CHROMATED COPPER ARSENATE (CCA) TREATMENT FOR RUBBER WOOD PRESERVATION AND ITS IMPACT ON THE AQUATIC BIOTA

Thesis submitted to the
Cochin University of Science and Technology
in partial fulfillment of the requirement for the award of the degree of
DOCTOR OF PHILOSOPHY

in

MARINE BIOLOGY

by

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August 2008

Dedicated to my family & friends……..

CERTIFICATE

This is to Certify that this thesis entitled **"Chromated Copper Arsenate (CCA) Treatment for Rubber Wood Preservation and its Impact on the Aquatic Biota"** is an authentic record of independent bonafide research work carried out by **Miss. Sreeja A. M.Sc.,** under my guidance and supervision in the Fishing Technology Division of Central Institute of Fisheries Technology, Cochin, in partial fulfillments of the requirements for the degree of **Doctor of Philosophy** (Faculty of Marine Sciences) and that no part thereof has previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title of any University or Institution.

Cochin - 29
August, 2008

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DECLARATION

I, **Sreeja A**., hereby declare that this thesis entitled "**Chromated Copper Arsenate (CCA) Treatment for Rubber Wood Preservation and its Impact on the Aquatic Biota**", is an authentic record of the research work carried out by me under the supervision and guidance of **Dr. Leela Edwin**, Principal Scientist, Fishing Technology Division, Central Institute of Fisheries Technology, Cochin, in partial fulfillment of the requirement for the Ph.D. degree in the Faculty of Marine Sciences and that no part thereof has previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title of any University or Institution.

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ACKNOWLEDGEMENT

First and foremost I convey my reverential salutation to the Almighty, for enabling me to take up and complete this task.

I would like to express my deep sense of profound gratitude and indebtness towards my guide, Dr.Leela Edwin, Principal Scientist, Fishing Technology Division, Central Institute of Fisheries Technology, Cochin, for her inspirational valuable guidance, sustained encouragement and critical assessment during the entire period of research

I sincerely thank Dr. K. Devadasan, Director, for his invaluable guidance, encouragement, timely assessment of research work and also for extending all the facilities at the Central Institute of Fisheries Technology for my research work.

My sincere thanks to Dr. B. Meenakumari, HOD, Fishing Technology Division, for worthful guidance throughout the work

I sincerely thank Dr. A.V. Saramma HOD, for her wholehearted encouragement and for being a member of the Doctoral Committee.

My sincere thanks are due to all Scientists of Fishing Technology Division of the Central Institute of Fisheries Technology for their fruitful comments and help during the work

I am grateful to Dr. Geethalakshmi V., Dr. T. V. Sankar and Shri. P. Muhamed Ashraf (Scientists, CIFT) for their valuable suggestions and help during the work. I would like to thank Shri. P.T. Sebastian, Smt. K.G. Sasikala, Shri. N. Krishnan and Shri. P. Raghavan (Staff of Material Science and Marine Pollution Section) for the immense help.

I am also indebted to the scientific, technical, administrative, supporting staff of the CIFT for their kind co-operation and goodwill.

I sincerely thank all Senior Research Fellows of CIFT, especially in the Fishing Technology Division for their support, candid and critical comments and goodwill.

I express my sincere gratitude to the Scientists and staff of Forest Research Institute, Dehra Dun; Institute of Wood Science and Technology, Bangalore and Marine Biodeterioration Division, IWST, Visakhapatnam, Rubber wood Testing Laboratory, Rubber Board, Kottayam for their help and providing me with library and testing facilities for the successful completion of work.

I owe loving thanks my entire family and friends especially to my parents, sister and my fiancé for without their encouragement, understanding and prayers it would have been impossible for me to finish this work.

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PREFACE

From time immemorial wood, the renewable resource has always remained as prime preference for traditional fishermen for various marine constructional purposes especially for boat building. Initially the type of wood that was used for aquatic applications included highly durable varieties like teak (*Tectona grandis*), aini (*Artocarpus hirsuta*) etc. Due to the hot and humid environmental conditions prevailing in the tropics only 10% of the wood is durable. Wood when exposed to marine environments is easily deteriorated by a large variety of marine biodeteriorating agents like marine bacteria, marine fungi and marine wood borers especially crustaceans and molluscs. More than 2 lakhs of fishing crafts are constructed using locally available wood species and the country still prefer wood for construction of boats. The attack of bacteria, fungi and marine woodborers necessitate frequent replacement of boat building timber. Here, the financial loss incurred due to the deterioration of wood is very huge. Towards the end of 20^{th} century the crisis such as dwindling forest resources, scarcity of naturally durable varieties of wood and the high cost of durable varieties forced to shift the focus towards the use of alternative varieties of non-durable wood species for constructional applications. Rubber wood (*Hevea brasiliensis*) is an agricultural by-product and is easily available at relatively low cost. Superior mechanical properties and workability makes rubber wood a preferred

alternative to durable varieties. Although rubber wood has many desirable qualities to replace a durable wood in the marine condition it is highly vulnerable to biodeterioration by a variety of marine biodeteriorating agents. The serviceability of rubber wood in marine conditions therefore relies on preservatives applied on wood. Initially the indigenous substances applied for protection of wood constituted plant and animal extractives including ground-nut oil, cashew-nut shell oil, poon seed oil, neem oil, Castor oil, coal tar, 'chandrus', dammar-batu, crude fish oils (sardine oil and shark liver oil) etc. But these preservatives provided protection only for a short duration and the maintenance became costly and labour intensive. A scientific approach on the formulation and application of preservatives came out with chemical wood preservatives like CCA (Chromated Copper Arsenate), CCB (Copper chrome borate), ACZA (Ammoniacal Copper Zinc Arsenate), ACQ (Ammoniacal Copper Quat) etc. The importance of CCA lies in the fact that till date no other preservative have been formulated that can be assigned for a broad spectrum of applications with the same efficiency.

CCA was first formulated and patented under the trade name ASCU, in 1933 by an Indian Scientist Sonti Kamesam, working in the Forest Research Institute, Dehra Dun (patent no. 19859 1933). The high efficacy CCA against fungi and woodborers are mainly due to the toxicity imparted by copper and arsenic. In India, from 1938 onwards it was extensively used for preservation of wood for railway sleepers and electric posts. Large-scale production of CCA first started in U. S in 1938. CCA was in use for the last 60 years in

countries like U.S., United Kingdom, U.S.A., Japan where it has been a major preservative for above ground applications etc. In India, over all rough estimates available shows that about 400 metric tons CCA preservative is used per annum. The use of CCA treated wood for aquatic purposes is recent and one of the major applications is in marine plywood manufacturing. According to the recent reports there are about 5747 plywood boats in operation all along the Kerala coast itself.

Like other wood preservatives, wood can be treated with CCA by brushing the CCA solution on the surface or by dipping the wood in the solution of CCA or by impregnating the CCA solution into the wood cells applying pressure. Although CCA is well established as a fixed preservative concern over the use of CCA treated wood started to be raised during the last decade. Studies conducted world over has shown that there exists a possibility of CCA components to leach out and can pose a threat to the environment. Regarding the emerging concerns over the hazardous chemicals especially arsenic in CCA, U.S. Environmental protection Agency have voluntarily banned the use of CCA treated wood for residential and domestic applications. The end-use of CCA is still permitted for wood under fresh water and marine conditions, plywood and veneers etc.

In India, a number of studies have been conducted in order to assess the durability of different species of wood when treated with CCA. Studies are being carried out in Central Institute of Fisheries Technology, Cochin; Institute of Wood Science and Technology, Bangalore regarding the effective

utilization of low grade wood species for aquatic purposes and the application of different preservatives for wood in aquatic environment. In view of of the environmental concern regarding the use of CCA in marine condition and contradictory conclusions made by the scientists who have conducted studies regarding the leaching of CCA from treated wood and its impacts in the aquatic environment, the present investigation is focused on the assessment of the extent of protection imparted to rubber wood by Chromated Copper Arsenate (CCA) treatment. The study also assesses alternative preservatives like dual preservative treatment and physical barriers like paint and Fibreglass Reinforced Plastic (FRP) sheathing on CCA treated wood. Under such circumstances where better options for conservation of forest and protection of wood in view of effective utilization of low cost, non-durable wood species like rubber wood after proper preservation, the present study is crucial and decisive.

Chapter I

1. INTRODUCTION

The practice of preservation of wood to protect it from deterioration in aquatic environment is perhaps as old as human civilization itself. Wood is a highly versatile material of prime importance that has been in use over centuries for a variety of constructional purposes. The ease of availability, high economic viability, the strength to elastic properties together with its environmental friendliness make wood the best choice for artisanal fishermen for maritime constructional purposes that includes the construction of marine crafts, jetties, walk boards, fish landing platforms etc. Wood remains a major raw material for preparation of small scale fishing vessels like canoes and catamarans to highly mechanized wooden fishing boats. One of the major factors that restrict the use of wood in a variety of aquatic conditions is its high biodegradability. Initially the type of wood that was used for aquatic applications included highly durable varieties like teak (*Tectona grandis*), aini (*Artocarpus hirsuta*), sal (*Shorea robusta*) etc. In tropical countries like India, where highly hot and humid environmental conditions prevail, only 10% of the wood is known to be durable. Towards the end of 20^{th} century there was a drastic decline in the supply of naturally durable wood varieties and it became necessary to opt for non-durable species of wood. According to Kumar (1985, 1986) in India, about 20,000 m^3 of wood is required for replacement in marine sector alone. According to the latest estimates, there are about 5547 wooden fishing crafts in service in India. In recent reports by Kumar (2005),

the current use of wood in India is estimated at 39.5 million m^3, out of which nearly 22.5 million m^3 is used in adverse conditions requiring protection against biodegradation.

1.1. Biodeterioration of wood

Although wood has been the prime preference for the construction purposes in the marine environment, the biodeterioration of wood in aquatic condition and its prevention have been a problem world over. The studies pertaining to various aspects of marine biodeterioration of wood were initiated from 1950s onwards. In the beginning, studies mainly focused on different biodeteriorating agents and biofoulers in the marine environment - their systematics, ecology, physiology and the anatomical peculiarities of biodeterioration. In India, basic research on marine biodeterioration started in the 1930s and the Forest Research Institute was the pioneer institution to work over it. Later, the Department of Defence Research; National Institute of Oceanography, Goa; University of Kerala; the Central Institute Fisheries Technology, Cochin; Institute of Wood Science and Technology, Bangalore has contributed much in this field.

Biodeterioration is defined as the 'biological processes that are destructive or yield undesirable products or both' (Starkey, 1976). The proceedings of 79th Annual Meeting of American Wood Preservers Association, Richards (1983) has given a historical reference of the problem of woodborers worldwide and the need for formulating preservatives and their effective usage in slowing the problems of biodeterioration. 'International

Conference on Marine Biodeterioration' with special reference to Indian Ocean that was held in Goa in 1986 dealt with the status of marine biodeterioration and future prospects of the biodeterioration research programme in the country (Turner, 1986). The programme also put forward some recommendations for Indo-United States collaborative research programme that specially deals with three major aspects of marine biodeterioration *viz.* biofouling, marine wood boring organisms and biocorrosion. A review on marine biodeterioration research carried out in Indian waters was given by Nagabhushanam and Alam (1988).

The degree of deterioration in the marine condition generally depends on the natural durability of the wood. The studies on the natural durability of different species of wood can be dated back to 1909. Rutherford *et al.,* (1980) summarized the durability studies conducted on some of the tropical hardwood species. Wood can be classified into five different classes according to their durability viz. highly durable, durable, moderately durable, non-durable and perishable (Findlay, 1985). In India, a number of studies were conducted pertaining to the natural durability of wood in the marine and estuarine waters. Cookson (1986) carried out similar studies in Australian waters. Oevering *et al.,* (2001) gave a detailed study on the biodeterioration of marine timbers exposed above and below the mean sea level along the coastlines of England and Wales. In India, about 85 species of wood were tested in Bombay harbour waters and about 60 species of wood including those which are commonly employed for wooden fishing craft construction,

were tested in Goa waters for their natural durability (Santhakumaran & Alikunhi, 1983; Santhakumaran & Rao, 1988) most of these species were found to be destroyed in two years due to the severe attack of marine wood borers.

The harsh tropical climate of the Indian subcontinent is conducive to rapid biodeterioration. Enormous amount of money had to be spent for maintenance of the deteriorated crafts. Santhakumaran and Jain (1983), Santhakumaran (1988) have discussed in detail the socio-economic aspects of the biodeterioration of wooden fishing craft materials in India and various biodeterioration control measures.

1.2. Agencies causing marine biodeterioration

Even the highly durable varieties of wood like teak (*Tectona grandis*), aini (*Artocarpus hirsuta*), sal (*Shorea robusta*) etc. are prone to biodeterioration on prolonged exposure in the estuarine condition under the influence of biotic and abiotic factors. Bacteria, fungi and marine woodborers constitute the biotic factors that contribute to the degradation of wood in the estuaries (Eaton, 1985). The initial colonization of marine bacteria and marine lignicolous fungi species in the primary film initialize the deterioration by softening the wood rather indirectly (Eltringham, 1971; Kohlmeyer *et al.*, 1995). In tropics, high temperature prevailing throughout the year and heavy rainfall during the monsoon and a highly productive nature of water creates a favourable condition for the action of various wood biodeteriorating agents especially for the woodborers. So far, studies have been conducted on these

organisms for a better understanding of the problem and to find effective methods of preventing biodeterioration.

A preliminary survey on marine wood boring organisms along the Indian coast was done by Erlanson in 1936. The wood boring organisms in the marine and estuarine waters mainly come under two phyla *viz.* Arthropoda and Mollusca. Marine arthropods especially those belonging to the Class Crustacea and molluscs of Class Bivalvia that bore into the wood enjoy a worldwide distribution. Crustaceans of genus *Sphaeroma, Limnoria, Chelura* and the bivalves of family Pholadidae that include two important genera *viz. Martesia* and *Lignopholas* and Teredinidae that include two genera *Teredo* and *Bankia* were identified as the major destroyers of wood in the marine environment**.** Each of these genera has two or more representative species. The taxonomical, anatomical and the distributional characteristics of the Teredinid molluscs were detailed by Turner (1984). 'A monograph on the wood boring crustacean in the Indian waters' was made by Pillai (1961) which was the pioneer attempt to provide a detailed view on the characteristic distributional characters ecology, and economy of different species of crustacean woodborers with a special reference to those that are associated with the submerged timber. Later on, a detailed description of various timber destroying organisms along the coasts of India was given by Nair (1968), specifying the need for biological, physiological and distributional data on marine borers which will help in developing effective methods of prevention of wood deterioration. Eaton and Hale (1993) were of the same opinion and

suggested the use of preservatives as a measure for an effective control over their action. In the Andaman waters, the distribution of crustacean woodborers was studied by Ganapati and Lakshmana Rao (1960). Karandae (1968) carried out investigations on the distribution and characteristics of marine wood boring organisms, in the Bombay harbour waters. At the Visakhapatnam coast, a series of studies were conducted by Naghabhushanam (1961,1962) on the factors like salinity, water currents, primary film formation etc. affecting the settlement and growth of wood boring molluscs, the anatomical and biochemical peculiarities that render the ability to cause destruction by boring into the wood and digestion of ingested wooden particles.

In the west coast of India, investigations carried out by Cheriyan (1964) showed that the occurrence and abundance of wood boring organisms depends on the prevailing seasonal hydrographical conditions. Based on their studies, Nair and Saraswathy (1969) have given a detailed account on the biology of Teredinidae. The vertical distribution of economically important woodborers was studied by Santhakumari and Nair (1984). An annotated bibliography of marine woodborers of Indian coast was given by Santhakumaran (1985). The systematics of pholads especially *Martesia* and *Lignopholas* was detailed by Turner and Santhakumaran (1989). Raveendran and Wagh (1988) provided a comparative account on the wood boring organisms in the coastal and off shore waters. The occurrence of four species of wood boring Pholads and their distribution along the Indian coast was

studied by Santhakumaran and Rao (1994). The growth aspects of Teredinid borers were studied by Radhakrishnan and Kasim (1997). A recent study along the west coast, in Goa coastal waters by Vishwakiran *et al.,* (2001) describes the spatial and temporal distribution of woodboring organisms. Laboratory studies by Henderson *et al.,* (1995) have examined the behavioral and sensory mechanisms that help crustacean woodborers *viz. Limnoria* spp. in locating the wood as their substrate of attack. An overview on the taxonomy, biology, ecology and the behavioral patterns of these crustacean woodborers were provided by Cragg *et al*., (1999).

1.3. Durability of rubber wood

When the service life of even the highly durable varieties of wood species are considerably affected by the severe attack of wood borers and the shortage of durable varieties wood species for the replacement, treated rubber wood (*Hevea brasiliensis*) emerges as an alternative to the durable wood varieties. Rubber wood is an agricultural by product that is available at a relatively low cost at about 40% cheaper than teak. Commercial rubber wood plantations are found in about thirty countries that are mainly confined to tropical and sub tropical regions. Malaysia, Indonesia, Thailand, Sri Lanka and India are the leading producers of rubber wood in Asia. According to Food and Agricultural Organization's (FAO) statistics in 1999, India is the 6^{th} largest producer of rubber wood. In India, about 10,600 ha of area is under rubber cultivation with a total production of 6,58,210 tonnes during 2004 - 2005 (Rubber Board, 2005). About 5 million m^3 of total rubber produced is

utilized by industries like packing cases and plywood manufacture and the rest is used as firewood. Although highly non-durable, because of its easy availability, low cost and good working qualities rubber wood is gaining importance now-a-days.

Rubber wood is a plantation grown timber and rubber wood is tapped for latex when it matures in 14 -17 years. As the latex yield decreases with the age of tree after 25 to 30 years the replantation has to be done. It is a light hard wood with a whitish yellow or pale cream colour when freshly cut. At 12 % moisture content the density of rubber wood ranges between 450 - 626 kg m^{-3} (Edwin, 2003). Rubber wood can be well compared with the other conventionally grown timbers due to its good mechanical properties and workability (Shukla & Lal, 1985). Rubber wood is moderately refractory so it can take up preservatives easily (Shukla & Lal, 1985) Rubber wood is classified as a light to moderately heavy wood and its specific gravity was estimated to be 0.557 at 12 % moisture content (Rubber board, 2005).

Although rubber wood has many desirable qualities to replace a durable wood it is highly vulnerable to biodeterioration by a variety of marine biodeteriorating agents. Rubber wood has relatively high content of starch and low content of lignin in the cell walls. Unlike highly durable varieties of wood like teak (*Tectona grandis*), rubber wood lack phenolic substances that usually render resistance against biodeterioration. The natural resistance of rubber wood to marine woodborers was studied by Rao *et al*., (1993) and Edwin and Pillai (2004). According to these studies rubber wood is found to

be highly perishable under marine conditions. According to Findlay (1985) such wood requires rapid seasoning that considerably reduces the moisture content in the wood and increases the life. The service life of rubber wood panels exposed under the marine condition was about 4-6 months wood (Rao *et al.*, 1993). The marine exposure trials conducted by Edwin and Pillai (2004) showed similar results where the panels were completely destroyed in 5-7 months. Preservation of rubber was studied by Hong *et al.*, (1982), Gnanaharan and Mathew (1982), Damodharan and Gnanaharan (1994) and Edwin and Pillai (2004).

1.4. Assessment of biodeterioration

Biodeterioration and degradation of wood can be assessed at different levels ranging from the very basic method of checking the changes in the external appearance and softening of the wood to the present day analysis using highly sophisticated microscopic to molecular level characterization. The wood under biodeterioration can be visually inspected, assessed and the extent of deterioration can be rated according to internationally accepted standards of American Society of Testing and Materials (ASTM-2481-81) or European Union (BS EN-275-1992) or Indian Standards (IS-1708). The damage in the wood specimens due to the marine wood boring molluscs *viz.* shipworms and pholads cannot be assessed by the examining the external destruction only, since the damage due to these organisms are more internal than external. Only the larval forms of these organisms are getting access into the wood, while development of these

larval forms is taking place inside the wood utilizing the wooden particles as food. In such cases the deterioration can be detected and assessed by applying X-ray radiography (BS EN- 275-1992). X-ray analysis is a non-destructive method to study the biodeterioration caused by teredinid borers and this technique has been well adopted and established to assess the damage due to molluscan woodborers (Rutherford *et al*., 1980; Barnacle & Ampong, 1983; Eaton, 1985; Edwin & Pillai, 2004).

Weight loss and corresponding changes in the specific gravity of wood is considered to be a first-rate index of deterioration of wood Green *et al*., (1999). The destruction on *Shorea leprosula* panels by tropical marine woodborers were studied both qualitatively and quantitatively using weight loss methods (Singh & Sasekumar, 1996). During the biodeterioration, the wood substance is lost that is ultimately shown as the loss of weight. Since the weight and specific gravity of the wood are directly related, any change in the weight brings about a corresponding change in the specific gravity.

Studies on the mechanical properties of the wood can be another index of the biodeterioration of wood. Cellulose, hemi-cellulose and lignin form the basic chemical constituents of wood. Though in different proportions, these constituents are arranged in a specific manner as wood fibers, vessels etc., so as to provide a basic structural integrity to wood. Microbial degradation of wood sometimes leads to the preferential loss of these chemicals from wood. There are some marine bacteria and fungi that invariably remove lignin or cellulose from wood (Cowling, 1961).

According to Winandy and Lebow (2001), Curling *et al*., (2002) the biodegradation was initiated with breakdown of the hemi-cellulose chains. Later on, as degradation progresses the main chain of hemi-cellulose, cellulose and lignin is removed. Thus, any change that alters the basic structure and chemical make up of wood brings about corresponding changes in the mechanical properties.

In the marine environment, deterioration of wood is mainly due to the action of woodborers. The action of marine wood boring crustaceans and molluscs directly destroy the integrity of the wood fibers. Thus ultimately the strength properties are reduced due to deterioration (Winandy & Rowell, 2005). According to the studies conducted by Edwin and Pillai (2004), Edwin and Ashraf (2006) the mechanical strength properties of wood can be used as indices to assess the biodeterioration of wood.

The basic anatomy of the wood varies according to the type and species of wood. In hardwoods like rubber wood, that are derived from the flowering plants called angiosperms, four basic cellular components *viz.* vessels, fibers, parenchyma cells and rays are present (Wiedenhoeft & Miller, 2005). These cells are interconnected throughout the wood in a much predictable manner in each species of wood that makes it possible to identify the wood precisely by checking the anatomy. During decay or deterioration of the wood, changes in the basic structure and chemical composition of the wood undoubtedly affect the anatomical peculiarities of the wood. The microscopical observations of the slices or slides of decayed or deteriorated

wood samples under light microscope or highly sophisticated Electron Microscopes like Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) etc. can explain the changes during deterioration processes. Wilcox (1970), Blanchette *et al.,* (1985), Nilsson *et al.,* (1989) has shown that there exists a specific correlation between the changes in chemistry of cell wall to its microstructure.

Any increase in the serviceability of wood in marine conditions therefore relies on preservatives applied on wood.

1.5. Prevention of biodeterioration

In the traditional fisheries sector initially the fishermen used plant and animal based preservatives for increasing the service life of wooden boats. These indigenous preservatives included crude fish oils (sardine oil and shark liver oil), ground-nut oil, cashew-nut shell oil, poon (*Calophyllum sp.)* seed oil, neem (*Azadirachta sp*.) oil, castor oil, coal tar, plant resins and extracts like 'chandrus' (solidified plant resin), dammar-batu (oleo-resinous substance obtained from trees of family Dipterocarpaceae, imported from Malaya) etc. (Balasubramanyan, 1964; Santhakumaran & Jain, 1983). The preservatives were applied either alone or in combinations. But the action of these preservatives was limited as they can only provide a hydrophobic surface without any prophylactic activity (Nair *et al.*, 1985). This superficial protection provided, was only for a short duration and the maintenance is costly and labour intensive. A scientific approach on the formulation and application of preservatives was initiated in early 1950s when studies were

focused on the physiological requirements of the wood boring organisms and the characteristics of the surrounding environment. These studies came out with chemical wood preservatives like CCA (Chromated Copper Arsenate), CCB (Copper chrome borate), ACZA (Ammoniacal Copper Zinc Arsenate), ACQ (Ammoniacal Copper Quat) etc. Among these preservatives CCA proved to be very efficient in protecting the wood against marine borers.

1.6. Chromated Copper Arsenate (CCA) as wood preservative

Chromated Copper Arsenate (CCA) as a wood preservative was first formulated and patented under the trade name ASCU, in 1933 by an Indian Scientist Sonti Kamesam, working in the Forest Research Institute, Dehra Dun (Patent no. 19859, 1933) (Aston, 1985). CCA is a waterborne wood preservative that is classified under 'fixed type' where the chemical components of CCA get fixed into the wood cell components and do not leached out. CCA was in use for the last 70 years in countries like United Kingdom, U.S.A., New Zealand, and Japan where it has been a major preservative for above ground applications. It was widely accepted in marine construction purposes for pilings, poles wooden jetties etc. Large-scale production of CCA first started in U. S in 1938. In Japan CCA has been well accepted since 1963. Based on the recent data provided by The Wood Preservatives Industry Association of Japan, increasing concerns about leaching of CCA, other preservative formulations has gradually resulted in the replacement of CCA.

In India, from 1938 onwards it was extensively used for preservation of wood for railway sleepers and electric posts. Rough estimates available shows that about 400 metric tons CCA preservative is used per annum (Kumar, 2005). The Institute of Wood Science and Technology have constructed catamarans, a common fishing craft in operation along the east coast made out of planks of *Albizia chinensis* and *Bombax ceiba* that were treated with CCA to retentions of 16 kg m^{-3}, 32 kg m^{-3} resisted borer attack for about 24 years (Santhakumaran, 1997). Studies are being carried out at the Central Institute of Fisheries Technology, Cochin to upgrade the wood species like rubber wood for aquatic purposes. Five rubber wood canoes are already under experimental operation. Of these two canoes are made of rubber wood treated with CCA and creosote and three canoes treated with CCA and sheathed with Fibreglass Reinforced Plastic (FRP)

One of the major applications of CCA is in marine plywood manufacturing. Marine plywood is a type of plywood that is used in marine construction applications especially in boat building. High economical viability and relatively low damage make marine plywood a good constructional material in aquatic conditions. Additionally, marine plywood has high strength-to-weight ratio and can withstand compression forces upto a considerable level. Marine plywood is waterproof and can be glued to any surface. It is relatively strong and can be painted or varnished. In India, the major producers of plywood are mainly Uttar Pradesh and southern states like Karnataka. According to the end uses specified, the plywood veneers are

manufactured from highly durable varieties like teak, rosewood, aini etc. to the less durable hard wood species like pine, rubber wood etc. It is prepared by gluing together a number of thin veneers of wood using a waterproof adhesive such as epoxy or phenol resourcinol. The marine construction purposes utilize plywood of not less than 5 veneers joined together. To ensure rot proofness, the plywood panels are treated in a vacuum pressure impregnation plant, using any of the preservative solutions. The most common treatment and retention level for plywood used in boat construction is CCA at approximately 6.4 kg m^{-3} retention. After preservative treatment the panels are kiln dried to moisture content of 18% so as to minimize the development of bends and cracks. Plywood is used in boats after laminating with fiberglass. The studies conducted by The Engineered Wood Association North America (American Plywood Association) have shown that the treated plywood has bond strengths similar to the untreated plywood and the CCA treatment does not adversely affect the bondability of fiberglass to plywood. In the south-west coast of India, the commercial application of plywood boats started in mid-80s. South Indian Federation of Fishermen Societies (SIFFS) a non-government organization had taken a major role in production and supply of plywood boats. According to the recent reports (2001) there are about 5747 plywood boats in operation all along the Kerala coast.

CCA is a mixture of salts or oxides of copper, chromium and arsenic. These chemical constituents are mixed in different proportions to prepare three different types of CCA *viz.* Type A, B, and C. According to American

Wood Preservers' Association (AWPA, 1996) the active ingredients of type A, B, and C are copper oxide, chromium trioxide and arsenic trioxide. The nominal composition of these ingredients varies in each type. The proportion of copper oxide by weight varies as 18.1, 19.6 and 18.5 while chromium trioxide as 65.5, 35.3 and 47.5 and arsenic trioxide as 16.4, 45.1 and 34.0 in each type respectively. The Indian Standards (IS-10013 (Part II) – 1981) specifies the proportion by weight of ingredients in CCA as copper sulphate - 37.5, sodium dichromate or potassium dichromate - 50.0 and arsenic pentoxide - 12.5.

The high efficacy CCA against fungi and woodborers are mainly due to the toxicity imparted by copper and arsenic. Copper is an effective fungicide while arsenic has high toxicity against insects. Apart from toxicity, chromium fixes the CCA components in the wood by a series of reactions initiated by the reduction of hexavalent chromium to trivalent state. CCA, being a water-soluble preservative it is diluted with water to obtain treating solution of required concentration and applied on the wood. Typically CCA is a golden yellow to brown coloured concentrated, odourless and chemically stable solution at normal temperatures with pH ranging from 1.9-2.6 (Aston, 1985). CCA treated wood has a number of advantages, as it is clean to handle, odourless and can be painted when once dried (Findlay, 1985). Like other wood preservatives, wood can be treated with CCA by brushing the CCA solution on the surface or by dipping the wood in the solution of CCA or by impregnating the CCA solution into the wood cells applying pressure. The

method of treatment is selected according to the species of wood to be treated, nature of the preservative and conditions in which the treated wood is to be used. In tropical marine conditions where highest hazard due to woodborers are recognized, the pressure impregnation of CCA solution that provides deep penetration and maximum retention of the preservative in the wood cells is regarded as the most effective method of treatment (Aston, 1985). Combining the oil borne and waterborne wood preservatives a multiple preservative treatment method were also adopted where the pressure impregnation of CCA and then creosote as dual preservative treatment was well established.

During the fixation of CCA in the wood cell components a series of chemical reactions are initiated that fixes the copper, chromium and arsenic components with the sugar components in the cell wall. These chemical changes are known to have some implications in the chemical structure, mechanical properties and microstructure of wood. The changes in these properties are greatly influenced by the retention of the preservative in the wood (Wood *et al.*, 1980; Winandy, 1985). The water borne preservatives that chemically react to wood cells are known to reduce the mechanical properties of wood (Winandy, 1995b, Green *et al.*, 1999). Studies conducted by Lahiry (1996) reported 30% higher strength in oil borne and waterborne preservatives. According to Edwin and Thomas (2000) creosote treatment does not bring any changes in the strength of wood when compared to untreated wood. Edwin and Pillai (2004) reported an increase in compressive strength of the dual preservative treated panels along with increased

protection from biodeterioration. A 7.5% CCA solution was taken for the study based on the results of studies conducted by Edwin *et al.,* (1993), Thomas *et al.,* (1998) and Edwin and Thomas (2000).

The studies conducted at the Central Institute of Fisheries Technology showed that a 7.5 % CCA solution is effective in protecting wood under marine conditions. According to AWPA (2003), wood used in fresh water pilings and columns should have 9.6 kg m^{-3} and 12.8 kg m^{-3} retention of CCA whereas, for marine conditions retention of 40 kg m^{-3} is recommended. In the marine conditions, a dual preservative treatment in which a wood is treated with 16 to 24 kg m^{-3} followed by creosote treatment to get retention of 320 kg m^{-3} to 400 kg m^{-3} was recommended (AWPA, 2003)

The degree of protection imparted by a particular preservative formulation was assessed by studying the toxicity of the preservative components to the target organisms, permanence, retention and depth of penetration of the preservative in the treated wood etc. (Ibach, 2005). The preservative applicable to a particular environment for a particular use was finalized according the conditions where the treated wood should be used. Eaton and Hale (1993) put forward field-testing strategy for the preservative treated wood to assess its efficacy in a particular environment condition. Agencies like European Committee for Standardization (CEN) BS EN 275:1992, American Society for Testing Materials ASTM 2481- 81 (1982), Indian Standard (IS) specifies the standard marine test methods to assess the effectiveness of the preservatives that are deeply penetrated into the wood.

In India, a number of studies have been conducted in order to assess the durability of different species when treated with CCA. The studies conducted in the Cochin harbour by Cheriyan and Cherian (1983), Krishnan *et al.,* (1983), Cheriyan *et al*., (1988) showed that CCA treated wood is effective against woodborers especially *Teredo spp.* and thereby the durability of less durable timbers can be increased by CCA treatment. Sreenivasan and Vallabhan (1988) conducted studies in Madras harbour waters using *Bombax ceiba, Terminalia alata* etc that are treated to CCA showed that CCA treatment to higher absorptions considerably increased the durability of wood. Dual preservative treated panels were known to prevent crustacean woodborers effectively (Richards, 1983). Studies conducted by Johnson and Gutzmer (1984) using Southern pine samples treated with dual preservative resisted borer attack for 6 years. Previous investigation carried out at Cochin estuarine waters for 19 months showed that the dual preservative treated panels of *Mangifera indica* were completely free of borer attack throughout the period (Edwin *et al.,* 1993). Previous investigations carried out on rubber wood treated with CCA and creosote to retention of 160 kg m^{-3} was shown to prevent the borer attack for more than 33 months (Edwin & Pillai, 2004).

1.7. Leaching of CCA

Although CCA is well established as a fixed preservative concern over the use of CCA treated wood started to be raised during the last decade. Studies have been conducted to assess the possibility of leaching of CCA

components from the treated wood. Leaching of CCA from the treated wood is dependent on many factors. Proper drying conditions and drying period provided to treated wood after CCA treatment to confirm proper fixation of preservative in wood is the primary factor that affects leaching of chemical components. The retention of the preservative in wood, size and surface area of the treated wood, the environmental conditions of exposure viz. the medium, pH, salinity, temperature water circulation etc. also adds up to the leaching process (Fahlstrom *et al*., 1967; Warner & Solomon, 1990; Lee *et al*., (1993); Van Eetvelde *et al*., 1995; Lebow, 1996; Hingston *et al*., 2000; Brooks, 2002; Kartal *et al.,* 2004). According to Cooper (1991), Lebow (1996), Lebow *et al.,* (2000), Brooks (1997, 2000, 2003) there exists a possibility of CCA components to pollute the marine eco-system. Several reviews discuss the environmental hazards of leachate from preservative treated wood, but all these reviews lack proper quantitative records to establish the fact that these preservatives provide a real threat to the aquatic environment (Brooks, 1997).

1.8. Impact of CCA components on non-target biofouling organisms

Aquatic environment is highly dynamic in nature and it harbours a wide variety of life forms. It is difficult to measure the impact of chemicals or heavy metals on aquatic ecosystem since a large majority of the aquatic organisms are mobile or else they are transported through water currents so

the extent to which the aquatic organisms get exposed to such pollutants always varies. Furthermore, from treated wood the metals leach out in various chemical forms into the surrounding environment and out of these forms some are toxic and bioavailable while others are not. Although these factors restricts the studies on aquatic biota, great number of attempts were made to assess the effect of CCA leachate in aquatic organisms According to the studies conducted by Albuquerque and Cragg (1995b), Baldwin *et al*., (1996), Cookson *et al*., (1996) the use of CCA treated wood causes little hazard to the marine environment and has no adverse effect on non-target aquatic biota. Some researchers suggested that the metal leachate do accumulate in the aquatic biota and can cause deleterious effects when exposed for more than several days (Adler-Ivanbrook & Breslin, 1999; Brown & Eaton, 2001; Weis *et al*., 1991). Studies conducted by Weis and Weis (1992), Brown and Eaton (2001) the organisms living associated with the CCA treated wood were negatively affected biodiversity with a decrease in total number of individuals, biomass and a retardation in growth. The conflicting conclusions made by the researchers on the impact of CCA on the aquatic biota highlight the relevance and the need of further studies on this topic.

Assessment of the impact of wood preservatives is based on the fact that the metals leached out from the treated wood before getting diluted with water, come in contact with the epibiotic community. Epibiotic organisms that include the fouling and wood boring organisms that live attached to the wood, the preservative components cause direct toxic effects by contact itself.

In those organisms swimming in the water column, leached metals are directly imbibed into the body. The leachate components deposited in the sediments becomes the source of metals for burrowing forms. In addition to all these, metals may indirectly enter into the body of an organism through food chain and food web (Brown & Eaton, 2001).

