CHAPTER 1

PHYSICO-CHEMICAL CHARACTERISTICS AND TOXICITY OF RUBBER WOOD PROCESSING EFFLUENT

1.1 INTRODUCTION

Rubber (*Hevea brasiliensis* Mull.Arg.) plantations were established by the Bangladesh forest Industries Development Corporation (BFIDC) and subsequently pursued by the private sector (Hossain, 2001). 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 (Killman and Hong, 2000).

The major problem in rubber wood utilization is its low durability. In natural state, rubber wood is highly susceptible 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. In order to maintain quality, freshly sawn timbers have to be protected with suitable preservatives, if they are not processed immediately.

Generally, the timber is momentarily immersed in a mixture of preservative solution containing fungicide and insecticide to protect the outer layer against infestation by wood borers and fungi. However, this procedure only provides temporary protection for the timber as it allows only shallow penetration of preservatives. The depth of preservative penetration will depend very much on the type and solution strength of wood preservatives used and the moisture content of the timber during the
treatment process (Tam and Daljeet, 1985; Salamah et al., 1987). When further processing of timber is carried out such as conversion into furniture components, the machinery process would remove the protective layer of preservative and expose fresh untreated surfaces. Hence, complete protection of timber is necessary and in-depth studies on the type of preservatives, appropriate solution strength and treatment process to achieve full preservative penetration are required (Zaitun et al., 1990).

Boric acid and Borax are the most common boron compounds that have found many application areas in the wood preservation industry (Hafizoglu et al., 1994). 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) (Lloyd, 1997; Grace and Yamamoto, 1997). Boron derived compounds are used for different purposes in nearly 250 industrial fields and important among them are areas such as fiber optics, glass, ceramics, nuclear industry, military armoured vehicles, electricity, electronics, computer, construction-cement, metallurgy, energy, automotive and textile (Guyaguler, 2001; Borokhov and Schubert, 2007).

Dip-diffusion process using boron-based compounds is the most simple and cheapest method of treating freshly sawn rubber wood, but it requires long treatment time to give complete preservative penetration throughout the cross-section of timber (Zaitun et al., 1990). Due to time constraints in sawn rubber wood treatment and industrial demand for a cost effective process, the vacuum-pressure impregnation method is the most viable option. Pressure treatment gives much better chemical loading, deeper and more uniform penetration of preservative in a comparatively
shorter period of time (Tan *et al.*, 1980 and 1983; Hong *et al.*, 1982 and 1987; Salamah *et al.*, 1987). After pressure treatment, the timber is kiln-dried to appropriate moisture content for further processing (Salamah and Dahlan, 2008).

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. The large amount of wood residues with the preservatives are dumped or thrown away in rural sites and burned illegally, causing soil contamination and generation of hazardous gases. Large quantities of wood wastes with the harmful preservatives are thrown away while the preservative itself is released as wastewater without any treatment, which causes various environmental problems (Ministry of Science, Technology and the Environment, 1999).

Boron is released into the environment mainly by industrial wastewater discharge (Howe, 1998., Coughlin, 1998). Boron-contaminated water is unfit for human consumption and irrigation due to its toxicity for plants and animals above the threshold concentration (Nable *et al.*, 1997).

The industrial wastes from wood processing industries generally contain high quantities of dissolved and suspended solids, inorganic chemicals and have high BOD and COD, besides carrying toxic metals which cause deleterious effects on freshwater fish, when discharged into water bodies. The release of boron-contaminated waste water is of concern due to its demonstrated phytotoxicity (Landauer, 1952), teratogenic (Birge and Black, 1977) reproductive (Saiki and May, 1988) and growth effects (Ohlendorf *et al.*, 1986) observed in laboratory studies and accumulation in the water.
sediments and biota of several aquatic and wetland ecosystems (Butterwick et al., 1989; Smith and Anders, 1989; Maier and Knight, 1991). Boric acid and borates are not metabolized beyond the boric acid structure B (OH)₃ because it needs high energy to break the boron-oxygen bond (Ku et al., 1991; Naghi and Samman, 1997; Murray, 1998). The permissible level of boron in drinking water is 0.5 mg/l (WHO, 1994; Weinthal et al., 2005).

