2.1 INTRODUCTION

Wood is a natural and traditional building material used for construction. It is relatively light and strong. Its appearance and properties differ a lot between species and even between trees, so there is a wide variety in material to choose from all kind of uses. Wood products are used indoors as well as for residential and other outdoor constructions. The weak point in wood usage is that the material is susceptible to degradation by termites, fungi, so that the good native qualities are challenged daily. To overcome shortcomings, wood is treated with different preservatives (Schultz et al., 2007).

For the protection of historic wood there are also repeatedly used preservatives on the basis of inorganic chemicals but there is not enough information about their possible corrosive effect on wood from the perspective of its long term exposure. The influence of inorganic chemicals on wood is observed mainly from the point of their application, fixation in wood and the mechanism of their protection effect (Winandy, 1995).

Wood preservatives, based on boron compounds are very effective for remedial treatment of wood as well. They can be applied by various techniques like spraying, dipping, injection. The most important advantage of boron based biocides is that they are soluble in water as well as in methanol and ethanol. Boron compounds are well
absorbed by the wood surface and then penetrate by diffusion deeper into the objects (Morrell, 1991).

Boric acid and sodium borate have been used as wood preservatives since 1930s (Murphy, 1990) and are valued for their protective capacity against decay fungi, wood boring insects, and as slightly higher retention levels, termites. In addition, boron-based preservatives are colourless, odourless noncorrosive, and non flammable (Hashim et al., 1994; Manning and Artur, 1995).

Although boron wood preservatives have many advantages (Laks, 1989 and Hyde, 1994), boron by itself doesnot adequately protect wood that is in ground contact because of the chemicals susceptibility to leaching (Williams and Amburgey, 1987; Nicholas et al., 1990). This is the main reason why boron cannot be applied for outdoor applications without additives or surface coatings that reduce boron leaching (Obanda et al., 2008).

Among a number of industrial activities that might pose harmful to aquatic ecosystems are industrial areas that handle and store wood materials such as log-yards, sawmills and other wood-based by-products, raise concerns due to potential release of organic and inorganic compounds (Libralato et al., 2007).

When preserved wood is used for in-water construction such as pilings, break walls, abutments, or other submerged or partially submerged structures, the potential exists for the toxic preservatives to leach from the wood. The early studies of preservative leaching tended to focus on the ability of a preservative to provide long-term protection. Preservative permanence in the wood is critical to efficacy, and
Leaching studies remain an integral part of research to evaluate potential new preservative systems. These types of leaching trials emphasize comparative evaluations of preservative formulations, and they typically use methods that accelerate leaching. More recently, emphasis has shifted to evaluate the environmental impact of wood preservatives. These later studies place greater emphasis on quantifying in-service leaching rates and measurement of environmental concentrations of leached preservative (Lebow et al., 2004).

Leaching is the term used to describe the gradual loss of preservative chemicals from wood through solution and removal in water. Leaching tests of wood preservatives provide a relative measure of the leaching loss from treated wood products in service (Harrow, 1959).

When investigating changes of preservatives compounds in wood, a possible identification and monitoring of physical aspects of leaching mechanism in wood is very difficult. The degree of leaching of active compound is strongly related to dimensions of tested samples or more precisely to the ratio surface-volume (Millerr, 1972; Leuritz, 1986). The remaining factor influencing the amount of leached components from impregnated wood depends on the degree of absorption and distribution of permeability and technological parameters of the impregnation process (Cockroft and Laidlaw, 1978).

The degree of leaching of preservative components is determined by the composition of the chemical agent, which directly influences the run of the fixing impregnant in wood. Temperature, wood moisture content and its duration also influence fixing (Lee et al., 1993).
The degree of protection achieved depends on the preservative used and the adequate penetration and retention of the chemicals. Once treated with chemical wood preservative, the timber represents a totally new habitat. These preservative themselves become the important abiotic factor determining the types of organisms that live in the wood. Some preservatives are effective than others, and some are more adaptable to certain use requirements. Not only different methods of treating wood are available, but also treatability varies among wood species. To obtain long-term effectiveness, adequate penetration and retention are needed for each wood species, chemical preservative and treatment method. Penetration is the amount of chemicals that get into wood through radial and longitudinal directions and retention is the amount of chemicals that is retained in the wood after treatment. Wood preservatives that are applied at recommended AWPA (American Wood Preservatives Association) retention levels and satisfactory penetration can greatly increase the life of wood structures. The annual replacement cost of treated wood in service is much less than that of wood without treatment (Forest Products Laboratory, 1999).

