2. MATERIALS AND METHODS

The experiments conducted can be broadly classified as field exposure studies and laboratory based toxicity studies. The field experiments were patterned so as to determine the efficacy of preservative treated, FRP sheathed and paint coated rubber wood samples in preventing the biodeterioration by wood boring organisms in the estuarine water. The experiments were conducted to assess the extent of biodeterioration of the rubber wood samples by studying the physical, mechanical, chemical and microscopical characteristics of untreated and preservative treated rubber wood samples. The impact of different preservative treated panels in occurrence and diversity of epi-biotic fouling organisms were also studied. The laboratory experiments were modeled so as to determine the acute toxicity of CCA in common benthic clam *Villorita cyprinoides* and to compare the results with the acute toxicity of copper, chromium and arsenic. Another experiment was carried out to determine the bioconcentration of CCA components *viz.* copper, chromium and arsenic in *Oreochromis mossambicus* exposed to water in aquaria tanks where untreated rubber wood panels and preservative panels were introduced.

2.1. Materials

2.1.1. Rubber wood

The freshly felled plantation-grown rubber wood tree samples were collected from local suppliers at Ernakulam area. The trees were of 30 years of age and with a girth of 400mm. The portions of the tree free from knots,
without visible evidence of infection from mould, stains or decay fungi, were used for the preparation of panels. The rubber wood panels of size 150 x 100 x 25mm were cut and the edges of the panels were smoothened using a planer. Shortly after returning to the laboratory, the panels were immersed in 2% CCA solution to prevent the fungal attack.

Air seasoning of the panels was carried out for a period of 4 weeks promptly after immersion in 2% CCA for minimizing the warping, cracking, splitting and decay through fungal agents. The panels were stacked in a clean and dry area under the shade. After the seasoning period moisture content of the wooden samples was determined by oven dry method. In this method, representative samples of size 25 x 50 x 50mm were cross cut along the grain from the selected wooden panels. The samples were weighed and dried in a ventilated oven maintained at a constant temperature of 102°C ± 1°C and allowed to attain a constant dry weight. The moisture content of the samples were calculated using the formula

\[
\text{Moisture content} = \frac{\text{Wet weight} - \text{Oven-dry weight}}{\text{Oven-dry weight}} \times 100
\]

The panels having below 25% moisture content and devoid of cracks were selected for the study.
2.1.2. Marine plywood

Marine plywood has been extensively used for marine construction purposes. Due to its commercial feasibility, high economical viability and relatively low damage in aquatic conditions marine grade plywood has been well-established as a boat building material. It comprises as much as 80\% of the material of any plywood vessel. To improve the quality of the plywood, CCA treatment is usually employed. CCA is known to provide a greater penetration and fixation into the veneers. These are the major reasons for selecting marine plywood for the present study. Marine plywoods from Green ply manufacturers were purchased from the local market. Commercially available marine grade plywood sample of 203.2 x 101.6 x 19mm was purchased. Representative samples powdered, digested and analyzed in Inductively Coupled Plasma Emission Spectrophotometry (ICP) showed retention of 4.05 kg m$^{-3}$ of CCA. Panels of size 150 x 100 x 19mm were cut and used for the experiment.

2.1.3. Preservative solutions

CCA: The commercially available CCA manufactured by ASCU was taken for the study. The 7.5\% (w/v) of CCA solution was prepared by dissolving CCA in water. The solution was heated to 45$^\circ$C to accelerate the dissolution. The precipitate was removed and the supernatant solution was cooled and used for preservative treatment.

Creosote: Commercially available light creosote oil containing 5 to 7\% tar acid with a specific gravity of 1.02 - 1.03 was used for the experiment.
2.1.4. Paint

Coal tar epoxy finish paint was (Asian paints) purchased commercially and used for the experiments. A total of twenty-five panels were coated of coal tar epoxy paint. The base and hardener was mixed together in a ratio 4:1 as specified. Two coats of the paint were given with an intermittent drying period.

2.1.5. Fibreglass Reinforced Plastic (FRP)

The FRP sheathing was done using Chopped Strand Mat (CSM) of weight 450 g m\(^{-2}\) used for boat building purposes. The resin used for reinforcement was general-purpose polyester resin. Twenty-five numbers of panels were given two layers of resin coating. The panels after proper curing were used for the experiment.