Fish and other aquatic biota may be harmed by boric acid contaminated water. The preservative enters water bodies as a consequence of rain and leaching from the soil or carelessly get discharged directly into the aquatic ecosystem. Therefore fish and other aquatic organism are considered to be indicators of the ill-effects of non-target wood preservatives (Schulz and Martins, 2001 and Hamed et al., 2003). Effluent runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream (Helfrich et al., 1996 and Battaglin and Fairchild, 2002).

Fish populations are greatly affected by the boric acid containing effluent that exerts a direct effect on them by causing nausea, histological damage and physiological disturbances leading to death. The behavioural changes in fishes are sensitive indicators of toxicity (Sharma et al., 1993) and among aquatic fauna, fishes are highly sensitive to pollutants (Karuppasamy, 1979). Birge and Black (1977) reported that, concentrations of 100 to 300 mg B/l killed all species of aquatic vertebrates tested, produced high level of embryonic mortality and teratogenesis in hard water than in soft water while the larval mortality of fish and amphibians was higher in soft than in hard water. Boron compounds were more toxic to embryos and larvae than to adults. However, any accumulations of boron in groundwater through wider uses of B-containing cleansing agents might adversely affect aquatic organisms and other species of plants and animals, as in areas where natural boron deposits existed (EPA 1975). Long-term
monitoring of ground and surface waters for boron levels was required to combat boron toxicity.

The safe concentrations at which effluents could be introduced into the aquatic environment without subjecting fish to any sort of stress should be documented to safeguard them from the various deleterious effects of the toxicants. This would be possible by subjecting the fish to toxicant stress and conduct preliminary mortality studies using the effluent (Pathan et al., 2009).

The common bioassay used in such studies is the static bioassay for calculating the concentration of the effluents which causes the death of 50 percent of the exposed fish over a specific period of exposure. Acute toxic effects varied depending upon the strength of the toxicant and tolerance of the organism tested and the susceptibility and survival potential of organisms to a particular toxic substance could be measured (Craddock, 1977). The importance of experimental exposure of fish to industrial waste for predicting its potential damage to aquatic ecology has been advocated and demonstrated (Mala and Babu, 2006) and the informations obtained from various toxicity test could be used in the management of water pollution.

Increased basic demand of water for human, agricultural and industrial uses urged the environmentalists to determine the chemical, physical and biological characteristics of most of the natural water resources and negate the effects of pollution to enhance the availability of useable water (Regina and Nabi, 2003). The concentrations of biologically available boron and its potential for toxicological problems were expected to increase in future (Butterwick et al., 1989). The present study explored the effect of Rubber wood processing effluent on a common freshwater fish, *Poecilia reticulata* Peters, a highly tolerant indicator species, commonly used in toxicity bioassay studies.
1.2 MATERIALS AND METHODS

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 collected samples were stored at low temperature (20°C) for further analysis. Care was taken, not to stir the effluent which turned it turbid.

1.2.1 Physico-chemical characteristics of the effluent

The pH, Electrical conductivity, Turbidity, Total hardness as CaCO₃, Total alkalinity as CaCO₃, Sulphate, Nitrate, Total dissolved solids, Total phosphorus, D.O (ml/l), B.O.D and C.O.D were determined by standard methods (APHA, 1998).

1.2.2 Estimation of Boric Acid

1ml of the effluent was pipetted into a 500ml conical flask. 2-3gms of mannitol powder was added and shaken well to make slurry. Then it was titrated with N/10 sodium hydroxide using bromocresol purple as indicator. The end point was yellow to blue. The amount of boric acid can be calculated by the following formula.

\[ \text{ml of N/10 NaOH used} \times 6.184 = \ldots \text{g B/l of effluent.} \]

1.2.3 Procurement of test fish

The fresh water fish, *Poecilia reticulata* Peters belonging to the same stock were procured from the local hatchery and brought to the laboratory in large aerated tanks. *P. reticulata* was selected because of its easy availability and hardy nature and could be acclimatized very easily. In the laboratory, they were maintained in large FRP tanks, where a continuous and gentle flow of tap water was maintained. 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.

