25, 18.99 and 18.75 ml/dl respectively. Mortality was observed in a dose dependant manner. Therefore, it is recommended that the effluent should be properly treated before being discharged into the environment.

Keywords: Fish, effluent, mortality, toxicity.

1. Introduction
Acute toxicity tests play an important role in assessing the effect of human activities on animals and such tests have wide applicability in evaluating the toxicities of pollutants in fish and other aquatic species [1]. The parameters of short-term (toxicity) exposure are the most common measures of toxicity [2, 3]. The importance of potential damage to aquatic ecology by effluent has been advocated and demonstrated [3], informing through various toxicity tests used in the management of water pollution as: to estimate environmental effect of waste, to compare the toxicity of different toxicants in animal, to regulate the amount of discharge pollutant [4].
The potential of rubber wood as a source of timber has already been recognized and an increasing volume of sawn rubber wood is being used for making furniture, parquet, flooring, wood based panels and indoor building components, as an alternative to other timber species [5]. The major problem in rubber wood utilization is its low durability and high susceptibility to insect and fungal infestations due to its high starch content and high humidity, which causes a severe sap stain and mould problem in rubber wood [6, 7]. Boron compounds like boric acid, borax or disodium octaborate tetrahydrate have proved their efficiency as wood preservatives for many years. These compounds are found to be highly toxic to fungi and insects (including termites) [8, 9].
The problems related to the process of rubber wood preservation are the use of toxic preservatives and generation of large amount of wood residues. The preservatives contain harmful compounds such as boric acid and mixture of borax pentahydrate resulting in high boron content in wastewater [10]. Boron is released into the environment mainly by industrial wastewater discharge [11]. Boron-contaminated water is unfit for human consumption and irrigation due to its toxicity for plants and animals above the threshold concentration [12]. The industrial wastes from wood processing industries generally contain high quantities of dissolved and suspended solids, inorganic chemicals and have high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), besides carrying toxic metals which cause deleterious effects on freshwater fish, when discharged into water bodies. The present study was carried out to evaluate the acute toxicity of the effluent exposed in different concentrations to the fish *Poecilia reticulata*.

2. Methodology
The rubber wood processing industrial effluent was collected in plastic cans from the discharge point of Borotik India Woodtech (P) Ltd, Shenbagaramanputhoor (8.245°N and 77.48°E) of Kanyakumari district and brought to the laboratory. The study period was carried out during the year 2009 The physico-chemical characteristics of the effluent were analyzed during experimentation [13].

The fresh water fish, P. *reticulata* Peters were procured from the local hatchery and brought to the laboratory in aerated tanks. *P. reticulata* was selected because of its easy availability and hardy nature and could be acclimatized very easily. The fish were acclimated for about 10 days before using them in the bioassay and feeding was stopped a day before the commencement of the experiment inorder to avoid faecal contamination. The toxic range of the effluent to the test fish was established by conducting pilot toxicity studies in diluted effluent at broad concentrations of (in ml/dl v/v) 1.0, 10.0, 15.0, 20.0 and 25.0. Based on the results of the pilot study, the narrow scale acute toxicity tests were designed exposing fish to different concentrations (18.0, 18.2, 18.4, 18.6, 18.8, 19.0, 19.2, 19.4, 19.6, 19.8, 20.0, 20.2 ml/dl) of the rubber wood processing effluent. A batch of 10 fishes was maintained in each tank. After every 12 h the polluted water was changed by the fresh solution of the same concentration without any disturbance of fishes by static method. The mortality of the fishes was recorded before each change of water. The nh LC_{50} values and their 95 percent confidence limits were calculated using probit analysis [14]. The ethological responses and mortality of fish were recorded after 12, 24, 48, 72, 96 and 120 h of exposure.

3. Results and Discussion

The pH of effluent sample was highly acidic, colourless and exhibited unpleasant smell. The concentrations of COD, BOD and boric acid were high (Table 1). The result of bioassay, however implicated the effluent to be unsafe to fishes.

A sharp decline in pH (3.25) and dissolved oxygen might have influenced stress of the fishes. The most pronounced stress of the fish caused by the effluent was the reduction in pH of the medium owing to the presence of boric acid. A change in pH following the discharge of an acid effluent would modify the toxicity of other poisons already present, while a reduction of pH below 4 would lead to mortality of many animals as a result of acidamia causing bicarbonate loss in the body fluid [15].