The concentration of leachate generated from wood residue is dependent on the diffusion rate and solubility of organic and inorganic wood residue constituents. Initial leaching of wood residue generally produces a relatively concentrated leachate and can be exacerbated by a high proportion of finely-divided wood-residue particles. Water soluble and biodegradable constituents are expected to predominate in leachate initially. The concentration of less soluble, reactive and biodegradable constituents and metabolites of decomposition processes will increase overtime and will ultimately reach a stabilized level. If wood residue is exposed to a high rate of water exchange the
concentration of leachate and the leaching rate are expected to be high initially, then
decline relatively rapidly. Pulses of leachate containing high concentrations of soluble
extractives can be generated when wood residue, stored in relatively dry conditions, is
exposed to heavy rainfall or snow melt (Samis et al., 1999).

Fungal and bacterial growths, which often accompany decomposition of wood
residue and leachate, can also affect fish and fish habitat. Such growths can restrict
water flow and oxygen exchange in a stream, affecting the survival of fish eggs and
developing alevins. Microbial growth can physically impair the movement of juveniles
fish in the water column. The microorganisms could even grow directly on the gills,
rendering the fish more susceptible to the water quality or habitat changes caused by
wood residue and its leachate (Samis et al., 1999).

According to Libralato et al. (2007) toxic effects of wood leachate on fishes and
invertebrates are related to naturally occurring wood-related compounds such as
aldehydes, phenols, terpenes and others. Furthermore, tannins, lignin, tropolones, resin
acids and toxic metals such as zinc, aluminium and copper are of greatest concern due
to their contribution to log-yards runoff toxicity (Bailey et al., 1999a and b; Hedmark
and Sholz, 2008). The impact of aquatic deposition of wood residue has been
investigated at a number of industrial log handing sites. Impacts include an overall
decrease in species diversity, abundance and biomass, particularly with regard to the
benthic invertebrate community. Most benthic organisms, particularly faunal
invertebrate species cannot survive in the anoxic conditions that are often created by
wood residue deposition (Pease, 1974). Suspension feeding bivalves, crustaceans and
polychaetes which rely on unobstructed access to the water column are negatively
affected by wood-residue deposits (Pease, 1974; Conlan and Ellis, 1979). The placement of wood residue in unstable area, such as within marine intertidal zones or near estuaries can result in the re-suspension of materials in the water column and subsequently, setting and smothering of benthic communities in adjacent areas.

Low levels of dissolved oxygen in the presence of toxicants, such as resin acids, may exacerbate the impact of those compounds on fish and other aquatic organisms (Kruzynski, 1979; Werner and Robinson, 1978; Davis, 1975b).

Hypoxic conditions generally induce metabolic stress that is typically associated with an increase in respiration rate, increased toxicant uptake and lowered resistance to toxicants (Davis, 1975b). Fish exposed to resin acids have been observed to exhibit symptoms of respiratory distress characterized by coughing and increased gill ventilation (Taylor et al., 1988). This response may be of critical importance in situations involving exposure to wood-residue leachate. When exposed to the hypoxic conditions that are typical of receiving waters containing leachate, fish may be stressed to the point that they succumb to that which would ordinary be a sublethal concentration of the toxicant (Kruzynski, 1979). A continuous layer of wood debris in aquatic habitat presents a significant obstacle to the settlement and recruitment of benthic organisms. The net result can be an area of significantly reduced fish productivity (Samis et al., 1999). In the present study, an attempt was made to analyse the leachability of the selected wood preservative (boric acid) and the phenolic compounds from the sawdust and to study the impact of the sawdust leachate on the selected fish.
2.2 MATERIALS AND METHODS

The boric acid treated rubber wood sawdust was collected in a plastic bag from the Borotik India Woodtech (P) Ltd, Kanyakumari and brought to the laboratory.