1.2.4 Probit analysis

The toxic range of the effluent to the test fish was established by conducting pilot toxicity studies in battery jars containing 2l of 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 in 100l FRP tanks. Three replicates were maintained for each concentration. A batch of 10 fishes was maintained in each tank. The nh LC\textsubscript{50} values and their 95 percent confidence limits were calculated using probit analysis [Finney, 1971]. The ethological responses and mortality of fish were recorded after 12, 24, 48, 72, 96 and 120 h of exposure.
1.3 RESULTS

1.3.1 Physico-Chemical Parameters

The effluent was clear and transparent from the point of effluent discharge but had a pungent odour. Acidic pH (3.25±0.02) was recorded at the effluent discharge point and very low electrical conductivity of 0.32±0.02 (\(\mu\)S/cm) and turbidity of 13.66±1.52 mg/l was recorded during the duration of the study. Dissolved oxygen, which is a critical parameter to the survival of biota showed reduced values (0.96 ± 0.02 ml/l). The mean concentrations of boron as boric acid equivalent (BAE) at the effluent discharge point was 0.33±0.25 g/l. High BOD of 840.33±2.51mg/l and COD of 1866±2.00 mg/l were recorded during the sampling period (Table 1.1).

1.3.2 Toxicity Studies

After 12h of exposure to effluent 10 percent mortality of *Poecilia reticulata* was recorded at an effluent concentration of 19.6 ml/dl (Table 1.2). Probit analyses of the toxicity response of *Poecilia reticulata* to effluent (Table 1.3 to 1.12) were used to find out the nhr LC\(_{50}\) values and their upper and lower confidence intervals (Table 1.13). The LC\(_{50}\) value for 12 h exposure was 20.149, the LCL, 19.833 and UCL, 20.45 ml/dl and X bar and Y bar values were 1.299 and 4.571 respectively (Table 1.3).

In *P.reticulata* exposed to effluent for 24h, 10 percent mortality was recorded in the concentration of 19.2 ml/dl and 20, 30, 60,100 percent mortalities at 19.4, 19.6, 20.2 ml/dl respectively. The 24h LC\(_{50}\) value was 19.77, the LCL, 19.61 and UCL, 19.93 ml/dl (Table 1.13). The 24h X bar was 1.294 and Y bar, 4.78 (Table 1.4).

In 36h exposure, 20 percent mortality was recorded at a concentration of 19.2 ml/dl, 50 percent mortality recorded at 19.8ml/dl and 80 percent mortalities at
concentration of 20 ml/dl. In this exposure, the X bar was 1.292; Y bar was 4.859 and b value, 87.369 (Table 1.5). The 36h LC₅₀ was 19.687 ml/dl (Table 1.13).

After 48h of exposure, the mortality recorded at 19.4, 19.6, 19.8 and 20.0 mg/dl were 40, 40, 60 and 100 percent respectively. For 48h exposure the X bar was 1.290, Y bar, 4.818 and b value, 136.473 (Table 1.6). The 48h LC₅₀ was 19.558 ml/dl (Table 1.13).

In 60h of exposure, 60 percent mortality was recorded at 19.4 and 19.6 mg/dl and 70 percent mortality at 19.8 ml/dl. In this exposure, the X bar was 1.286, Y bar, 4.810 and b value, 82.731 (Table 1.7). The 60h LC₅₀ was 19.437 ml/dl (Table 1.13).

In *P. reticulata* exposed to effluent for 72h, 70 percent mortality was recorded at 19.6 ml/dl; 100 percent in 19.8 ml/dl. Probit analysis of 72h response of *P. reticulata* recorded a X bar of 1.28, Y bar of 4.99, b value of 123.07 and LC₅₀ of 19.25 (Table 1.8 and Table 1.13).

After 84h of exposure, the mortalities recorded at a concentration of 19.2 ml/dl was 50 percent; 19.4 and 19.6 ml/dl was 70 percent. The LC₅₀ value for 84h was 19.183 mg/dl, the LCL, 18.97, the UCL, 19.37 ml/dl (Table 1.13). The 84h X bar value was 1.28 and Y bar 4.89 and b value, 77.22 (Table 1.9).

In 19.6 ml/dl concentration of effluent 100 percent mortality was recorded after 96h of exposure, 80 percent recorded in 19.4 ml/dl and 50 percent in 19.0 ml/dl. The 96h LC₅₀ value of effluent to *P. reticulata* was 18.99 ml/dl, X bar, 1.27, Y bar, 5.09 and b value, 127.265 (Table 1.10). The 96h LCL value was 18.82 and UCL, 19.13ml/dl (Table 1.13).
After 108h of exposure of *P. reticulata* to effluent, 60 percent mortality was recorded at 19.0 ml/dl; 80 percent mortality was recorded at 19.2 ml/dl and 90 percent mortality was recorded at 19.4 mg/dl (Table 1.11).