CCA is formulated as an anti-wood borer preservative that effectively gets fixed into the wood and those organisms that bore and digest the wood particles are affected. While in the context of release of chemical components from CCA, the uptake and toxicity responses would be expected to occur in a higher level in fouling organisms that are generally considered as non-target to CCA. When any of the metal components get access to the organisms it get either stored in their body or get eliminated or may get entry into the food chain and food web through the process of eating and being eaten up. In such cases it should be either bioaccumulated in the organism or transferred initiating consequent adverse effects in higher organisms except those living attached to the treated wood (Adler-Ivanbrook & Breslin, 1999). The uptake of the CCA leachate by fouling organisms and other forms inhabiting water column and sediments is the first step to trophic transfer.

All the constituent metals of CCA are found to be the natural parts of earth's lithosphere and hydrosphere. The average crustal concentrations are: $50\mu g.g^{-1}$ for copper, $125\ \mu g.g^{-1}$ for chromium and $7\mu g.g^{-1}$ for arsenic (Brooks, 2002). All the fractions of these metals are not available to the aquatic organisms. In the aquatic environment the metals exist as their soluble

fraction that is readily absorbed (Walthert, 2003). In the aquatic environment arsenic exists as arsenite (As III), while arsenate (As V) is present in much lower levels. Arsenate is chemically similar to phosphate, and so is readily taken up by plants and animals for normal cellular metabolism. Chromium exists in two oxidation states *viz.* chromium (III) and chromium (VI), where chromium (III) forms stable complexes with inorganic and organic compound and are nor easily bioavailable. Copper and chromium are essential micronutrients for plants and animals and so the uptake and metabolism of these metal forms are normal biological process. All these metal forms are found to be bioconcentrating in the aquatic organisms and in some forms they get biomagnified. According to Warner and Solomon (1990) out of CCA components, copper tends to leach out most and hence bioaccumulated in higher amounts than chromium and arsenic. Gill surfaces of aquatic organisms are highly permeable to copper compounds especially to uncomplexed cupric ions and hence invertebrate larval stages are highly susceptible for copper toxicity. Arsenic in the aquatic environment ultimately get incorporated in the sediments and hence the toxic effects of arsenic is evident in those forms inhabiting the sediment and those incidentally take up the sediment particles while feeding. The studies regarding the uptake and assimilation of chromium from seawater suggests that direct uptake is a far more important pathway than assimilation through the food chain (Baldwin *et al.*, 1996; Brooks, 1996, 1997, 2000, 2003)

The studies have shown that metals existing as their soluble fractions or those stored as granules in the body of organisms is transferred through the food chain or food web to the organisms in the higher trophic levels (Nott & Nicolidon, 1990; Reinfelder & Fisher, 1991). A number of studies have been conducted on the mechanism of bioconcentration and biomagnification of CCA constituents. Studies conducted by Weis and Weis (1992) showed that algae, *Ulva lactuca* and *Enteromorpha intestinalis*, collected from CCA treated wood and from rocks nearby when fed to mud snails (*Ilyanassa obseleta*); the snails took up the metals mainly copper, from the algae and suffered adverse effects. Brooks (2002) in his review discusses that in the study conducted by Weis and Weis (1992), epibiota analyzed was scraped of from CCA treated wood in such a way that the possibility of contamination of tissue samples with wooden particles. The background levels of copper, chromium and arsenic in the study site was also not reported and so the mortality of snails may be due to combination of the factors including very limited water volume (Brooks, 1996, 1997, 2000, 2003). According to Weis and Weis (1999) the epibiotic organisms living on CCA treated wood take up the metals and transfer it through the food chain to higher trophic levels. The study was conducted for three months with CCA treated panels kept in a cage where various consumer organisms were reared. It was found that the metals get bioaccumulated in grazing organisms like amphipods. But the metal concentration in glass shrimp (*Palaemonetes pugio*) and two fish species, naked goby (*Gobiosoma bosci*) and mummichog (*Fundulus heteroclitus*) that

usually consume on amphipods, was unaffected by CCA treated wood. None of the above consumers showed bioaccumulation of metals except amphipods. When the test organism *Lumbriculus variegatus* was exposed for about 28 days to the CCA leachate near a sawmill area, arsenic is found to be bioaccumulating in the tissue of the organism. Oligochaetes near the site showed very high concentration arsenic in their tissues (Lyytikainen, 2001). A significant increase in copper concentration was observed in the tissue of predatory snails, *Thais sp.* that was fed with oysters collected from residential canals bulk-headed with CCA treated wood (Weis & Weis, 1993). But significant elevation in metal concentration was not found when polychaete worms collected from the sediments adjacent to the CCA treated bulkheads was given as food to juvenile pin fish (*Lagodon rhomboides*) and spot (*Leiostomus xanthurus*) for one month. Brooks suggests that the increase in copper concentration may be due to other sources of copper and other contaminants in such in these waterways (Brooks, 1997). A report by the Environmental Protection Agency, demonstrated that residential canals contain numerous contaminants that can have adverse effects on aquatic life and suggested that copper, chromium and arsenic that leached out from CCA treated wood contribute nothing to the potential adverse effects (Chaillou & Weisberg, 1995).

The protection to wood by CCA treatment is mainly due to the direct toxic effects of the chemical components of CCA. Copper is essential for the normal physiological and biochemical functioning of the body whereas

chromium and arsenic are needed in minute quantities. But at higher concentrations, all these are known to be toxic. Chromium (VI) and arsenic (III) are not only toxic but also carcinogenic and can alter the genetic makeup of an organism by functioning as mutating agents (Bodek *et al.*, 1988a; Wong & Chang, 1991; Havens, 1994; Weis & Weis, 1999). It is also known that when compared to the other organisms, aquatic organisms can tolerate arsenic and chromium at moderately high levels.

A number of laboratory toxicity tests and field exposure tests were conducted to explain the possible deleterious effects of CCA leachate on aquatic biota. According to Cragg *et al.*, (1996) the toxicity of the leachate depends on the nature of wood sample and the nature and volume of the water in which leaching occurs. In the laboratory, toxicity studies are usually conducted under controlled environmental and hydrographic conditions in aquaria. LC_{50} values for each metal to specific organisms are known. Lethal Concentration (LC_{50}) studies conducted on *Daphnia magna* showed that the chromium and arsenic concentrations in CCA leachate were very low as to have no effect but at 2% level lethality caused by the leachate was mainly due to copper (Buchanan & Solomon, 1990). LC_{50} studies provide the basic toxic limits of chemicals. Laboratory based studies have a drawback as there is a possibility of overestimation of toxicity effects, even then aquarium based laboratory studies may be of much importance in those cases where there is a chance of comparison of data obtained from field and laboratory (Alder-Ivanbrook & Breslin, 1999).

The leaching and subsequent toxicity of CCA leachate depends on the volume of water in which the treated wood is immersed and the volume of leachate in which the organisms are exposed. In seawater, due to tidal action there is greater possibility of leachate to get diluted and the toxicant components are less available to the organisms. The toxicity of leachate gradually decline with increase in time of exposure. Apart from the bioaccumulation, the physiological responses of organisms in the polluted environment can also be taken as an index of toxic effects of a pollutant. Studies conducted by Weis *et al.*, (1991) showed that fiddler crabs (*Uca pugilator*) showed suppression in limb regeneration when it is exposed to CCA leachate. It was also reported in the results that in the presence of CCA treated wood, 90% of the sperms of sea urchin (*Arbacia punctulata*) failed to fertilize the eggs and further development of fertilized ones were totally inhibited. Lower concentration of these metals did not have any effect either on fertilization or on larval development (Weis *et al.*, 1991; Weis & Weis, 1992). The mud snails (*Ilyanossa obseleta*) became inactive and retracted into their shells when exposed to CCA leachate and in clear water they were recovered (Weis & Weis, 1992a).

The studies conducted by Weis *et al.*, (1995) showed that the gill cells of the oysters (*Crassostrea virginica*) had increased. It was also reported that the digestive diverticula of oysters living in the canals lined with CCA showed the presence of severe lesions in digestive diverticula compared to control oysters. Studies have shown that the barnacles living in the close

proximity of the leachate have elevated levels of copper and chromium in their shells. The major physiological activities like feeding and respiration can be taken as an index to determine the degree of metallic pollution. When *Thias sp*. (carnivorous snail) was given a diet of oysters collected from CCA treated canals, the growth rate is highly reduced Weis *et al.,* 1992; Weis & Weis, 1992a. Barnacles growing on CCA treated piles showed reduction in over all growth rates when compared to that on the untreated ones. It was found that the diameter of the barnacles growing on treated panels were about 8mm, while that on untreated ones were 11mm. A decrease in bryozoans (*Membranipora* sp.) colonies was also apparent on treated wood (Weis *et al.*, 1991).

The change in the biodiversity of organisms in a particular area is effectively used as tool for the pollution monitoring and impact assessment studies. The status of a particular organism in an ecosystem is closely linked with the biotic and abiotic conditions adjacent to it. Any alteration in these factors is ultimately expressed as changes in the biodiversity in the population. Due to these changes most sensitive organisms are easily eliminated from the polluted environment while the tolerant ones became established due to decreased interspecific competition, resulting in a more simplified ecosystem. To measure the community changes diversity indices are commonly employed. Many studies conducted showed that the metal leachate from the CCA treated wood has adverse effects on early fouling community (Weis & Weis, 1996; Brown & Eaton, 2001). It was found that the

species diversity, abundance of certain species and biomass levels were significantly reduced on CCA treated panels. The studies showed that many of the larval forms of macrofouling organisms like bryozoans, serpulid polychaetes, barnacles and ascidians are prevented from settling on treated wood. Individuals of certain species was found to be more in number on CCA treated panels than on untreated ones and in the case of serpulid forms like *Ficopomatus enigmaticus* abundance of individuals increased with increase in CCA preservative loadings. The studies on early colonization by macroalgal species showed that percentage cover of most species of algae was almost similar on surface of both treated and untreated panels except *Hincksia granulosa* and *Ceramium nodulosum* which was found to be decreasing (Weis & Weis, 1996; Brown & Eaton, 2001; Brown *et al.*, 2001; Brown *et al.*, 2001b; Brown *et al.*, 2003).

Studies by Brown and Eaton (2001) reported the dominance of certain species like *Eluminius modestus, Hydroides ezoensis* and *Electra pilosa* on CCA treated panels while total number of species remained same. However, the study does report decrease in both the abundance and diversity of biota on control panels compared with the treated panels. It was hypothesized that due to the structural weakness, during retrieval of the panels some wood along with the organisms get cast off from untreated ones leaving an inadequate data for analysis (Brooks, 2000).

The actual cause of the dominance of some organisms on CCA treated panels is not yet defined. The reasons cited were that treatment procedures

may change the surface properties of the wood like texture, colour, etc that may in turn affect the attachment of the organisms. It was concluded that the leaching of CCA from treated wood is insufficient to disturb the fouling community development living at sites with normal tidal water circulation (Brown & Eaton, 2001). However, the organisms are found to be adversely affected by the close proximity of the leachate by Weis during her studies (Weis & Weis, 1992a; Weis & Weis, 1996).

But according to some studies the CCA leachate has no adverse effects on non-target aquatic biota. Studies were conducted to examine the effect of CCA and other preservatives on aquatic organisms at Krishnapatnam on the east coast of India (Tarakanadha & Satyanarayana Rao, 2002). According to the study results it was reported that impact of CCA preservative on epibiotic community in the sea is negligible, where total number of individuals, biomass and growth were actually higher on CCA treated panels when compared to the control ones. According to him, heavy settlement of barnacles, bryozoans, oysters and moderate settlement of serpulids on CCA treated panel is an indication that preservatives have less impact on fouling organisms or the organisms may be tolerating the toxicants. It was already reported that variation in the physical, chemical and biological properties of wood to a greater extend determines the settlement and abundance of certain species of organisms especially barnacles. CCA preservative treatment changes the physical properties of timber, giving it a

different texture and the colour of wood that may enhance barnacle settlement (Tarakanadha & Satyanarayana Rao, 2002)

1.9. Alternatives to CCA

The growing awareness about pollution caused by these chemicals called attention to the research on eco-friendly wood preservatives. Alternatively, protection of wood in the aquatic environment has been achieved using physical barriers, based on sheathing and coating that can prevent leaching. The surface finishes paint, varnishes and other protective stains have been in use for coating CCA treated wood primarily because it improves the a appearance of treated wood. Studies were conducted on the performance of surface finishes that are applied on CCA treated wood (Feist, 1989; Ross *et al.*, 1992). When compared with mere treatment of waterborne or solvent borne preservatives on wood, a coating of paint over the treated wood surfaces enhances the decay resistance and weather resistance. Though scientific research on the leaching of CCA components through these surface finishes were not much, the studies have shown that the leaching of chemicals especially of arsenic is lower through these coatings (Cooper *et al.*, 1997; Stilwell, 2004).

Fibreglass Reinforced Plastic (FRP) is a composite made from Fibreglass Reinforcement in a plastic (polymer) matrix, which can act as an effective barrier to protect wood from biodeterioration. Historically, the development of FRP can be traced to 1940s when the North Americans first used FRP for coating the hull of boats. Innovative application of FRP as a

boat building was initiated on the development of catalysts that made the FRP resins to cure much faster and that imparted certain improved characteristics and the use of glues that effectively bonded the FRP resins to wooden base. According to the chemical composition of the resin and varying the reinforcement certain characteristics like water absorption, resistance to UV radiations and strength properties are improved. Lower production cost and faster and less skilled production systems made the developed countries to opt for large-scale use of FRP for boat building. While in developing countries like India where the insufficient supply of raw materials, relatively high cost involved in the production and lack of technical knowledge became impediments to the wide use of FRP in fishing sector (Venugopal, 1979; 1980). The primary function of sheathing of FRP on wooden boats is to protect wood from attack of marine woodborers and the biodeteriorating agents. One layer of FRP sheath having a thickness approximately of one millimeter can prevent the entry of water into the wood and there by reduce the possibility of fungal growth. FRP sheath can also improve the appearance of the structure, reduce maintenance. The studies on the application of FRP sheathing of wooden fishing canoes are being carried out in the Central Institute of Fisheries Technology, Cochin. CIFT has already made four prototype canoes, with rubber wood as a base material that is sheathed with two layers of FRP that are under experimental operation.

Even after 70 years of history of use of CCA and other chemically treated wood in aquatic environments there is little or no convincing evidence

of adverse effects. But even now only limited information is available about the ultimate fate potential environmental hazards of CCA treated wood in the aquatic environment. In tropical countries like India where the use of these chemically treated timbers are increasing the study on the impact of CCA on the aquatic environments assumes great relevance.

1.10. Scope of the study

The impact of a chemical added into a particular environment can be studied by different methods. The metal pollutants are highly toxic to the aquatic organisms in some form or the other these are soluble in seawater. The free ions, the most bio-available forms of metals are readily taken up by the organisms and either accumulated in the body tissues or released. If the metal gets accumulated, both the essential metals in higher quantities and the non-essential metals in minute quantities are harmful to the normal functioning of the organisms. Chromated Copper Arsenate (CCA) is a waterborne preservative containing metals like copper, chromium and arsenic mixed in their oxide forms. The insecticidal properties of arsenic pentoxide and the fungicidal properties of copper sulphate make CCA one of the most efficient wood preservatives. Although CCA is considered to be a fixed type of preservative, the studies have shown that there is a possibility of leachning out of constituent metals into the surrounding waters and the rate of leaching depends on many factors. According to the reports there are about 208 preservative treatment plants in India, using about 400 tonnes of CCA and CCA like preservatives. The marine plywood manufacturers are

the major consumers of CCA. So the possibility of direct toxicity of CCA as the run off from CCA treatment plants or by leaching from the treated wood cannot be ignored. The present investigations were carried out in order to elucidate the possibility of the ecological risks associated with the use of CCA treated wood in the aquatic conditions.

1.11. Objectives of the study

The present study covers two aspects of prime importance the extent of protection offered to rubber wood by CCA preservative treatment and the impact of CCA treatment on the aquatic organisms. The main objectives are to study

- the biodeterioration of rubber wood under aquatic conditions and the extent of protection offered to rubber wood by CCA preservative treatment
- the biodeterioration of untreated and CCA treated rubber wood by visual observations and X-ray photography
- the biodeterioration by studying the changes in the microstructure of untreated and treated rubber wood
- the biodeterioration by physical analysis, chemical analysis and mechanical strength tests
- the acute toxicity of CCA and its chemical components *viz.* copper, chromium and arsenic separately

- the bioaccumulation of CCA components in fishes reared in aquaria under laboratory conditions
- the bioaccumulation of CCA components in barnacles growing on the treated wood under the field conditions
- the impact of CCA treated wood in the biodiversity and the total biomass of fouling organisms growing on the treated wood
- the efficacy of dual treatment and protective barriers *viz.* FRP and paint in preventing the biodeterioration of CCA treated rubber wood and its effect on biodiversity of fouling organisms.

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 102ºC ± 1ºC 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^{o}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).

$$\text{Retention, kg m}^{-3} = \frac{1000\ GC}{V}$$

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 in to 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^o 57.759' N and 76^o 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 ± 1^{o}C. 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 p^{H} was determined using p^{H} Testr (Eurotech Instruments) calibrated to P^{H} 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 size1 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 $CuSO_4.5H_2O$ (M. W.- 249.53), $CrCl_3.6H_2O$ (M.W. - 266.48), As_2O_3 (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.

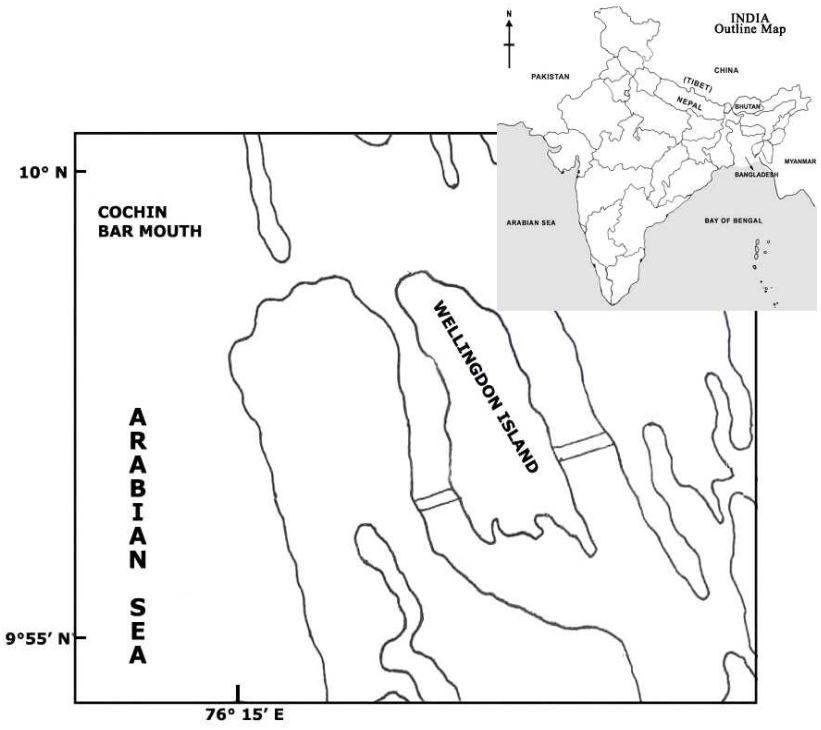
In the laboratory, the experiments were conducted in order to determine the bioconcentration possibilities of copper, chromium and arsenic that leach out from CCA treated panels. The studies were conducted in Tilapia

(*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

Retention ($kg\ m^{-3}$)		Initial vacuum (cm of Hg)	Time (min)	Pres-sure (kPa)	Time (min)	Final vacuum (cm of Hg)	Time (min)
CCA	16	56	30	172.37	90	38	15
	29	56	30	448.16	30	38	15
	42	56	30	517.11	15	38	15
Creosote	150	56	30	344.74	45	38	15

Figure 2.1. Map showing the location of the test site



Chapter III

3. RESISTANCE OF CCA TREATED RUBBER WOOD TO MARINE BIODETERIORATION

3.1. Introduction

Biodeterioration of wood under aquatic condition is mainly due to the attack of woodborers and degradation due to fungi was considered negligible. Earlanson in 1936 conducted a preliminary survey on marine wood boring organisms along the Indian coast. About sixty-three species of woodborers are already reported from the Indian waters that belongs mainly into two groups *viz*. crustaceans (Phylum: Arthropoda), Pholads and Teredinids (Phylum: Mollusca). All along the west and east coast studies were conducted on different aspects of biodeterioration of wood under marine condition and the distribution and characteristics of marine wood boring organisms. In the west coast investigations were carried out by Cheriyan (1964), Santhakumaran and Jain (1983), Santhakumari and Nair (1984), Raveendran and Wagh (1988), Santhakumaran and Rao (1994) and along the east coast by Nagabhushanam and Alam (1988), Karande (1968), Vishwakiran *et al.*, (2001). Nair (1968) has given a detailed description of various timber destroying organisms along the coasts of India specifying the need for the information on marine borers.

Biodeterioration of wood in the aquatic condition can be effectively minimized by the use of chemical preservatives. Chromated Copper Arsenate (CCA) is water borne preservative and has been well recognized as protecting

the wood against marine borers. In India, in the present scenario of depleting forest resources, studies were focused on the use of non-conventional timber species like rubber wood that is easily available as an agricultural by-product. It was established that, although rubber wood has mechanical properties comparable to other conventional timbers, it is highly vulnerable to biodegradation (Shukla & Lal, 1985; Gnanaharan & Damodharan, 1992). Preliminary studies conducted in rubber wood showed that CCA treatment can impart better protection to rubber wood under marine condition (Rao *et al.*, 1993; Edwin & Pillai, 2004). CCA also finds its application in commercially available marine grade plywood that has applications in marine boat building. According to the recent reports there are about 5547 marine plywood boats working along the Kerala alone (SIFFS, 1998). There are species of marine borers like *Sphaeroma* spp. (Phylum: Arthropoda), which are found to bore into the CCA treated wood. Combining the action of waterborne as well as the oil borne preservatives a dual preservative treatment method was also introduced that involves the pressure impregnation of CCA followed by creosote. The dual preservative treatment was recommended in the areas of high borer hazard. According to the recommendations by American Wood Preservers' Association the retention of CCA preservative for marine application is 40 kg m^{-3} and dual preservative in which wood is treated with 16 to 24 kg m^{-3} followed by creosote at a retention of 320 to 400 kg m^{-3} (AWPA, 2003).

It was only in the last decade, the problem of leaching of constituents copper, chromium and arsenic from CCA treated wood on exposure to aquatic environment came into view. In recent years, the studies were focused on recommending new alternative materials to replace CCA that can impart better protection to wood in the aquatic environment and at the same time able to prevent the leaching of CCA components from the treated wood. Eaton (1985), Eaton and Hale (1993) discusses the use of physical barriers like metals, concrete, plastics, Fibreglass Reinforced Plastic (FRP) as possible materials to protect wood in extreme marine conditions.

The objectives of the study are to: (i) assess the nature and extent of biodeterioration of untreated, CCA and dual preservative treated rubber wood and to compare it with the performance of commercially available CCA treated marine grade plywood (ii) assess the performance of rubber wood panels protected using physical barriers such as paint and FRP in the estuarine condition**.**

3.2. Materials and Methods

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. The preparation of the rubber wood panels, preservative treatment procedures is detailed in Chapter II (Table 2.1.). Six replicates of all the panels were

installed on iron racks and immersed in the test site. The details regarding in the test site and immersion of the panels at the test site are described in Chapter II (Fig. 2.1).

3.2.1. Examination and evaluation of the panels

The experiment was patterned as per the standard method BS EN 275:1992 intended to study the protective effectiveness of preservatives applied to wood by vacuum pressure impregnation. As per the standard, six replicate samples were used in the experiment. The experiment was started in June 2005. Half the number of the panels immersed in the test site was scraped monthly to remove the fouling in order to facilitate the action of wood boring organisms. After 18 months of continuous exposure in the estuary these panels were returned to the laboratory for analysis. The other half, which was left as such, included three sets of panels each consisting of eight different treatment types. Each of these sets consisted of panels of eight different treatment types that were removed from the frames after exposure periods of six, twelve and eighteen months. Panels were washed in seawater and transported into the laboratory in polythene bags having sufficient seawater. The fouling organisms were scraped off from the panels and examined dominant foulers were noted. The wood boring organisms on the panels were identified, sorted out into different classes.

3.2.1.1. Visual observations

The panels were visually examined for the presence of borer attack. The number, diameter and depth of borer holes on each type of the panel was

counted and measured. The destroyed superficial area was traced on to a graph sheet and estimated. The crustacean attack on each panel was graded as, the panels with no signs of attack was rated as 0, the panels showing galleries covering not more than 10% of total surface area rated as 1, the panels with more than 10% of the total area deteriorated was rated as 2, the panels with severe attack with substantially reduced cross- sectional dimensions was rated as 3 and the panels with more than half of the total volume of the panel was lost was rated as 4.

3.2.1.2. X - ray radiographic analysis

The internal damage of the panels was assessed by X-ray analysis after 6, 12 and 18 months of exposure in the estuarine condition (BS EN 275:1992). The X-ray radiographs of the panels were taken under 60 MA Elpro X-ray machine. The distance between the panel and radiation unit was about 76 cm the panels were exposed to X-ray (45 kV, 20 mA) for 15 sec. The darker regions in the X-ray radiograph represent the tunnels in surrounding wood matrix. The length and diameter of the tunnels were measured and the area/volume of the tunnels was calculated. The panels were graded according to area of the tunnels in the X- ray radiograph for the attack of molluscan woodborers. The panels without any attack was rated as 0, the panels with not more than 15% area deteriorated was rated as 1, the panels with moderate attack of not more than 25% of area deteriorated was rated as 2, the panels with severe attack of 25% to 50% area deteriorated was rated as 3 and those panels with tunnels covering more than 50% of the area deteriorated was rated

as 4. For each of the panels the total area and volume destroyed was calculated from the data and reported as percentage area and percentage volume of the panel destroyed per total area and volume. Eaton (1985) suggested X-ray analysis as a non-destructive method to study the biodeterioration due to teredinid borers. This technique has been well adopted and established to assess the damage due to molluscan woodborers (Rutherford *et al.,* 1980; Barnacle & Ampong, 1983; Edwin & Pillai, 2004)

Water samples were collected fortnightly to study and to find out the influence of hydrographical parameters on biodeterioration of wood. The hydrographical conditions prevailing in the test site throughout the period of study was monitored. Atmospheric temperature and water temperature were measured in the field using centigrade thermometer corrected to ± 0.1ºC. Water samples brought to the laboratory were analyzed for dissolved oxygen, salinity, P^H and turbidity as per standard methods (Strickland & Parsons, 1965).

3.3. Results and Discussion

3.3.1. Visual observation

All the panels were overgrown with various fouling organisms during the entire period of the study. The major macrofoulers observed included barnacles, hydroids, mussels, and polychaete worms. Small crabs and fishes like goby were also found in association with the panels. The major wood boring organisms observed during the period of investigation included, crustacean woodborer *Sphaeroma* spp. (Family: Sphaeromidae) and molluscan

woodborer *Teredo* spp. (Family: Teredinidae). The Pholads were represented only by *Martesia* spp. *Sphaeroma* was the only wood-boring organism observed on the periphery while *Martesia* and *Teredo* spp. resided well inside the panel.

It is a well-known fact that the hydrography of Cochin backwater system is related to a greater extent to the monsoon and rainfall. The hydrographical data collected during the study showed marked difference between the pre monsoon, monsoon and post-monsoon periods (Fig.3.1.and Fig. 3.2.). The changes in the atmosphere temperature and water temperature during this period varied within a range 28.87 ± 1.97 (Fig. 3.1.). The salinity, dissolved oxygen and turbidity showed significant variations according to the prevailing season. Two monsoon seasons marked the 18 months study. The study was started during onset of monsoon in June and as the south-west monsoon and north-east monsoon intensified the salinity at the site reduced gradually (Fig. 3.1). During the monsoon, heavy wave action and churning up of the sediments increased turbidity of water and reduced the amount of dissolved oxygen (Fig. 3.2.). In the present investigation crustacean woodborers was present on all the panels irrespective of the preservative in use and seasonal changes. It was the only species observed during the monsoon season when there was a sudden drop in salinity. The occurrence of *Sphaeroma* spp. throughout the study period may be due to the fact that it breeds throughout the year and can tolerate wide ranges of salinity and temperature (Pillai, 1961; Cherian, 1964). In the present investigation there

was a progressive increase in the occurrence of *Teredo* spp. in the panels with increase exposure periods *viz.* 6, 12 and 18 months.

The results of the visual observation of the panels are summarized in Table 3.1., Table 3.2. and Table 3.3. In the present study the untreated rubber wood samples failed between 6-12 months on continuous exposure in estuarine condition. The marine plywood panels retrieved after an exposure period of 18 months also failed. Preservative treated panels to a lower retention of CCA *viz.* 16 kg m^{-3} showed severe attack after 18 months while the panels with a higher retention of 29 kg m^{-3} CCA showed trace attack. The panels treated with CCA to retention of 42 kg m^{-3}, was free of attack even after 18 months of continuous exposure in the estuarine condition. The dual treated, painted and FRP sheathed rubber wood panels provided 100% protection during the entire period of estuarine exposure.

In the present investigation, the panels that are scraped clean of fouling for 18 months showed a higher intensity of damage than the panels with fouling that are kept continuously for 6, 12 and 18 months (Table 3.4.) The untreated control samples failed between 6-12 months. After exposure for 18 months, CCA treated panels to three retentions *viz.* 16, 29 and 42 kg m^{-3} and dual treated panels that are scraped monthly showed borer attack. When fouling organisms were allowed to grow on the panels continuously, the dual preservative treated panels and panels with 42 kg m^{-3} retention of CCA were free of borer attack. The periodical removal of fouling organisms facilitated the attack of woodborers and the panels of 42 kg m^{-3} retention of CCA and

dual treated panels showed superficial damage. One of the painted samples also showed borer attack on continuous removal of fouling from its surface. This may be due to the fact that during scraping, the paint layer on the surface might have been removed that facilitated attack by woodborers. FRP sheathed panels were completely free of borer attack. From the results it can be assumed that the extensive growth of fouling assemblages limit the chances of attack of wood by woodborers. The results were in agreement with the finding that the heavy fouling assemblages inhibit the wood boring molluscs (Nagabhushanam, 1960).

The superficial damage of the panels was due to the attack of *Sphaeroma* spp. Their burrows were shallow and cylindrical with an average diameter of 0.5 cm and heavy infestations were shown as honeycomb like excavations. The intensity of damage due to *Sphaeroma* was high only on the surface, but the major agent causing damage was *Teredo* spp. According to the studies conducted, marine crustaceans damaging the wood superficially attack wood merely for shelter and rely on filter feeding (IWST, 1997). It was known that the borer holes of shipworms are very small since the young larval stages are attacking the wood. Once the organisms entered into the wood, they tunnel deep inside and utilize the fine particles of wood as food (IWST, 1997). In the present investigation, the superficially visible excavations made by *Teredo* spp. were of maximum diameter 0.1 cm. The burrows produced by *Teredo* spp. and *Martesia* spp. were similar in size and shape externally but a much higher intensity of internal damage due to these organisms were observed

through X-ray radiographic studies. Fig. 3.3. show the internal destruction of a estuarine exposed panels, where the characteristic deteriorative borer holes due to *Sphaeroma* spp., *Teredo* spp. and *Martesia* spp.

After three months of immersion the untreated and the marine plywood panels showed presence of borer holes. The intensity of damage was very low as the number of burrows was less with a maximum diameter of 2 mm. When compared to the untreated panels higher number of borer holes was observed in marine plywood. On retrieval after 6 months, 22 borer holes in the untreated rubber wood panel and 14 borer holes in marine plywood were visible externally. The borer holes on both the panels were of an average diameter of 5 mm. Average depths of borer holes on untreated and marine plywood panels were 8 mm and 11 mm respectively. A single borer hole was observed on CCA treated panel of 29 kg m^{-3} while CCA 16 kg m^{-3}, CCA 42 kg m^{-3}, dual treated, FRP and paint coated panels were without trace of attack. After continuous immersion at the test site for a period of 12 months, the panels showed increased intensity of borer holes by *Sphaeroma* spp. The untreated rubber wood panel showed 4.5 times increase in number of borer holes, while in marine plywood no significant change was observed. Numerous side branches were observed from the main burrow completely damaging certain localized areas on the wood surface. FRP sheathed, painted and preservative treated panels except CCA retention of 16 kg m^{-3} were free of damage after exposure for 12 months. The untreated rubber wood samples retrieved after 18 months of exposure were completely destroyed making it difficult to count

and measure the dimensions of borer holes. The preservative treated panel with CCA retention of 16 kg m^{-3} showed localized damage removing a part of the panel making it difficult to count the number of borer holes. About 45 borer holes were observed in CCA treated panel with 29 kg m^{-3} retention. The painted FRP sheathed preservative treated to CCA retention of 42 kg m^{-3} and dual treated panels were free of borer attack.

3.3.2. X-ray radiographic studies

The damage caused by *Teredo* spp. was not clearly visible superficially but X-ray radiographs revealed the presence of long tunnel like burrows deep into the wood (Fig. 3.4). These tunnels lined with calcareous shell that are characteristic to *Teredo* spp., appeared darker than the surrounding wood (Fig. 3.4). Active infestation of the species was observed on untreated rubber samples than the treated ones. Even after an exposure period of six months tunnel like burrows with an average diameter of 9 mm was observed on untreated and marine plywood panels. Three such long burrows of length 122 mm, 86 mm and 70 mm were observed in the untreated panels while two such burrows of length 61 mm and 200 mm was observed in marine plywood. The treated panel with 16 kg m^{-3} retention of CCA showed internal damage due to shipworms after a continuous exposure of twelve months in the estuary. Although these panels showed no signs of borer attack externally, X-ray radiographs revealed two burrows each of length 47 mm and 26 mm with an average diameter of 4 mm. The untreated panel showed higher numbers of borer holes while the other panels *viz.* CCA 29 kg m^{-3}, CCA 42 kg m^{-3}, dual

treated panels, FRP and paint coated panels were without any internal tunnels due to the attack of ship worms. The untreated rubber wood samples retrieved after 18 months was completely infested with *Teredo* spp. were totally disintegrated that the area destructed was difficult to measure using X-ray radiograph. Marine plywood and panels treated to CCA retention of 16 kg m^{-3} showed the presence of long tunnels deep inside. An X-ray radiograph is showing internal destruction of the panels due to the attack of *Teredo* spp. is presented in Fig. 3.4.

3.3.3. Reduction of Surface area and volume

It was observed that extent of biodeterioration caused by *Sphaeroma* spp. was rather insignificant when compared to that of *Teredo* spp. The total superficial area destroyed due to the attack of *Sphaeroma* spp. was small when compared to the total volume deteriorated due to *Teredo* spp. During 6, 12 and 18 months of exposure in the field the untreated rubber wood samples showed a progressive increase in destruction. The periodical damage in total superficial area observed was 1.78 cm^2, 14.35 cm^2 and 300 cm^2 and total volume destroyed was 80.16 cm^3, 330.79 cm^3 and 375 cm^3. Marine plywood panels showed destruction in total area of 1.22 cm^2, 3.24 and 4.25 cm^2 with a corresponding loss in volume of 80.2 cm^3, 42.8 cm^3 and 248.3 cm^3. After 18 months of exposure in the field, the area of the panel destroyed was 18.84 cm^2, 5.8 cm^2 for CCA 16 kg m^{-3} and CCA 29 kg m^{-3} with a corresponding loss in volume of 81.6 cm^3 and 1.2 cm^3 respectively. None of the panels treated with CCA to a retention of 42 kg m^{-3} and dual preservative showed deterioration.

FRP sheathing and paint coating accorded a 100% protection to the underlying rubber wood panel. The percentage area and volume reduced due to the attack of wood borers during 6, 12 and 18 months of immersion of the panels in the field are given in the Table 3.5.