2.2.1 Leaching of Treated Saw Dust

The leaching test was conducted according to ENV1250 (1994) to reproduce the effect of weathering. The procedure involves immersing the saw dust in different water media (Tap water, Distilled water and Sea water), stirring with a magnetic stirrer (400-450 rpm) for 8h, followed by drying at 60°C for 16h. This cycle was repeated 4 times. Leachate samples were collected after 24, 48, 72, 96 and 120h of exposure. After each leaching cycle, the water was replaced with fresh water at a ratio of 10 volumes of water to 1 volume of wood sawdust. After the leaching process, the water was filtered using Whatman filter paper No.1 and leachability of boric acid in each water medium was analysed.

The reason that boron analysis was expressed in terms of BAE because boric acid was the borate used for the wood preservation.

2.2.2 Analysis of Boric Acid in Leachate

Boric acid present in the leachate was analysed by following the method prescribed in 1.2.2.

2.2.3 Estimation of total phenol content

The total phenolic contents in leachate were determined by Folin Ciocalteau Assay (Leamsomrong et al., 2009). Aliquots of standard gallic acid solution or 1 ml of test sample solution were mixed with 5ml of 0.2ml Folin Ciocalteau reagent and 4.0ml of 75g/l sodium carbonate. The blue colour product was measured at 765nm in Spectrophotometer after standing at room temperature for 30 minutes. Total phenol
content was expressed as mg gallic acid equivalent/g lettuce (mg GAE/g). The pH of the leachate was analyzed using a water quality analyzer (ELICO, India). Dissolved oxygen of the leachate was also analysed using Winkler’s method.

2.2.4 Lethal Toxicity Study

The filtered saw dust leachate collected in each water medium was used to find the lethal toxicity on fishes. *P. reticulata* were exposed to the sawdust leachate. Each concentration was maintained intriplicate. Behavioural abnormalities associated with exposure to different hours of the leachate were recorded. Mortality of the exposed fish was recorded after 24, 48, 72, 96,120h of exposure to the saw dust leachate. Ten healthy fish were introduced into each exposure system.mortality was recorded as a percentage of the total number of fish exposed.the dead fish were removed immediately to rule out contamination of the exposure system. Percent mortality for each leachate was calculated (Luoma *et al.*, 1983).

Percent mortality for each leachate was corrected for control mortality using the equation:

$$Net\ mortality = \frac{x - y}{x} \times 100$$

where $x$ = percent alive in the control and $y$ = percent alive in the treatments.

2.2.5 Data Analysis

Two way ANOVA was used to find the significant leachability of boric acid, phenolic compounds for different hours of exposure and different water media used.
2.3 RESULTS

2.3.1 Assayed Boric Acid Equivalent (BAE)

The leaching results were obtained by averaging four replications for different leaching time (Table 2.1). The majority of leaching from boric acid treated sawdust occurred upon initial exposure to the water medium. Assayed BAE (boric acid equivalent) is a function of leaching time for the selected three water medium (tap water, distilled water and sea water). There was a maximum leaching rate at the initial stage (24 hour leaching time) and the rate decreased as the leaching time increased. After 24 hour leach, the assayed BAE level of tap water was 0.046±0.02 g/l and after 96 hour leach, BAE level was 0.007±0.04 g/l. In distilled water, after 48 hour of leachability, BAE level was 0.025±0.01 g/l after 120 h, it was 0.006±0.02 g/l. In sea water after 24 h of exposure mean leachability of 0.047±0.01 g/l boric acid was observed and the corresponding value observed after 120 hr of exposure was 0.021±0.01 g/l. Two way ANOVA revealed that the leachability of boric acid was significant for different hours of exposure (F=16.63; df=4; P<0.05) but in the different water medium used the leachability of boric acid was not significant (Table 2.2 and Fig. 2.1).

2.3.2 Rate of leachability of total phenolic content

In the untreated sawdust leachate, after 24 h of leachability the rate of total phenolic content was 0.0147 mg/g/h in distilled water (untreated saw dust) and in tap water 0.022 mg/g/h, after 48 h, the sea water leachate contained 0.009 mg/g/h total phenol content (Table 2.4 and Fig. 2.2).

In boric acid treated saw dust leachate, after 24 h leach the rate of leachability of total phenol content was 0.027 mg/g/l in distilled water and the percent change was
66.6 which increased from the untreated sawdust in distilled water (Table 2.3). The corresponding value observed in tap water was 0.0241mg/g/l and the percent change was 7.25 which increased from the untreated sawdust. In tapwater, after 48 h of exposure, the rate of leachability was 0.0062mg/g/l and in sea water leachate contained 0.00666mg/g/l of total phenol content after 96h (Table 2.4). After 24h of leachability the total phenol content was 4.15±0.31mg/g which decreased to 0.95±0.02mg/g in sea water after 120 hours of exposure in boric acid treated saw dust (Table 2.3 and Fig. 2.3).