2.2. Methods

2.2.1. Preservative impregnation procedure

The selected panels were treated with 7.5% (w/v) CCA solution to get retentions of 16 kg m\(^{-3}\) (type I), 29 kg m\(^{-3}\) (type II) and 42 kg m\(^{-3}\) (type III). The above-mentioned retentions were selected in such a way that they cover the minimum and maximum retentions recommended by AWPA for aquatic purposes. The wet weight retention of the preservative in the panel was calculated as per ASTM D2481-81. After air seasoning for a period of two weeks, 25 panels from type I category were selected and pressure treated with creosote (type IV).
Preservative treatment was done by Full Cell or Bethell process according to IS – 401:1960. The process called pressure impregnation was carried out in vacuum pressure impregnation chamber of 400 l capacity which is fixed vertically. The impregnation chamber was connected to a supplementary tank for storing the preservative. The panels were loaded in the treatment chamber and screwed airtight. A vacuum of 56 cm of Hg was applied for 30 min with a vacuum pump in order to remove the air present in the wood cells. The preservative solution from the supplementary tank was passed into the treatment chamber under vacuum. When the chamber was filled with the preservative solution the vacuum was released. The valves were closed and pressure was applied to so that preservative solution gets imbibed in to the wood cells. The conditions provided in the preservative chamber to get retentions of 16 kg m\(^{-3}\), 29 kg m\(^{-3}\) and 42 kg m\(^{-3}\) of CCA and retention of 160 kg m\(^{-3}\) for dual preservative are given in the Table 2.1. The time and quantity of pressure applied varies according to the required net wet weight retention. A final vacuum of 38 cm of Hg for 15 min was applied to drain the excess of preservative from the panels and to facilitate drying.

The retention of the preservative in the panels on wet weight basis was calculated as per ASTM D2481- 81 (ASTM, 1981).

\[
\text{Retention, kg m}^{-3} = \frac{1000 \, \text{G}}{V}
\]

Where, \(G = T_2 - T_1\), weight in grams of the treating solution absorbed by the wood.
C = Grams of preservative in 100 grams of treating solution.

V = Volume of the block in cm$^3$.

The qualitative estimation of preservative penetration in wooden panels was conducted to confirm the extent of penetration of CCA into the panels. In this method, a piece of preservative treated wood sample is sprayed with a solution freshly prepared by dissolving 0.5 g of diphenyl carbazide in 50 ml isopropyl alcohol and made up to 100 ml (IS: 2753, 1991). The reagent treated surface was examined after 15 minutes. The purple coloration indicated the area where the preservative solution has penetrated.

2.2.2. Field exposure of preservative treated panels

The estuarine exposure experiments were conducted at test site located in the North Oil Tanker Berth, 9° 57.759' N and 76° 16.869' E in Cochin estuary (Fig.1). This site is situated in Ernakulam channel, which is a part of Cochin backwater system of Vembanad lake. It is a tropical positive estuarine system extending between 9° 40' and 10° 12' N and 76° 10' and 76° 30' E that is connected to Arabian sea and receives fresh water from Periyar and Muvattupuzha rivers. The average depth of water at the test site was 10 -12m. Due to the permanent connection with Arabian sea and major rivers, the hydrology of Cochin backwater system is highly influenced by tides and currents. The seasonal variations in the hydrographical parameters are also observed due to the pronounced influence of monsoon seasons. The Cochin backwaters receive run off during the south-west monsoon and north-east monsoon season.
The test site located in the Cochin backwater system was selected for the study, since it is one of the highly productive ecosystems that harbor rich fishery resources. The ecological importance of the Cochin backwater lies on the fact that it is a breeding ground for many of the marine and estuarine fishes, fin fishes and shellfishes. The salinity gradient existing in the area together with high organic content in the sediments creates a favourable condition for the luxuriant growth, reproduction and development of a large variety of species.

2.2.3. Hydrographical conditions of the test site

The hydrographical parameters of the test site were monitored during the entire period of the study. The surface water samples were collected fortnightly throughout the period of study to get background information on the hydrographical conditions prevailing in the study area. Atmospheric temperature and water temperature were measured in the field using centigrade thermometer corrected to ± 1ºC. Water samples were brought to the laboratory for further analysis of dissolved oxygen, biological oxygen demand, salinity, as per standard methods Strickland and Parsons (1970). The pH was determined using pH Testr (Eurotech Instruments) calibrated to pH 4, 7 and 10 using NIST standard solutions. The turbidity was measured using Nephelo-turbidity meter 131 (Systronics). Nitrate content of the water was estimated by colorimetric method as outlined in Strickland and Parsons (1965) Dissolved Oxygen (D.O.) and Biological Oxygen Demand (B.O.D.) was
determined according to standard procedures by Strickland and Parsons (1965).

2.2.4. Arrangement of the panels and sampling strategy

Eight sets of panels each set carrying six replica of eight different treatment types were tied on to two iron racks and immersed at the test site 1m below the low tide level. Panels were arranged on the rack in statistically approved Completely Randomized Design (CRD). The racks carrying the experimental panels were immersed in test site located in the North Oil Tanker Berth, Kochi. This site is situated in Ernakulam channel, which is a part of Cochin backwater system (Fig 1.). The depth of water ranges between 6-12m and the tides and currents have pronounced influence on the water characteristics of the site. Six replica of each of these eight types of panels were tied using polyethylene ropes of 2mm diameter onto two different iron racks of size 1 x 0.4m. The arrangement of the panels was according to the statistically acceptable completely randomized design. The racks were immersed 1m below the water level in such a way that it the panels are not exposed during the low – tide.