The results of the present investigation showed that preservative treatment could increase the service life of rubber wood to a greater extent. Earlier studies conducted have shown that rubber wood without any treatment gets completely destroyed after an exposure period of six months (Rao *et al.*, 1993; Edwin & Pillai, 2004). In the present investigation, the total volume of the untreated rubber wood deteriorated was 21% within six months and 88% within one year. In India, studies pertaining to the use treated wood with chemical preservative for marine application were initiated in 1960s. Cheriyan and Cherian (1983), Krishnan *et al*., (1983) reported that the durability of less durable timbers like *Albizzia sp*. *Tetrameles nudiflora*, *Pinus longifolia* etc. in the marine condition increased when treated with CCA. According to the studies conducted in Goa waters by Sanathakumaran and Krishnan (1991), CCA is very effective in preventing the attack of woodborers. They were of the opinion that same type of preservative treatment offers different degrees of protection to different wood species. Although CCA treated panels prevented the deterioration of wood the panels were destroyed after prolonged service in the field (Rao *et al.,* 1993; Kuppusamy *et al.*, 2004; Cookson & Barnacle, 1987). It was reported that crustacean woodborers become tolerant to copper as these organisms store copper as granules in the hepatic caeca and hereby

lower retentions of CCA can be nonresistant to the attack of woodborers (Cragg & Icely, 1982; Barnacle *et al.*, 1983; Cookson & Barnacle, 1987). Studies were conducted by Sreenivasan and Vallabhan (1988) in Madras harbour waters using a variety of durable wood species like *Bombax ceiba*, *Terminalia alata* etc. that are treated to CCA to two different retentions 16 kg m^{-3}, 32 kg m^{-3}. The results showed that CCA treatment to higher absorptions considerably increased the durability of wood. The present investigation confirmed that CCA is very effective in preventing the attack of woodborers and higher preservative retentions give higher degree of resistance to biodeterioration by woodborers.

Dual preservative treatment is known to prevent crustacean woodborers effectively (Richards, 1983). Studies conducted by Johnson and Gutzmer (1984) using Southern pine samples treated with dual preservative resisted borer attack for 6 years. Previous investigation carried out at Cochin estuarine waters for 19 months showed that the dual preservative treated panels of *Mangifera indica* were completely free of borer attack throughout the period (Edwin *et al.*, 1993). American Wood Preservers' Association (2003) recommends a final dual preservative retention of 320 to 400 kg m^{-3} in the areas of heavy borer attack. Previous investigation carried out on rubber wood treated with CCA and creosote (dual treatment) with a final average creosote retention of 160 kg m^{-3} was shown to prevent the borer attack for more than 33 months (Edwin & Pillai, 2004). In the present investigation, CCA retention of 16 kg m^{-3} followed by creosote retention of 150 kg m^{-3} was

found to be efficient in preventing both crustacean and molluscan woodborers. The studies regarding the use of paint to prevent the settling of fouling organisms were known while very little has been known about anti woodborer paint formulations. According to Highley (1999) the copper containing antifouling paints protects boat hulls against woodborer until the coating remains unbroken. In the present investigation as long as the paint coating remained as such without exposing the underlying wood, it provided 100% protection against woodborers. While the untreated and treated rubber wood panels without any physical protection showed internal damage that are evaluated in terms of microstructural deteriorative aspects that are detailed in chapter IV.

3.4. Conclusion

From the investigation carried out it can be concluded that the service life of rubber wood can be increased by CCA as well as dual preservative treatment. It was also observed that extensive growth of fouling assemblages on the panels hinder the action of woodborers to a considerable extent. The preservative treated panels performed well in preventing the attack of marine woodborers. But on prolonged exposure for 12 to 18 months the panels treated to lower retentions of CCA *viz.* 16 kg m^{-3}, 29 kg m^{-3} showed susceptibility to borer attack especially due to *Teredo* spp. But the deterioration of panels with 29 kg m^{-3} retention of CCA is negligible where only 1.2 % total volume of wood was destroyed in 18 months. Higher retention *viz*. 42 kg m^{-3} of CCA preservative in wood imparted higher degree of protection. Dual treated panels

performed equally well as CCA treated panels of 42 kg m^{-3} retention. The physical barriers that were applied on CCA treated rubber wood panels accorded a 100 % protection against woodborers.

Table 3.1. Performance rating of panels for attack by Teredinids, other molluscs and crustaceans after an exposure period of six months in the estuary

Type of the panel	Rating for attack of crustaceans			Rating for the attack of molluscs		
	Rating	Classification	Condition and appearance of test specimen	Rating	Classification	Condition and appearance of test specimen
Rubber wood (Untreated)	1	Slight attack	Few scattered galleries	2	Moderate attack	Tunnels covering <25% of the area of the specimen as it appears on the X-ray film
CCA 16 kg m^{-3}	0	No attack	No sign of attack	0	No attack	No sign of attack
CCA 29 kg m^{-3}	1	Slight attack	Few scattered galleries	0	No attack	No sign of attack
CCA 42 kg m^{-3}	0	No attack	No sign of attack	0	No attack	No sign of attack
Dual	0	No attack	No sign of attack	0	No attack	No sign of attack
Paint	0	No attack	No sign of attack	0	No attack	No sign of attack
FRP		No attack	No sign of attack	0	No attack	No sign of attack
Marine plywood	1	Slight attack	Few scattered galleries	2	Moderate attack	Tunnels covering <25% of the area of the specimen as it appears on the X- ray film

Table 3.2. Rating for attack by Teredinids, other molluscs and crustaceans after an exposure period of twelve months in the estuary

Type of the panel	Rating for attack of crustaceans			Rating for the attack of molluscs		
	Rating	Classification	Condition and appearance of test specimen	Rating	Classification	Condition and appearance of test specimen
Rubber wood (Untreated)	1	Slight attack	Few scattered galleries	4	Failure	Tunnels covering more than >50% of the area of the specimen as it appears on the X-ray film
CCA 16 kg m^{-3}	1	Slight attack	Few scattered galleries	1	Slight attack	Few scattered tunnels covering <15 % area of the specimen as it appears on the X-ray film
CCA 29 kg m^{-3}	0	No attack	No sign of attack	0	No attack	No sign of attack
CCA 42 kg m^{-3}	0	No attack	No sign of attack	0	No attack	No sign of attack
Dual	0	No attack	No sign of attack	0	No attack	No sign of attack
Paint	0	No attack	No sign of attack	0	No attack	No sign of attack
FRP		No attack	No sign of attack	0	No attack	No sign of attack
Marine plywood	1	Slight attack	Few scattered galleries	2	Moderate attack	Tunnels covering <25% of the area of the specimen as it appears on the X-ray film

Table 3.3. Rating system for attack by Teredinids, other molluscs and crustaceans after an exposure period of eighteen months in the estuary

Type of the panel	Rating for attack of crustaceans			Rating for the attack of molluscs		
	Rating	Classification	Condition and appearance of test specimen	Rating	Classification	Condition and appearance of test specimen
Rubber wood (Untreated)	4	Failure	More than half of the original volume lost	4	Failure	Tunnels covering more than >50% of the area of the specimen as it appears on the X- ray film
CCA 16 kg m^{-3}	2	Moderate attack	More than 10% of the total surface area of the specimen covered with galleries	3	Severe attack	Few scattered tunnels covering <15 % area of the specimen as it appears on the X- ray film
CCA 29 kg m^{-3}	1	Slight attack	Few scattered galleries covering not more than 10% of the total surface area	1	Slight attack	Single tunnel covering <15% of the area of the specimen
CCA 42 kg m^{-3}	0	No attack	No sign of attack	0	No attack	No sign of attack
Dual	0	No attack	No sign of attack	0	No attack	No sign of attack
Paint	1	Slight attack	Single gallery	0	No attack	No sign of attack
FRP		No attack	No sign of attack	0	No attack	No sign of attack
Marine plywood	1	Slight attack	Few scattered galleries	4	Failure	Tunnels covering >50% of the area of the specimen as it appears on the X-ray film.

Table 3.4. Rating system for attack on panels (that are scraped of monthly to remove fouling) by Teredinids, other molluscs and crustaceans after an exposure period of eighteen months in the estuary

Type of the panel	Rating for attack of crustaceans			Rating for the attack of molluscs		
	Rating	Classification	Condition and appearance of test specimen	Rating	Classification	Condition and appearance of test specimen
Rubber wood (Untreated)	4	Failure	More than half of the original volume lost	4	Failure	Tunnels covering >50% of the area of the specimen as it appears on the X- ray film
CCA 16 kg m^{-3}	2	Moderate attack	More than 10% of the total surface area of the specimen covered with galleries	3	Severe attack	Few scattered tunnels covering <15 % area of the specimen as it appears on the X- ray film
CCA 29 kg m^{-3}	1	Slight attack	Few scattered galleries covering not more than 10% of the total surface area	1	Slight attack	Single tunnel covering <15% of the area of the specimen
CCA 42 kg m^{-3}	1	Slight attack	Few scattered galleries covering not more than 10% of the total surface area	0	No attack	No sign of attack
Dual	1	Slight attack	Few scattered galleries covering not more than 10% of the total surface area	0	No attack	No sign of attack
Paint	1	Slight attack	Single gallery	0	No attack	No sign of attack
FRP		No attack	No sign of attack	0	No attack	No sign of attack
Marine plywood	1	Slight attack	Few scattered galleries	4	Failure	Tunnels covering >50% of the area of the specimen as it appears on the X-ray film.

Table 3.5. The percentage area and volume of the panels deteriorated during 6, 12 and 18 months of immersion in the field

Panel Type	Six months		Twelve months		Eighteen months	
	% area destroyed	% volume destroyed	% area destroyed	% volume destroyed	% area destroyed	% volume destroyed
Untreated	0.418	21.377	3.376	88.210	70.58	100
CCA (16 kg m^{-3})	ND	ND	ND	2.445	4.43	81.6
CCA (29 kg m^{-3})	0.369	0.502	ND	ND	1.36	1.2
CCA (42 kg m^{-3})	ND	ND	ND	ND	ND	ND
Dual (150 kg m^{-3})	ND	ND	ND	ND	ND	ND
FRP	ND	ND	ND	ND	ND	ND
Paint	ND	ND	ND	ND	ND	ND
Marine plywood	0.287	22.21	0.76	11.41	1.0	66.23

***(**ND - Not Detected)

Figure 3.1. Fortnightly variations in the water temperature, atmospheric temperature and salinity during the study period

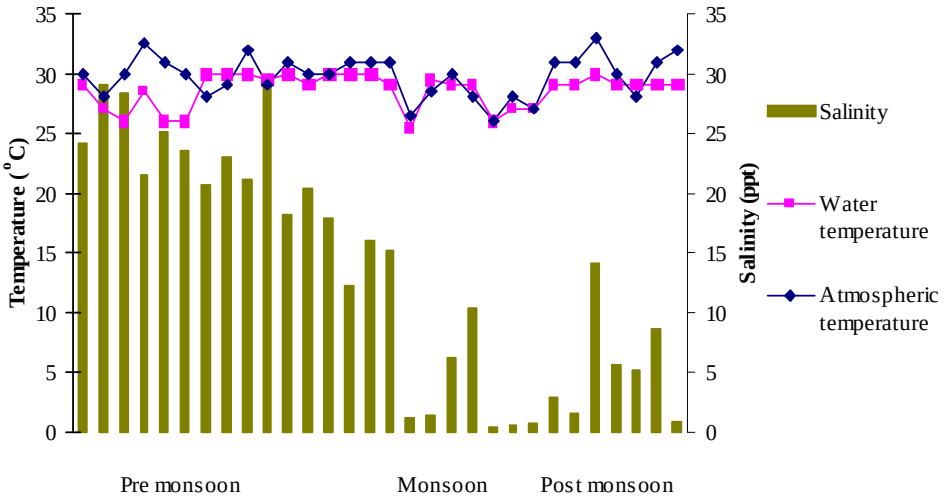


Figure 3.2. Fortnightly variations in dissolved oxygen and turbidity during the study period

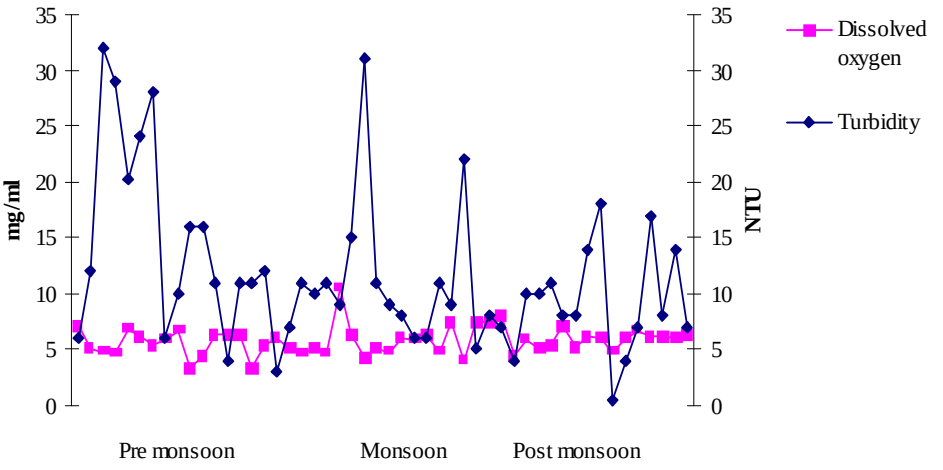


Figure 3.3. Internal destruction of the panels after exposure for 18 months in the estuary

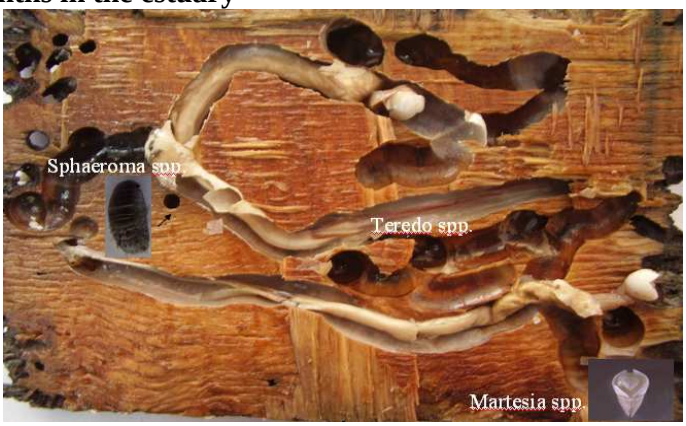
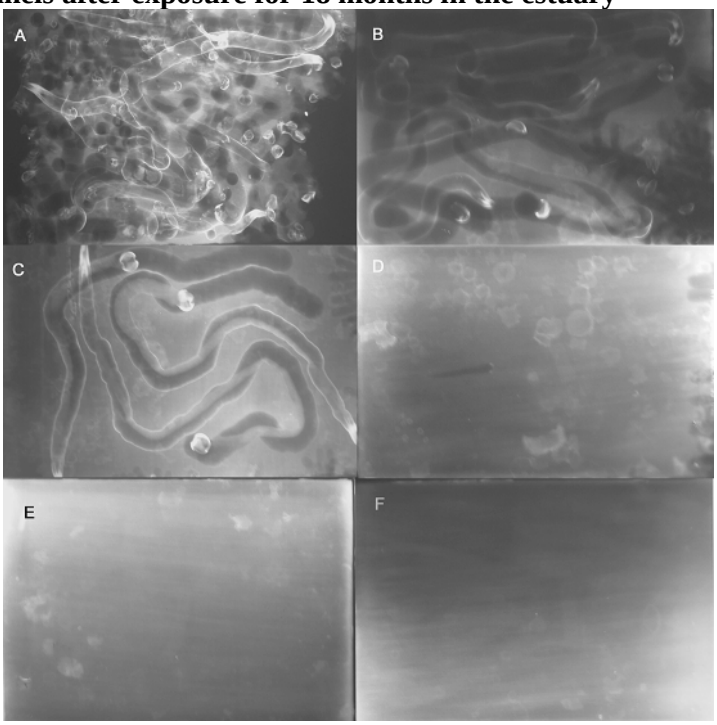


Figure 3.4. X-ray radiograph showing internal destruction of the panels after exposure for 18 months in the estuary



*A. untreated rubber wood, B. Marine plywood, C. CCA treated (16 kg m^{-3} retention), D. CCA treated (29 kg m^{-3} retention), E. CCA treated (42 kg m^{-3} retention) F. Dual

Chapter IV

4. MICROSTRUCTURAL CHANGES IN UNTREATED AND PRESERVATIVE TREATED RUBBER WOOD ON ESTUARINE EXPOSURE

4.1. Introduction

The wood exposed to different environmental conditions is always under the threat of attack by a number of organisms that include microorganisms like bacteria, fungi and woodborers. The changes during decay and deterioration of wood due to the attack of these organisms are characteristic to the species attacking the wood. The changes during deterioration are identified and characterized at different levels that range from the visually observed alterations to the basic anatomical changes. Studies have been conducted on the microstructure of the wood using Light microscopic and Electron Microscopic observations and the changes corresponding to decay and deterioration are characterized. Although highly sophisticated Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) studies occupy the fields, the light microscopic examination get much attention since it is much more rapid and requires minimum specimen preparation. The changes during wood decay was studied by Wilcox (1970), Blanchette *et al*., (1985), Nilsson *et al*., (1989) succeeded in making out some correlations with corresponding changes in the chemistry of cell wall components. The microstructure analysis has been also used as a tool to

understand and assess changes in preservative components in wood during the process of fixation (Petric *et al*., 2000; Peters & Parameswaran, 2004).

The objectives of the present study is to identify the changes in the anatomical structure of rubber wood under pressure treatment using preservatives, the changes occurring during biodeterioration of untreated and preservative treated rubber wood in the estuarine environment at different exposure periods and to correlate the changes in anatomy and changes in biodeterioration. The anatomical changes during the deterioration of the wood are studied in relation to the strength and compared with the chemical changes that are detailed in Chapter V.

4.2. Materials and Methods

The preparation of rubber wood panels, CCA and dual preservative treated panels and exposure of the panels in the test site are detailed in Chapter II. Panels treated with CCA to three different retentions viz. 16 kg m^{-3} (type I), 29 kg m^{-3} (type II), and 42 kg m^{-3} (type III) and the panels treated with creosote to a retention of 150 kg m^{-3} (type IV), untreated rubber wood panels together with the exposed panels for six, twelve and eighteen months were selected for the study. The treated rubber wood panels that are physically protected with FRP and paint experienced little damage in the aquatic condition and so these panels are not considered in the present study.

All the samples were cut in to small cubes of size 50 x 10 x 10 mm and kept in distilled water so that samples are completely immersed in water. Using a sliding microtome thin sections of thickness of 20µ and of uniform

size were cut from each of the samples. Cross sectional, longitudinal and radial sections were taken from each of the samples. The thin sections were thoroughly washed in distilled water and stained through a series of toludine-blue-O and passed through tertiary butyl alcohol (TBA) - Xylene series. The stained sections were washed thoroughly in distilled water and mounted on DPX. The slides were observed under a light microscope (Olympus 3X60) with a video camera attached to it.

4.3. Results and Discussion

The microscopical examination of Cross Section (CS), Radial Longitudinal Section (RLS), and Tangential Longitudinal Section (TLS) of degraded samples supported the visually observed changes during estuarine exposure, later on confirmed with the X-ray radiographs. The deteriorative changes observed in the microstucture of wood were compared with the changes in compression parallel to grain strength and FTIR analysis. The single stain used for the experiments differentially stains the cell wall components like cellulose and lignin. The stain chemically reacts to the cell wall components giving a light blue colour with cellulose and a deep blue color with lignin. The major cell elements distinguished in the sections from untreated unexposed rubber wood samples were parenchyma cells, vessels and fibers. In the cross section, the parenchyma cells were observed as thin walled polygonal cells, which are distributed sparsely in between the fiber cells. The fibers were identified as polygonal cells with thick lignified walls and narrow lumen. The vessel members were observed as large oval cells with tyloses

projecting in to the vessel lumen. These cells were intact and the walls of the cell elements were well defined. In the radial, longitudinal and the cross sectional analysis have shown that the fiber walls were smooth without any cracks. The ray parenchyma cells were filled with starch. The tension wood regions when present were revealed as fibers with thickened walls and with detached G-layer of the secondary cell wall that stains violet in toludine blue.

The sections from preservative treated panels showed a golden yellow coloured solution inside the cells (Fig 4.2., Fig 4.3., Fig 4.4., Fig 4.5.), which indicates that during pressure impregnation of rubber wood with CCA and dual preservative, the solution reaches well into the cells. Much of the solution was observed in parenchyma cells indicating a differential absorption of the solution by parenchyma cells when compared to fibers and vessel members. It was also observed that in dual treated panels the parenchyma cells bordering the vessels are scattered from the adjoining cells. This may be due to the fact that during dual preservative treatment the extra high pressure applied may be causing injury to the parenchyma cells. The granular material observed in the untreated unexposed samples probably because of the infiltrated water that was not removed even after washing in ethanol (Fig.4.1a).

Although light microscopic studies for ensuring preservative penetration were not reported electron microscopical studies to explain the changes in cell wall during fixation of chemical preservatives have been conducted. According to Bariska *et al.,* (1988) during the preservative treatment there occurs the chemical dissolution of cell walls Winandy and

Rowell (2005). When pressure treated, most of the injected preservative remains in the cell lumen and that during the process of fixation the metal components of CCA get deposited as their salts on the cell wall, which can be observed through the photomicrographs. The studies conducted by Peters and Parameswaran (1980) showed that copper particles are getting adsorbed onto the wood cell wall. TEM and X-ray microanalysis of the wood treated with metallic waterborne and organic solvent wood preservatives have shown that the metal components are preferentially adsorbed onto the lignin component of the wood that can be observed with localized distribution at cell corners and middle lamella (Petric *et al.,* 2000).

In the present investigation, it was confirmed that biodeterioration of rubber wood under estuarine condition can be evidenced through microstructure of the cell elements. In Fig. 4.1., the micrographs of untreated unexposed rubber wood samples were compared with that of untreated panels exposed for six, twelve and eighteen months. The deterioration observed was severe after 18 month as reported in Chapter III. As the deterioration progressed there was an increase in the intensity of damage to cell components. The distorted nature and loss of continuity of fiber cells were readily observable in deteriorated samples. The compact nature of the cells was lost and the inter-cellular spaces were increased. The fiber walls were found eroded and such cells were found detached from each other. The secondary layers of the fiber walls were also found detached.

When compared to untreated rubber wood samples the characteristic changes during deterioration was not well evident in preservative treated samples. The intensity of deterioration observed was less during exposure. The visual observation has shown that the CCA treated panels to lower retention of 16 kg m^{-3} (Type I) showed deterioration. The pits and cracks were observed in the walls of fibers Fig. 2. At the region of severe degradation the parenchyma cells were detached from each other. In some of the marine exposed samples the residual cell wall material is penetrating into the cell lumen. In CCA treated panels of 29 kg m^{-3} (Type II) retention and 42 kg m^{-3} (Type III) and dual treated samples the degradative changes observed was minor. With increased exposure in the field it was also noted that the golden yellow coloured solution retained in the parenchyma cells were gradually removed. It was also observed that some cells were filled with starch that stained black in the toludine blue Fig 4.1., Fig 4.2., Fig. 4.3., Fig. 4.4. and Fig. 4.5. In the dual treated panels the vessel walls were destroyed and the parenchyma cells lining the vessels were also found detached. The relative content of the solution inside the parenchyma cells were also high. This may be due to the fact that during dual treatment, CCA solution is impregnated into the cells applying pressure and after drying the wood is again pressure treated with creosote. The high pressure applied twice may be the factor that destroyed the vessel members and cells (Fig.4.5.).

In the present investigation it was observed that with increase in duration of estuarine exposure there occurs a progressive increase in

destruction of the panels (Chapter III). The loss of weight observed in exposed panels can be attributed to ultimate loss of wood substance due to destruction of wood by marine borers. The observed percentage weight loss of untreated rubber wood panels retrieved after estuarine exposure of 6, 12 and 18 months was 3.17, 27.97 and 64.43 respectively. The percentage weight loss in CCA treated panels was not significant up to 18 months of exposure. After twelve months of exposure, the weight loss observed for CCA treated panel with retention of 16 kg m^{-3} was 0.81% and for retention of 29 kg m^{-3} was 0.14%. After 18 months of exposure the percent loss of weight was 26.69 for CCA 16 kg m^{-3} and 3.42 for CCA 29 kg m^{-3}.

Loss weight of wood samples can be an effective index to denote the decay and biodeterioration. It was reported that the loss of compressive strength and stiffness owing to loss of wood components could be primarily indicated by loss of weight (Winandy *et al.,* 2000; Winandy & Lebow, 2001; Curling *et al.,* 2002; Edwin & Pillai, 2004). According to Winandy and Rowell (2005) the basic physical and mechanical properties of wood have a correlation with the chemistry of cell components that can be well explained at three distinct levels viz. macroscopic or cellular, microscopic or cell wall and molecular or polymeric. A number of studies have been reported where the changes in the anatomical structure of the wood was used as a tool to assess the decay of wood due to fungal attack. The loss of weight of rubber wood during fungal attack and corresponding changes in cell wall structure have been studied by Florence *et al.,* (2002). In the marine environment the decay

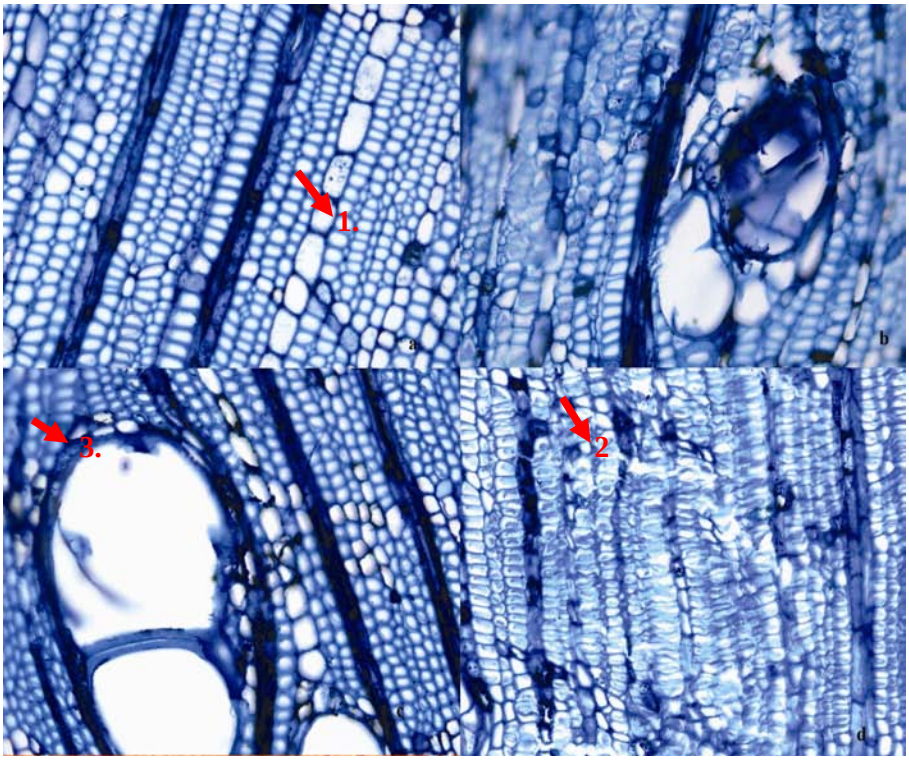
due to marine fungi is rather insignificant and the weight loss with corresponding loss of wood substance was attributed to the attack by wood boring organisms. In the present investigation it was observed that a differential absorption of wood preservatives took place in parenchyma cells, which are the basic living and food containing cells of wood. The higher toxicity imparted by the panels with high retention of CCA and dual preservative may be due to the presence of preservative solution in the parenchyma cells. The microscopical examination of the sections from CCA treated *Pinus radiata* pile after 12 years of marine exposure showed extensive loss of carbohydrate fractions of cell wall with corresponding changes in the cell wall structure including middle lamella and trachaeids (Singh & Hedley, 1990). The electron microscopic studies on degraded sample have shown that thickness of the fiber cells were reducing during decay (Hoffmann *et al*., 2004).

4.4. Conclusion

In this study, it was confirmed that the deterioration experienced by rubber wood under aquatic condition damages the wood both externally and internally as the anatomical structural components are damaged. As CCA treated panels to higher retentions *viz.* 29 kg m^{-3} and 42 kg m^{-3} and dual preservative treated panels experienced very little deterioration externally, the internal anatomical damage was also negligible. While untreated rubber wood panels and CCA treated panels to lower retention of 16 kg m^{-3} showed degradation of parenchyma cells and fiber cells resulting in the loss of

chemical integrity of wood and ultimate reduction in strength. The degradative changes observed during the study are later on supported by mechanical strength tests and chemical analysis as explained in Chapter V.

Figure 4.1. Light Microscopic image of untreated rubber wood samples
*(all micrographs at 200 μm scale, 1. Parenchyma cells 2. fibre cells 3.Vessels and tyloses)



a.) unexposed b.) exposed for 6 months, c.) exposed for 12 months, d.) exposed for 18 months

Figure 4. 2. Light microscopic image of CCA treated panel with retention of 16 kg m^{-3}

a.) (unexposed), b.) exposed for 6 months, c.) exposed for 12 months, d.) exposed for 18 months

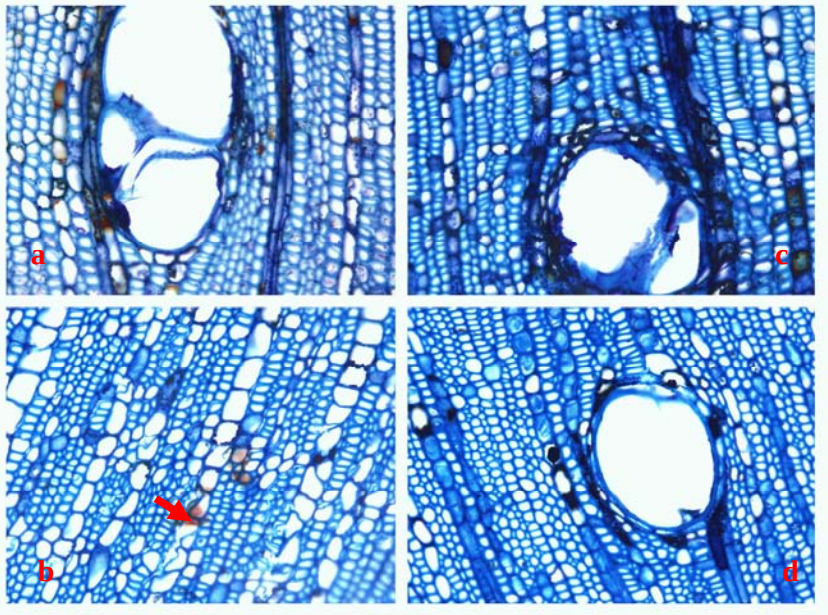


Figure 4. 3. Light microscopic image of CCA treated panels with retention of 29 kg m^{-3}

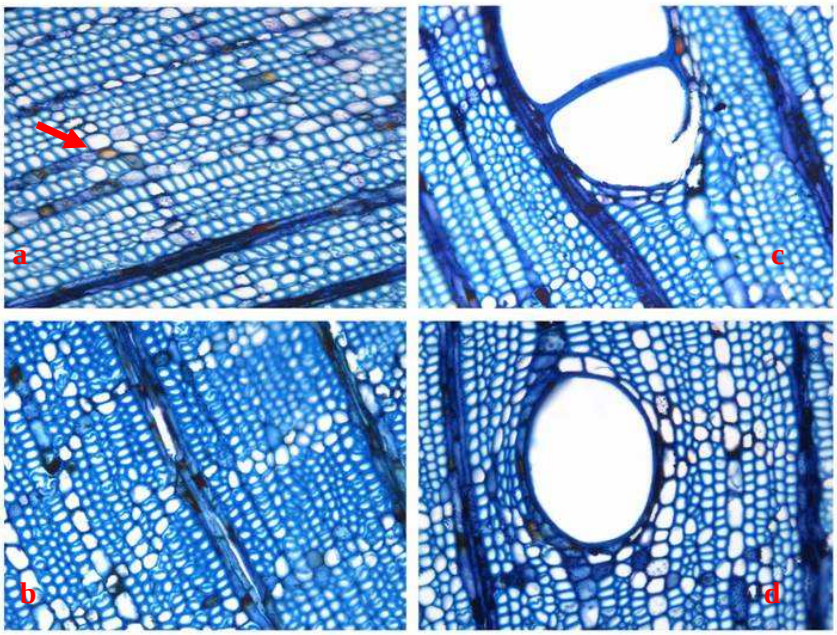


Figure 4. 4. Light microscopic image of CCA treated panels with retention of 42 kg m^{-3}
a.) (unexposed), b.) exposed for 6 months, c.) exposed for 12 months, d.) exposed for 18 months

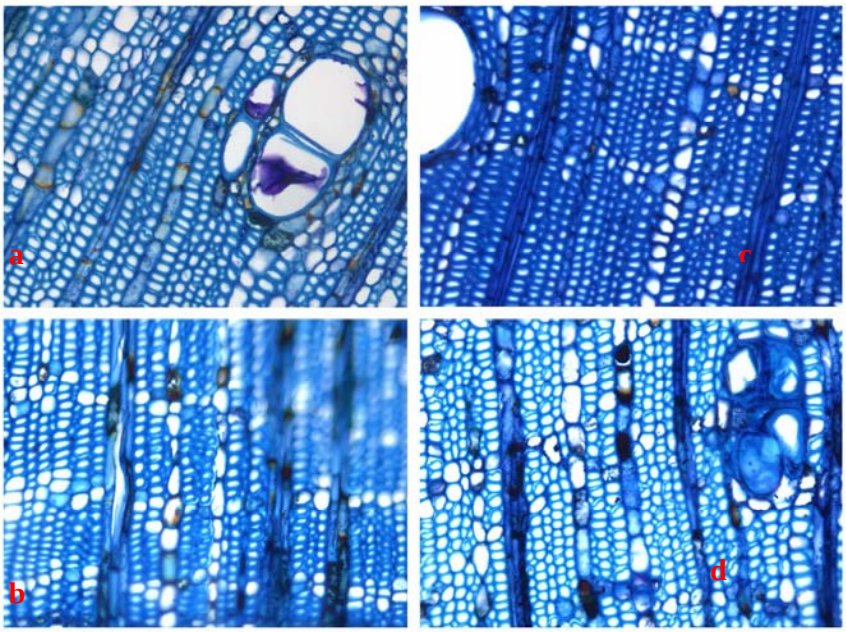
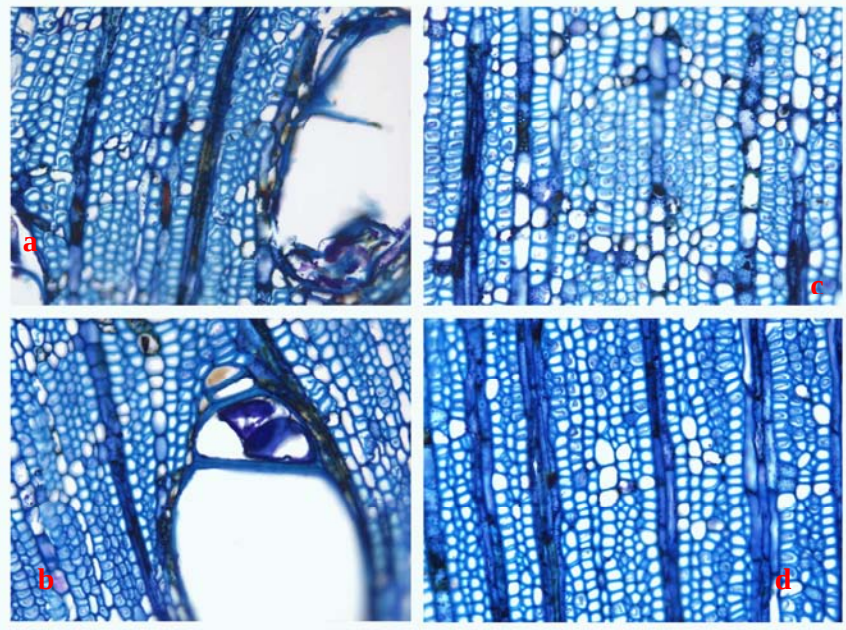


Figure 4. 5. Light microscopic image of dual treated panels



Chapter V

5. BIODETERIORATIVE CHANGES IN THE PHYSICAL, CHEMICAL AND MECHANICAL PROPERTIES OF CCA TREATED RUBBER WOOD DUE TO ESTUARINE EXPOSURE

5.1. Introduction

Wood is made up of inter-connected sugar based polymers and is highly susceptible to biodeterioration under various environmental conditions. In the tropical estuarine conditions a much higher risk is reported, where the wood is acted upon by a large variety of decay fungi and woodborers. In general, several methods have been adopted to assess the deterioration of wood, far from the simplest method of visual examination and rating based on the external degradation to the latest and highly multifaceted methods involving InfraRed (IR) Spectroscopic techniques. During decay and biodeterioration, the chemical integrity of the wood is lost that in turn is reflected as changes in its physical and mechanical properties.