According to Two-way ANOVA (Table), the rate of leachability of phenolic compounds in different hours of exposure was highly significant at 5% significant level, both in untreated sawdust leachate (F=43.93; df=4; p<0.05) (Table 2.5) and treated sawdust leachate (F=408.41; df=4; p<0.05) (Table 2.6) but the interaction between the leachability of phenolic compounds and the water medium was significant in only untreated sawdust leachate (F = 6.66; df = 2; p < 0.05).

2.3.3 pH of the leachate

In untreated saw dust leachate the initial pH was 6.62± 0.0081 during 24h of exposure and the corresponding value observed after 120h was 7.612±0.05 in tap water. In distilled water the pH ranged from 5.98±0.041 during 24 hours of exposure to 7.38± 0.082 in 120 hours of exposure and in sea water the pH was 6.63±0.082 during 48 hr of exposure and 7.83±0.057 during 120h of exposure of untreated sawdust (Table 2.7).

In treated sawdust leachate, the pH of the leachate ranged from 5.48±0.054 to 6.15±0.082 in tap water and in distilled water it ranged from 5.43±0.047 (after 24 hours
of exposure) to 6.48±0.057 (after 120 hours of exposure). Low pH was noted in 24h leachate and then high pH of was observed during 120h of exposure (Table 2.7).

According to Two-way ANOVA, there is significant difference between the pH of leachate and the different hours of exposure in untreated sawdust leachate (F=33.17; df=4; p<0.05) (Table 2.8) and treated sawdust leachate (F=4.27; df=4; p<0.05) (Table 2.9) but the interaction between the pH and the water medium was significant only in untreated sawdust leachate (F=4.76; df=2; p<0.05)

### 2.3.4 Dissolved Oxygen

In untreated sawdust leachate, the dissolved oxygen was 1.11±0.033 ml/l in 96h leachate and it was 1.55±0.029 ml/l in120h leachate in tap water. In distilled water it ranged from 1.42±0.021 to 1.97±0.020ml/l. In treated sawdust leachate, the dissolved oxygen was 1.42±0.025ml/l in 96hr leachate in distilled water. The dissolved oxygen of 120hr leachate in sea water was below detection limit (BDL) (Table 2.10).

According to Two-way ANOVA (Table), there was significant difference between the dissolved oxygen and the different hours of exposure of the untreated sawdust (F=24.49109; df=4; p<0.05) and the interaction between the pH and the water medium was also significant (F=7.496279; df=2; p<0.05) (Table 2.11) in untreated sawdust leachate.

In boric acid treated sawdust leachate, there was significant difference between the dissolved oxygen of leachate and the different hours of exposure (F=3.91; df=4; p<0.05) and the interaction between the pH and the water medium was not significant (Table 2.12).
2.3.5 Toxicity Studies

The toxicity bioassay of the leachate was also carried out and after 24 hour exposure 100% mortality was recorded for the 24 hour leachate (Table 2.13).

Poecilia reticulata, Peters exposed to rubber wood saw dust leachate for a short-term exposure were studied in terms of general behavior and mortality. After 24h of exposure to 24 h leachate of distilled water, 100 percent mortality was recorded. (Table 2.13)

After 48h exposure to 48h leachate of distilled water, 60 percent mortality was recorded (Table 2.13). In 96h of exposure, 75 percent mortality was recorded.

When P. reticulata was exposed to 96h leachate fro 96h, 20 percent mortality was recorded. In 120h leachate, no mortality was recorded.

In 24h period of exposure to 24h leachate of tapwater, 100 percent mortality was recorded (Table 2.14). In 48h leachate, 50 percent mortality was recorded after 120h period of exposure. In 72h leachate, 25 percent mortality was recorded after 96h period of exposure. In 120h leachate, no mortality was observed till 120hr of exposure.
2.4 DISCUSSION

Wood residue deposits in fish habitat have resulted from forest-harvesting operations, log dumping, log sorting and wood processing. When deposited in water, residue will quickly absorb water and sink. The placement of wood residue into or adjacent to fish habitat can result in the physical alteration, disruption of the habitat. Wood waste fibres form sludge which decomposes through activities of microorganisms, depleting oxygen from the bottom waters and forming noxious gases. In addition, aquatic resources may be affected by the formation of leachate (Toews and Brownlee, 1981).