Dissolved oxygen is the measure of the degree of pollution by organic matter, the destruction of organic substances as well as the self-purification capacity of the water body [16]. In the present study, low level of DO (0.96±0.02 ml/l) was observed in the effluent. Low range of DO in polluted sites was probably due to the inflow of effluents and sewage which minimize the capacity of atmospheric oxygen getting dissolved in water [17].

The dissolved oxygen was found to decrease with increasing BOD and COD [18]. BOD is the measure of the oxygen required by microorganisms while breaking down organic matter. In the present study, BOD of the effluent was 840.33±2.51 mg/l while WHO guidelines of BOD value was 50 mg/l. Similar range of BOD of 959.66 mg/l was observed in tanning industrial effluent [19]. The high BOD levels are indications of the pollution strength of the waste waters. The high BOD and low oxygen content of effluent will affect the survival of the receiving water body [20].

Chemical oxygen demand is the measure of amount of oxygen required to breakdown both organic and inorganic matters. The COD level of the effluent was 1866±2.00 mg/l. This value was higher than that of WHO guideline value of 1000 mg/l [21]. COD of 1830±81.85 mg/l was observed in paper industry effluent [22]. High COD level indicate toxic state of the waste water along with the presence of biologically resistant organic substances [23].

In the present study the boron concentration as boric acid equivalent (BAE) in the effluent was 0.33± 0.016 g/l. Similar observations were made in ceramic waste water which contained boron of 15 mg/l [24]. Borate and boric acid are in equilibrium depending on the pH of the water. At an acidic pH, boron exists in solution mainly as undissociated boric acid, whereas at alkaline pH it is present as borate ions [25]. WHO guidelines declared 0.5 mg/l as the permitted maximum of boron concentration [26, 27] in drinking water. A concentration between 0.75 and 1.0 mg B/l is recommended as an environmentally acceptable limit for boron in aquatic systems [28].

The effects of pollutants are generally characterized on survival, reproduction or alteration in the animal. The physical, chemical and biological components of the environment play an important role in manifestation of biological response to pollutants. The toxicity of particular pollutants depend upon many factors such as animal weight [29], developmental stages [30] period of exposure and temperature, pH, hardness of water and dissolved content of the medium [31, 32].

Fishes exposed to lethal concentration of rubber wood processing effluent for a short term period were studied. In the present study, the LC_{50} values were calculated for different concentration of effluent for 24 to 120 h exposure period. The LC_{50} values of freshwater fish for 24, 48, 72, 96 and 120 hours were 19.77, 19.55, 19.25, 18.99 and 18.75 ml/dl respectively (Table-2). The fish, *P. reticulata* when exposed for 24 h exhibited abnormal behaviour. It is noticed that at this concentration a sudden terse was laid on the animal, which entailed in erratic swimming, convulsion, jerky movement and rapid opercular movement. The fishes tried to leap out the toxic medium and a thick mucous covering was observed over the whole body surface.

The resistance of the fish to different effluents was found reduced with increase in concentration and exposure time. The data indicate that decrease in LC_{50} concentration is associated with increase in duration of exposure. Survival rate of *Rasbora daniconius* gets decreased as the concentration of paper mill effluent increases [33]. Toxicity of the effluent mostly depends on the uptake of the effluent by the body. The rate of uptake is determined by the ratio of the permeability of body surface in contact with the medium to volume or weight of exposed animal and similar with relationship persists between the rate of metabolism and weight of animal [34]. In the present study LC_{50} values of effluent to *P. reticulata* exposed for 24 h and 96h was 19.77 and 18.99 ml/dl. The lowest 96 hours LC_{50} values for coho and Chinook salmon (*Oncorhynchus tshawytscha* Walbaum, 1792) were 447 and 566 mg/L, respectively [35]. Toxicity level is noted for low boron concentration of 0.01 mg/l in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) [36, 37] and highest concentration of about 88mg/l is observed for fathead minnow (*Pimephales promelas* Rafinesque, 1820) [38]. The lowest reported 96 hour LC_{50} values for coho salmon and rainbow trout were 304.1 mg/L [39] (MELP, 1996). Water containing 5600 mg/l of boron caused 50% mortality (LC_{50}) at 96 hour for mosquito fish (*Gambusia affinis* Baird and Girard, 1853) [40]. In both catfish and rainbow trout, embryonic mortality and teratogenesis increased in hardwater and boric acid produced higher frequencies than borax [36].