The mechanical strength of wood is accorded by the presence of ligno-cellulosic components in a well structured manner. The differential loss of these chemicals significantly reduces the weight, specific gravity and mechanical strength. Considerable work has been done on relating the changes in the weight loss, specific gravity and mechanical properties of different types of wood specimens with microbial decay and marine borer attack (Curling *et al*., 2001; Curling *et al.,* 2002). It is a well-known fact that the wood can be

protected from deterioration by using different preservatives. Chromated Copper Arsenate (CCA) is a chemical, wood preservative that has been well established in marine applications. The changes in the physical, chemical and mechanical status of different types of wood during preservation have been a matter of discussion during the last few years. The mechanical properties are affected by a number of factors including the defects like knots on wood, age, moisture content, specific gravity, chemical preservative treatments, fungal decay and biodeterioration (Gnanaharan & Dhamodaran, 1992; Krishna Rao & Kamala, 1993; Green *et al.,* 1999; Matan & Kyokong, 2003; Winandy & Rowell, 2005). The mechanical properties of chemically treated wood are in turn dependent on the species and chemistry of the wood, retention of the preservative in treated wood and the post treatment drying conditions (Winandy, 1995). Studies conducted by Edwin and Pillai (2004), Edwin and Ashraf (2006) used mechanical strength as an index to assess marine biodeterioration.

The application of IR spectroscopy is based on the fact that during deterioration or decay of wood there occur changes in the intensity of absorption of IR owing to the changes in the chemical structure of major components like lignin, cellulose and hemi-cellulose. The practical uses of FTIR spectroscopy in different aspects of wood science was detailed by Faix (1988). The Fourier Transform Infrared Spectroscopic (FTIR) studies were conducted by Ostemeyer *et al*., (1989), Mitchell (1993) Pandey *et al*., (1998), Pandey and Pitman (2003), Pandey and Pitman (2004, Edwin and Ashraf

(2006) that help in differentiating the basic chemical nature of different species of wood and assist in distinguishing the changes during decay and deterioration.

The objectives of the present study are to quantify the extent of biodeterioration of untreated and preservative treated rubber wood exposed to estuarine conditions for 6, 12 and 18 months, through the changes in the specific gravity, compressive strength and chemical nature.

5.2. Materials and Methods

The experimental panels categorized under Series I was selected for the study. CCA treated panels to three retentions *viz.* 16 kg m^{-3} (Type I), 29 kg m^{-3} (Type II), 42 kg m^{-3} (Type III) and dual preservative (Type IV) treated panels that are retrieved after six, twelve and eighteen months of exposure in the estuarine condition was used for the experiment. The details regarding preparation of the rubber wood panels, preservative treatment, test site and the field exposure set up are given in Chapter II.

The panels after exposure for the specified duration were removed carefully from the frames, washed in seawater and transported into the laboratory in polythene bags having sufficient seawater. In the laboratory, the panels were scraped off to remove the fouling organisms. The panels were weighed and allowed to dry at room temperature and conditioned at 12 % moisture content. The analysis was done with reference to the following properties.

5.2.1. Specific gravity

The Specific Gravity (SG) is given as the ratio of oven-dried weight of the sample to its volume at 12% moisture content. A piece of wood was cut from the untreated and preservative treated rubber wood panels that are unexposed and exposed in the estuary for specified durations. These samples were oven dried at 105 ± 2°C, weighed and SG was calculated.

5.2.2. Compressive strength

Three replicate blocks of size 20mm X 20mm were from the conditioned panels. The compression parallel to grain strength was conducted as per IS: 1708-1969. The tests were conducted at 12% moisture content and at 20 ºC in the Zwick Universal Testing Machine of 200 kN capacity. The samples were mounted on the panes of UTM and the load was applied parallel to the grain of wood. The parameters studied included Compression Parallel to Grain, Maximum Load, Compressive Stress at Limit of Proportionality (CS at LP), Compressive Stress at Maximum Load (CS at ML) and Modulus of Elasticity (MOE), the dimension of wood compressed.

5.2.3. FTIR analysis

The chemical changes during biodeterioration were assessed using Fourier Transform Infrared Spectroscopy (FTIR). The FTIR study was performed using Nicolet Avatar 360 Esp FTIR Spectrophotometer using KBr. The wood samples retrieved after 6, 12 and 18 months of exposure were allowed to dry at room temperature. The panels were chipped and powdered in wood pulveriser (Pulverisette 14 Variable Speed Rotor Mill, Fritsch). The

powdered samples were dried in hot air oven at 102 ± 2 ^{o}C. The dried samples were mixed with KBr and were introduced in to Avatar Diffuse Reflectance Slides. The spectra of samples were collected followed by a background spectrum of KBr. The spectra in the region of 400 to 4000 cm^{-1} were recorded using Avatar Diffuse Reflectance Smart Accessory (DTGS) at a resolution of 4 cm^{-1}. A total of 150 scans were taken per sample with 80% sensitivity. The spectra were plotted as wave numbers (cm^{-1}) along the X-axis and absorbance along the Y-axis. Band positions characteristic to the major chemical components like cellulose, hemi-cellulose and lignin was studied in detail. The relative change in the amount of cellulose, hemi-cellulose and lignin of untreated, CCA treated and dual preservative treated panels were compared for unexposed and estuarine exposed samples.

5.3. Results and Discussion

5.3.1. Changes in specific gravity

Rubber wood, classified as a light to moderately heavy wood has a specific gravity of 0.557 at 12% moisture content (Rubber Board, 2005). In the present investigation rubber wood used were having an initial specific gravity of 0.605. Since the weight and specific gravity of specimen are strongly dependent on each other, on preservative treatment an increase in weight and a corresponding increase in specific gravity were noted. The ratio of percentage increase in weight to the percentage increase in specific gravity remained near unity for both CCA and dual treated panels. In the study, it was observed that with increase in duration of estuarine exposure there occurs a

progressive increase in destruction of the panels (Chapter III). When wood undergoes biodeterioration in the aquatic environment especially in marine or estuarine condition, loss of wood substance in turn loss of weight and finally reduction in the specific gravity resulted which can be attributed to the destruction of wood by marine borers.

According to Winandy and Rowell (2005), the specific gravity of wood is strongly dependent on the cell wall material. In the present investigation the rate of loss of weight and loss of specific gravity for untreated rubber wood samples were sharp. Within 6, 12 and 18 months the reduction in specific gravity was 3.75%, 27.98% and 64.43% for untreated panels. By 12 months, with a reduced specific gravity 27.98% more than half of the wood substance was lost from the untreated panels. Almost whole of the wood sample was destroyed within 18 months when 64.43% reduction in specific gravity was observed. In the case of preservative treated panels, reduction in specific gravity was rather slow since a lower rate of loss of wood substance. After 18 months of exposure percent loss in specific gravity for treated panels with CCA of 16 kg m^{-3} was 26.69, for 29 kg m^{-3} was 3.42 and for panels with 42 kg m^{-3} CCA retention the loss in SG was 1.2%.

It was reported that the loss of weight and a corresponding loss in specific gravity owing to loss of wood components is one of the excellent indices of mechanical properties of wood (Green *et al.,* 1999). With decrease in specific gravity a corresponding decrease in mechanical properties was

noted (Panshin & deZeeuw, 1964; Curling *et al*., 2002; Winandy *et al*., 2000; Winandy & Lebow, 2001).

5.3.2. Effect of preservative treatment on mechanical strength

The compression parallel to grain strength of rubber wood was studied from the recorded values during the test which included Maximum Load (ML), Compressive Strength at maximum load (CS at ML), Modulus of Elasticity (MOE), Compression Strength at Limit of Proportionality (CS at LP) and deformation produced during the compression. Results of the present investigation showed that CCA and dual preservative treatment slightly changes the mechanical properties that are studied here. However, when statistical tests were applied on the data, these changes were not found significant ($p<0.5$) when compared to the untreated control samples. Summary statistics of Tukey's test results regarding the parameters studied are given in the Table 5.1. The Compression Strength at Maximum Load for untreated rubber wood sample was 44.21 N mm^{-2}. With regard to preservative treated panels, the CS a ML values remained between 36.09 - 43.69 N mm^{-2}. However, these changes in the CS at ML values were not statistically significant between the differences in the ultimate retention and treatment types (Table 5.1a. and Fig. 5.1.). Considering the MOE values, no significant difference was observed between untreated control sample and preservative treated ones (Table 5.1b.). MOE values remained with in a range of 2703.60 – 3741.03 N mm^{-2} where for untreated control rubber wood samples the value was 3491.26 N mm^{-2}. From the results obtained it can be concluded that

neither of these preservative treatment types significantly affect the mechanical properties of rubber wood.

When treated with a preservative, the basic structure and chemical composition of the wood is altered that brings about subsequent changes in physical and mechanical properties (Wangaard, 1979). The water borne preservatives are generally known to reduce the mechanical properties of wood (Winandy, 1995b; Green *et al.*, 1999). CCA is a waterborne wood preservative that when impregnated into the wood, get fixed into cell wall and thereby oxidize the reducing sugars in the cell wall. During the fixation process, the metallic salts of the preservative undergo hydrolytic reduction and alter the existing chemical bonds. According to Winandy (1985), Winandy and Rowell (2005) these changes were ultimately expressed as a reduction in the basic mechanical properties like compression strength and bending strength. In the present investigation it was observed that, although not statistically significant higher retentions of CCA *viz.* 42 kg m^{-3} slightly reduces the CS at ML and MOE of rubber wood. It is known that the usable concentration of these waterborne preservatives is slightly acidic or alkaline in nature. In the present study, 7.5% solution of CCA used for the treatment was found to have a pH of 2.7. This high acidic nature of the preservative solution can be another factor that brings about slight reduction in compression parallel to grain strength. According to Green *et al.*, (1999) the MOE values remains unaffected or slightly increased on treating the wood specimens with different

water borne preservatives. The preservative applied at lower retention has little effect on the strength of wood while higher retentions were found to reduce the bending strength (Wood *et al.*, 1980; Winandy *et al.,* 1985). The reduction in mechanical strength of wood during preservative treatment was explained as, during the process of fixation the metallic salts in the preservative cause the hydrolytic reduction of the sugar components and a simultaneous oxidation of the cell wall layers that leads to reduction in strength (Winandy & Rowell, 2005). Green *et al.,* (1999) is of the opinion that water borne preservatives affects different mechanical properties differently where, according to retention of the preservative and the drying conditions the properties like Modulus Of Elasticity (MOE), compressive strength parallel to grain, and compressive stress perpendicular to grain are unaffected or slightly increased while modulus of rupture (MOR) and tensile strength parallel to grain are reduced from 0% to 20%. The above-mentioned study rather substantiates the present findings. As far as organic oil borne preservatives are concerned that merely fill cavities in the cell and remains inert towards the cell wall, does not bring about any significant change in the strength of wood (Green *et al.*, 1999; Winandy & Rowell, 2005). According to Mitchell and Barnes (1986) the chemical dissolution of the cell wall is the reason for reduction in strength. Lahiry (1996) are of the opinion that the oil borne preservatives resist water to a greater extent, reduce moisture content, prevents chemical hydrolysis and make it unfit for the action of decay fungi and there by do not affect the strength properties.

5.3.3. Effect of biodeterioration on mechanical strength

Changes in the mechanical properties are directly related to the changes in the chemical components in the wood. As weathering process due to the action of UV, Infra Red radiations or temperature, decay process due to the attack of fungi or bio-deterioration process due to the attack of bacteria or wood boring organisms progresses, an ultimate loss of strength and stiffness of the wood is resulted. In the present investigation, the untreated rubber wood panels showed a progressive decrease in compressive strength as biodeterioration progressed. The percentage reduction in strength of untreated panels after 6 and 12 months was 42.3% and 70.9% respectively. About >90% of the total volume of untreated panel was lost in 18 months of estuarine exposure and the panels could not be tested for the compressive strength (chapter III).

During the initial 6 months, loss of strength was more obvious than the periods after that. Initially, within 6 months the percentage reduction in CS at ML was 20.41, 13.87, 9.86 for CCA treated panels of retentions 16 kg m^{-3}, 29 kg m^{-3} and 42 kg m^{-3} respectively. While during the next 6 months of the study, percentage reduction in compressive strength was 13.29 %, 10.39 %, 4.33 % for the same type of panels. As far as the mechanical properties are concerned, loss of stiffness of wood is indicated by the loss in Modulus of Elasticity (MOE). As deterioration progressed gradual reduction in MOE values are observed. For untreated panels, a significant change was observed in MOE values after 6 and 12 months of immersion. There was a reduction of

42.2% and 60.88% in MOE value in 6 and 12 months respectively. The panels retrieved after 18 months could not be tested due to loss of >90% of the wood substance. In the case of CCA 16 kg m^{-3} upto 18 months, the loss in MOE was not significant. About four-fold (79%) reduction in the MOE values was observed in 18 months. In case of CCA 29 kg m^{-3}, upto 18 months the changes in MOE was not apparent while 6 % reduction was observed in 18 months. The panels with a higher retention of 42 kg m^{-3}, where a slight decrease of 3% in MOE were observed. In the case of dual treated panels the reduction in MOE was 10 %. CS at ML for untreated unexposed sample was 44.21 N mm^{-2} after 12 months of exposure 70.9% reduction was observed in untreated panels. In the case of CCA 29 panels 8.52 % reduction was observed.

Exposure to chemicals, decay and insect damage are some of the factors that directly affect wood strength (Winandy, 1994; Green *et al.*, 1999). According to Cowling (1961) and Findlay (1985) the biological decay process to a greater extent influences the mechanical strength properties of wood. As decay or degradation proceeds, the changes the nature and composition of the wood fibers are affected which ultimately results in measurable strength losses (Winandy & Morrell 1993; Winandy *et al*., 2000; Curling *et al*., 2001; Winandy & Lebow, 2001; Curling *et al*., 2002). The above mentioned factors justify the reduction in Compression strength and MOE values as the degradation proceeds.

5.3.4. FTIR analysis

5.3.4.1. Spectra of preservative treated panels

The FTIR spectra of untreated and preservative treated panels are given in Fig.5.2. In the untreated rubber wood sample three prominent bands were observed at band position above 2800 cm^{-1}. The band observed at 3547 cm^{-1} was due to the presence of hydrogen bonded O-H stretch. The bands observed at positions 3182 cm^{-1}, 2892 cm^{-1} were due to the C-H stretching vibrations in lignin, cellulose or hemi-cellulose. In preservative treated panels these peaks have shifted into well-differentiated broad stretch of O-H vibrations at around 3400 cm^{-1}. When compared to untreated sample, a change in shape and reduction in the intensity of band at 2892 and loss of band at 3182 cm^{-1} was observed in the spectra of preservative treated panels. This suggests a change in sp^3 and sp^2 hybridized C-H stretch in polymerized lignin or cellulose. A number of bands were observed between 1450 cm^{-1} and 800 cm^{-1} that are known as the finger print region of IR spectra. It was observed that on preservative treatment significant changes occur in carbonyl groups associated with lignin, cellulose or hemi-cellulose. In preservative treated panels a significant reduction was observed in band intensity at 1736 cm^{-1} characteristic to unconjugated C=O stretch of hemi-cellulose. This may be due to the hydrolysation of hemi-cellulose by acidic CCA solution. When compared to that of untreated rubber wood panels, characteristic band of conjugated C-O stretch at 1660 cm^{-1} was lost in preservative treated samples. In untreated panels the aromatic skeletal stretch of lignin was observed as

strong absorption peaks at 1593 cm^{-1} and 1503 cm^{-1}. In preservative treated panels peak at 1593 cm^{-1} was broadened together with a reduction in the intensity of peak at 1503 cm^{-1}. These finding are in agreement with the fact that on treating wood with solutions containing inorganic salts like chromium, the aromatic skeletal structure of lignin (Pandey *et al*., 1998). In preservative treated panels the C-H deformation band at cellulose and hemi-cellulose at 1375 was reduced on preservative treatment while it was completely lost in dual treated samples. The peak at 1245 cm^{-1} denoting the C-O stretch in lignin and xylan was lost in preservative treated panels.

The microscopic studies have revealed that the preservative solution have reached into the interior of the wood cells (Chapter IV). The changes in the compressive strength of preservative treated panels show that the preservative components have chemically reacted with the chemical constituents of wood cell. According to Goldstein (1991) hemicellulose is the major component of wood that get hydrolysed easily by the acidic solutions. Humphrey (2002) is of the opinion that during chemical preservation the CCA chemically binds to the wood cell walls fixing the preservative components. According to Ostemeyer *et al*., (1989) less prominent bands at 1593 and cm^{-1} 1503 cm^{-1} together with the reduction in intensity at carbonyl peaks indicates that there might have occurred a chemical reaction between the preservative components and the major wood polymers like lignin, cellulose and hemi-cellulose out of which the chemical integrity of the polymers have lost

(Pandey *et al*., 1998). The major change might have occurred in the conjugated carbonyl groups in lignin that lead to shift in spectra

5.3.4.2. Spectra of biodeteriorated panels

The present study reveals that as the biodeterioration progresses there occurs a change in the spectral pattern of rubber wood. Either the decrease or increase in the intensity of IR spectral bands corresponding to carbohydrates and lignin indicates the changes in the chemistry of these components during deterioration together with the release of degradation products.

The FTIR spectra of untreated rubber wood at different levels of biodeterioration in estuarine environment are given in the Fig 5.3. The spectra (Fig. 5.3.) reveal that the relative intensity of bands at 1740 cm^{-1}, 1376 cm^{-1}, decreased as degradation increased the bands at 1157 $cm^{-1}$and 890 cm^{-1} were absent. That are known to be the reference bands for carbohydrates are lowered as deterioration increased. The typical hardwood band positions represented as 3419 cm^{-1}, 2919 cm^{-1}, 2850 cm^{-1}, 1711 cm^{-1}, 1610 cm^{-1}, 1502 cm^{-1}, 1462 cm^{-1}, 1425 cm^{-1}, 1315 cm^{-1}, 1267 cm^{-1}, 1218 cm^{-1}, 1113 cm^{-1}, 1026 cm^{-1}, 912 cm^{-1} (Pandey, 1999). The relative intensities of many of these bands decreased as biodeterioration progressed. The lignin assigned bands *viz.* 1590 cm^{-1}, 1505 cm^{-1}, 1245 cm^{-1}, 1113 cm^{-1}, 1130 cm^{-1}, 1376 cm^{-1} etc. also decreased. The band at 1660 cm^{-1} showed an overall increase at different levels of estuarine exposure. This shows an increase in carbonyl groups that are liberated from lignin or carbohydrates during chemical degradation. Studies conducted by Edwin and Ashraf (2006) have shown that when

compared to unexposed rubber wood samples the relative intensities of lignin and carbohydrate bands lower in marine exposed samples. Accelerated weathering and photodegradation tests conducted in rubber wood have shown that the relative intensities at band positions at 1596 cm^{-1}, 1505 cm^{-1}, 1465 cm^{-1} and 1245 cm^{-1} decreased as weathering process progressed (Pandey, 2003).

The same pattern was observed for CCA treated panels of three retentions namely 16 kg m^{-3}, 29 kg m^{-3} and 42 kg m^{-3} (Fig. 5.4., Fig. 5.5., Fig. 5.6.). It was observed that CCA treated panels to 16 kg m^{-3} retention, the band intensity at 1733 cm^{-1}increases as duration of exposure increased. There was an increase in peak position at 1660 cm^{-1} due to carbonyl release. But the intensity of the peaks showed was related to the type of preservative, retention of the preservative in wood and the extent of degradation (Fig. 5.4.). In CCA treated and dual treated panels since the degradation was very low the intensity changes were not prominent (Fig. 5.4., Fig. 5.5., Fig. 5.6., Fig. 5.7.). Intensity changes at 1660 cm^{-1} showed that during first six months of exposure, as with the unexposed samples the carbonyl addition to this region was almost absent, while in 12 months a significantly higher amount of carbonyl groups were added. In CCA treated panels with 29 kg m^{-3} of preservative in it, the most significant change was observed in band positions at 1120 cm^{-1}, 1323 cm^{-1} corresponding the lignin showing that the lignin degradation have started (Fig. 5.5.). The decrease in intensity at 1660 cm^{-1} with an increase in1735 cm^{-1} was observed due to the changes in carbonyl groups. The C-H deformation band at 898 cm^{-1}cellulose decreased as decay

proceeded. In the case of dual treated panels the bands at 1425 and 1455 cm^{-1} were marked in the spectrum while as the degradation increased the band at 1455 cm^{-1} was lost and shifted towards 1425 cm^{-1} (Fig. 5.7.). The peak at 897 $cm^{-1}$was present in unexposed and exposed for 6 months sample while for 12 and 18 month's sample it was absent.

The results of the FTIR analysis confirms increase in deterioration and corresponding reduction in mechanical strength values. The increase in peak intensity in all the panels at band position 1660 cm-1 denotes that as the deterioration progresses there is an increased addition of carbonyl groups as the degradative product of chemical components of wood. During the process of degradation the initial changes are occurring in the hemicellulose of the wood and later on the advanced stages of degradation can be assessed by chemical changes in cellulose and lignin (Winandy & Lebow, 2001). Oevering *et al.*, (2003), Huang *et al.*, (2004) and Guerra *et al.*, (2004) have employed FTIR spectroscopy as a means to analyse fungal deterioration in wood. The characteristic changes in the FTIR spectral intensity of wood on fungal decay were studied by Pandey and Pitman (2003), Pandey and Pitman (2004). The present study is also in agreement with the results provided by Edwin and Ashraf (2006), where the marine exposed rubber wood panels showed significant decrease in the band intensities corresponding to lignin and carbohydrates.

5.4. Conclusion

The present study confirm that the higher retentions of CCA *viz.* 29 kg m^{-3} and 42 kg m^{-3} protects the wood effectively as there is minimum loss of wood substance, reduction in specific gravity and corresponding decrease in compressive strength when compared to the untreated rubber wood panels and CCA treated rubber wood panels to low retention of 16 kg m^{-3}. The effective protection offered by the preservative treatment can be ascribable to the resistance offered by the chemicals to degradation of lignin and carbohydrate that form fibers and parenchyma cells in the main frame work of wood.

The visual observation, X-ray radiographic analysis, microstructural observations, chemical analysis and compressive strength tests of untreated and CCA treated, dual preservative treated and physically protected panels have shown that the CCA treated panels protects the wood from deterioration in the aquatic conditions. Effective protection is imparted by the CCA treated panels to higher retentions of 29 kg m^{-3} and 42 kg m^{-3} and FRP sheathed painted panels. So further studies were conducted to assess the toxicity of CCA to aquatic organisms to identify the most effective treatment method with the effect of which deterioration of wood effectively prevented at the same time the impacts on non- target organisms are minimized. The studies are explained in the following Chapters.

Table 5. 1. Tukey's test results for the (a) Compression Strength at Maximum Load of (1) untreated rubber wood panels, CCA treated panels to retention of (2) 16 kg m^{-3} (3) 29kg m^{-3} (4) 42 kg m^{-3} (5) dual treated panels

a)

Compression Strength at Maximum Load (CS at ML)

Tukey HSD [a,b]

Treatment types	N	Subset 1
2	3	36.0933
5	3	42.1167
4	3	42.2733
3	3	43.6967
1	3	44.2133
Sig.		.060

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 9.792.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

b)

Modulus of Elastisity (MOE)

Tukey HSD [a,b]

Treatment types	N	Subset 1
2	3	2703.6000
3	3	3409.8900
1	3	3491.2633
5	3	3538.1600
4	3	3741.0367
Sig.		.078

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 179091.668.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Delta

c)

Tukey HSD[a,b]

Treatment types	N	Subset 1
5	3	.7500
4	3	.7867
3	3	.7967
1	3	.8333
2	3	.8367
Sig.		.425

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.487E-03.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Table 5.2.Tukey's test results for the Compression Strength at Maximum Load of (a) untreated rubber wood panels, CCA treated panels to retention of (b) 16 kg m^{-3} c) 29kg m^{-3} (d) 42 kg m^{-3} (e) dual treated panels; exposed in cochin estuary for (durations (1) unexposed (2) 6months (3) 12 months (4) 18 months

(a)

Tukey HSD[a,b]

EXPOSURE	N	Subset 1	2	3	4
4	3	.0000			
3	3		12.8533		
2	3			25.5100	
1	3				44.2133
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 19.808.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

(b)

Tukey HSD[a,b]

EXPOSURE	N	Subset 1	Subset 2
4	3	8.5533	
1	3		38.0933
2	3		38.3100
3	3		41.3267
Sig.		1.000	.775

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 17.053.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

(c)

Tukey HSD[a,b]

EXPOSURE	N	Subset 1
4	3	41.9600
2	3	44.3000
1	3	44.3633
3	3	44.9233
Sig.		.702

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 10.961.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

(d)

Tukey HSD[a,b]

EXPOSURE	N	Subset 1
3	3	41.8800
4	3	41.9367
1	3	42.2733
2	3	42.5433
Sig.		.984

Means for groups in homogeneous subsets are displa
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 5.327.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

(e)

Tukey HSD[a,b]

EXPOSURE	N	Subset 1
2	3	39.6967
4	3	40.8567
1	3	42.1167
3	3	44.0033
Sig.		.335

Means for groups in homogeneous subsets are displaye
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 8.474.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure 5.1. Compressive strength of untreated and treated rubber wood panels of exposed and estuarine exposed for 6, 12 and 18 months

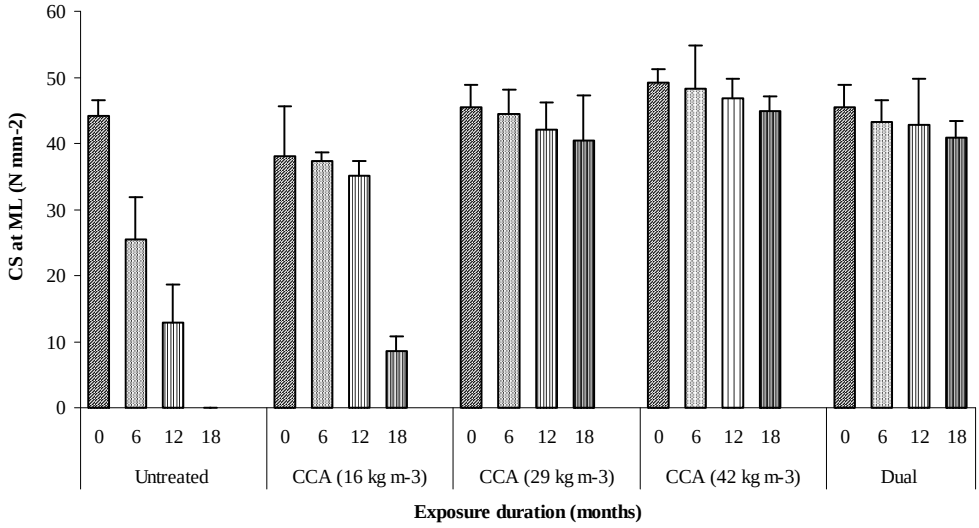


Figure 5.2. FTIR spectra of a. untreated rubber wood samples b. CCA (16 kg m^{-3} retention) c. CCA (29 kg m^{-3} retention) d. CCA (42 kg m^{-3} retention) e) dual treated samples

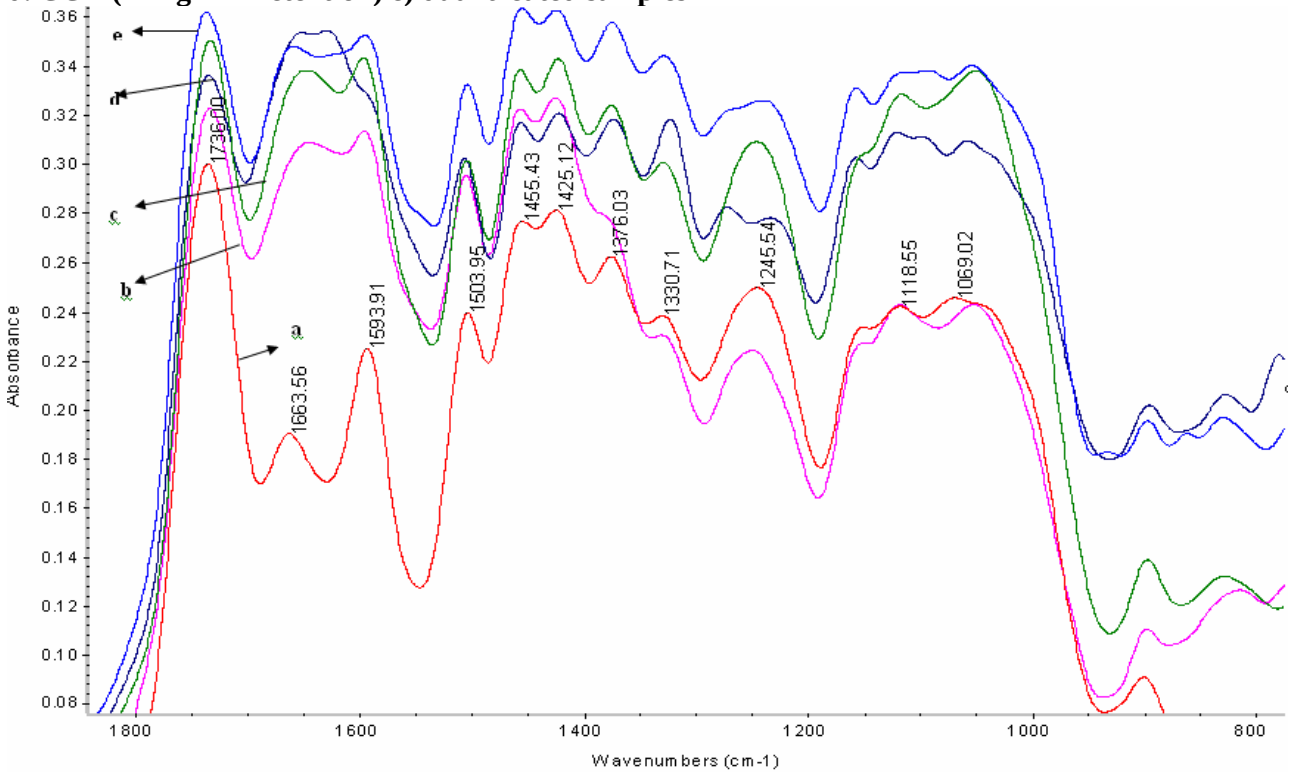


Figure 5.3.FTIR spectra of untreated rubber wood samples (a. unexposed, b. marine exposed 6 months, c. marine exposed 12 months d. marine exposed 18 months)

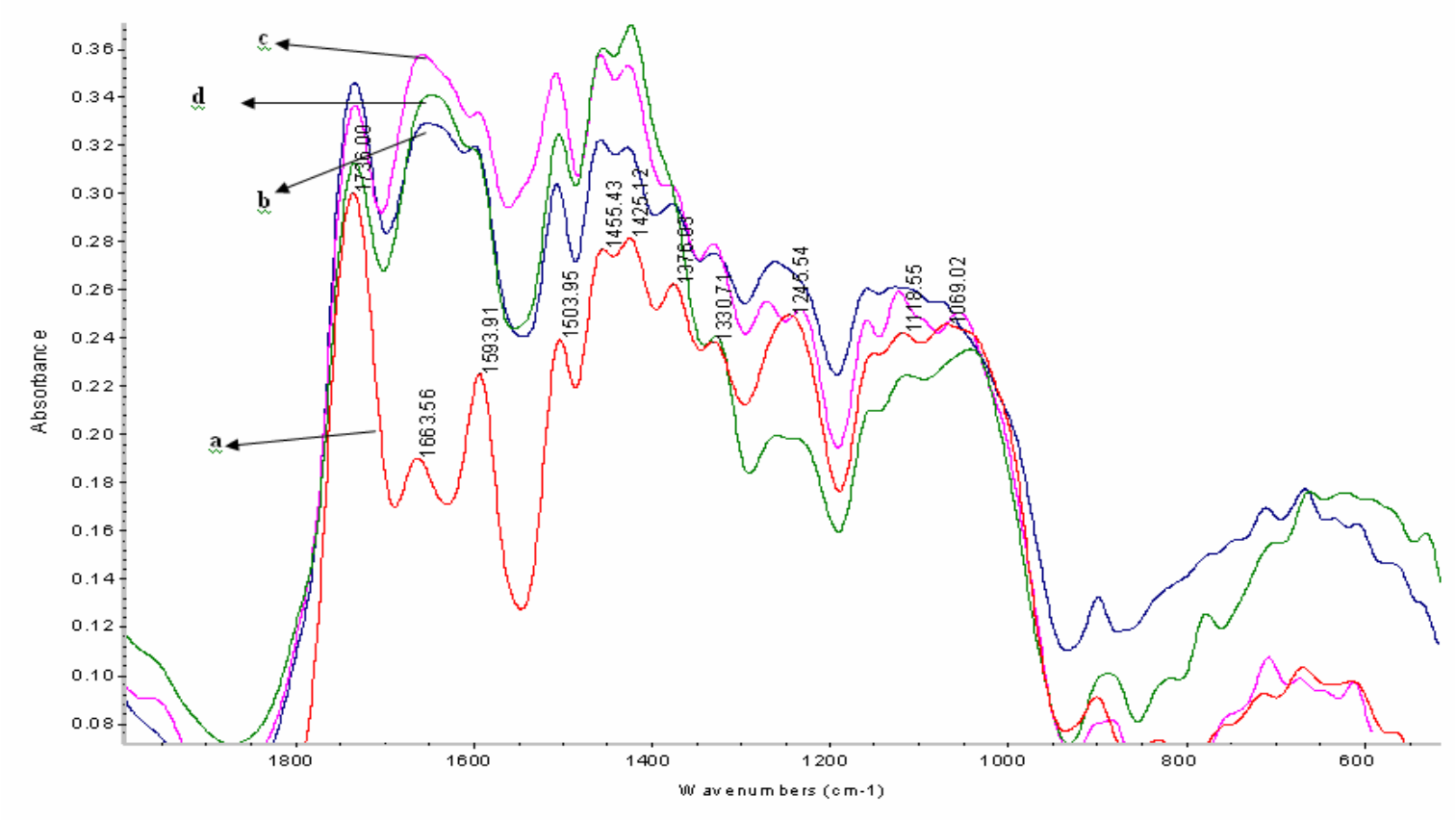


Figure 5.4. FTIR spectra of CCA (16 kg m^{-3} retention) treated rubber wood samples (a. unexposed, b. marine exposed for 6 months, c. marine exposed for12 months d. marine exposed for 18 months)

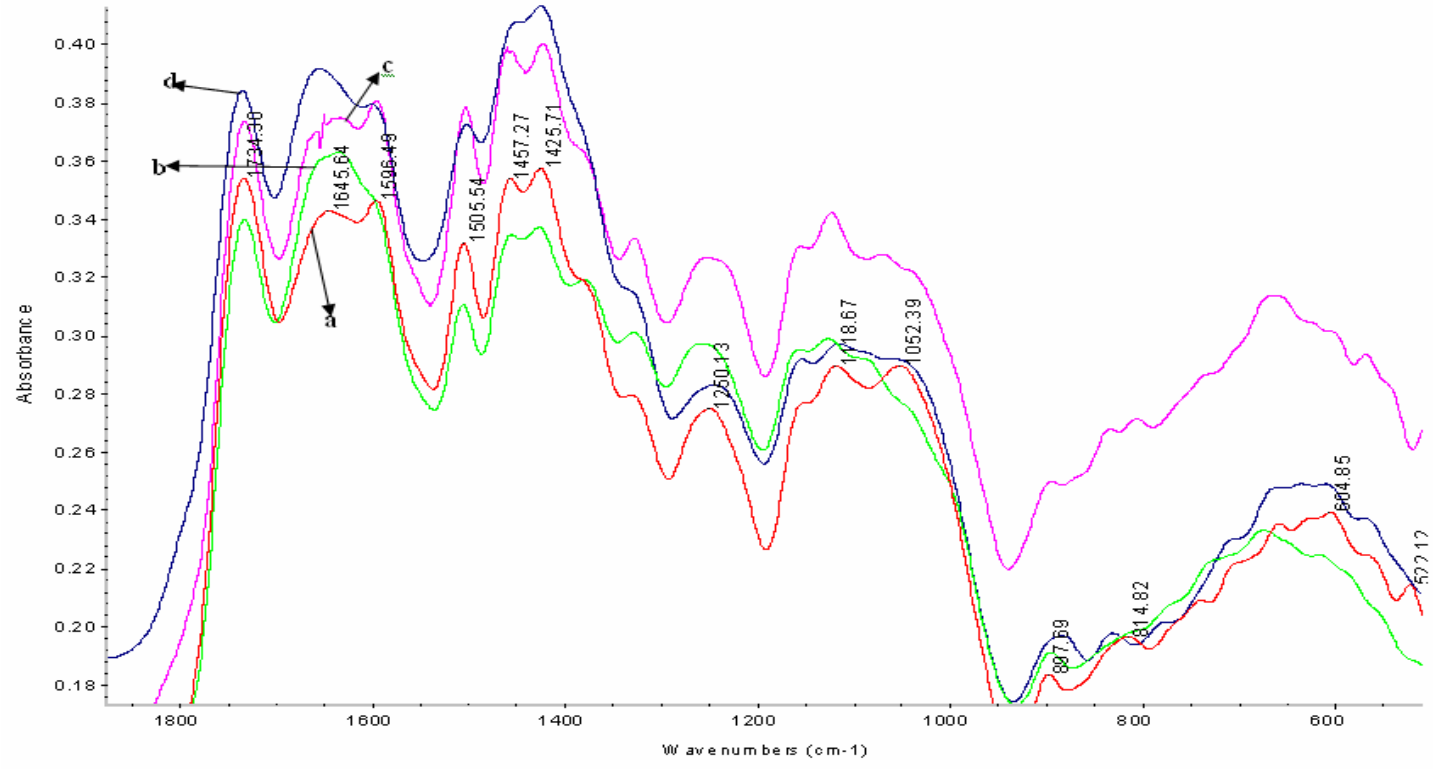


Figure 5.5. FTIR spectra of CCA (29 kg m^{-3} retention) treated rubber wood samples (a. unexposed, b. marine exposed for 6 months, c. marine exposed for 12 months d. marine exposed for 18 months)