The leaching medium to which preservative treated wood is exposed is one of the most important factors for evaluation of the leaching of preservative components from wood, since its type and properties determine the amount of the components released from treated wood (Terzi et al., 2012).

In the present study, the majority of leaching from the treated sawdust occurred upon initial exposure to the water. Maximum of 0.047 g/l of boric acid leached out in sea water and distilled water while 0.046g/l in tapwater during the initial 24 hours of exposure. Similarly, Nair (2006) also reported the leachability of sodium tetraborate of 156.88mg/l from sodium tetraborate decahydrated (Na$_2$B$_8$O$_{13}$ .4H$_2$O) treated Hawaiian Albezia wood (Albezia falcataria) during 6 h of exposure. It was also supported by Lee and Wu (2007) who reported that there was a larger initial leaching rate (upto 24 hour leaching time) and the rate decreased as the leaching time increased. As the leaching time increased, the ratio of boric acid leachability decreased significantly, indicating that boron element leached out a higher rate. Two way ANOVA revealed that there was
significant leachability of boric acid for different hours of exposure ($F=16.62; \text{ df}=4; p<0.05$) but in the different water media used the leachability of boric acid was not significant.

Among the three different water media tested, the leachability was high in sea water and distilled water followed by tapwater. Lebow et al. (1999) found that sea water always caused more copper losses from CCA-treated wood than de-ionized water. These findings highly agree with the present study which shows that the boric acid losses were maximum in sea water and distilled water than tap water. In contrast, Kartal and Imamura (2004) indicated that more boron was released from the wood specimens with tap water leaching. The higher leaching rates are caused by the changes in wood structure during the weathering process (Townsend et al., 2005). Lebow and Kartal (1999) showed that arsenic loses were somewhat greater in distilled water than in sea water.

A number of factors may be involved with the temporal trends for metalloid (boric acid) leaching from boric acid sawdust. The increased surface area caused by chipping, resulted in the exposure of new surfaces for rapid leaching and weathering (Cockroft and Laidlaw, 1978; Hingston et al. (2002); Townsend et al., 2003a; Lebow et al., 2004; Townsend et al., 2004. Metalloid leachability increased with decreasing particle size (Townsend et al., 2004) and so small shavings and particles resulting from the chipping process may have also added to the higher metalloid levels in the leachate. Lloyd (1997) reported that the rate of boron loss get significantly reduced at retention below 0.4% (Boric acid Equivalent) BAE to a point where it appears to become negligible. According to Lloyd (1997), essentially 100% of the disodium octaborate
tetrahydrate was lost after only a few days of leaching, regardless of the treatment. 98.44% of disodium octaborate tetrahydrate was lost from Alaskan spruce (*Picea glauca* Moench Voss) by leaching and 97.50% of disodium octaborate tetrahydrate was lost from Hawaiian albezia (*Albezia falcataria* (L.)) by leaching. The results of the leaching study with boric acid solution indicate that the boron was easily leached from the samples, suggesting that minimal fixation occurred with this chemical (Nair, 2006). Compounds such as resin acids, fatty acids and phenols may be released from wood into the environment via the natural process of leaching. The major toxic component of leachate is phenol augmented by low pH and dissolved oxygen concentration (Taylor, 1994).

The toxicity of wood leachate is mostly attributed to phenolic compounds like tannins, lignins, tropolones, terpenes, zinc, low pH and other unidentified constituents (Peters *et al.*, 1976; Field *et al.*, 1988; Temmink *et al.*, 1989; Taylor *et al.*, 1996; Bailey *et al.*, 1999a; Taylor and Carmichael, 2003). Very few studies have revealed that water-soluble phenolic compounds are the main toxic constituents in leachate generated from aspen wood chips (Goudey and Taylor, 1992).