After 120h of exposure, 10 percent mortality was recorded at effluent concentration of 18.2ml/dl; 50 percent, 18.8ml/dl and 100 percent at a concentration of 19.4 ml/dl. Probit analysis of 120h response of *P. reticulata* recorded an X bar of 1.273; Y bar of 4.974 and b value of 124.236 (Table 1.12). The LC$_{50}$ value for 120h exposure was 18.753 ml/dl, the LCL, 18.603 and UCL, 18.879 ml/dl (Table 1.13).
1.4 DISCUSSION

Water quality parameters like dissolved oxygen, temperature, and ammonia are more likely to be involved with fish losses. Others, such as pH, alkalinity, hardness and clarity affect the fish, but usually are not directly toxic. Each water quality parameter interacts with and influences other parameters, sometimes in complex ways. The non-availability of oxygen created by high BOD and the fluctuating pH of the industrial effluent might affect the normal metabolism of fish.

In the present study, the negative impact of rubber wood processing effluent discharged was confirmed by the highest values in the physico-chemical parameters with a concomitant decrease in pH and DO at the effluent discharge point. pH is the measurement of intensity of acidity and alkalinity and measures the concentration of hydrogen ion in water. Variation in pH values of the effluent can affect the rate of biological reactions and survival of various microorganisms (Sankpal and Naikwade, 2012). The pH of the rubber wood processing effluent was 3.25±0.02. The low pH might be due to the presence of boric acid used as wood preservative. Similar observations were supported by Mohapatra et al. (2008) who reported a low pH of 0.9 in liquid crystal display manufacture (LCD) industrial waste water. Zinatizadeh et al. (2006) reported that fresh palm oil mill liquid effluent was acidic with pH ranging from 3.8 to 4.5.

When fish are exposed to a low pH, chloride cells in the gill tissue take up bicarbonate (HCO₃⁻) ion from the outside to neutralize the hydrogen (H+) ion flowing in the body. At this time, the loss of sodium (Na+) and chloride (Cl⁻) ions from the body fluids occurs, and plasma osmotic pressure decreases (Iwata et al., 1990). This
process is considered to be one of the major reasons why freshwater fish die under acidic conditions. Low pH values in water have been reported to affect aquatic life and alter toxicity of other pollutant in one form or the other (DWAF, 1996).

Electrical conductivity is a function of total dissolved solids (TDS) known as ions concentration, which determines the quality of water (Hem, 1989). In the present study, the electrical conductivity of the effluent was 0.32mS/cm which coincided with the findings of Srisuwan and Thongchai (2002) who reported electrical conductivity of 1.0mS/cm in Zinc rinsed electroplating industrial waste water. This value was less than WHO recommended threshold of 250 μS/cm, for effluents discharged into receiving water bodies.

Turbidity is the measure of suspended matter in water. The turbidity was 14 NTU in the rubber wood processing effluent. This trend is in agreement with Chukwu et al. (2012) who reported turbidity value of 4NTU in the effluent discharge point in Effurunctor river in Uhegheli South Lga of Delta State Nigeria. The maximum permissible levels of turbidity vary from 0-10 NTU (WRC, 2003). Also, the excessive turbidity in water can cause problem with water purification processes such as flocculation and filtration, which may increase the treatment cost (DWAF, 1996).

The hardness of water is not a chemical parameter but it indicated the water quality mainly in terms of Ca$^{2+}$ and Mg$^{2+}$ and expressed as CaCO$_3$ (Mohabansi et al., 2011). In the present study, the total hardness of the effluent was 140mg/l. It was found that total hardness of the rubber wood processing effluent was within the maximum permissible limit (500 mg/l) but was higher than the desirable limit for discharge (100 mg/l) according to WHO standard. These high levels of hardness can cause some
problems if the wastewater is to be reused and before reusing this wastewater, treatment for elimination of the hardness is essential.

In water, total dissolved solids (TDS) are composed mainly of carbonates, bicarbonates, chlorides, phosphates and nitrates of calcium, magnesium, potassium and manganese, organic matter salts and other particles. Total dissolved solids recorded for the rubber wood processing effluent was 276mg/l. The TDS value was within the desirable range (below 500 ppm) as standardized by World Health Organization (WHO).