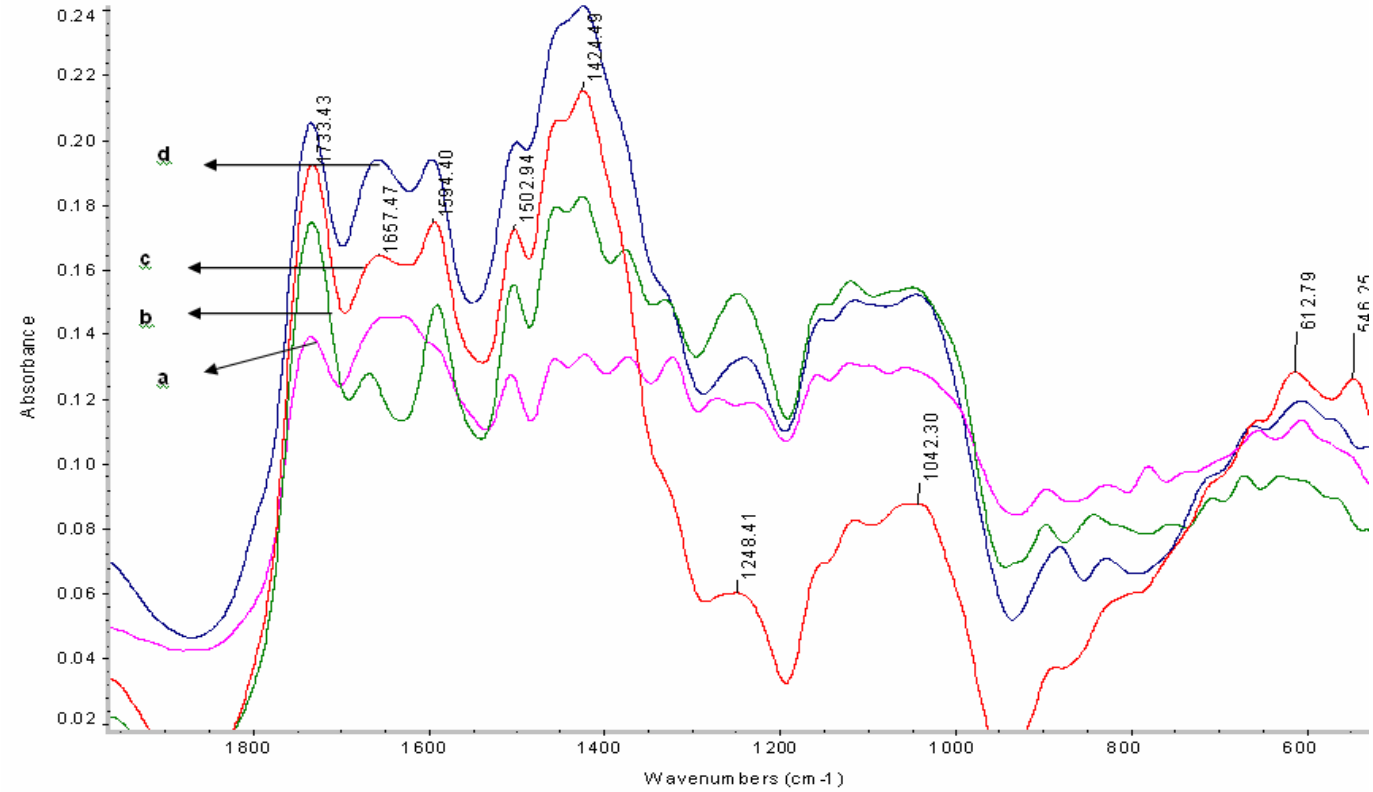


Figure 5.6. FTIR spectra of CCA (42 kg m^{-3} retention) treated rubber wood samples (a. unexposed, b. marine exposed for 6 months, c. marine exposed for 12 months, d. marine exposed for 18 months)

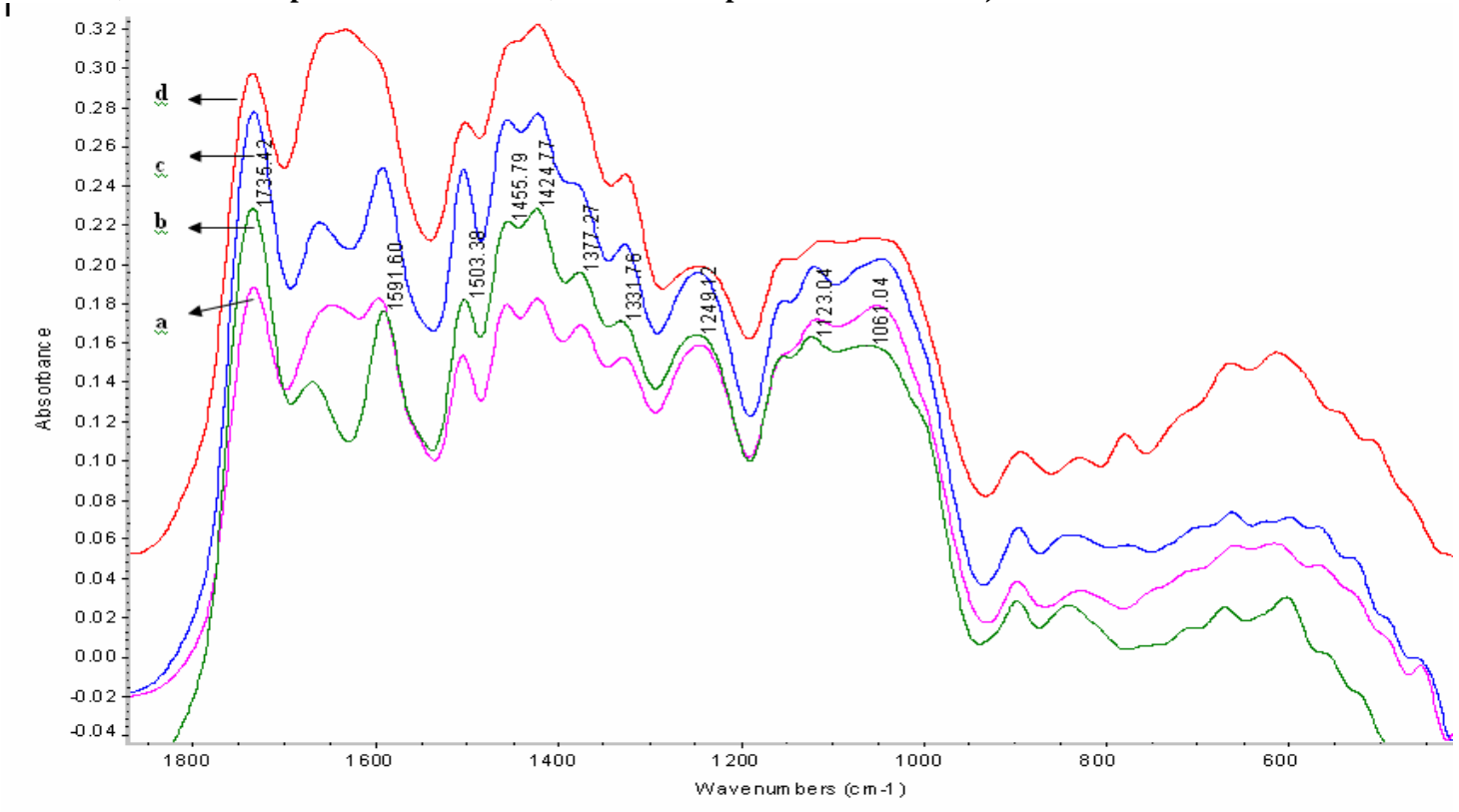
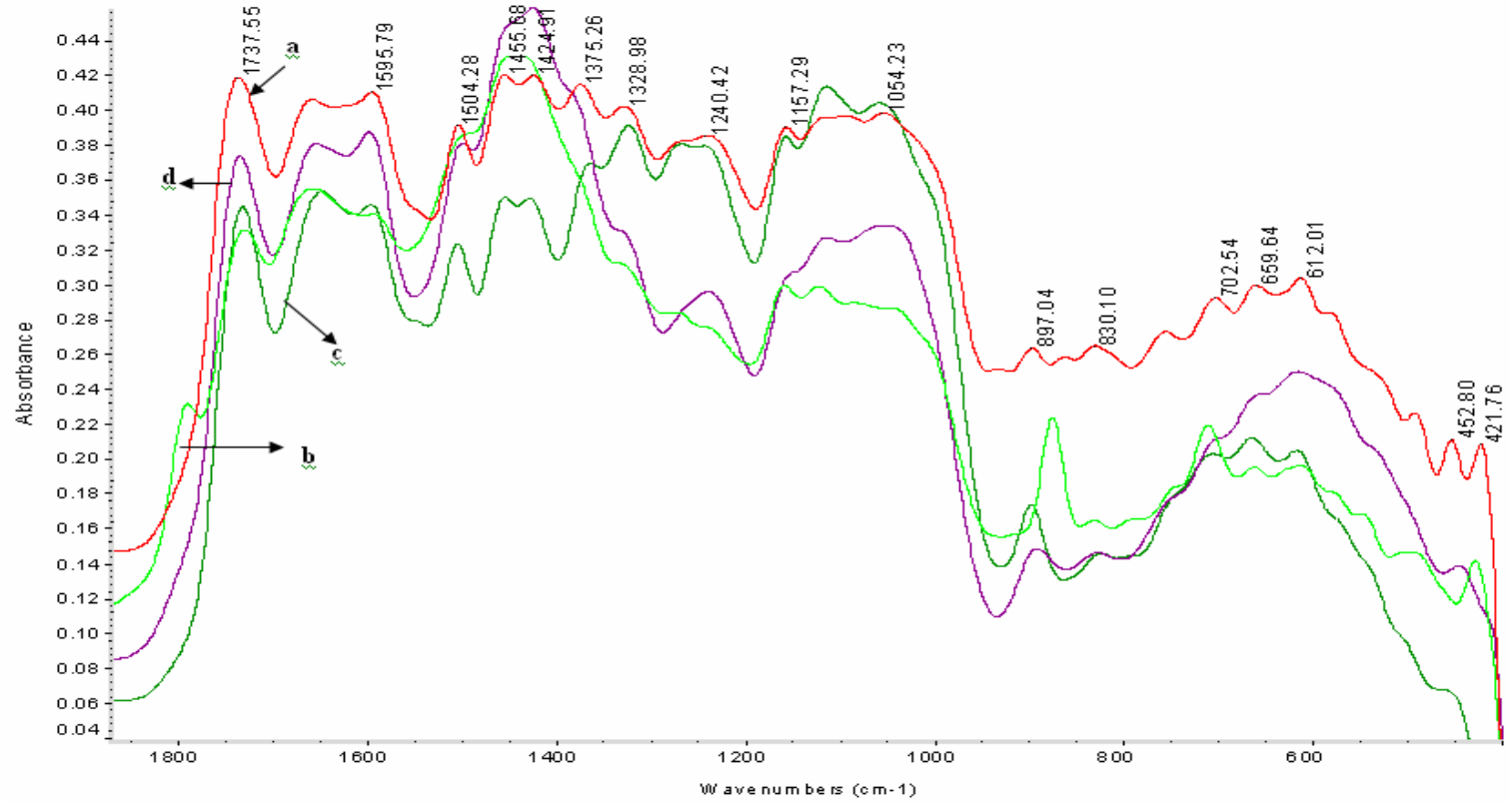


Figure 5.7. FTIR spectra of dual treated rubber wood samples (a. unexposed, b. marine exposed for 6 months, c. marine exposed for 12 months, d. marine exposed for 18 months)



Chapter VI

6. ACUTE TOXICITY OF CCA AND ITS CONSTITUENTS COPPER, CHROMIUM AND ARSENIC IN *VILLORITA CYPRINOIDES*

6.1. Introduction

The Group of Experts on Scientific Aspects of Marine Environmental Protection (GESAMP) defines marine pollution as 'the introduction by man, directly or indirectly, substances or energy into the marine environment (including estuaries) resulting in deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of seawater and reduction of amenities. The estuaries and coastal areas with maximum population density are more prone to the deleterious implications of pollution as it becomes the primary source for pollutant release. The heavy metals, released through the domestic sewage, industrial wastes volcanic eruption and weathering of rocks forms one of the major class of pollutants added into the estuaries and coastal waters. The metal pollutants are highly toxic to the aquatic organisms since in some form or the other these are soluble in seawater. The free ions, the most bio-available forms of metals are readily taken up by the organisms and either accumulated in the body tissues or released. If get accumulated, both the essential metals in higher quantities and the non-essential metals in minute quantities are harmful to the normal functioning of the organisms.

The impact of a chemical added into a particular environment can be studied by different methods. The ecological risk assessment and pollution-monitoring programme essentially employ toxicity studies as a tool to assess the immediate and direct effects of the particular toxicant. The aim of the toxicity test is to define the concentrations at which the toxicant is producing a selected deleterious response in a population (Ward & Parrish, 1982). The aquatic toxicity tests are conducted in a controlled condition with selected organisms especially marine or fresh water algae and macro invertebrates and fishes. The bivalve molluscs *viz.* mussels, clams have been well recognized as tools for a widely accepted biological pollution monitoring programme called 'Mussel watch' introduced world over in 1975 by Goldberg. These organisms are cosmopolitan in occurrence and are hardy enough to survive under laboratory conditions. These organisms are sessile, and can accumulate certain heavy metals without suffering mortality (Beaby & Eaves, 1983). The acute and chronic effects of a toxicant were determined by conducting a time dependent and time independent renewal or static acute toxicity tests or by conducting chronic toxicity studies. Although not environmentally unrealistic the results can be extrapolated to predict toxicant concentration that may be allowed in waters without adverse effects on living resources. *Mytilus, Perna, Crassostrea, Ostrea spp., Villorita spp.,* etc. are the common species of molluscs used in toxicity studies (Rainbow, 1995).

The present investigation was on *Villorita cyprinoides* (black clam), which is a benthic bivalve found in estuaries of Kerala. The species was

selected for the study because of the fact that these are commonly cultured and commercially important species in Kerala. These animals can easily be sampled, transported and maintained in the laboratory (Boening, 1999). Necessary background information on the species was available since studies were conducted under laboratory condition by Lakshmanan and Nambisan (1977), Abraham *et al.,* (1986), Sathyanathan (1996) to assess the depuration and toxicity of various metals. Pillai *et al.,* (1986) used *Villorita* as a tool to assess the environmental pollution at Vembanad Lake.

Chromated Copper Arsenate (CCA) is a waterborne preservative containing metals like copper, chromium and arsenic mixed in their oxide forms. The insecticidal properties of arsenic pentoxide and the fungicidal properties of chromium oxide make CCA one of the most efficient wood preservatives. There are about 208 preservative treatment plants in India, using about 2980 tonnes of preservatives equivalent to CCA (Gairola & Aggarwal, 2005). The marine plywood manufacturers are the major consumers of CCA. The wood when treated with this preservative fixes well into the wood. So the only possible source for direct toxicity is the run off from CCA treatment plants. The experiments are being conducted all over the world to find out the potential toxicity of this preservative. The run-off from such CCA treatment plants are known to contain high concentration of arsenic (III), chromium and copper (Cox, 1991). So the possibility of contamination of aquatic system by this source of heavy metals is indeed a matter of concern. Though studies regarding the acute toxicity of CCA the larvae of wood boring teredinids that

are target to the CCA were known, very little has been known about the direct acute toxicity of CCA on non-target organisms (Balaji *et al.*, 2004). In the view of the known hazards and possible deleterious effects of copper, chromium and arsenic in the aquatic environment, an attempt is made to study the acute toxicity of CCA and its constituent metals copper, chromium and arsenic separately and their bioconcentration in black clam.

In the view of the known hazards and possible deleterious effects of copper, chromium and arsenic in the aquatic environment, an attempt is made to study the acute toxicity CCA and its constituent metals separately and their bioconcentration in black clam.

6.2. Materials and Methods

The test was conducted in order to compare the toxicity of CCA with the toxicity of its constituent heavy metals copper, chromium, and arsenic (Ward and Parrish, 1982) and to determine the possibility of bioaccumulation of these metals in selected species of clam. The experiments were conducted using *V. cyprinoides* as the test organism. The test organisms were collected from Cochin backwaters, where the salinity during the period of collection ranged between 24 ± 2‰ and pH 7.6 ± 0.2. The clams were transported to the laboratory in plastic containers of 50 l capacity with minimum stress to the organism. In the laboratory, the collected test organisms were acclimatized in well-aerated tap water at 28 ± 2°C for 48 to 72 h prior to the experiment. The specimens from the same population were used for a single experiment. Size

of clams was measured using vernier calipers and sorted. The average length of shells of clams used in the study ranges between 22 mm – 28 mm. The experiment was conducted in 10 l trays with 5 l water. Water was kept aerated for two days so as to minimize the effect of chlorine in it. The physico-chemical parameters of water were checked before and after the experiment are given in Table 6.1.

Analar grade of salt of each toxicant, $CuSO_4.5H_2O$ (M.W.- 249.53), $CrCl_3.6H_2O$ (M.W.-266.48) and As_2O_3 (M.W.- 197.83) is used for the experiment. Stock solutions of each of these were prepared by dissolving the salts in distilled water not more than two days in advance of the test. Stock solutions were serially diluted to get required concentrations in $\mu g\ l^{-1}$.

The present investigation was carried out using 7.5% solution of CCA to treat rubber wood samples. The Central Institute of Fisheries Technology has standardized a 7.5% solution of CCA for treating boat-building timbers since it imparted sufficient protection under marine condition. The solution is diluted through several steps to conduct range-finding studies in such a way that the concentration of toxicant in each tank is 50% of the next higher concentration. From the preliminary range finding studies the concentrations for final toxicity studies were selected. In case of CCA, the concentrations selected for the studies were 0.5 ppm, 0.625 ppm, 1 ppm, 1.25 ppm, 2.5 ppm, 5 ppm and 10 ppm. For each test a duplicate tank was run for reference. A control tank was maintained in order to check the acceptability of the results (Fig. 6.1.).

Ten clams were exposed to 5 l toxicant solution in each tray. During the period of experiment clams were not fed and water was not aerated. Every 24 h mortality was monitored and water was changed maintaining the concentration of the toxicant. The criterion for mortality was the valve gaping of 5 mm and the lack of response of the organism when gently prodded. After 96 h of exposure the live organisms were collected. The soft tissues of the clams were dissected out and shells were separated. Both the tissue and shell samples were dried at 80-82°C till to attain a constant dry-wt. The 0.2g of the dried samples was digested in HNO_3 - $HClO_4$ mixture in microwave acid digester. The concentration of copper, chromium and arsenic in the digested samples were analysed by ICP - AES (Perkin Elmer-Optima 2000 DV).

6.2.1. LC_{50} values and BCF values

LC_{50} values and their 95% upper and lower confidence limits were calculated using Trimmed Spearman - Karber estimate, which is a recent modification of Spearman-Karber estimate (1978). The software for this method obtained from Civil and Environmental Engineering, Old Dominion University (CEE/ODU) Civil/Environmental Model Library (CEML). The percent mortality at each toxicant concentration is determined and plotted in a graph.

Bioconcentration Factor is a measure of distribution of the heavy metal between biota and water. It is calculated using the equation:

$B.C.F = C_{biota}/C_{water}$

Where, C_{biota} is the total metal concentration in biota (ppm)

C_{water} is total metal concentration in water (ppm)

6.3. Results and Discussion

Although the metal components of CCA are essential for the normal functioning of the organism, the results show that above a particular threshold level they can be acutely toxic. In all metals studied and in CCA, number of survivors decreased with increase in concentration.

The results indicate that copper is more toxic than all other metals studied. It was found that 0.5 ppm of $CuSO_4$ was found to be lethal to 50% of the population (Fig.6.2.). Concentrations above 0.3 ppm were found to be causing much stress on the organism. Mucus secretion was significantly high in all these concentrations. The results of the study show that LC_{50} value for chromium and arsenic is 3.6 and 3.72 (Fig. 6.3., Fig. 6.4.). In all concentrations of CCA taken for the study, the shells are found to be disintegrating. At higher concentrations of CCA the disintegration is found to be more. The dead organisms after 96 h was dissected and the tissue were found to be green in colour (Fig. 6.5.). In the case of CCA even 1 ppm solution showed 50% mortality and 10 ppm solution showed more than 50% death in the specified duration (Fig.6.6.).

The analysis of tissue of clams in the control tank showed presence of 38 ppm of copper, 33 ppm of chromium and 23 ppm of arsenic. When concentration in the water increased the uptake and bioconcentration of copper in the tissue also increased. The tissue concentration of copper shows that the clams can concentrate and tolerate increased body burden of copper when

compared to the concentration of other metals studied here. At the LC_{50} of 0.5 ppm the concentration of copper was 49.26 that was the highest value observed. The bioconcentration factor for copper was 77.40, 82.11 and 98.14 respectively for 0.3, 0.4 and 0.5 ppm of copper concentrations. But in the case of chromium the concentration of metal in the tissue sample was found decreasing as the concentration in the water increased. The range of concentrations studied near the LC_{50} value of chromium showed that no significant increase in concentration. The bioconcentration studies showed that as the concentration increases the bioconcentration factor for chromium decreases. In the case of arsenic no such trend was observed while the concentration in the tissue increased to an amount of 263.05 ppm on exposure to 3 ppm. But at 2 ppm and 4ppm the value was very low when compared to that at 3 ppm. The bioconcentration factors also showed the same trend. The data analysis showed that copper has the highest potential to get concentrated in the tissues than chromium and arsenic referring to the fact that the bioconcentration potential depends on the properties of the chemical.

The toxicity of a particular toxicant on particular organism varies significantly with changes in the conditions of the test. Salinity is one of the major factors that affect toxicity. The toxicity studies conducted in the same species of clam of 25 mm size, at a salinity of 1‰ showed that mortality was too rapid above 2 ppm and extensive mucus secretion was observed in copper concentrations above 0.5 ppm (Lakshmanan & Nambisan, 1977). Static toxicity studies conducted in the same species resulted in an LC_{50} value of

1.21μg l^{-1} (Abraham *et al.*, 1986). This very low value when compared with the results of the study shows that the methodology used also affects the resultant toxicity values. The LC_{50} value of copper in green mussels for a period of 96h was reported as 0.175 ppm (Nambisan & Lakshmanan, 1986). The studies conducted by Meenakumari and Nair (1993) showed that in 48 h 50% mortality has occurred in green mussel population that is exposed to a copper concentration of 0.83 and reported that copper is found to be the most toxic metal studied.

The results of the study show that LC_{50} value for chromium and arsenic are 3.6 and 3.72. According to Brooks, (1997) arsenic and chromium are tolerated at moderately high levels by aquatic species. Chromium seems to be somewhat less toxic to aquatic organisms. The static 96h studies conducted in *V. cyprinoids* var *cochinensis* using chromium oxide resulted in an LC_{50} value of 11.5 μg l^{-1} (Abraham *et al*., 1986). Green mussels are found to be more efficient in tolerating toxicity of chromium since 48 h toxicity range was 7-12.8 ppm (Govindarajan *et al*., 1993), which was higher than the results obtained in this study in *Villorita sp*. In *Mytilopsis sallei* LC_{50} of chromium at a salinity of 10 ppt is 5.34 ± 0.22 ppm. Arsenic can exist in two different oxidation states, out of which Arsenic (III) was found to be more toxic than Arsenic (VI). LC_{50} range for arsenic (III) is given as 3000-7500 μg l^{-1} for molluscs. 48-h LC_{50} value for *Perna viridis* for arsenic was 2.39 ppm

(Meenakumari & Nair, 1993). In blue mussels 100% mortality was observed for arsenite concentration of 16 ppm.

Studies have been conducted to assess the impact of CCA on bacteria fungi and borers that attack the wood, there has been very little attempt to study the acute toxicity of CCA on non- target organisms (Buchanan & Solomon, 1990). But the acute toxicity of this chemical to *Daphnia magna* and to a species of algae *Selenastrum capricornatum,* shows that metals acts jointly to cause toxicity since the toxicity of CCA was greater than that of its individuals metals (Cox, 1991). Experiments conducted by Keith and Warner (1990) shows that copper and chromium acts synergistically to cause toxicity. The study conducted on teredinid wood borers showed that arsenic of 1.5 ppm concentration was sufficient to cause 50% death in 72h and chromium was found to be least toxic since all animals active at 2 ppm even after 96h (Balaji *et al*., 2004)

The analysis of the tissue of *V. cyprinoides* collected from the Cochin area of the Vembanad Lake ranged between 12-30 ppm (Pillai *et al.,* 1986). The depuration and bioconcentration studies conducted in *V. cyprinoides* showed that the organism can live with significantly higher content of copper in their body (Sathyanathan, 1996). The results showed that the clams concentrate fairly high content of copper. Studies conducted showed that B.C.F. for chromium in oysters and blue mussels ranged between 125 -192 (Train, 1979). Copper is found to have a higher bioconcentration potential than chromium (Won & Tack, 1994). The B.C.F values of arsenic were low

except in the case of algae (Eisler, 1988a). Bioconcentration factor arsenic was 350 for *Crassostrea virginica* when it was exposed for 112 days in arsenic solution (Zaroogian & Hoffman, 1982).

6.4. Conclusion

Of the CCA components copper is found to be more toxic than chromium and arsenic. Copper can be acting along with arsenic and chromium to impart an added effect on its toxicity. All the three metals copper, chromium and arsenic of CCA have a tendency to get concentrated in the tissues of invertebrates like clams. The B.C.F value indicating the bioconcentration potential of a metal in an organism is higher for copper than the other two metals studied. In the case of CCA possibility of heavy metal pollution that it is imparted cannot be ignored. Combined toxicity studies are still to be done in order to conclude that whether these metals act in a synergistic way to cause toxicity in organisms. Since CCA is used after impregnating the wood with the chemical solution, the chemical components leached out from CCA is studied for its toxicity and bioconcentration characteristics are detailed in chapter VII

Table 6.1. Hydrographical parameters of water used for the study

Water parameters	Value
Temperature	28 ± 2°C
Dissolved oxygen	90 - 110 %
pH	8 ± 0.2
Salinity	0 ‰

Table 6. 2. Bioconcentration factor (B.C.F.) for copper, chromium and arsenic in black clam tissue

Metal	Metal concentration in tissue of clams in control tank (ppm)	Conc. of metal in water (ppm)	Conc. of metal in tissue (ppm)	Bioconcentration Factor (B.C.F.)
Copper	38	0.3	232.19	773.98
		0.4	328.44	821.12
		0.5	492.68	985.36
Chromium	33	2	41.48	20.74
		3	32.78	10.92
		4	15.89	3.97
Arsenic	23	2	91.40	45.70
		3	263.05	87.68
		4	61.23	15.30

Figure 6.1. a) Laboratory set up of toxicity studies b) clams exposed to CCA solution

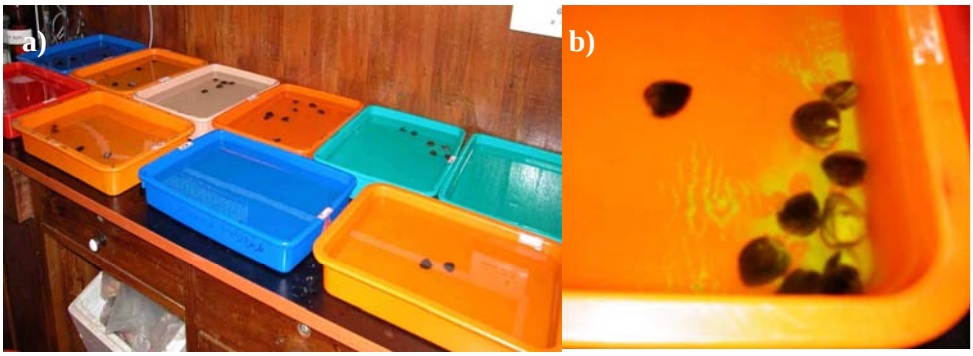


Figure 6.5. *Villorita cyprinoides* exposed in (a) control tank and (b) CCA solution

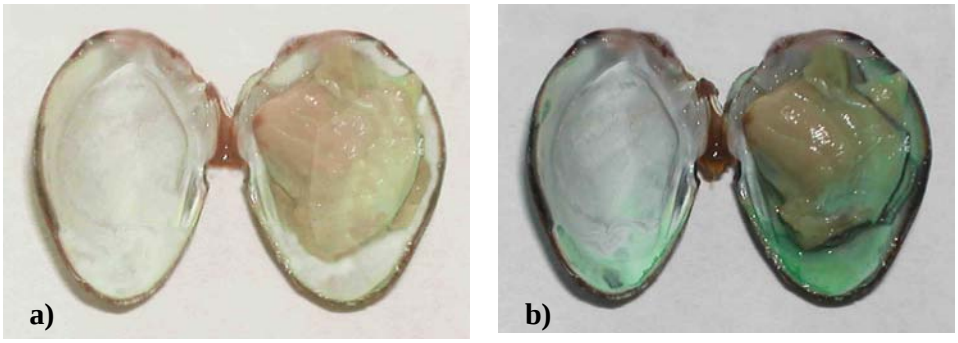


Figure 6.2. The percentage mortality of black clams at different concentrations of copper

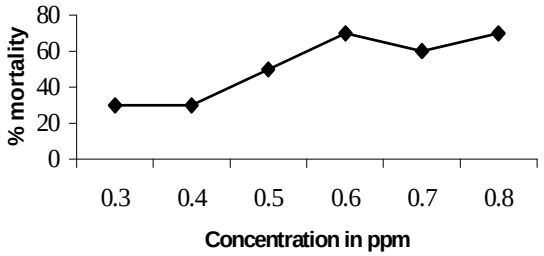


Figure 6.3. The percentage mortality of black clams at different concentrations of chromium

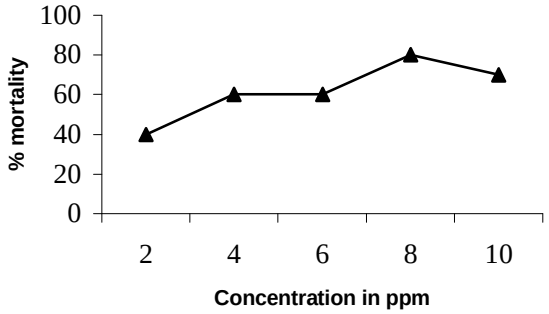


Figure 6.4. The percentage mortality of black clams at different concentrations of arsenic

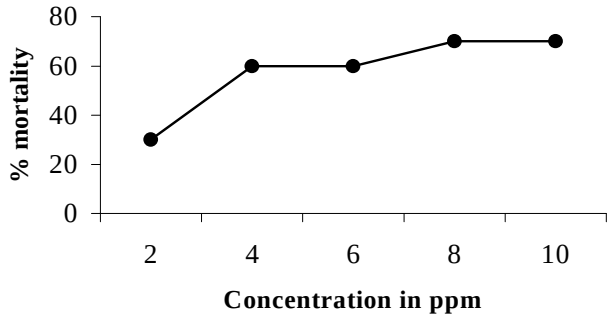
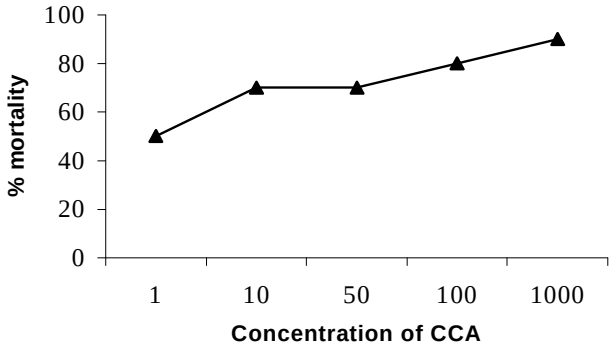


Figure 6.6. The percentage mortality of black clams at different concentrations of Chromated Copper Arsenate (CCA).



Chapter VII

7. BIOACCUMULATION OF CHROMATED COPPER ARSENATE COMPONENTS IN *OREOCHROMIS MOSSAMBICUS*

7.1. Introduction

All living organisms require minerals for the normal physiological functioning of the biological system. Although essential, when exposed to higher concentration, the minerals can upset the normal functioning of the system, can accumulate in various tissues of the organism and can cause detrimental effects (Van Vuren *et al*., 1999; Turkmen *et al*., 2005). Fishes have an ability to accumulate the metals and can provide valuable information pollution in the water bodies. When exposed to polluted water, uptake of metal by fish occurs actively or passively via. gills, skin, orally through food and water (Norey *et al*.,1990; Battaglini *et al*.,1993). Subsequently, the metal gets circulated through blood in various organs, get metabolized, detoxified, stored or excreted. Tilapia (*Oreochromis mossambicus*) was selected for the study. This fish seen in fresh water and in estuarine areas, is hardy and can tolerate wide fluctuations in the salinity and temperature and pH. Studies on the bioaccumulation of toxicants and its impact on the physiology of *Oreochromis mossambicus* have been extensively studied by Pelgrom *et al*., 1995; Kotze *et al*., 1999; Nussey *et al*., 1995; Abdelmeguid *et al*., 2002; Allinson *et al*., 2002; Heerden *et al*., 2004; Begum *et al*., 2005. The present study aims to evaluate

bioaccumulation of the metals copper, chromium and arsenic leached out from CCA treated wood in *Oreochromis mossambicus.*

7.2. Materials and Methods

7.2.1. Fish

Tilapia, of comparable size (length 30 - 40 mm and weight 3.8 - 4.5 g) were collected from rather pollution free areas of the upstream reaches of the Vembanad lake and were acclimatized in the laboratory, in aquaria tanks of 80 l capacity (Fig. 7.1.). Fishes were stocked in each of the tanks filled with fresh water. The tanks were continuously aerated. The photoperiod in the aquarium was 12h light and 12h dark. After one month of acclimation the experimental panels were introduced into the tanks. One tank was maintained as control with proper aeration and without panels. Fishes were fed daily 10% (dw/ww). Temperature, pH, turbidity, salinity, dissolved oxygen (D.O.) and Biological Oxygen Demand (B.O.D.) of the water was monitored on monthly basis during the period of experiment the detailed procedures are given in Chapter II.

7.2.2. Experimental panels

The rubber wood panels dip treated with CCA, treated with CCA to retention of 16 kg m^{-3} (Type II), dual treated panels (Type V), painted panels (Type VI), FRP sheathed panels (Type VII) and marine plywood panels (Type VIII) along with untreated rubber wood panels as control were used for the experiment. In addition, the dip treated panels where rubber wood panels were dipped in 7.5% CCA solution for 5 minutes were also used in the experiment

which represents treatment type IX in the experiment. The details regarding preparation of rubber wood panels, conditioning of the panels, pressure impregnation procedure and the conditions provided in the preservative treatment chamber was given in Chapter II (Table 2.1.). The preservative treated samples were dried at room temperature for 4 weeks and commercially available marine plywood were purchased and cut to the specified size (150x 100x 25 mm) and were used for the experiment.