The panels selected for the study included rubber wood panels treated to retention of 16 kg m$^{-3}$ (Type I), 29 kg m$^{-3}$ (Type II) and 42 kg m$^{-3}$ (Type III), Type I dual treated with creosote (Type IV), Type I panel coated with epoxy paint (Type V) and sheathed with FRP (Type VI) and marine plywood panels (Type VII) along with untreated rubber wood panels (Type VIII) as control.
For convenience in analysis the panels were categorized into three series viz., Series I, II and III.

Series I: The panels of Series I were used to study the extent of biodeterioration due to marine borers and to study the biodiversity of fouling organisms on the panels. Three sets of panels were considered as series I, in which each set included panels of eight treatment types. The racks containing the panels were immersed in the test site in June 2005. Each set of these panels was retrieved periodically over 6 months, 12 months and 18 months. The retrieved panels were brought to the laboratory and were assessed quantitatively and qualitatively.

2.2.4.1. Qualitative assessment

Visual observations: Experiment was patterned as per the standard method BS EN 275:1992. The panels were visually inspected. The superficial area and volume of the panel deteriorated was estimated. Except for the visually observed defects the panels were assessed for internal damage since the attack of ship worms is not superficial. The X-ray photographs of the panels were taken under 60 MA Elpro X-ray machine. Details are given in Chapter III.

Microscopic studies: The samples were cut into small cubes and sectioned using a microtome. The sections of 30 stained in toluedene blue and observed under light microscope. Details are given in Chapter IV.
2.2.4.2. Quantitative assessment

The extent of biodeterioration of untreated rubber wood and preservative treated panels was studied by physical, mechanical and chemical methods. Details are given in chapter V

Physical properties: The main physical property studied was the specific gravity. The wood loss due to biodeterioration was assessed by noting the differences in weight of the panel before and after exposure in the test site and the specific gravity changes are calculated.

Mechanical properties: The unexposed and marine exposed panels were subjected to mechanical strength tests using 200kN ZWICK Universal Testing Machine (UTM). The compression parallel to grain stress of the panels was studied.

Chemical properties: The changes in wood chemistry during preservation and biological deterioration were studied using Fourier Transform Infrared Spectroscopy (FTIR). The FTIR study was performed using Nicolet Avatar 360 Esp FTIR Spectrophotometer using KBr pellets. The spectra in the region of 400 to 4000 cm\(^{-1}\) were recorded using Avatar Diffuse Reflectance Smart accessory (DTGS). The spectra of all the panels were analyzed for relative change in the amount of cellulose, hemi-cellulose and lignin.

Series II: The panels of Series II were used to experiment on the effect of fouling assemblages on the attack of marine woodborer. One set of panels were examined monthly and scraped to remove the fouling organisms growing on the panels. The panels were reinstalled in the frame in the same position so
as to determine the effect of fouling assemblages in preventing the attack of wood boring organisms. Details of the experiment are given in Chapter III.

Series III: The panels of Series III were used for the bioaccumulation of copper, chromium and arsenic in dominant epibiotic fouling organism, barnacles collected from the treated panels. It comprised of two sets of panels used for the bioaccumulation studies. Half the area of fouling organisms of the panels was scraped off every month. The common biofouling organism viz. the barnacles were separated out. The shells and tissue samples from each type of the panel were digested separately. The sample digestion was carried out in Microwave Acid Digestor. The digested samples were analyzed for the amount of copper, chromium and arsenic in shells and tissues separately in ICP-AES. Details are given in Chapter VI.

The acute effects of the preservative solution viz. CCA and its constituents copper, chromium and arsenic was studied in benthic Black clam (Villorita cyprinoides) maintained in the laboratory. The toxicants viz. copper, chromium and arsenic was provided as respective salts CuSO₄·5H₂O (M. W.- 249.53), CrCl₃·6H₂O (M.W. - 266.48), As₂O₃ (M.W.- 197.83) and CCA each was added into 5l of water in which organisms are maintained. The experimental procedure and the results of 96h renewal acute toxicity are details are given in Chapter VII.

In the laboratory, the experiments were conducted in order to determine the bioconcentration possibilities of copper, chromium and arsenic that leach out from CCA treated panels. The studies were conducted in Tilapia
(Oreochromis mossambicus), maintained in aquarium tanks. The untreated rubber wood panels and preservative treated panels to different retentions were exposed in each of these tanks. The gills, liver, gonads and muscle samples were dissected out, digested and analyzed in ICP-AES for the concentration of copper, chromium and arsenic. The detailed procedure and results of the study is given in Chapter VIII.
Table 2.1. Conditions of vacuum - pressure impregnation

<table>
<thead>
<tr>
<th>Retention (kg m⁻³)</th>
<th>Initial vacuum (cm of Hg)</th>
<th>Time (min)</th>
<th>Pressure (kPa)</th>
<th>Time (min)</th>
<th>Final vacuum (cm of Hg)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA</td>
<td>29</td>
<td>56</td>
<td>30</td>
<td>448.16</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td>Creosote</td>
<td>150</td>
<td>56</td>
<td>30</td>
<td>344.74</td>
<td>45</td>
<td>38</td>
</tr>
</tbody>
</table>

Figure 2.1. Map showing the location of the test site