The results of the present study showed that there is leachability of phenolic compounds from untreated and boric acid treated sawdust in the selected water media (distilled water, tap water, sea water). Maximum phenolic content of 4.15±0.13mg/g and 3±0.82 mg/g leached from treated sawdust and untreated saw dust respectively in the sea water medium. This study coincides with the work of Taylor and Goudey (1992) who reported high concentration of phenol upto 83mg/l in aspenwood leachate. Taylor and Carmichael (2003) also reported various levels of phenolic compounds in
the range of 2-27 mg/L in aspen wood (*Populus tremuloides* Michx.) leachate. Laboratory studies demonstrated that phenol is produced from the degradation of parahydroxy benzoic acid (PHBA) that is associated with lignins and lignin precursors in certain hardwoods including poplar, willow, cherry and cranberry (Shariff *et al*., 1989). Two-way ANOVA results indicated that the leachability of phenolic compounds in different hours of exposure was highly significant at 5% significant level, both in untreated wood (F=43.92; df=4; p<0.05) and treated wood (F=408.41; df=4; p<0.05) but the interaction between the leachability of phenolic compounds and the water medium was significant untreated saw dust leachate (F=6.66; df=2; p<0.05).

The discharge of wood-residue leachate may result in an increase in acidity in the receiving waters, depending on the volume and concentration of the leachate and the available dilution. The pH of leachate significantly influences its toxicity (Mcleay *et al*., 1979; Cameron, 1982; Haygreen and Bowyer, 1989).

Wood residue leachate is acidic due to the reaction of carbon dioxide (generated from biological decomposition) with water to produce carbonic acid (Peters *et al*., 1976). Low pH of the leachate observed during the initial period of exposure may be due to the leachability of boric acid from the treated sawdust. Peters *et al*. (1976) reported that leaching of plicatic acid from western red cedar heartwood was responsible for the sharp drop in leachate pH during the initial leaching phase.

In the present study, the initial pH of the leachate was acidic but increased to neutral in the water media used. In the untreated sawdust, the pH of leachate in tapwater was 6.62 ± 0.081 and 7.61 ± 0.05 during 24 h and 120 h of exposure respectively whereas in distilled water and sea water the pH raised from 5.98± 0.041 to
7.38±0.082 and 6.02±0.05 to 7.83±0.057 respectively. In the treated sawdust, the pH of
the leachate in tapwater, distilled water and sea water was 5.48±0.054, 5.43±0.096 and
5.34±0.047 respectively during 24 hour period of exposure. There was a significant
difference between the pH of the leachate and the different hours of exposure of the
sawdust both in untreated wood (F=33.16; df=4; p<0.05) and treated wood (F=4.27;
df=4; p<0.05) but the interaction between the pH and the water medium was significant
in untreated saw dust leachate (Two-way ANOVA).

The increase in acidity overtime in the leachates was probably related to
continued release of organic acids from the logs (Goudey and Taylor, 1992). Similar
observations were made by Peters et al. (1976) who reported acidic pH of 3 to 6.5 in
western red cedar heartwood leachate.

The pH range of the undiluted leachate samples may themselves prove toxic to
aquatic life. Acidic leachates have varying effects on aquatic life depending on the
characteristics of the receiving waters. pH can change the ionic form of many chemical
and this can affect toxicity (Haygreen and Bowyer, 1989).

The presence of wood-residue leachate in fish habitat can result in localized
depletion of dissolved oxygen and therefore may influence the distribution of fish and
other aquatic organisms. Habitat shifts and other changes in fish behavior occurring in
response to sublethal concentrations of leachate may be associated with a variety of
biophysical factors, such as reduced food availability and increased risk of predation
that ultimately determine the fitness and survival of fish. In general, most fish avoid
aquatic habitats that are characterized by low levels of dissolved oxygen
(Kramer, 1987).
Most wood leachates have dissolved organic material concentrations (Taylor, 1994) which may be estimated by BOD, COD. Due to their high organic content, wood leachates generally have high biological oxygen demands and even low dissolved oxygen concentrations (Greenberg et al., 1992; Taylor, 1994; Liu et al., 1996). However, low DO and pH levels influence leachate toxicity to fish in an indirect manner. Low dissolved oxygen levels may induce increased fish respiration resulting in greater exposure of fish to dissolved toxicants as water passes over the gills more frequently.