7.2.3. Sampling procedure

During the acclimation period the fishes that did not respond when touched were considered to be dead and removed from the tank (Fig. 7.2). Three fishes from each tank was netted and killed monthly for a period of six months. The fishes were weighed, measured and kept in frozen condition until dissection. The specimens mainly the gills were visually inspected for the presence of lesions and deformities (Fig. 7.3.). Gill, liver, muscle and gonadial tissues were dissected from both control and experimental fishes, cleaned with distilled water and weighed (Fig. 7.4). The tissue samples were digested with 5ml nitric acid/perchloric acid mixture in Microwave Digestion System (Milestone ETHOS PLUS). The tissue digests were filtered through 0.4 mm cellulose nitrate filter paper and diluted with Milli-Q grade water. The metal concentrations were determined using ICP-AES (Optima 2000 DV Optical Emission Spectrometer, Perkin Elmer Instruments, USA). Blanks were run (Merck, Germany) along with the samples. The concentration of the samples

was determined using standard solutions Merck, Germany traceable to NIST prepared in the same analytical matrix.

7.2.4. Data analysis

Bioconcentration Factor (BCF) is a measure of distribution of the heavy metal between biota and water and is calculated using the equation:

$B.C.F = C_{biota}/C_{water}$

Where, C_{biota} is the total metal concentration in biota (ppm)

C_{water} is total metal concentration in water (ppm)

Statistical analysis was performed using SPSS version 10 windows. Tukey's test for multiple comparisons was used to compare concentration of copper, chromium and arsenic in gill, liver, muscle and gonadial tissues at different treatment types at statistically significant level ($p < 0.05$).

7.3. Results and Discussion

7.3.1. Hydrographical parameters

During the acclimatization the conditions provided in the aquaria like aeration, light and food were found sufficient since no mortality was observed after the period of acclimatization. Mortality was not observed in any of the experimental tanks during the entire period of the study. The hydrographical parameters like atmospheric temperature, water temperature, pH, salinity, turbidity, Dissolved Oxygen (D.O.) and Biological Oxygen Demand (B.O.D.) determined, showed no significant variation throughout the period of the study (Table 7.1.). Results showed that even though highly acidic solution CCA was impregnated into the wood, leaching of chemical components of CCA from

panels cause no significant change in the pH of the surrounding medium. Turbidity values in the tanks remained within a range of 9.50 ± 0.965 NTU. The D.O. and B.O.D. values when compared between the tanks where panels of Types II, V, VI, VII, VIII, IX and untreated rubber wood was introduced, showed no significant difference (Table 7.1.).

7.3.2. Concentration of CCA components in fish tissues

Visual observation of the fish specimens showed excess of mucus on all the fishes except for control specimens. Usually excess of mucus secretion is observed when fishes are under some stress. In the present investigation, the leaching of metals into a limited volume of water might have created some stress on the organisms. The analysis of metals in various organs of fish showed that highest accumulation is in liver for copper, chromium and arsenic that leached out from the exposed panels of Type II, Type V, Type VI, Type VII, Type VIII and Type IX. The muscles, that form the edible part from the human consumption point of view, showed the least accumulation of metals. The gills and gonads showed comparatively low concentration of copper, chromium and arsenic than liver. The mean concentration of copper chromium and arsenic in liver, muscles, gills and gonads of the fishes reared in the different tanks where the panels are exposed is given (Table 7.2. & Table 7.3.). The fishes reared in the control tank where no experimental panels were exposed, showed the presence of copper, chromium and arsenic in gills. Mean concentrations of the metals in gills were 3.89 ± 1.79µg Cu/g wet wt., 3.83 ± 0.70µg Cr/g wet wt. and 3.34 ± 1.26 µg As/g wet wt. In liver tissues and

gonads copper was detected while chromium and arsenic remained below the detectable level. The mean values were 22.09 ± 1.03µg Cu/g wet wt. in liver and 2.10 ± 2.46µg Cu/g wet wt. in gonads.

Copper accumulated in higher amounts in liver and the observed pattern was liver>gills>gonads>muscle. Same pattern of accumulation was followed by chromium and arsenic as well. The highest accumulation of copper was observed in liver of fishes exposed to Type IX panels. Copper concentration in liver varied significantly between the treatment types and was in the order of Type IX>Type II>Type VIII> Type VI> Type VII> Type V. In the gills, gonadial tissues and muscles highest accumulation of copper was observed in fishes exposed to Type II panels. In gills, the observed pattern of accumulation of copper between treatment types was Type II>Type VIII> Type V >Type IX> Type VI> Type VII. In muscles the copper concentrations were around 2µg Cu/g wet wt. in fishes exposed to all the different treatment types. Statistical analysis was conducted considering copper concentration in tissues between different treatments. Copper concentration in the tissues of fishes exposed to Type IX was significantly higher when compared to the other treatment types ($p<0.05$) (Table 7.8.). While tissues from fishes exposed to Type II, Type VIII was significantly higher than Type VI and Type VII but lower than Type IX. But difference in copper concentration between Type VI and Type VII was not significant ($p<0.05$). As far as the tissue types were concerned, copper concentration varied significantly (Table 7.11.).

Concentration of chromium in liver tissues exposed to Type VIII panel was the highest. The observed pattern of accumulation in liver was Type VIII >Type II> Type V > Type VI> Type IX >Type VII. In gills the pattern of accumulation was Type VIII> Type IX> Type VII> Type VI> Type V> Type II. In gonads, the accumulation pattern was Type VIII> Type IX> Type II> Type VI> Type V. Chromium was not detected in gonadial tissues exposed to Type VII panel. In muscles chromium remained below the detectable range initially for two months in all the treatment types. The mean concentration remained below 2 ppm in fishes exposed to all these types. In muscles of fishes exposed to the leachate from Type VI, Type VII panels, chromium concentration remained below the detectable limit. Tukey's test results are displayed in Table 7.9. show that when considering all the tissue types as a single factor, chromium concentration varies significantly between all the treatment types. As far as the tissue types are concerned, chromium concentration varied significantly between all the treatment types.

Being the detoxifying center, liver tissues showed highest accumulation of arsenic also. In liver, mean concentration of arsenic showed a pattern of Type II > Type IX > Type V > Type VII > Type VI > Type VIII. Arsenic concentration remained below the detectable level in muscle tissues of fishes exposed to all the treatment types except for Type II and Type V. In gonads the mean concentrations were 0.0.018 µg As/g wet wt. in Type II, 0.08µg As/g wet wt. Type IX, 0.16 µg As/g wet wt. Type V, 0.06 µg As/g wet wt. Type VII, 0.013 µg As/g wet wt. Type VIII. Statistical analysis to identify

the variation in arsenic accumulation between the treatment types show that there is no significant variation in arsenic concentration in the tissues exposed to between Type V, Type VI and Type VII panel leachate, while the value is significantly lower when compared to Type IX and Type II and significantly higher than Type VIII. A significant difference was observed in arsenic concentration when considering the tissue types where highest value reported from liver tissues and lowest from muscle tissues. The concentration of copper, chromium and arsenic in gills, liver, muscle and gonad and the bioconcentration factors are given in the Table 7.4., Table 7.5., Table 7.6. and Table 7.7.

7.2.3. Concentration of metals in the leachate

The leaching of CCA components from Type IX panels was relatively high during the first month (Fig. 7.5.). The leachate from this tank showed the presence of copper, chromium and arsenic during the entire period of the study but the observed concentrations were lower than the leachate of Type II panel and higher than Type V, Type VI, Type VII and Type VIII (Fig 7.5.). Copper concentration was highest after one month of exposure of Type II panels and the concentration in the leachate gradually decreased in the leachate as the exposure duration increased. The mean concentration of copper, chromium and arsenic in the leachate of Type II panels was 0.26 ppm, 0.02 ppm, 0.15 ppm. In Type IX and type VII tank copper, chromium and arsenic concentration were low after one - month exposure.

The copper concentrations in water where Type IX panels were exposed remained with in a range 0.11- 0.022 ppm. The highest value (0. 11ppm) was observed after an exposure of one month and the values decreased thereafter. During the six months study, the copper concentration in type II tank reduced from an initial value of 0.27 ppm to 0.076 ppm. The copper concentrations in all the tanks showed a gradual reduction as the duration of exposure increased while no such specific pattern was observed with chromium and arsenic. In leachate of Type VIII tank, copper concentration when compared to chromium and arsenic remained high throughout the study period. The mean value of copper concentration was 0.026 ppm while for chromium and arsenic the mean value observed was 0.066 ppm. The mean copper concentration in the leachate where Type V, Type VI and Type VII panels were exposed was below 0.02 ppm.

The chromium concentrations in the leachate of Type IX panel remained within a non-detectable level to a maximum of 0.005 ppm after five months of exposure. In the leachate of Type II tank showed an increase in chromium concentration upto five months and reduced thereafter and the values remained within a range 0.005 - 0.009 ppm. Arsenic concentration was highest in the leachate where Type II panel was exposed. The mean value was 0.15 ppm. The general pattern of arsenic concentration in the leachate of panels was Type II> Type V> Type VII > Type VI> Type VIII >Type IX. Arsenic concentration in the leachate of Type IX tank showed maximum value of 0.026 ppm during the first month. Arsenic concentration in type I tank

increased 8 times from an initial low value. Leaching of chromium was the lowest in the case of dual treated panels, painted panels and FRP sheathed panels, while in the case of marine plywood panels the observed pattern was Cu>Cr>As where arsenic remained below detectable level during the initial five months of exposure.

The Bio Concentration Factor (BCF) for copper, chromium, arsenic in various tissues viz. liver, gills, gonads and muscle of *Oreochromis mossambicus* exposed to CCA and marine plywood leachate are given in the Table 7.2., Table 7.3., Table 7.4. and Table 7.5.

The wood can be treated with CCA preservative using different methods as dip treatment, vacuum pressure impregnation etc. In dip treatment the preservative get loaded in the superficial layers of the wood while in vacuum pressure impregnation the cell cavities are filled with preservatives (Findlay, 1985). In the present study the quick leaching of preservatives from dip treated panels when compared to pressure treated ones may be due to the fewer adherences of preservatives in wood during dipping.

The toxicity of metals in fishes depends on many factors like characteristics of the surrounding water including temperature, pH, salinity, dissolved oxygen, presence of organic ligands and the presence of other metals. Increase in the salinity was found to decrease the accumulation of copper in tilapia (Newell & Sanders, 1986; Karakoc, 1999). Acute toxicity studies on blue tilapia (*Oreochromis aureus*) have shown that toxicity of copper increases as pH increases (Straus, 2002). In the present investigation

increase in metal accumulation due to the changes in the hydrographical conditions were not expected since the conditions were almost constant in all the three tanks throughout the period of the study.

In the present investigation it was observed that the fishes exposed CCA and marine plywood showed an increase in metal concentrations when compared to the control samples. While the FRP sheathed panels, painted and dual treated panels effectively reduced the arsenic leaching from CCA treated panels. Chromium was the lowest detected metal in the leachate showing that chromium act as an effective fixative of CCA that fixes CCA components in wood. Although some studies have been reported on accumulation possibilities of metals from CCA treated panels, the studies on fishes under static aquaria conditions are not reported. The laboratory flow through studies and field exposure studies conducted with *Mytilus edulis* exposed to CCA treated panels showed that the laboratory fed mussels have significantly higher levels of copper in their tissues when compared to mussels collected from the field (Ivanbrook & Breslin, 1999). Copper showed significant accumulation in the tissue of blue mussel when compared to chromium and arsenic (Ivanbrook & Breslin, 1999). This showed that the metals copper, chromium and arsenic of CCA have the potential to get accumulated in the tissues of aquatic organisms. Field trials conducted by Weis and Weis (1999) to assess the copper, chromium and arsenic concentration in grass shrimp (*Palaemonetes pugio*), and two teleost fish, the naked goby (*Gobiosoma bosci*) and mummichog (*Fundulus heteroclitus*) showed that the fishes were unaffected

by the presence of CCA treated wood. This may be due to the fact that in the field conditions the CCA leachate has higher possibilities to get diluted. In a static aquarium the fishes have higher risk to get access to the metals in much higher concentration.

In accordance with the present data, the concentration of copper, chromium and arsenic was highest in liver signifying the detoxification role of the liver. The studies by Pelgrom *et al*., (1995) showed that most of the copper in the water originally enters through the gills of the fish and get transported *via.* blood plasma in to the liver. Liver forms a major site of detoxification mechanisms by metallothionein (MT) production, and storage site to the pollutants. The accumulation of metals was highest in liver (McCarter, 1983; Hilmy, 1987; Karakoc, 1999). Studies conducted by Karakoc (1999) in *Tilapia nilotica* showed that copper accumulation was highest in the liver tissues while lowest in the muscle. In *Tilapia mossambicus* copper was found accumulated for 11 days and then attained a steady value (Dang *et al*., 1999). The gills of the fishes being the primary site to get in contact with the metal through the respiration and excretion process showed a lower accumulation rate. Only at very high concentration of metals in the gills there is a possibility of impairment in the function. In this study, concentration of all the metals remained below the lethal level in much lower levels than liver indicating a lower possibility of damage to the gills. Studies conducted by Pelgrom *et al*., (1995) *Oreochromis sp*. was found more copper tolerant and gills were affected by increased accumulation. According to Boeck *et al.,* (2004) the

copper get concentrated in gills or other tissues only when it spills over the copper concentration in the liver. This explains observed pattern of accumulation in the present study as liver>gills>gonads>muscle. The results were agreeable with the studies conducted by Kotze *et al.*, (1999) where a similar the pattern for accumulation of copper was observed.

Bioaccumulation studies conducted by Begum *et al.*, (2005), Allinson (2002) considered only the muscle tissues for analysis that forms the major part for human consumption. In the present study the concentration of metals copper, chromium and arsenic were the lowest in muscles. According to the USFDA and EPA Safety levels (US FDA, 2001), the permissible limits for arsenic and chromium for fish and fishery products are 76 ppm and 12 ppm respectively. Export Inspection Agency of India recommends the same standard as a criterion to assess the export standard for fish and fishery products to EU countries. According to water quality criteria for copper by Agency for Toxic Substances and Disease Registry, the water and organisms having copper concentrations below 1300 µg/l is permitted for the human consumption (ATSDR, 2000). In the present investigation where the studies were carried out in stagnant condition the metal concentrations were well below the standards levels indicating a much lower possibility of risk due to CCA to fishes in natural aquatic condition. Studies by Kotzae (1999) have shown that the lowest concentration of copper is in muscle of *Oreochromis mossambicus* collected from the field. The higher levels of metals in gills, viscera and liver than muscles (Zhou, 1998) may be due to the fact that the

most of the heavy metals are accumulated in the liver after ingestion or excreted through kidney or skin (Sorensen, 1991) or retained in viscera. Studies showed that the metals could get accumulated in gonads also (Shah and Altinda, 2005).

7.4. Conclusion

Based on the studies carried out, we can conclude that the metals copper, chromium and arsenic that leached out from CCA treated panels (both rubber wood dip treated and pressure treated with preservative and marine plywood) have the potential to get bioaccumulated in the tissues of organisms living in the vicinity. The accumulation pattern of copper, chromium and arsenic in the tissues of *Tilapia* has shown that the liver, being the detoxifying center of the body accumulates body burden of the absorbed metals. From the human consumption point of view, the metals accumulated from CCA treated panels remained below the permissible level pointing out a lower risk. The study showed that the dip treated panels are more prone to quick leaching. FRP sheathing, painting and dual treatment with CCA and creosote reduces the risk of exposure of CCA components as it reduces leaching.

Table 7.1. Hydrographical parameters in the aquarium tanks

Parameters	Control	Type II	Type V	Type VI	Type VII	Type VIII	Type IX
Atmospheric temperature (°C)	28.6 ± 0.78	28.62 ± .79	28.60 ± 0.78	28.62 ± 0.79	28.62 ± 0.69	28.62± 0.74	28.62 ± 0.76
Water temperature (°C)	28.62 ± 0.48	28.62 ± .48	28.62 ± 0.46	28.62 ±0.50	28.62 ±0.48	28.62 ±0.44	28.52 ± 0.52
pH	7.61 ± 0.20	7.60 ± .21	7.71± 0.24	7.59 ± 0.27	7.60 ± 0.19	7.71 ±0.11	7.47 ±0.19
Salinity (ppt)	0.17 ± 0.05	0.18 ± .06	0.22 ± 0.12	0.18 ± 0.08	0.17 ± 0.06	0.21 ±0.22	0.16 ±0.19
Turbidity (NTU)	6.14 ± 4.41	6.14 ± .41	5.7± 1.05	5.2 ±2.17	5.1± 1.65	5.0 ±3.65	5.57 ±3.73
Dissolved Oxygen (D.O.) (mg l^{-1})	5.90 ±1.22	5.94 ± .23	5.62± 0.29	5.62 ±0.29	5.62± 0.29	5.62 ±0.29	5.31 ±1.57
Biological Oxygen Demand (B.O.D.) (mg l^{-1})	3.64 ± 1.00	3.65 ±1.03	4.11± 1.67	4.11 ±1.67	4.11± 1.67	4.11 ±1.67	3.83 ±1.75

Table 7.2. Mean concentration of copper, chromium and arsenic in liver and muscles of *Oreochromis mossambicus* over 6 months (Concentration of metals in µg.g^{-1} wet wt.)

Preservative treatment type	Liver			Muscles		
	Copper	Chrom-ium	Arsenic	Copper	Chrom-ium	Arsenic
Type II	57.64	2.365	15.19	1.69	0.04	0.013
Type V	17.11	2.35	1.59	2.25	1.9	0.09
Type VI	20.38	1.75	0.86	1.34	ND	ND
Type VII	19.93	1.02	1.49	ND	ND	ND
Type VIII	36.43	12.86	0.018	1.78	0.82	ND
Type IX	121.42	1.58	2.51	1.82	0.04	ND

Table 7.3. Mean concentration of copper, chromium and arsenic in gills and gonads of *Oreochromis mossambicus* over 6 months (Concentration of metals in µg.g^{-1} wet wt.)

Preservative treatment type	Gills			Gonads		
	Copper	Chrom-ium	Arsenic	Copper	Chrom-ium	Arsenic
Type II	28.73	0.46	0.11	13.68	1.56	0.018
Type V	5.37	0.77	ND	0.32	0.41	0.16
Type VI	2.38	1.07	0.86	2.02	0.76	ND
Type VII	1.39	1.09	1.02	1.98.	ND	0.06
Type VIII	3.26	1.64	ND	7.89	1.74	0.013
Type IX	2.89	1.32	0.76	1.07	1.66	0.8

Table 7.4. Bioconcentration of copper, chromium and arsenic in gills of *Oreochromis mossambicus* (Concentration of metals in µg g^{-1} wet wt.)

Treatment types	Months	Copper	BCF	Chromium	BCF	Arsenic	BCF
	1	3.9	35.45	0.83	835.66	3.34	128.56
	2	0.73	9.12	ND	ND	ND	
	3	4.01	77.17	ND	ND	ND	ND
	4	3.96	180.37	ND	ND	ND	ND
	5	3.51	79.8	ND	1095.5	ND	ND
Type IX	6	1.26	48.73	1.65	1656.9	ND	181
	1	7.95	29.45	ND	ND	ND	ND
	2	4.51	22.55	ND	ND	ND	ND
	3	4.45	27.83	ND	ND	ND	ND
	4	11.02	101.14		ND	0.33	1.37
	5	35.24	329.38	2.73	55.63		ND
Type II	6	43.25	569.08	ND	ND	0.34	1.38
	1	1.5	250.33	ND	ND	ND	ND
	2	3.55	295.83	ND	ND	ND	ND
	3	3.22	103.88	ND	ND	ND	ND
	4	6.37	135.52	ND	ND	ND	ND
	5	2.37	87.74	2.49	829.37	ND	ND
Type VIII	6	2.57	75.68	1.35	192.57	ND	ND
	1	4.31	359.17	ND	ND	ND	ND
	2	6.79	357.69	ND	ND	ND	ND
	3	4.17	355.2	0.55	551	ND	ND
	4	2.6	292.52	0.97	970.87	ND	ND
	5	1.73	387.68	1.47	1473	ND	ND
Type V	6	12.65	527.08	1.6	1602	ND	ND
	1	0.49	28.824	0.53	530	0.23	7.6667
	2	0.78	43.333	0.27	270	0.46	11.5
	3	1.26	60	0.35	350	0.89	29.667
Type VII	4	0.95	43.182	1.78	ND	1.23	43.929
	5	1.82	140	1.24	ND	1.94	194
	6	2.98	156.84	2.36	1180	1.42	48.966

	1	0.79	65.833	0.65	650	0.23	25.556
	2	1.26	96.923	1.46	1460	0.59	59
	3	0.98	75.385	0.98	490	1.58	175.56
	4	2.65	203.85	1.5	ND	1.23	ND
	5	4.57	457	2.19	ND	0.86	ND
Type VI	6	3.98	398	3.65	ND	0.67	35.263

Table 7.5. Bioconcentration of copper, chromium and arsenic in liver of *Oreochromis mossambicus* (Concentration of metals in µg g^{-1} wet wt.)

Treatment types	Months	Copper	BCF	Chromium	BCF	Arsenic	BCF
	1	50.23	456.62	0.05	52	0.57	0.145
	2	266.75	5625	0.19	ND	ND	ND
	3	30.55	587.5	1.66	ND	5.55	1.38
	4	81.25	3693.2	3.24	ND	5.8	ND
	5	14.58	331.44	ND	ND	ND	ND
Type IX	6	101.96	3921.6	4.36	4356	3.19	455.38
	1	24.32	90.09	ND	ND	ND	ND
	2	77.27	386.37	ND	ND	2.73	30.33
	3	46.8	292.47	ND	ND	5.08	338.6
	4	52.12	478.17	ND	ND	23.68	97.87
	5	69.23	647.02	5.63	114.84	12.17	44.09
Type II	6	76.13	1001.8	8.56	778.09	47.53	192.43
	1	16.66	490	ND	ND	ND	ND
	2	10	37.04	ND	ND	ND	ND
	3	26.32	131.58	4.15	460.57	ND	ND
	4	32.88	205.48	11.84	1480.3	ND	ND
	5	72.74	667.36	28.08	9360.7	ND	ND
Type VIII	6	60	560.75	33.12	4731.4	0.09	2.39
	1	5.19	433.16	1.26	1268	ND	ND
	2	13.63	717.7	1.75	1759	ND	ND
	3	17.24	749.62	1.32	1325.4	1.5	30.61
	4	18.36	706.5	3.24	3245	2.7	42.85
	5	20.02	527	2.35	294.25	2.3	30.26
Type V	6	28.26	1177.9	4.23	2117.5	1.9	26.38
	1	9.56	562.35	0.59	590	0.98	32.66
	2	13.68	760	0.87	870	1.26	31.5
	3	26.85	1278.5	1.69	1690	2.17	72.33
Type VII	4	17.65	802.27	0.58	ND	1.56	55.71
	5	18.95	1457.6	1.42	ND	0.76	76
	6	30.69	1615.2	1.08	540	1.42	48.96

Type VI	1	8.56	503.52	0.76	760	0.23	7.66
	2	19.49	1082.78	1.65	1650	0.56	14
	3	12.45	592.85	1.98	1980	0.91	30.33
	4	30.28	1376.36	1.63	ND	0.89	31.78
	5	25.14	1933.84	2.09	ND	1.24	124
	6	21.76	1145.26	2.46	1230	1.09	37.58

Table 7.6. Bioconcentration of copper, chromium and arsenic in muscles of *Oreochromis mossambicus* (Concentration of metals in µg g^{-1} wet wt.)

Treatment types	Months	Copper	BCF	Chromium	BCF	Arsenic	BCF
	1	ND	ND	ND	ND	ND	ND
	2	2.34	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND
	4	3.96	ND	0.25	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND
Type IX	6	4.64	2.8	0.01	ND	ND	ND
	1	1.8	6.66	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND
	3	2.79	17.46	0.29	16.06	0.08	5.33
	4	1.29	11.83	ND	ND	ND	ND
	5	1.95	18.23	ND	ND	ND	ND
Type II	6	2.31	30.39	ND	ND	ND	ND
	1	2.32	385.95	ND	ND	ND	ND
	2	1	83.09	ND	ND	ND	ND
	3	2.29	74	ND	ND	ND	ND
	4	2.6	55.24	2.4	300	ND	ND
	5	2.51	92.89	2.5	833.33	ND	ND
Type VIII	6	ND	ND	ND	ND	ND	ND
	1	0.46	38.23	ND	ND	ND	ND
	2	0.88	46.08	ND	ND	ND	ND
	3	1.52	66.16	ND	ND	ND	ND
Type V	4	3.25	125	0.69	690	ND	ND
	5	4.65	122.37	3.35	418.75	ND	ND
	6	8.75	364.58	7.36	3678.9	0.56	7.78
	1	ND	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND
Type VII	3	ND	ND	ND	ND	ND	ND
	4	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND

Type VI	1	0.45	26.47	ND	ND	ND	ND
	2	0.86	47.77	ND	ND	ND	ND
	3	1.53	72.85	ND	ND	ND	ND
	4	1.56	70.909	ND	ND	ND	ND
	5	2.02	155.38	ND	ND	ND	ND
	6	1.68	88.42	ND	ND	ND	ND

Table 7.7. Bioconcentration of copper, chromium and arsenic in gonads of *Oreochromis mossambicus* (Concentration of metals in µg g^{-1} wet wt.)

Treatment types	Months	Copper	BCF	Chromium	BCF	Arsenic	BCF
	1	ND	ND	0.08	ND	ND	ND
	2	0.06	0.75	2.73	ND	0.01	0.43
	3	0.04	0.74	3.4	ND	0.06	8.11
	4	1.45	65.74	0.02	4	1	ND
	5	2.75	62.5	2.35	ND	ND	ND
Type IX	6	1.91	73.53	1.43	ND	4.19	598.74
	1	1.43	5.3	ND	ND	ND	ND
	2	11.65	58.25	0.14	11.67	ND	ND
	3	28.63	178.94	1	55.56	0.08	0.53
	4	2.91	26.7	1.04	41.6	ND	ND
	5	3.28	30.65	2.37	48.37	0.03	0.11
Type II	6	34.21	450.14	4.86	441.55	ND	ND
	1	3.4	566.67	ND	ND	ND	ND
	2	6.9	575	1.14	190	ND	ND
	3	3.08	99.35	1	142.86	ND	ND
	4	11.22	238.72	1.12	140	0.02	ND
	5	8.05	298.15	2.37	790		ND
Type VIII	6	14.71	432.65	4.86	694.29	0.02	0.56
	1	0	ND	ND	ND	ND	ND
	2	0.77	40.35	ND	ND	ND	ND
	3	ND	ND	ND	ND	1	20.41
	4	ND	ND	0.58	583.33	ND	ND
	5	0.38	10	0.89	111.63	ND	ND
Type V	6	0.75	31.35	1	500	ND	ND
	1	0.86	50.58	ND	ND	0.03	1
	2	0.99	55	ND	ND	0.06	1.5
	3	1.25	59.52	ND	ND	0.09	3
Type VII	4	1.69	76.81	0.04	ND	0.06	2.14
	5	2.89	222.30	0.06	ND	0.14	14
	6	3.85	202.63	ND	ND	0.04	1.37

Type VI	1	0.86	50.58	0.42	420	ND	ND
	2	2.23	123.88	1.22	1220	ND	ND
	3	1.74	82.85	0.84	840	ND	ND
	4	1.58	71.81	0.68	ND	ND	ND
	5	2.35	180.76	0.56	ND	ND	ND
	6	3.34	175.78	1.03	515	ND	ND

Table 7.8. Tukey's test results showing significance of changes in copper concentration between treatment types studied

***(Treatment type code used in statistic analysis 1.Dip treated, 2. CCA treated, 3. Marine plywood, 4. Dual, 5.FRP, 6. paint*)**

Tukey HSD[a,b]

Treatment types	N	Subset 1	2	3	4	5
6	12	5.83				
4	12		6.5125			
5	12		6.5300			
3	12			12.340		
2	12				25.4350	
1	12					32.0500
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 4.167E-02.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = .05.

Table 7.9. Tukey's test results showing significance of changes in chromium concentration between treatment types studied

Tukey HSD[a,b]

Treatment types	N	Subset 1	2	3	4	5	6
6	12	.5275					
5	12		1.075				
2	12			1.1063			
1	12				1.150		
4	12					1.36	
3	12						4.26
Sig.		1.000	1.000	1.000	1.000	1.00	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .000.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = .05.

Table 7.10. Tukey's test results showing significance of changes in arsenic concentration between treatment types studied

Tukey HSD[a,b]

Treatment types	N	Subset 1	2	3	4
3	12	.008			
5	12		.4300		
4	12		.4600		
6	12		.6425		
1	12			1.2675	
2	12				3.83
Sig.		1.00	.130	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 4.167E-02.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = .05.

Table 7.11.Tukey's test results showing significance of changes in copper concentration between tissue types studied

Tukey HSD[a,b]

Tissue	N	Subset 1	2	3	4
4	18	1.6467			
2	18		4.6600		
1	18			7.3367	
3	18				45.4850
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 4.167E-02.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Table 7.12. Tukey's test results showing significance of changes in chromium concentration between tissue types studied

Tukey HSD[a,b]

Tissue	N	Subset 1	2	3	4
4	18	.4667			
2	18		1.0217		
1	18			1.1783	
3	18				3.6542
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .000.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Table 7. 13. Tukey's Test results showing significance of changes in arsenic concentration between tissue types studied

Tukey HSD[a,b]

Tissue	N	Subset 1	2	3
4	18	1.7E-02		
2	18		.3418	
1	18		.4583	
3	18			3.6097
Sig.		1.000	.329	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 4.167E-02.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

Figure 7.1. Experimental panels immersed in aquaria where fishes are grown

Figure 7.2. Tilapia removed from the tank

Figure 7.3. Visual observation of gills for deformities

Figure 7.4. Gills, liver, muscle and gonadial tissues dissected from Tilapia

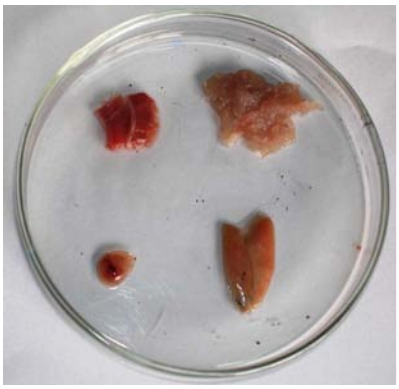
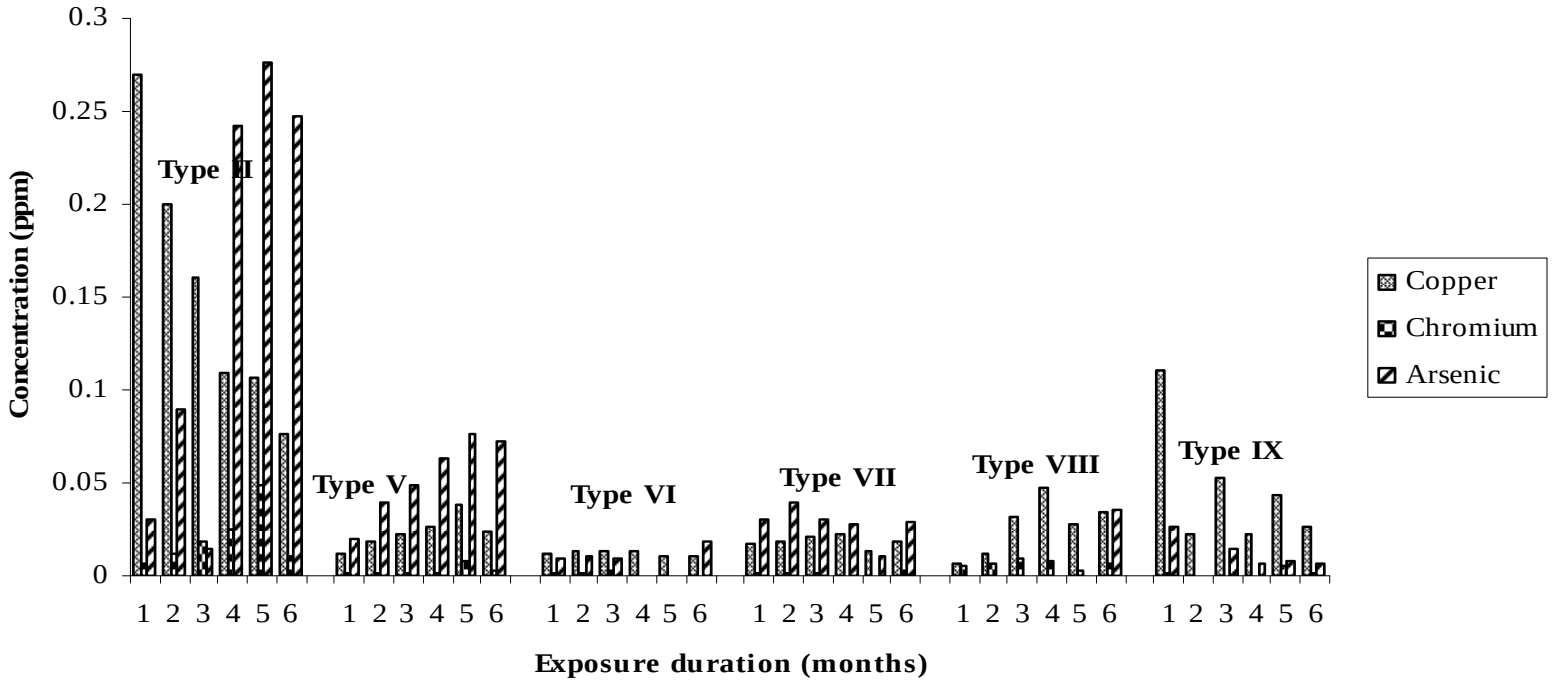


Figure 7.5. Concentration of copper, chromium and arsenic in water exposed to dip treated CCA and marine plywood panels (all values in ppm)



Chapter VIII

8. BIOACCUMULATION OF CCA COMPONENTS IN NON-TARGET ORGANISMS GROWING ON RUBBER WOOD PANELS

8.1. Introduction

Wood under marine or estuarine condition can act as a substrate for biofouling organisms and at the same time they are acted upon by biodeteriogens especially by marine woodborers. Chromated Copper Arsenate (CCA) has been well established as a wood preservative that protects wood from a variety of biodeteriorating agents including marine woodborers. Although it has been known as a fixed type of preservative, studies reveal that the constituent metal components copper, chromium and arsenic from CCA treated wood leaches into water as wood wets (Lebow, 1996). In the context of release of metal constituents from CCA treated wood, the epibiotic fouling organisms that are growing attached to the surface of the treated wood are the major groups affected by the released metal in different ways. The metals leached out can get accumulated in the tissues and shells of foulers growing attached to the treated wood, which is detailed in the present study. While the metals leached out can also interfere with the settlement, growth and life cycle of various fouling organisms by altering the normal physiological and biochemical mechanisms in the body that in turn is expressed as an ultimate change in the total biodiversity of foulers on the substratum and in the near vicinity which is dealt in detail in the following chapter.

In the long history of the sea sailing the fouling assemblages with a higher proportion of barnacles have been a problem for ships. According to the studies conducted, the barnacles are the dominant biofouling organisms all along the coastal waters of India (Nair, 1965; Meenakumari & Nair, 1984; Anil & Wagh, 1988a). They can grow on any hard substratum submerged in marine and estuarine conditions. These are sessile sedentary organisms attaching themselves to the substratum by cement glands and the outer shell constituted by calcareous plates protects the soft body from the tides and currents (Avelinmary & Sarojini, 1997). The barnacles are identified as potential bioaccumulators of pollutants and can be used as biomonitors of pollution (Anil & Wagh, 1988b; Philips & Rainbow, 1988; Fialkowski & Newman, 1998; Blackmore, 1998; Blackmore, 1999; Blackmore, 2001; Blackmore, 2004). A number of studies have been reported regarding the use of barnacles as an indicator and possible accumulators of preservative components leached out from the preservative treated wood (Scown & Cookson, 1999; Weis & Weis, 1999). According to the studies conducted, the epibiotic fouling organisms like barnacles (*Balanus eburneus*), oysters (*Crassostrea virginica*), algae (*Enteromorpha intestinalis*) etc. living on the CCA treated panels showed increase in copper, chromium and arsenic content in their tissues (Weis *et al.*, 1995; Weis & Weis, 1996; Scown & Cookson, 1999; Weis & Weis, 1999). But the studies conducted by Albuquerque and Cragg (1995b), Baldwin *et al*., (1996), Brown and Eaton (1997), Ivanbrook

and Breslin (1999) are of the opinion that treated wood have little adverse impact on the organisms attached to it or in the vicinity.