Sulphates are common in natural waters, but levels can be increased from industrial contamination with sulphuric acid, bisulphate and aluminum sulphate used in water purification. Sulphate may undergo transformations to hydrogen sulphide depending largely upon the redox potential of water. This is also an important anion imparting hardness to the water (Mohabansi et al., 2011). The sulphate ion concentration in the rubberwood processing effluent was found to be 9 mg/l. It was within the WHO permissible limit (200 mg/l).

The lowest nitrate value of 1.0mg/l was recorded in the effluent. The nitrate level was within the WHO permissible level which is 10mg/l (Jakhrani et al., 2009). The introduction of phosphorus in form of phosphates in aquatic environment is a major cause of eutrophication. Phosphorus occurs naturally, almost solely as phosphate (Wagner, 1974; Lindsy et al., 1960). In the present study, lowest phosphate value of 0.14mg/l was recorded for the effluent. This result agreed with the findings of Rupani et al. (2010) who reported low level of phosphorus (0.9mg/l) in palm oil mill effluent.
The level of phosphate was lower than the WHO limit of 5mg/l for the discharge of wastewater into river.

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 (Kuzhali et al., 2012). In the present study, low level of DO (0.96±0.02 ml/l) was observed in the effluent. Similar observation were reported by Hughes and Morgan (1973); Fernandes et al. (1994), who reported that low level of DO has direct consequences for the survival of fish. 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 (Moitra and Bhattacharya, 1965).

The dissolved oxygen was found to decrease with increasing BOD and COD (Ravaniah et al., 2010). 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 were 50mg/l. This work agreed with the findings of Ayoub et al. (2011) who reported BOD of 959.66 mg/l in tanning industrial effluent. 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 (Yusuff and Sonobare, 2004).

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 (Dutta, 1999). Kuzhali et al., (2012) reported COD of 1830±81.85 mg/l in paper
industry effluent. High COD level indicate toxic state of the waste water along with the presence of biologically resistant organic substances (Sawyer and McCarty, 1978).

Recently boron has been classified as a pollutant of drinking water in national, European Union and international drinking water directives. The recent European Union drinking water directive defines an upper limit of 1 mgB/l. In the present study the boron concentration as boric acid equivalent (BAE) in the effluent was 0.33±0.016 g/l. Similar findings were made by Chong et al. (2009) who evaluated boron contamination in ceramic waste water containing boron of 15mg/l. 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 (Howe, 1998). WHO guidelines declared 0.5mg/l as the permitted maximum of boron concentration (WHO, 1998; WHO, 2006). Black et al. (1993) recommended a concentration between 0.75 and 1.0 mg B/l as an environmentally acceptable limit for boron in aquatic systems.

The physical, chemical and biological components of the environment play an important role in manifestation of biological response to pollutants. The effects of pollutants are generously characterized on survival, reproduction or growth due to physiological alteration in the animal. The toxicity of particular pollutants depend upon many factors such as animal weight (Pickering,1968) developmental stages (Kamaldeep and Joor,1975), period of exposure and temperature, pH, hardness of water and dissolved content of the medium (Mc Leese,1974; Brungs et al.,1977).

The fishes were commonly used for toxicity studies. In the present study, *Poecilia reticulata* Peters, a freshwater fish was selected to test the toxicity of
rubberwood processing effluent containing boric acid under laboratory condition. Guppies (P. reticulata) have the capacity of tolerating wide fluctuations in water (Stalin et al., 2008).

In the present study, LC$_{50}$ values of rubberwood processing effluent were calculated for 12 to 120 h. In P. reticulata exposed to effluent for 24 h, 10 percent mortality was recorded in the concentration of 19.2 ml/dl and 20, 30, 60, 100 percent mortalities at 19.4, 19.6, 20.2 ml/dl respectively. The resistance of the fish to different effluents was found reduced with increase in concentration and exposure time. Similar results were obtained by other investigators using other effluents (Ayotunde et al., 2011). The data indicate that decrease in LC$_{50}$ concentration is associated with increase in duration of exposure.