Dissolved oxygen content of the wood leachate increased with the hour of exposure but was very low during the initial period of exposure. Similar observations were noted by Waldichuk (1974) in the saw mill wood waste site with low DO forming noxious gases. In the untreated sawdust, the DO of leachate in tapwater was 0 and 1.55±0.029 ml/l during 24 hours and 120 hours of exposure whereas in distilled water and sea water, the DO raised from 0 to 1.97±0.020 and 0 to 1.33±0.027 respectively. In the leachate from treated sawdust, the DO levels were 1.68±0.029, 1.97±0.020 ml/l and BDL (Below Detection Limit) after 120 hours of exposure to tapwater, distilled water and sea water respectively. There was a significant difference between the dissolved oxygen of leachate and the different hours of exposure of the sawdust in untreated wood (F=24.49; df=4; p<0.05) and treated wood (F=3.91; df=4; p<0.05) and the interaction between the pH and the water medium was significant (F=7.49; df=2; p<0.05) only in untreated saw dust leachate but no significance was observed in treated wood leachate (Two-way ANOVA). Low dissolved oxygen levels may induce increased fish respiration resulting in greater exposure of fish to dissolved toxicants as water passes over the gills more frequently (Haygreen and Bowyer, 1989).
Wood leachates are source of inert solid as well as toxic pollutants that directly clog fish gills and indirectly reduced light penetration which limits productivity. Contamination of the aquatic environment makes aquatic organisms vulnerable (FAO, 1991). Leachate impacted water medium was brownish in colour with an unpleasant odour. Similar observations were reported by Waldichuck (1974) in saw mill wood leachate. Wood-residue leachate that has not come into contact with soil or air is anoxic and has a dark colour. Rapid change of the anoxic leachate to black on exposure to air is caused by the oxidative condensation of tannins (Schermer and Phipps, 1976). In the present investigation, after 24h exposure of *P. reticulata* to 24 h leachate of distilled water and tap water, 100 percent mortality was recorded whereas 75 percent mortality and 25 percent mortality was recorded in distilled water and tap water respectively.

Increased hydrogen ion concentration (lower pH) will result in precipitation of mucous on the gill epithelium and precipitation of proteins within the epithelial cells. The toxic actions of low pH may or may not be responsible, depending on the extent of damage of low pH occurred. In addition, the toxic actions of low pH will likely make the fish more susceptible to the effects of other toxicants, diseases and hypoxic conditions. Hypoxic conditions generally induce metabolic stress that is typically associated with an increase in respiration rate, increased toxicant uptake and lowered resistance to toxicants (Davis, 1975b).

Behavioural responses like rapid and erratic swimming, darting up and down with occasional jumpy movement, and rapid opercular movement were observed when *P. reticulata* was exposed to wood leachate. Temmink *et al.* (1989) noted subtle behavioural changes including delayed feeding, surface swimming were observed in carp exposed to sublethal concentrations of bark extracts from Norway spruce wood.
Such physiological changes can result in the eventual death of the fish. Other effects are that, wood waste deposited in water bodies smothers spawning ground areas, decreasing fish variety and abundance (Arimoro et al., 2006).

The response of fish to wood leachate discharge depends on the fish species and its life-cycle stage. Researchers have studied toxicological effects using various bioassay methods that have involved the introduction of aquatic organisms to dilutions of wood-residue leachate or specific constituents for various periods of time (Samis, 1999). The bioassay provides an indication of the toxicity of wood-residue leachate and constituents.

In the present study, 100% mortality was observed for 24h period of leachate (distilled water and tap water leachate) after 24 h period of exposure of *Poecilia reticulata* Peters. Similar findings coincides with the work of Usta et al.(2009) who found that in termite mortality rate was found as 20.8% at 1% concentration of boric acid treated wood leachate and it reached to 100% at 1.5% concentration. Su et al. (1994) determined the LD$_{50}$ of boric acid as 721.29 f.Lg/g in *Coptotermes formosanus* Shiraki, 1909 workers and 264.02 f.Lg/g in *Reticulitermes flavipes* (Kollar). Habes et al. (2006) reported that the toxicity of boric acid is time-dependent and effects increase with dose and the duration of the treatment. Pease (1974) found that the LC$_{50}$ of pink salmon fry was 75 – 90 mg/l in freshwater western hemlock wood leachate at 96-h of exposure. Buchanan et al. (1976) observed that in sea water western hemlock wood leachate the LC$_{50}$ at 96-h of exposure was 56 mg/l pink salmon fry.

Leachate generated from weathered wood residue may cause environmental problems from operations where residue is continually being replenished. So proper measures must be taken to prevent leachate generation at wood-residue sites.