The present investigation focuses on the possibility accumulation of copper, chromium and arsenic in barnacles growing on rubber wood panels treated with CCA to different retentions. The study also focuses on the possibility of changes in accumulation of leachate constituents, when multiple preservative treatment methods physical barriers are adopted for covering the CCA treated wood treated wood.

8.2. Materials and Methods

The details regarding the preparation of the rubber wood panels, preservative treatment, and experimental set up, study location, arrangement and immersion of the racks at study location are given in the Chapter II. The rubber wood panels of series II were used for the regular monitoring of the metals in barnacles attached to it. The series included three sets of panels of eight different treatment types *viz.* CCA treated to three different retentions 16 kg m^{-3}, 29 kg m^{-3}, 42 kg m^{-3}, dual preservative with an ultimate retention of 150 kg m^{-3}, CCA treated wood (16 kg m^{-3}) coated with paint and CCA treated wood (16 kg m^{-3}) sheathed with Fiberglass Reinforced Plastic (FRP), commercially available CCA treated marine plywood and untreated rubber wood samples as control. The study was performed for a duration of eighteen months. The first sampling of barnacles was done after 6 months of immersion of treated panels in the estuary so as to provide sufficient time for the settling of barnacles on the panels. Sampling was done continuously for one year from

December 2005 to December 2006. The fouling assemblages were scraped off monthly from each type of the panels, using a stainless steel scraper. The barnacles were separated out and were washed thoroughly in distilled water. The collection of the barnacles was performed randomly without considering any specificity in the species and size of the barnacles. The samples were kept frozen at 4^{o}C until analysis. During analysis, the frozen samples were thawed; soft tissue including the mantle tissue, membranous base, egg masses etc. was dissected and separated out. The tissue and shell samples from each of the panel were macerated separately. Approximately 0.5 g of the wet sample of tissue and shells were weighed out and were digested separately in 7ml nitric acid - perchloric acid mixture (1:7). The acid digestion was carried out in a Microwave acid digester (Milestone Ethos Plus). Copper, chromium and arsenic in digested samples were analysed in ICP - AES (Perkin Elmer - Optima 2000 DV). The standard samples were run throughout the analysis. The metal concentrations were given in $\mu g.g^{-1}$ wet wt. of the sample.

Water samples were collected monthly from the study site. The hydrographical parameters like water temperature (^{o}C), atmospheric temperature (^{o}C), dissolved oxygen (mg ml^{-1}), salinity (ppt), turbidity (NTU) and pH were checked throughout the experiment. The results of the hydrographical observation for the period of one year from December 2005 to December 2006 are summarized (Chapter III. Table 3.1. & Table 3.2.). Water samples were also analysed in ICP-AES (Perkin Elmer-Optima 2000 DV) to

collect a basic data regarding the status of the concentration of the metals *viz.* copper, chromium and arsenic.

The statistical analysis regarding the metal loadings *viz.* copper, chromium and arsenic was conducted using SPSS version 12.0. The data was treated statistically using Multivariate Analysis and Tukey's HSD and the variation in the copper, chromium and arsenic concentrations were analysed using preservative treatment types as the fixed factor. The statistical significance was measured at $p \leq 0.5$

8.3. Results and Discussion

In the present investigation, barnacles made their first appearance after three weeks of immersion of the panels in the test site. Pinpoint like shells of barnacles having a diameter of 1 mm – 2 mm was observed during this time. The panels where immersed in June during the onset of monsoon. As the monsoon season prevailed the salinity at the test site was less than 1 ppt. During the entire period of investigation profuse growth of barnacles were observed on all the type of panels contributing a major proportion to the total biomass of foulers. Due to the drastic changes in salinity observed during the monsoon about 30% of the barnacles attached to the panels were sloughed off. Enough samples were observed even in the monsoon season for the analysis. After six months of continuous immersion the average basal diameter of the barnacles was 11 mm. After 12 months of exposure, the barnacles attained an average height of 17 mm and an average basal diameter of 21 mm. The crown of the barnacles at the time was of average 9 mm dia. After 18 months of

exposure the basal diameter attained was of 24 mm with an average height of 19 mm.

8.3.1. Metals in water

The hydrographical parameters that prevailed in the test site during the entire period of the study were recorded. A well-marked difference was observed in salinity, turbidity and dissolved oxygen during the pre monsoon, monsoon and post-monsoon periods (Fig.3.1.and Fig.3.2). The details regarding the changes in hydrographical parameters are detailed in Chapter III. Salinity, dissolved oxygen and turbidity showed significant variations according to the prevailing season. Two monsoon seasons marked the 18 months study. The investigation was started during onset of monsoon in June and as the southwest monsoon and northeast monsoon intensified the salinity at the site reduced gradually, turbidity of water increased and a reduction in the amount of dissolved oxygen was observed (Fig. 3.1. & Fig. 3.2.). The panels when retrieved after 6, 12 and 18 months of exposure are visually monitored and results of the visual observation are summarized in Table 3.1., Table 3.2. and Table 3.3. Barnacles and hydroids constituted majority of this fouling assemblage providing relatively enough specimen for investigation.

During the entire period of investigation the water samples collected from the study site showed the presence of copper, chromium and arsenic. Copper was observed in higher quantities that remained in a mean range of 2.2 - 5.0 ppm. The concentration of arsenic and chromium remained with in a range of 0.04 - 0.093 ppm and 0.003 - 0.005 ppm respectively. According to

Rajendran & Kurian (1986) the range of copper concentration in Cochin backwaters was 0.10 - 1.2 ppm. Meenakumari (1989) reported an average concentration of 4.37 ppm of copper in the Cochin harbour waters. It can be observed that there is not much variation in the copper concentration in the water due to the anthropogenic or any other such activities.

The monthly variations in the observed concentration of concerned metals copper, chromium and arsenic were rather insignificant in tissues and shells of barnacles collected from the panels and so statistical analysis was performed by taking into account the mean concentration of respective metals derived from the values for eighteen months. A slight increase in the metal concentration especially in the case of copper and chromium was observed in the monsoon season when the salinity of the water drastically lowered. But these changes were rather insignificant when compared to the metal concentration for the adjoining months. Nugegoda and Rainbow (1995) are of the opinion that metals are more bioavailable when salinity decreases. But in the present investigation, there was no significant change in the body burdens of concerned metals with the changes in the salinity of surrounding water.

8.3.2. Metals in barnacle tissues

Mean concentration of copper, chromium and arsenic over eighteen months study in the soft tissues of barnacles collected from untreated and preservative treated panels is given in Fig. 8.1. In general, it was observed that the tissues of barnacles collected from all the panels irrespective of the treatment types showed a higher concentration of copper, chromium and

arsenic when compared to that in water. The general pattern observed for was copper > chromium > arsenic.

A significant increase was observed in the concentration of copper accumulated in the tissues of barnacles collected from CCA treated panels of three different retentions *viz.* 16 kg m^{-3}, 29 kg m^{-3} and 42 kg m^{-3} between themselves and with the control samples (Table 8.1.). The variation in the copper concentration for a period of one and a half years was much more obvious in the case of barnacles collected from CCA treated panels to 29 kg $m^{-3}$and 42 kg m^{-3} retention where about 2.5 times and 3.5 times increase in concentration was recorded. As the results show, there was no significant variation in the copper concentration in samples collected from FRP sheathed, painted panels and marine plywood panels when compared to the Control samples (Table 8.1.). Even though copper concentration in the samples collected from CCA treated panels with retention of 16 kg $m^{-3}$and dual preservative treated panels varied significantly high when compared to the control samples, the concentration remained well below the mean levels observed in samples collected from the panels of higher CCA retention (Table 8.1.). There was no significant variation in chromium concentration in the tissues collected from any of the preservative treated or physically protected panels except CCA treated to higher retentions of 29 kg $m^{-3}$and 42 kg m^{-3} (Table 8.2.). When these two panels were compared, there was no significant variation in the concentration of chromium accumulated while when compared to the control samples, concentration increased 2 times in both the cases. The

mean arsenic concentration in the tissues was not statistically different in the treatment types and physically protected samples except in the case of CCA treated panels to retention of 42 kg m^{-3} (Table 8.3.). When compared with the control samples, double the amount of arsenic was obtained in the tissue of barnacles collected from panels of 42 kg m^{-3} retention.

In the present investigation, although the barnacle tissue samples collected from dual treated panels showed increase in the concentration of copper, chromium and arsenic, a significant increase was observed only for copper. The concentration of copper and chromium in the tissues of barnacles collected from the treated rubber wood panel coated with paint and sheathed with FRP have slightly increased from that of the samples collected from the control panels but the increase was not significant. The tissue burden of copper, chromium and arsenic in barnacles collected form marine plywood panels was not significantly increased when compared to other treatment types.

8.3.3. Metals in shells

In general, it was observed that the concentration of the copper, chromium and arsenic in shells of barnacles is less than the concentration in their tissues. The general pattern of observed concentration of metals was chromium>copper> arsenic. Concentration of copper and chromium in the shells in the samples collected from FRP sheathed and painted was not varied significantly (Table 8.4. & Table 8.5.). While arsenic concentration was significantly higher in both tissue samples and shells collected from FRP

sheathed panels (Table 8.6.). The possible explanations can be that the Fibreglass Reinforcement used may contain arsenic as one of its constituent that was not studied in the present investigation. The copper, chromium and arsenic concentration in the shell samples collected from CCA treated panels of higher retentions *viz.* 29 kg m^{-3} and 42 kg m^{-3} was significantly increased when compared with the control values (Table 8.4., Table 8.5. & Table 8.6.). However in the case of CCA treated panels of 16 kg m^{-3}, the accumulation of these metals in the shells of barnacles were not significant. The statistical analysis of the data showed that the concentration of copper, chromium in the shells of barnacles collected from dual treated panels were significantly higher than the concentration in the samples collected from control panels while no significant variation observed for arsenic. The variation in the copper and chromium concentration between the samples from untreated control, paint and FRP were not significant.

The impact of chemical constituents leached out from the chemically treated wood has been a matter of concern for the past few years. Over the extensive use of CCA for the past 70 years CCA has proved out to be a very efficient wood protecting preservative even in the hazardous aquatic conditions. For being a water-soluble preservative, if the fixation of the preservative chemical constituents is not proper these chemicals are likely to enter in to the surrounding medium. As with the wood submerged under the marine condition, these chemicals are most likely to affect the epibiotic fouling organisms living attached to the wood. In the tropical waters, the

major fouling organism attached to the substances immersed in the water was a barnacle.

Rainbow (1995) briefly reviews the potential use of barnacles as the biomonitors for checking the bioavailability of various metal constituents in the marine environment. These are considered to be the ideal crustaceans for bioavailability and bioaccumulation studies since they strongly accumulate the newly absorbed metals into the body burdens of detoxified metals giving an integrated measure of the total available metal for a known period of time (Rainbow, 1995; Rainbow, 1987; Rainbow *et al.*, 2000). The barnacles are found to be excellent monitoring organisms for the metal profiles in a particular area especially in the coastal and estuarine waters. The studies conducted by Anil and Wagh (1988a), Philips and Rainbow (1988), Zauke *et al.*, (1992), Fialkowski and Newman (1998), (1998, 1999), Rainbow *et al.*, (2000), Blackmore (2001, 2004) studied barnacles as possible accumulator for various metals like copper, zinc, cadmium, chromium, iron, manganese, nickel lead etc. Studies conducted in subtropical coastal waters by Paez-Osuna *et al.*, (1999) and Ruelas-Inzunza and Paez-Osuna (2000) showed that copper is accumulated in higher quantities from the surrounding water than chromium.

The fouling barnacles living on treated wood can thereby be used as biomonitors to assess the negative impacts of anti-woodborer formulations. One of the major aspects tackled in such studies were the occurrence, biodiversity of barnacles attached to the preservative treated panels that directly point out the negative impact of these preservative formulations

(Scown & Cookson, 1999; Weis & Weis, 1999; Cookson *et al*., 1996). Some of the studies reported that the chemical constituents leached out from CCA treated wood reduces the species diversity of the epibiotic fouling organisms living on it while some others are of the opinion that the CCA treated wood has little impact on biofoulers (Brown *et al*., 2003). The possibility of potential threat of preservative treated wood in the aquatic environment can also be assessed by studying the bioavailability of the preservatives components to the fouling organisms like barnacles. The study conducted by Weis and Weis (1993), Weis and Weis (1996) have shown that the barnacles take up and accumulate constituent metals *viz.* copper, chromium and arsenic from CCA treated wood. It was reported that copper concentration in the epibiotic organisms *viz.* barnacles (*Balanus eburneus*), oysters (*Crassostrea virginica*), algae (*Enteromorpha intestinalis*) etc. collected from the CCA treated wood is significantly higher than that of chromium and arsenic. The concentration reported in the dried tissue of *Balanus eburneus* in seven years was 83 ppm of copper, 25 ppm of chromium and 20 ppm of arsenic. In the present investigation also a higher concentration of copper was observed when compared to chromium and arsenic.

The studies conducted by Cookson *et al*., (1996) showed that according the characteristics of the wood to be treated and also according to the preservative used, the leaching and accumulation of the metal components in the tissues of barnacles varies. It was also reported in his studies that the double treated panels that are treated initially with CCA and then with

emulsified creosote significantly reduced the accumulation of copper, chromium and arsenic from *E.maculata* but not from *P. elliottii.* The results of two to four years of inspection combined in the studies showed that the CCA treated and double treated panels have higher copper content when compared to that of untreated panels Cookson *et al*., (1996). In the present investigation it can be observed that the copper concentration in the barnacles tissues from the dual preservative treated panels were significantly higher than that from the barnacles of control panels while the chromium and arsenic concentration showed no significant change. According to the studies conducted by Albuquerque and Cragg (1995) the algal mat covering the CCA treated panel had elevated content of copper, chromium and arsenic. He was of the opinion that the higher copper content observed when compared to chromium and arsenic may be due to the increased background levels of copper or since being essential metal it gets a better access than chromium and arsenic.

In the present investigation least accumulation of metals observed in the barnacles collected from FRP sheathed and painted panels shows that the physical barriers used would have reduced the leaching of CCA components through them owing to a less impact on the organisms growing on it. Surface coatings like paint, sealants, plastic encapsulation etc. significantly reduced the dislodgable arsenic from the CCA treated wood (Stillwell, 1998; Lebow, 2002; Lebow *et al*., 2003; CPSC, 2005). The polyurethane, acrylic, oil based and water repellent coatings were found to reduce arsenic leaching for 60 - 100%.

8.4. Conclusion

In general it can be concluded from the present investigation that the metal constituents copper, chromium and arsenic leached out from CCA is getting accumulated in the tissues and shells of barnacles living attached to the treated wood. With increase in the ultimate retention of the preservative in the wood, the accumulation in the tissues and shells were also increasing pointing out the possibility of increased leaching. The samples from dual treated panels showed lower accumulation of metals. The physical barriers like FRP and paint experimented in the study were probably effective in preventing the leaching of the chemical constituents of CCA into the surroundings since the concentration of the concerned metals the tissue and shells of the barnacles collected from these panels not significantly varied from that of control samples.

Table 8.1. Tukey's test results showing statistical significance of copper concentration in barnacle tissues collected from eight different treatment types

(Treatment Types - 1. Control, 2. CCA 16 kg m^{-3}, 3. CCA 29 kg m^{-3}, 4. CCA 42 kg m^{-3} , 5. Dual, 6. Paint, 7. FRP, 8. Marine plywood)

Tukey HSD[a,b]

Treatment Types	N	Subset 1	2	3	4
1	13	5.2069			
7	13	5.7269			
6	13	6.1362	6.1362		
8	13	6.9723	6.9723		
5	13		7.9962		
2	13		8.2992		
3	13			13.5892	
4	13				17.8062
Sig.		.217	.059	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.296.

a. Uses Harmonic Mean Sample Size = 13.000.

b. Alpha = .05.

Table 8.2. Tukey's test results showing statistical significance of chromium concentration in barnacle tissues collected from eight different Treatment types

Tukey HSD[a,b]

Treatment Types	N	Subset 1	2
6	13	4.0700	
7	13	5.1592	
1	13	5.1715	
5	13	6.2500	
2	13	6.8562	
8	13	6.9531	
3	13		10.2792
4	13		11.9708
Sig.		.068	.655

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 6.076.

a. Uses Harmonic Mean Sample Size = 13.000.

b. Alpha = .05.

Table 8.3. Tukey's test results showing statistical significance of arsenic concentration in barnacle tissues collected from eight different treatment types

Tukey HSD[a,b]

Treatment Types	N	Subset 1	Subset 2	Subset 3
1	13	1.8323		
8	13	2.5146	2.5146	
5	13	2.7100	2.7100	
6	13	3.1877	3.1877	
2	13	3.2515	3.2515	
3	13	3.2600	3.2600	
7	13		4.1408	4.1408
4	13			5.3738
Sig.		.243	.118	.428

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 2.260.

a. Uses Harmonic Mean Sample Size = 13.000.

b. Alpha = .05.

Table 8.4. Tukey's test results showing statistical significance of copper concentration in barnacle shells collected from eight different treatment types

Tukey HSD[a,b]

Treatment Types	N	Subset 1	Subset 2	Subset 3
1.00	13	1.3923		
7.00	13	1.8708		
6.00	13	2.5338	2.5338	
8.00	13		3.7069	
2.00	13		4.1162	
5.00	13		4.1608	
3.00	13			5.9192
4.00	13			6.0446
Sig.		.467	.085	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 2.054.

a. Uses Harmonic Mean Sample Size = 13.000.

b. Alpha = .05.

Table. 8.5. Tukey's test results showing statistical significance of chromium concentration in barnacle shells collected from eight different treatment types

Tukey HSD[a,b]

Treatment Types	N	Subset 1	2	3	4
1.00	13	2.1123			
7.00	13	2.4823	2.4823		
6.00	13	3.1554	3.1554		
2.00	13	4.1777	4.1777	4.1777	
5.00	13		4.3569	4.3569	4.3569
3.00	13			5.3223	5.3223
8.00	13			5.5285	5.5285
4.00	13				6.3431
Sig.		.059	.118	.496	.079

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.002.

a. Uses Harmonic Mean Sample Size = 13.000.

b. Alpha = .05.

Table. 8.6. Tukey's test results showing statistical significance of arsenic concentration in barnacle shells collected from eight different treatment types

Tukey HSD[a,b]

Treatment Types	N	Subset 1	2	3	4
1.00	13	1.0862			
6.00	13	2.0431	2.0431		
5.00	13	2.3992	2.3992	2.3992	
2.00	13	2.4346	2.4346	2.4346	2.4346
7.00	13		3.2838	3.2838	3.2838
8.00	13		3.5208	3.5208	3.5208
3.00	13			3.9008	3.9008
4.00	13				3.9585
Sig.		.140	.076	.067	.060

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.641.

a. Uses Harmonic Mean Sample Size = 13.000.

b. Alpha = .05.

Figure 8.1. Mean concentration of copper, chromium and arsenic in tissues of barnacles collected monthly for 18 months from preservative treated estuarine exposed panels (Mean ± SD)

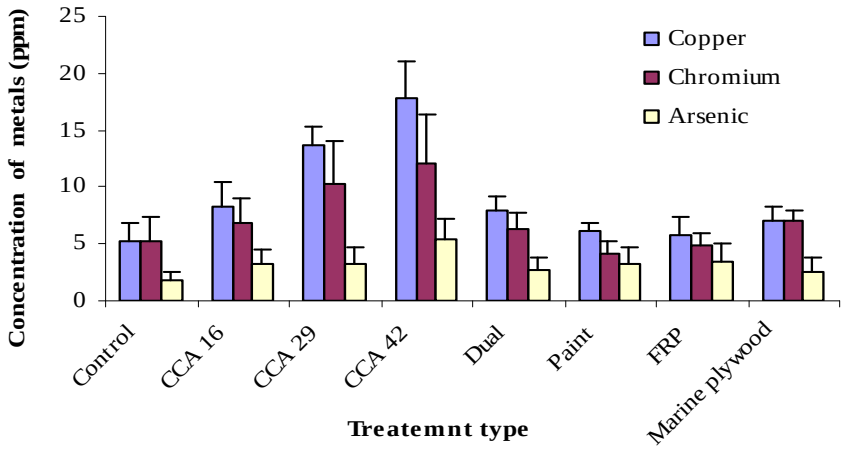
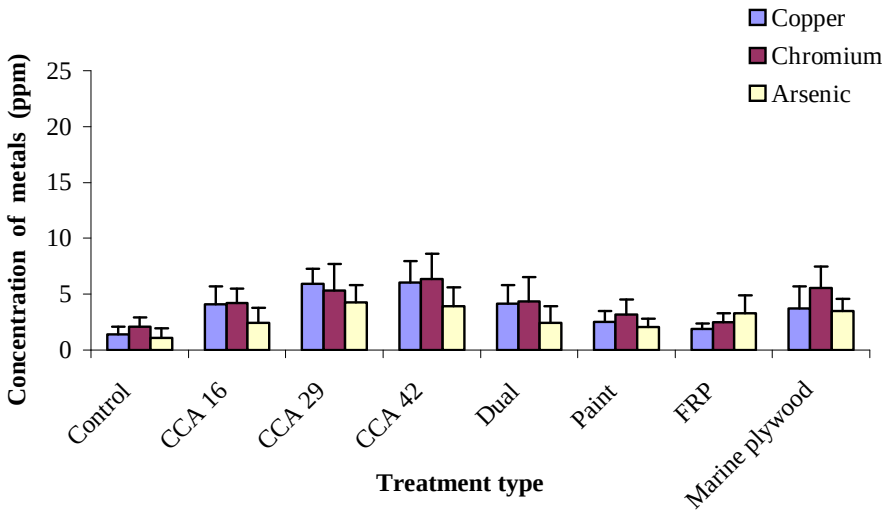


Figure 8.2. Mean concentration of copper, chromium and arsenic in shells of barnacles collected monthly for 18 months from preservative treated estuarine exposed panels (Mean ± SD)



Chapter IX

9. IMPACT OF CHROMATED COPPER ARSENATE ON NON - TARGET ORGANISMS

9.1. Introduction

Any solid substance when exposed under aquatic environment can harbour variety of organisms that are commonly called fouling organisms. Fouling assemblages growing attached to the moving objects like ships and boats poses a hindrance to their movement by decreasing speed, increasing friction thereby decreasing the fuel efficiency. A large variety of antifouling chemical formulations and antifouling paints are being used nowadays to manage with the problem. Even though copper constitutes one of the major ingredient of the antifouling formulations, CCA because of its high efficiency in preventing the attack of termites, was well accepted as a very effective anti wood borer formulation. Studies have been conducted from 1970 onwards in assessing the efficiency of CCA in preventing the wood borers under the aquatic environment especially under marine conditions. The use of cheaper and low quality wood for aquatic applications necessitated the studies on proper selection of preservatives according to the purpose to which it is put to, proper preservative retentions and treatment procedures and the impacts of the preservatives from treated wood on the variety of organisms living in the vicinity.

Contradictory conclusions were put forward by researchers regarding the after effects of use of CCA in aquatic environment. Weis and Weis (1992), Brown and Eaton (2001), Brown *et al.,* (2003) showed that leachate from CCA treated wood cause toxic changes in the organisms living in the immediate vicinity while Albuquerque and Cragg (1995b), Baldwin *et al.,* (1996), Cookson *et al.,* (1996) were of the opinion that CCA treated wood does not pose any threat to the environment. The present study can be considered as a lean-to the impact studies conducted world over regarding the use of CCA preservatives for marine applications. The main objective of the study is to assess the changes in occurrence of biofouling assemblages over CCA treated wood exposed in tropical estuarine condition. The study also assesses the changes in the foulers growing on physically protected CCA panels where the minimization of after effects of CCA leachate in the occurrence of fouling organisms is expected.

9.2. Materials and Methods

The panels under all the seven different treatment category viz. type I to type VII together with untreated rubber wood panels as control were selected for the study. The details regarding the preparation of the rubber wood panels, preservative treatment of the panels is detailed given in Chapter II (Table 2.1.). The details regarding the test site is given in the Chapter II (Fig. 2.1.). The experiment was started in June 2005. Six replicates of each of seven different treatment types along with the untreated rubber wood as control samples were exposed in the estuary for a period of 18 months. Each

set carrying each of these panels were carefully removed from the frames after exposure periods of six, twelve and eighteen months. Fig.9.1. shows the panels before and after exposure in the estuary. Panels were washed in seawater and transported into the laboratory in polythene bags having sufficient seawater. In the laboratory, the percentage coverage of the fouling organisms on the panels was noted. The thickness of the fouling was measured. The panels were kept in agitating water so that the moving organisms were easily separated out. The panels were kept in a solution of 1% formalin so that the wood boring organism *Sphaeroma* spp. can be easily be removed from the holes. The fouling organisms were identified up to the lowest possible taxon under low power microscope. The total number of species and number of organisms in each species was counted. When the settlement became very heavy the number of individuals per 2 cm^2 of panels was recorded. Barnacles and bivalves were weighed along with their shell. The fouling organisms were scraped off from the panels and thoroughly washed to remove the debris over it. The Fig. 9.1a. show the condition of the panels after the foulers are removed from the panels. The wet weight biomass of the fouling organisms on each of the panel was also noted. The changes in the biomass of fouling assemblage were analyzed. The wood boring organisms and fouling organisms on the panels were identified, sorted out into different groups. For giving a more balanced representation of the dominant organisms in the fouling community, the organisms were grouped under five most abundant groups *viz.* hydroids, polychaetes, crustaceans including wood borers, barnacles and

bivalves. The representative samples identified from each group is washed and transferred to 5% formalin for further clarifications in identification.

The statistical acceptability of the data on the changes in the wet weight biomass of foulers with reference to the treatment types and the duration of exposure of the panels in the estuarine condition were assessed using SPSS version 12.0.1. (SPSS Inc.). The Multivariate Analysis (MANOVA) was undertaken to check the statistical significance. The data was processed using the software package PRIMER version 5.2.9. (Plymouth University, U.K.). S (total number of groups or species), N (total number of individuals), d (margalef's index), J' (pielou's evenness index), Brilloin, H' (log2), λ' and N1 were calculated for the data sets. The similarity matrix was generated on the most abundant taxa and biomass on each of the panels during the retrieval after 6, 12 and 18 months. The cluster analysis was also carried out and the dendrogram was generated defining the distance between each of the treatment types with respect to the abundance and biomass of foulers on them. To depict the changes in the total biomass and occurrence of most abundant taxa on each of the panels, the Multi Dimensional Scaling (MDS) was done and illustrated as bubble plot. The bubble plot also overlays the total number of organisms categorized in five most abundant groups that were found associated with each of these panels during the 18 months study. Water samples were collected fortnightly to analyze the influence of hydrographical parameters on the occurrence of fouling organisms. Atmospheric temperature and water temperature, dissolved oxygen, salinity, pH and turbidity were

analyzed for as per standard methods Strickland and Parsons (1965). The details are mentioned in Chapter II.

9.3. Results and Discussion

The present study has indicated that the CCA preservative treatment does not cause any significant changes in the biodiversity of the fouling organisms attached to it while it was observed that the number of barnacles were reduced on CCA treated panels.

9.3.1. Occurrence of wood boring organisms

It is known that the changes in the hydrographical conditions have a pronounced influence on the survival, settlement, growth and breeding habits of biofouling and wood boring organisms. The major wood boring organisms observed during the period of investigation included, crustacean woodborer *Sphaeroma* spp. (Family: Sphaeromidae) and molluscan woodborer *Teredo* spp. (Family: Teredinidae). The Pholads were represented only by *Martesia* spp. (Chapter III, Fig. 3.3.) The panels showed attack of *Sphaeroma* spp. within an exposure period of three weeks in the estuary. Sphaeroma was the only wood-boring organism observed on the periphery of the panels while *Martesia* and *Teredo* spp. resided well inside the panel and could be detected through the X-ray radiographs (Chapter III). In number, *Sphaeroma* spp. predominate all the other species observed on the panels retrieved after six, twelve and eighteen months. In the study, major organisms that deteriorated wood were *Sphaeroma* spp. and *Teredo* spp. (Chapter III, Fig 3.3.). The occurrence of *Martesia* spp. were infrequent and completely absent during the

initial 6 months. The panels retrieved after 12 and 18 months showed the presence of *Martesia* spp. on untreated rubber wood samples.

Erlanson (1936) conducted a preliminary survey on the marine wood boring organisms at Cochin area. The major marine wood boring organisms in the Cochin estuary was constituted by three different species of crustacean wood borers *Sphaeroma* and molluscs comprising *Martesia* spp., *Teredo* spp. and *Bankia* spp. (Cheriyan, 1964). Later on the occurrence, distribution and damage caused by these organisms were confirmed and reported by Santhakumari and Nair (1984), Santhakumaran and Krishnan (1991), Santhakumaran and Rao (1994), Edwin and Pillai (2004). The studies conducted in Goa by Santhakumaran and Krishnan (1991) showed that when compared to the destruction caused by crustacean wood borers, the deterioration due to the attack of *Martesia* spp. is extremely high. Santhakumari and Nair (1984) studied the vertical distribution of the marine wood boring and fouling organisms in the Cochin backwaters. The results of the study indicated the extreme abundance of *Martesia* spp., *Teredo* spp., and Sphaeroma as destroyers of the exposed wooden panels.

In the present study, the major organisms that deteriorated wood were *Sphaeroma* spp., *Teredo* spp. The occurrence of *Martesia* spp. were rare and completely absent during the initial 6 months. The panels retrieved after 12 and 18 months showed the presence of *Martesia* spp. on untreated rubber wood samples. During the present investigation crustacean woodborers was

present on all the panels irrespective of the preservative in use and seasonal changes. It was the only group of organisms observed during the monsoon season when there was a sudden drop in salinity. Observed occurrence of *Sphaeroma* spp. throughout the study period may be due to the fact that it breeds throughout the year and can tolerate wide ranges of salinity and temperature (Pillai, 1961; Cherian, 1964). Despite the fact that *Sphaeroma* spp. is euryhaline, number of organisms was highly reduced during the monsoon season when compared to the pre monsoon and post monsoon periods. The numerical occurrence of crustacean woodborers with changes in salinity during pre monsoon, monsoon and post monsoon period is given in Fig. 9.2. In the present investigation there was a progressive increase in the occurrence of *Teredo* spp. in the panels as exposure duration increased *viz.* as 6, 12 and 18 months. Larval forms of the species are capable of attacking wood without any observable deterioration on the wood surface and could have easily penetrated through the wood with out much obstruction by the fouling assemblage over it (Karande, 1968). Once if inside these organisms can use wood for shelter as well as for food. These organisms burrow deep into the wood as it grows forming long tunnels of 1 m to 1.5 m. However, the organisms collected from FRP sheathed and painted panels showed presence of Sphaeroma spp. the damage due to these organisms was not observed. Teredo spp. and Martesia spp. was completely absent on these panels.

9.3.2. Occurrence of fouling organisms

All the panels were overgrown with various fouling organisms during the entire period of the study. During the initial days of exposure the panels were slimy due to the primary film formation. The pinpoint like shells of barnacles of diameter 1 mm - 2 mm made their first appearance after three weeks of exposure. Nearly all taxa were observed on all the panels. The major macrofouling organisms observed included barnacles, hydroids, mussels, and polychaete worms. Small crabs and fishes like goby were also found in association with the panels. Barnacles were predominant over all the other type of fouling organisms. Empty shells of the barnacles were occupied by wood boring crustaceans like Sphaeroma.

Extensive research has been carried out in Indian waters from the beginning of 20^{th} onwards when fouling organisms attached on to the ships, boat hulls and harbour structures posed hindrance to the sea sailing. The major trust areas of study during that time included biological aspects of fouling with special emphasis to the distributional characteristics, settlement, growth patterns of fouling organisms and the management of the problems posed by these foulers for navigation seeking for a very effective anti-fouling chemical and paint.

Considerable information regarding fouling in Indian waters has been furnished in the works by Nair (1965; 1967), Rao, (1978); Anil and Wagh (1988), Balaji (1988). Fouling in Cochin harbour waters have been discussed by Nair (1967), Nair and Nair (1985), Meenakumari and Nair (1984)

Meenakumari (1989). Serpulids in the Indian harbour waters was detailed by Lakshmana Rao (1969). The water characteristics can (Howard *et al.*, 2004) affect the fouling type and the population density. Much of these details documented are compiled and presented in a book 'Fouling organisms of the Indian Ocean – Biology and Control Technology' by Nagabhushanam and Thompson (1997). The compilation comprises the detailed description of biology, distribution, settlement, growth and reproduction patterns and the significance of major biofouling organisms like barnacles, hydroids, serpulid worms, mussels and oysters (Avelin Mary & Sarojini, 1997; Mane, 1997; Nagabhushanam & Sarojini, 1997). Fig. 9.3. show the major organisms observed in association with the estuarine exposed panels.

The hydrographic conditions prevailing in the study area showed profound influence on the settlement and growth of fouling organisms. Heavy settlement was observed during the pre monsoon and post monsoon seasons while during the monsoon only highly resistant forms survived through the drastic changes in salinity. Owing to the heavy rainfall persisted during the monsoon, about 30% of the barnacles got removed from the panels, leaving behind only the rest of the barnacles and hydroids for analysis. Hydroids were abundant on all the panels in pre monsoon and post monsoon seasons while during the onset of monsoon the hydroid colonies were less in number. Highest numerical abundance of fouling organisms was observed on FRP sheathed panels (Table. 9.1.). The total numerical abundance was lowest in the case of CCA treated panels of 29 kg m^{-3} and 16 kg m^{-3}. CCA treated panels of

42 kg m^{-3} retention showed slightly higher abundance than dual treated and CCA treated panels of other two retentions, however the total biomass of foulers on CCA treated panels of 42 kg m^{-3} retention was the lowest. The average of total numerical abundance of fouling organisms collected from the exposed panels during 6, 12 and 18 months are given Table 9.1. Owing to the lower retention of CCA (4.5 kg m^{-3}) in marine plywood panels, the abundance of fouling organisms on Marine plywood panels was higher than CCA and dual treated panels but less than physically protected panels. As far as the numerical abundance was concerned, the untreated rubber wood panels showed the lowest abundance of barnacles after 18 months of exposure in the estuarine condition (Fig. 9.4). The attachment and growth of barnacles requires substantially enough substrata that were not available since untreated panels failed completely after 12 months of exposure. The pattern of abundance of barnacles on different treatment types was in the order as FRP > paint > marine plywood > CCA 42 kg m^{-3} > CCA 29 kg m^{-3} > dual > CCA 16 kg m^{-3} > control (Fig. 9.4a.). However, in the case of polychaete worms, the pattern was FRP > paint > marine plywood > dual > CCA 16 kg m^{-3} > CCA 29 kg m^{-3}> control > CCA 42 kg m^{-3} (Fig. 9.4b.). The crustaceans other than barnacles were equally abundant on all the panels irrespective of the treatment types (Fig. 9.4c.). The study showed that hydroids prefer untreated rubber wood panels rather than treated ones, while on FRP sheathed, painted and marine plywood panels these organisms was equally abundant as that of untreated control panels (Fig. 9.4d.). The pattern of numerical abundance of

bivalves on the panels was FRP > paint > marine plywood > dual > CCA 16 kg m^{-3} > control > CCA 29 kg m^{-3} > CCA 42 kg m^{-3} (Fig. 9.4e.)

9.3.3. Changes in percent coverage

When compared to the untreated and preservative treated panels intensity of fouling was higher on painted and FRP sheathed panels. During the initial six months of immersion, the untreated panels retrieved showed higher percentage coverage than CCA treated panels. However, after 12 and 18 months the untreated panels were significantly damaged. After 18 months the loss of wood substance was severe and most of the fouling organisms attached were sloughed of from the panel (Chapter III). It was observed a significant reduction in the fouling percentage when compared to CCA and dual preservative treated panels. CCA and dual preservative treatment and coating/sheathing prevented the deterioration of rubber wood reducing the loss of wood substance these by providing enough substrata for the attachment. When compared to CCA treated panels, dual preservative treated panels showed higher percentage of fouling organisms during the entire study period. In the case of marine plywood, during the initial 6 and 12 months of exposure higher coverage of foulers was observed while after 18 months, about 66% of total plywood substance was lost that reduced the coverage of foulers (Table 3.5.). The areas between two panels showed the maximum fouling assemblage when compared to the other sides on the panels. Total percentage coverage of the organisms on the panels exposed for 6, 12 and 18 months are given (Fig. 9.4.).