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 the 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 (Bertalonffy, 1957). In the present study LC$_{50}$ values of effluent to P. reticulata exposed for 24 h and 96 h was 19.778 and 18.990 ml/dl. Similar results were obtained by Hamilton and Buhl (1990) who reported the lowest 96 hours LC$_{50}$ values for coho and Chinook salmon (Oncorhynchus tshawytscha Walbaum, 1792) of 447 and 566 mg/L, respectively. Birge and Black (1977 and 1981) have consistently found low boron concentration toxicity levels in rainbow trout (Oncorhynchus mykiss Walbaum, 1792) at about 0.01mg/l and highest concentration toxicity levels for fathead minnow (Pimephales promelas Rafinesque, 1820) at about 88mg/l (Butterwick et al., 1989).
The lowest reported 96 hour LC$_{50}$ values for coho salmon and rainbow trout were 304.1 mg/L (MELP, 1996). Wallen et al. (1957) studied that water containing 5600 mg/l caused 50% mortality (LC$_{50}$) at 96 hour for mosquito fish (Gambusia affinis Baird and Girard, 1853). In both catfish and rainbow trout, embryonic mortality and teratogenesis increased in hardwater and boric acid produced higher frequencies than borax (Birge and Black, 1977).

Survival rate of *P. reticulata* decreased with the increase in the concentration of rubberwood processing effluent. The exact cause of death is ill defined as there are number of channels. The death might be the result of severe physiological stress at cellular level. The physiological stress may be responsible for the death of fish (Abel and Skidmore, 1975).

Oh et al. (1991) attributed the selective action of toxicants on fish species to inhibition of acetylcholinesterase, different detoxification and absorption patterns. The factors may be operative in the toxic reaction of the effluent on the experimental fish. The reactions were more pronounced at higher concentrations due to increased inhibition of acetylcholinesterase which eventually results in the death of the fish (Adedeji et al., 2008).

In the present study, the ethological responses of the fish, *P. reticulata* treated with wood processing effluent was found to depend on its concentration and duration of exposure time. Fish were exposed to different concentrations of wood processing effluent for different period of treatment. They show altered behavioural responses, increase in its operculum movement and decrease in bottom dwelling activity. The drastic subsequent increase and decrease in surface activity such as linear movement,
distance travelling, jumping, equilibrium, movement of fins were also observed. The movements of eyes were observed to be very slow. The operculum movement increased considerably with increase in the effluent concentration. The hypoxic condition in fish causes increase in the breathing rate, which in turn is caused by decreased efficiency in oxygen uptake. Similar observations were also reported for *Gasterosteus aculeatus* Linnaeus, 1758 (Jones, 1956) and *Mystus keletius* Valenciennes, 1840 (Singh and Singh, 1979; Stephen *et al*., 1987).

Altered respiratory and osmoregulatory functions due to non-specific changes/responses in gill tissue of fish subjected to hypoxic conditions is a reflection of the inability of most fish species to effectively adapt to such conditions (Scott and Rogers, 1980). These responses have been reported to involve systemic changes in which fish become incapable of adapting to stressors thereby resulting in adverse effects on the overall health of the fish, including their performance, growth, reproduction, disease resistance and behaviour (Barton, 2002).

Studies revealed that fish exposed to toxicants usually exhibited changes in opercular rate, displayed erratic, sudden and jerky swimming movements and different behavioural activities (Ayotunde *et al*., 2011). Similar findings in relation to respiration were also recorded by Sen *et al.* (1991) for the common guppy, *P. reticulata* for Ni and Cr exposure. Almost all these changes were considered to be sub-lethal concentration of pollutants (Maikai *et al*., 2008).

Indirect death could also result from changes in the physico-chemical conditions of their immediate external environment (Ayoola, 2008 and Olufayo, 2009). The respiratory distress may be due to decrease in the dissolved oxygen contents (Dede and
Kaglo, 2001). In the present study, death of fish due to decreased oxygen content could not be ruled out. The exact factor which causes mortality in fishes is hard to define as industrial effluents are mixtures of different poison (Mason, 1991). However, high BOD, COD, dissolved and suspended solids could be other reasons for fish mortality (Haniffa and Thanislaus, 1989). Warren (1977) reported that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration of the waterbody impairing respiration leading to asphyxiation.

In order to protect ecosystems in the environment, the introduction of treatment or waste reduction at source could be used in addition to the regulations in order to control levels of pollution agents being released into the environment. Changes in behaviour of fish, *Poecilia reticulata* due to Rubber Wood Processing effluent stress could be used as a biological indicator of pollution as biological early alarm system of Rubber Wood Processing effluent.