Survival rate of *P. reticulata* decreased with the increase in the concentration of rubberwood processing effluent (Fig 1). The exact cause of death is ill defined as there are number of channels. The death may be the result of severe physiological stress at cellular level. The physiological stress may be responsible for the death of fish [41]. It is also noticed that, the toxicity of the effluent is attributed synergistically to the physical factors of medium i.e. high COD and BOD values besides low pH and low dissolved oxygen (DO). Thus it is concluded that the effluent is not safe to non-target organisms like fishes. This type of study can be useful to compare the sensitivity of various species of aquatic animals and potency of effluent using LC_{50} values and to derive safe concentration. It is observed that the treated rubber wood processing effluent impart toxicity to *P. reticulata*.

Table 1: Physico-chemical characteristics of the effluent

S. NO	Parameters	Mean	Permissible Limit [26]
1.	pH	3.25±0.02	6.5-9.2
2.	Electrical conductivity (µs/cm), (25 °C)	0.32±0.02	250
3.	Turbidity (NTU)	13.66±1.52	5
4.	Total hardness as $CaCO_3$ (mg/l)	140±2.00	500
5.	Total alkalinity as $CaCO_3$ (mg/l)	110.33±1.53	-
6.	Sulphate (mg/l)	9.0±2.00	200
7.	Nitrate (mg/l)	0.9±0.1	-
8.	Total dissolved solids (mg/l)	276±2.00	500
9.	Total phosphorus (mg/l)	0.14±0.02	5
10.	D.O (ml/l)	0.96±0.02	
11.	B.O.D (mg/l)	840.33±2.51	50
12.	C.O.D (mg/l)	1866±2.00	1000
13.	Boron (as BAE) (g/l)	0.33±0.016	0.5 mg/l

Table 2: nhr LC_{50} and confidence intervals of rubber wood processing effluent to *P. reticulata*

S. No	Hours of exposure	LCL	LC_{50}	UCL
1	12	19.83	20.14	20.45
2	24	19.60	19.77	19.92
3	36	19.48	19.68	19.87
4	48	19.38	19.55	19.70
5	60	19.23	19.43	19.62
6	72	19.07	19.25	19.40
7	84	18.97	19.18	19.37
8	96	18.82	18.99	19.13
9	108	18.74	18.89	19.02
10	120	18.60	18.75	18.87

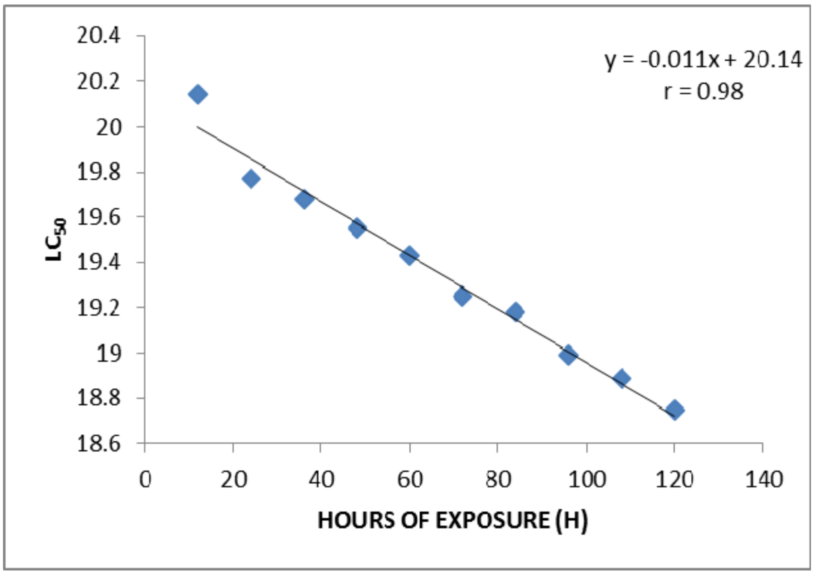

Fig 1: Relationship between hours of exposure and LC_{50}

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