9.3.4. Changes in the total biomass

Total biomass sample taken in to consideration for the present investigation included hydroids, polychaete and serpulid worms, barnacles with their shells, crustaceans other than barnacles, bivalves etc. that were removed from each panel. Overall, results of 18-month study showed higher abundance of organisms on FRP sheathed and painted panels when compared to the untreated control panels, while the lowest incidence was observed in CCA treated panels to 42 kg m^{-3} retention. The differences in biomass of foulers on the panels were analysed statistically ($p<0.05$). Multivariate analysis indicated significant changes between eight different treatment types during after 6, 12 and 18 months of exposure ($p<0.05$) (Fig. 9.5.). The biomass levels were high on the panels collected after 12 months of exposure that accounted fouling organisms that settled and grew during the pre monsoon season.

The richness of key taxonomic groups of fouling organisms represented as Margalef's index (d) remained between 0.611 - 0.651. The richness was lower on FRP sheathed and painted panels. This may be due to the higher abundance barnacles when compared to other treatment types and because of the absence crustaceans of family *Sphaeromidae* that does not bore wood. The similarity matrix generated with the biomass values showed that the similarity between all the possible pairs of treatments ranged between

74.79 – 98.68%. The total fouling biomass aggregated on the FRP sheathed and painted panels showed maximum similarity (98.68%) between themselves (Fig. 9.6.). While the lowest similarity was observed between CCA treated panels of 42 kg m^{-3} retention and painted panels. It was also noted that there existed an average of 85% similarity between the untreated control panels and CCA treated ones. The total biomass on each of the panels retrieved after 6, 12 and 18 months were overlaid in MDS and represented as bubble plots. Size of bubbles indicates the total biomass on each of the treatment type. For 12 months of exposure, there was no considerable change in the total biomass on the untreated rubber wood panels, while after 18 months the relative biomass on the panel reduced considerably. Owing to the severe attack of woodborers, structural integrity was lost and untreated rubber wood panels retrieved after 12 and 18 months were fragile. The lack of enough substrata for the attachment of biofoulers resulted in lowest abundance of biofouling organisms. There was no significant change in the biomass on CCA treated panels of 16 kg m^{-3} and 29 kg m^{-3} after 18 months of exposure in the estuarine condition. While CCA treated panels to higher retention of 42 kg m^{-3} showed gradual reduction in total biomass after 12 and 18 months may be due to the toxic effect of components leached out from the panels. The study showed that marine plywood panels having approximately 4 kg m^{-3} retention of CCA in it, have much more biomass on it than the other treated panels having 16, 29 and 42 kg m^{-3} CCA retained in them (Fig. 9.6.). After 18 months of exposure, total biomass on marine plywood panel was considerably reduced that can be

attributed to the loss of enough substrata for the attachment of biofoulers, as after 18 months the marine plywood panels failed (Fig. 9.6.). The cumulative dominance of most abundant groups of foulers on the panels exposed for 6, 12 and 18 months are given (Fig. 9.7.). It was observed that changes in the cumulative dominance of five major groups of fouling organisms *viz.* hydroids, polychaete worms, crustaceans including wood borers, barnacles and bivalves in were minimal.

In the present investigation it was observed that the numerical abundance of all the five key taxonomic groups of fouling organisms is high on FRP sheathed and painted panels. This likely be due to the fact that the FRP sheathing or paint coating over CCA treated wood possibly reduces leaching of CCA components into the surroundings and minimizing the toxic effects on the surface. Another possible explanation is the changes in surface properties of wood with FRP sheathing and paint coating. In the present investigation it was also observed that barnacles shows higher abundance on CCA treated panels than untreated ones. Some studies conducted earlier have reported higher numerical abundance of some species of epibiotic organisms on CCA treated panels that agree with the present results. Certain species of barnacle *Elminius modestus*, bryozoan *Electra pilosa* and serpulid worms like *hydroides ezoensis, Ficopomatus enigmaticus* showed higher abundance on CCA treated panels when compared to untreated ones (Brown & Eaton, 2001; Brown *et al*., 2001a). The study also reported a higher abundance of barnacles and serpulid worms as CCA retention increased. Higher abundance of fouling

organisms on CCA treated panels when compared to the untreated Scot pine control panels was explained as the lower larval recruitment of the organisms during the early stage of colonization or primary film formation (Brown & Eaton, 2001). According to Albuquerque and Cragg (1995b), Brown *et al*., (2001b) barnacle larvae prefer to settle on dark coloured substratum. Dark green colour of CCA treated panels when compared to the creamish yellow colour of untreated rubber wood may be another factor that attributed a higher larval recruitment on CCA treated panels. In the present study, species abundance data was not taken into consideration. However, the barnacles showed highest numerical abundance on the CCA treated panels and as the retention of CCA decreased the numerical abundance also decreased. It was reported that the texture, colour and other physical and chemical characteristics of the substrata greatly influence the primary film formation and the subsequent adherence, settling and abundance of the biofoulers (Taki *et al*., 1980; Mitchel & Kirchman, 1984; Wethey, 1986; Richmond & Seed, 1991; Thomson & Davenport, 1995). The study conducted by Brown *et al*., 2001 in order to a assess the impact of CCA treatment in the formation of early fouling community showed that after four weeks of exposure there was no significant change in the density of algal fouling communities, barnacles and serpulid worms but certain species of organisms also showed lower abundance. According to the studies conducted by Brown *et al.,* (2003) by exposing CCA treated panels with different loadings at seven European sites showed that there is no significant difference in the structure of fouling

assemblages between the different loadings exposed at most of the selected sites.

Along the east coast of Indian waters Tarakanadha *et al.*, (2002) studied the impacts of preservative treated panels by comparing the occurrence of biofouling organisms. Semul samples treated to a CCA retention of 32.4 kg m^{-3} was used for the study that reported a heavy settlement of barnacle, bryozoans and algal species was indicating less impact of CCA on the biofoulers when compared to the untreated control samples. As seen by the results of present investigation the percent coverage and the richness of fouling organisms was least affected by preservative treatment while total biomass of the fouling organisms settled on the panels were significantly reduced. According to the studies conducted by Weis and Weis (1996) the total biomass of the samples collected from CCA treated boards were showed 50% reduction and species richness and diversity of the organisms on CCA treated panels were significantly reduced when compared to untreated control samples.

9.4. Conclusion

The intensity of fouling was higher on painted and FRP sheathed panels when compared untreated and preservative treated panels. After 18 months of exposure, significant reduction in the percent coverage of fouling organisms on untreated rubber can be attributed to the significant loss of wood substance and subsequent sloughing off of fouling organisms from the panel. Coating/sheathing prevented the deterioration and the loss of wood substance

thereby providing enough substrata for the attachment. Statistically significant reduction in percent coverage of foulers was observed only for CCA. It was also proved that the CCA treatment does not pose any change in the total biomass of fouling organisms living on the panels.

Table 9.1. Primer analysis results

Treatment Types	S	N	d	J'	Brillouin	H'(log2)	Lambda'	N1
Type I	5	472.278	0.650	0.950	1.514	2.207	0.224	4.616
Type II	5	467.278	0.651	0.944	1.485	2.191	0.231	4.567
Type III	5	467.222	0.651	0.929	1.480	2.158	0.241	4.461
Type IV	5	477.111	0.649	0.906	1.435	2.104	0.258	4.298
Type V	5	489.667	0.646	0.949	1.503	2.202	0.228	4.602
Type VI	5	663.167	0.616	0.907	1.441	2.105	0.259	4.302
Type VII	5	693.222	0.611	0.917	1.452	2.130	0.254	4.378
Type VIII	5	584.333	0.628	0.923	1.457	2.142	0.246	4.415

Table 9.2. Average % similarity in total biomass between treatment types

Treatment types	Type I	Type II	Type III	Type IV	Type V	Type VI	Type VII	Type VIII
Type I	0	0	0	0	0	0	0	0
Type II	97.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Type III	96.04	98.50	0.00	0.00	0.00	0.00	0.00	0.00
Type IV	94.20	96.65	98.14	0.00	0.00	0.00	0.00	0.00
Type V	97.15	98.71	97.65	95.83	0.00	0.00	0.00	0.00
Type VI	92.12	92.03	91.72	91.76	93.29	0.00	0.00	0.00
Type VII	90.94	90.64	90.33	90.37	91.92	98.60	0.00	0.00
Type VIII	94.62	94.86	94.54	94.59	95.82	97.16	95.76	0.00

Figure 9.1. The Panels before and after immersion in the Cochin estuarine waters for 18 months

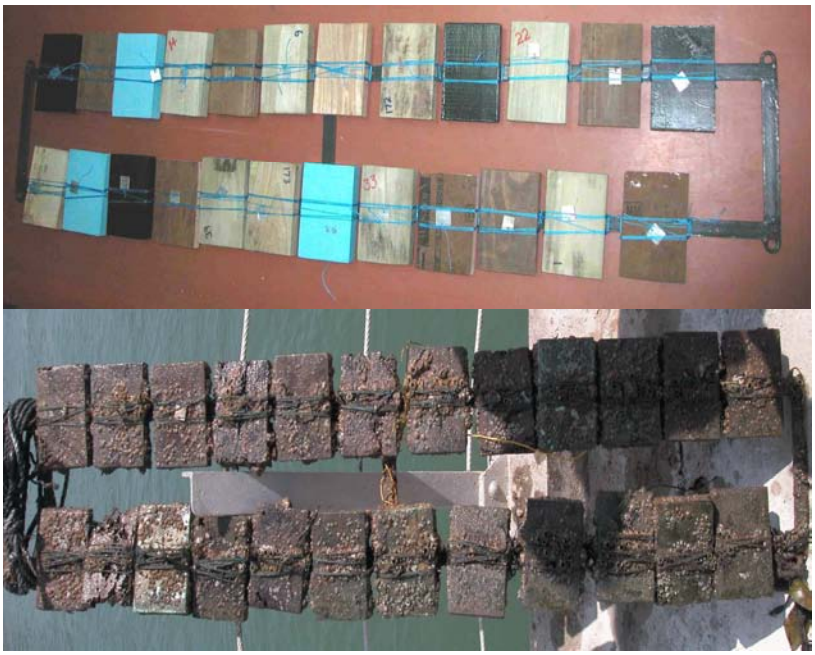


Figure 9.1. a) Panels after immersion in the Cochin estuarine waters for 18 months b) a close view of foulers on the panels

Figure 9.2. Numerical occurrence of *Sphaeroma* spp. on the preservative treated panels exposed in the Cochin estuary at Pre monsoon (Jan - May), Monsoon (June – Sep) and Post monsoon (Oct - Jan) periods – average salinity respectively 8.5 ‰, 1.45 ‰, 5.8 ‰.

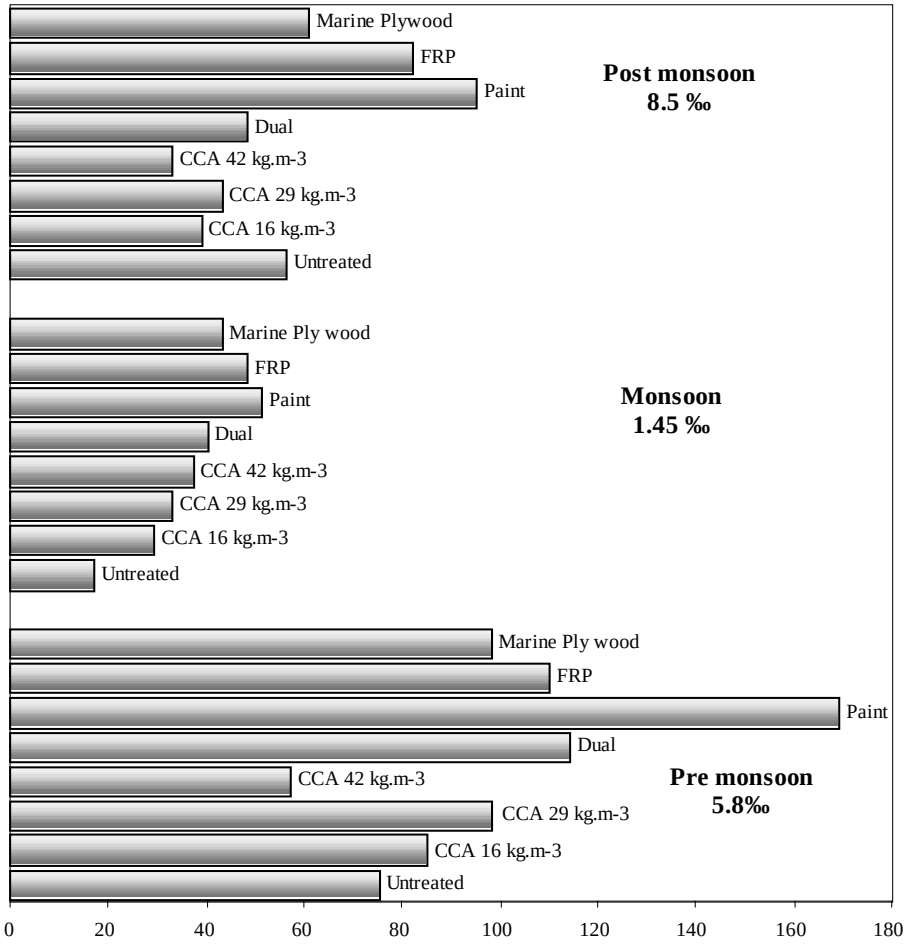
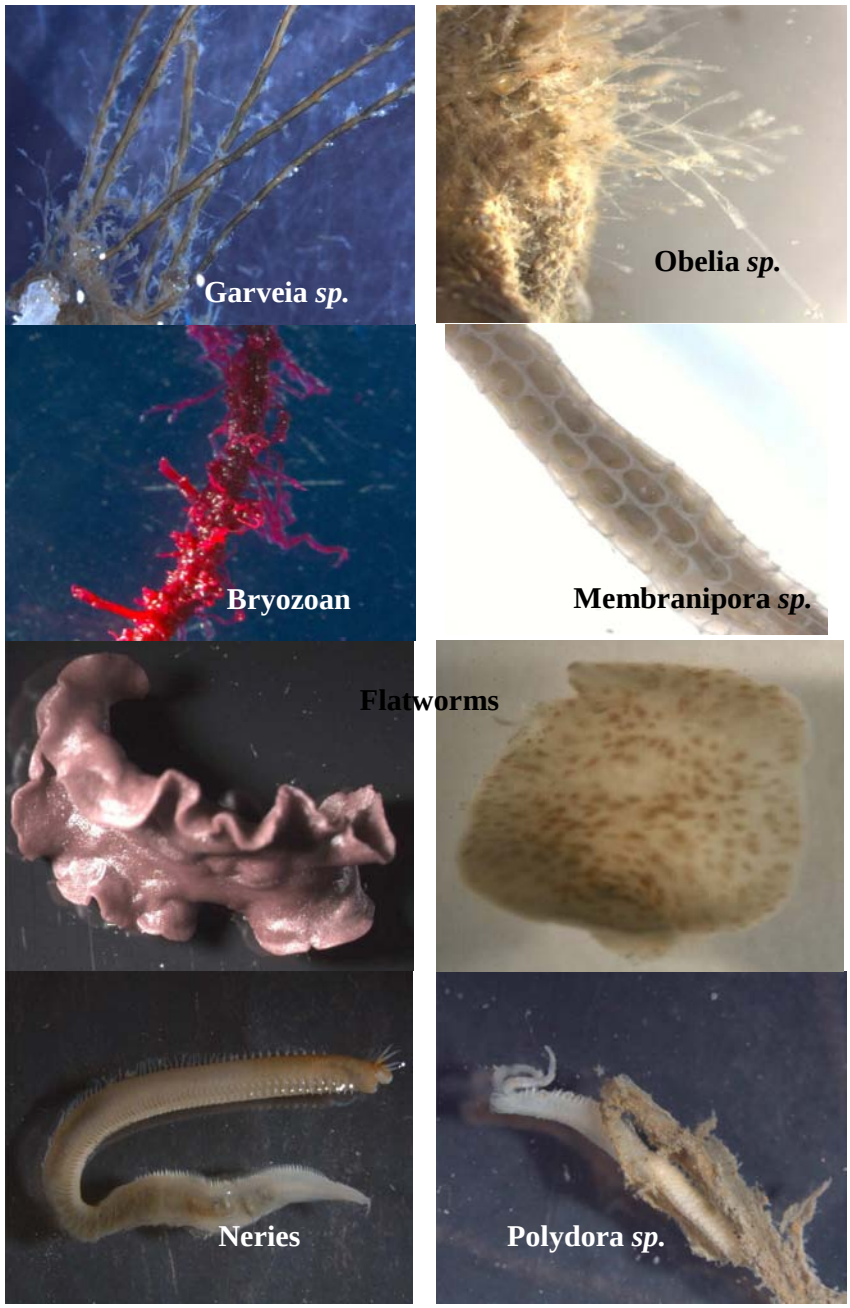


Figure 9. 3. Major biofouling organisms observed on the panels




Crab


Barnacle


Mussels


Fish

Figure 9.4. Bubble plot depicting the numerical abundance of major taxonomic groups of fouling organisms (a) barnacles b) polychaetes c) crustaceans d) hydroids e) bivalves.

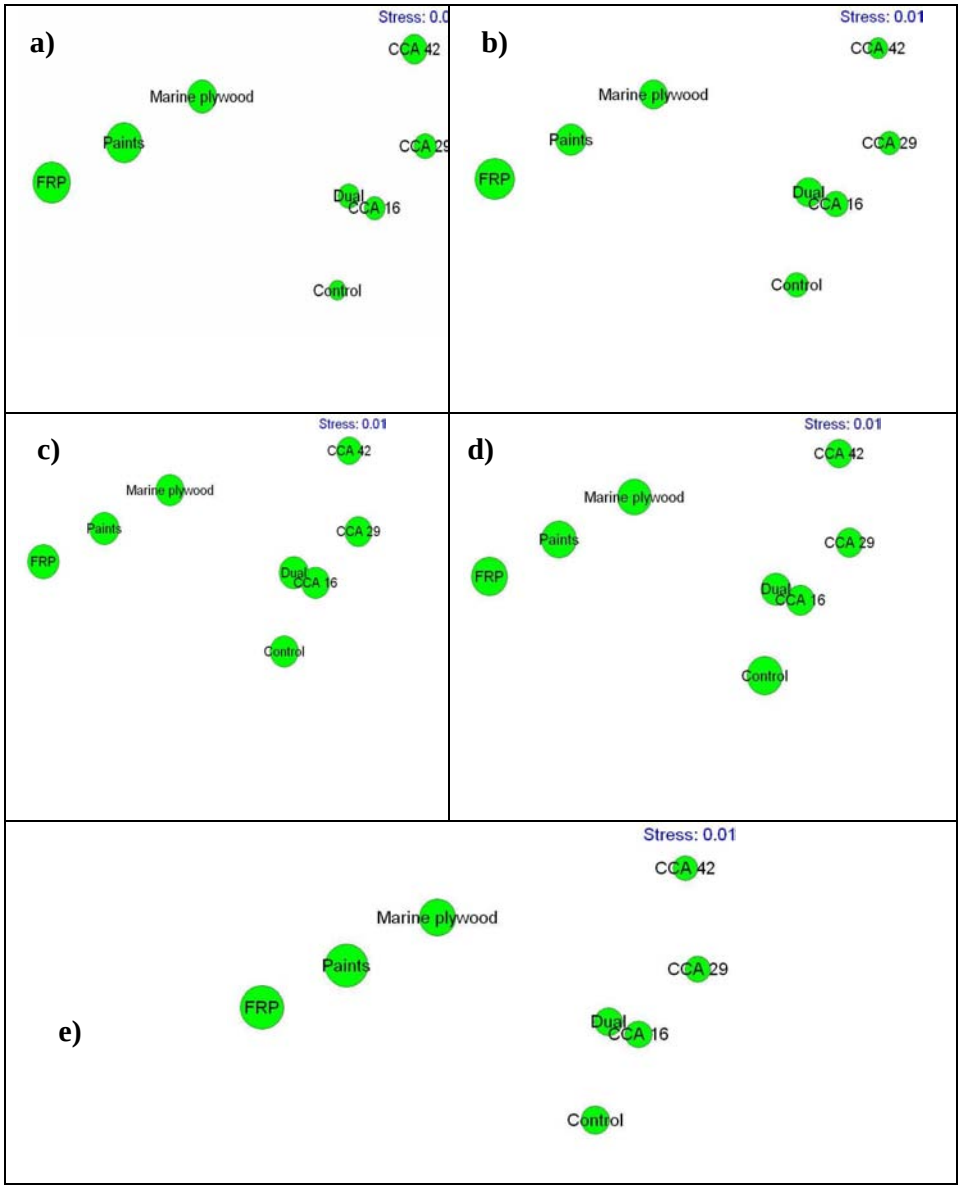


Figure 9. 4. Percentage coverage of fouling organisms on exposed panels

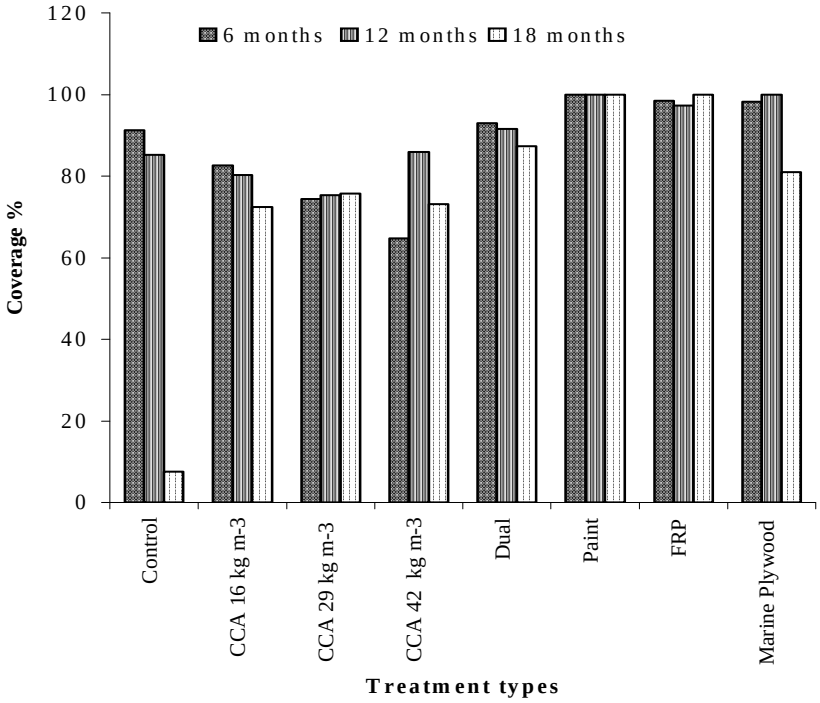


Figure 9.5. Wet weight biomass of epibiotic organisms collected from different type of panels exposed for 6, 12 and 18 months (Mean ± Std. Error)

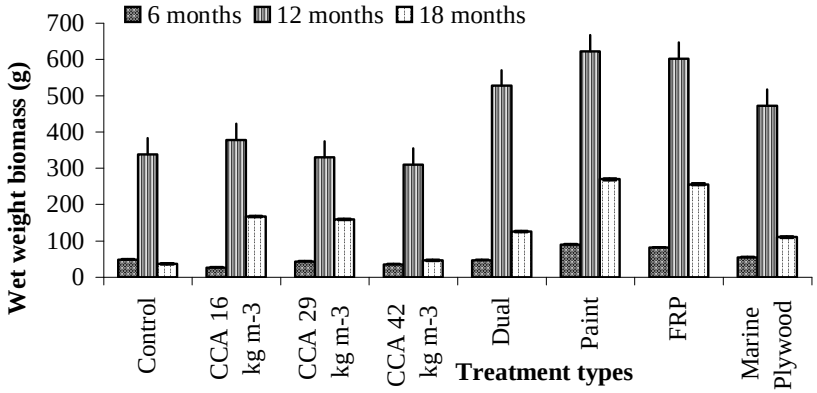


Figure 9. 6. Bubble plot depicting the biomass of fouling organisms on the exposed panels for a) 6 months, b) 12 months, c) 18 months d) dendrogram showing the average similarity between the treatment types.

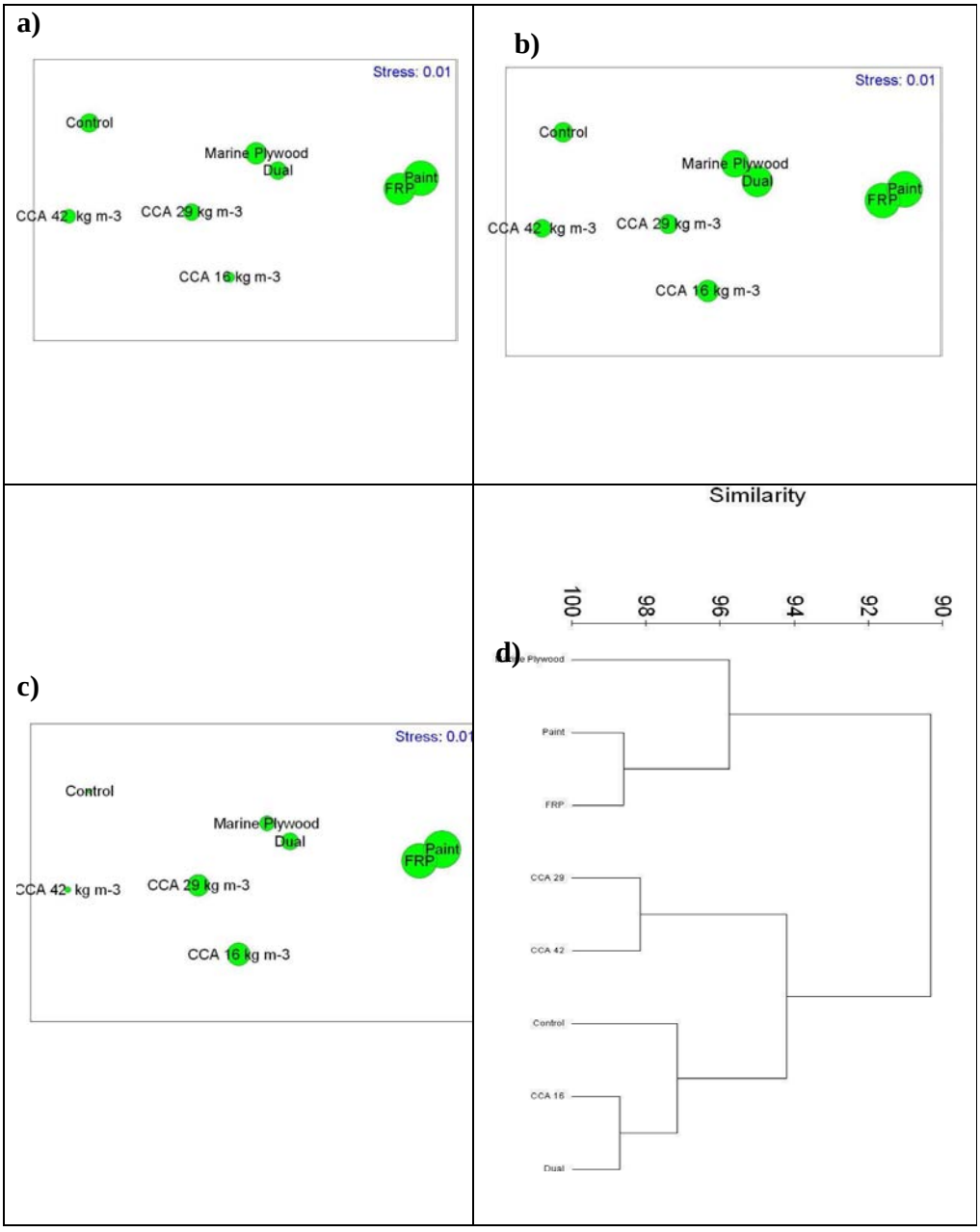


Figure 9.7. Dominance plot depicting the dominance of five key abundant groups *viz.* hydroids, polychaetes, crustaceans including wood borers, barnacles and bivalves after 6, 12 and 18 months of exposure

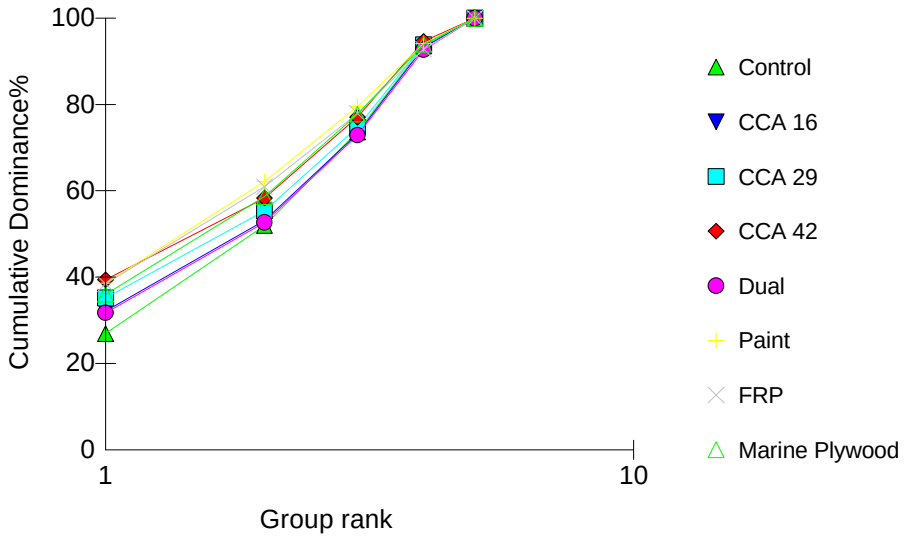
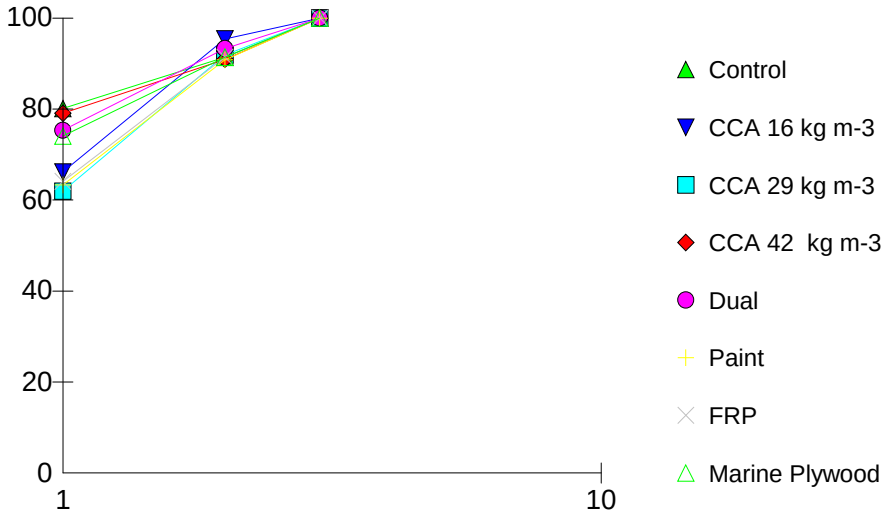


Figure 9.8. Dominance plot depicting the abundance of organisms during 6, 12 and 18 months



Chapter X

10. SUMMARY

Artisanal fisherman in India preferred highly durable varieties of wood like teak, aini, semul etc for marine construction purposes especially for boat building. However, when used in the aquatic conditions especially under marine and estuarine environment, the service life of even highly durable varieties of wood species is considerably affected by the severe attack of wood borers and other biodeteriorating agents like marine bacteria and fungi. In India, current use of wood is estimated to be 39.5 million m^3, out of which nearly 22.5 million m^3 is used in adverse conditions requiring protection against biodeterioration. Rubber wood (*Hevea brasiliensis*) emerges as an alternative when a shortage of durable varieties to replace exists. Despite the fact that rubber wood is versatile in its use and its mechanical properties are comparable with that of durable varieties of wood, rubber wood is yet not recognized among the fisheries community as a boat building material. This is because rubber wood is highly susceptible to biodeterioration. However, the treatability to different chemical preservatives possibly projects rubber wood as an alternative to durable varieties. The present investigation analyse the possibilities of making a less durable wood like rubber wood. The study is the first one in this respect where the extent of protection provided to rubber wood by Chromated Copper Arsenate (CCA) preservative when it is loaded with three different retention levels are investigated. The study also focuses on the possible alternative preservative treatment methods that can provide a better

chance to prevent deterioration of rubber wood rather than when treated alone with CCA. These alternative methods studied includes, rubber wood treated with comparatively lower retention of CCA is pressure treated with Creosote (dual treatment), the CCA treated panels coated with epoxy paint, CCA treated panels sheathed with Fibreglass Reinforced Plastic (FRP). In the context of the possibility of release of metal constituents *viz.* copper, chromium and arsenic from CCA treated panels the study also focuses on the impacts of CCA on aquatic organisms and also the effectiveness of paint and FRP sheathing in minimizing the impacts of CCA on the biofoulers. The check out for commercial feasibility of the study, CCA treated marine grade plywood available in the market that forms a major boat building material was also tested for its biodereiorative aspects.

Materials selected for the study included the rubber wood panels treated with preservatives like CCA and creosote; CCA treated panels of lower retention coated with physical barriers like paint and FRP. Marine grade plywood panels available in the market are also used for the study. Laboratory studies were conducted in order to assess the impacts of preservative treatments on aquatic organisms *viz. Villorita cyprinoides*, *Tilapia mossambicus* and the data available is furnished so as to support the findings in the field trials using barnacles and other biofouling organisms living on the preservative treated panels. The statistical analysis using SPSS version 10 Windows was also conducted on the available data so as the check out for the scientific acceptability of the results provided. The changes in the biodiversity

of the fouling organisms growing on estuarine exposed panels were studied using software programme PRIMER.

The nature and extent of biodeterioration of untreated, CCA and dual preservative treated rubber wood panels exposed in Cochin estuary for 18 months was studied. The major tools of assessment include the visual observation of the exposed panels and the X-ray radiographs. The chapter also explains the performance of commercially available CCA treated marine grade plywood in comparison with other treatment types. The performance CCA treated panels protected with physical barriers such as paint and FRP is also studied. The results showed that biodeterioration of the rubber wood under the marine tropical condition can be prevented by using CCA retention of about 29 kg m^{-3}. The physical barriers that were applied on CCA treated rubber wood panels accorded a 100 % protection against woodborers.

The visually observed deteriorative changes are explained through microstructural changes in Chapter IV and later on confirmed through physical, chemical and mechanical indices in Chapter V. Microstructural analysis of treated wood show that the preservative solution enters the cell lumen during pressure impregnation. It was also observed that during biodeterioration the integrity of xylem vessels and parenchyma cells are lost and fibre cells were damaged.

Chapter V further elaborates on the results furnished in Chapters III and IV. In the Chapter, the changes in specific gravity, compression strength parallel to grain and the changes in the FTIR spectral bands corresponding to

hemi-cellulose, cellulose and lignin is discussed with reference to the preservative treatment and biodeterioration. The present study confirm that higher retentions of CCA *viz.* 29 kg m^{-3} and 42 kg m^{-3} protects the wood effectively as there is minimum loss of wood substance, reduction in specific gravity and corresponding decrease in compressive strength when compared to the untreated rubber wood panels and those treated with lower retentions of CCA. So, further studies were conducted to assess the toxicity of CCA to aquatic organisms in order to find out a preservative treatment method that can provide enough protection at the same time least impact on the surroundings.

In the view of the known hazards and possible deleterious effects of copper, chromium and arsenic, an attempt was made to study the acute toxicity of CCA and its constituent metals copper, chromium and arsenic separately and their bioconcentration in black clam. The study showed that of the CCA components copper is found to be more toxic which can be acting along with arsenic and chromium to impart an added effect on its toxicity.

In continuation of the acute toxicity studies conducted in the previous Chapter where all the three metals copper, chromium and arsenic of CCA have a tendency to get concentrated in the tissues of invertebrates like clams when added as concentrated solutions, an attempt was made to study the bioconcentration of copper, chromium and arsenic leached out from CCA treated panels.

Chapter VIII details with the field trails conducted to analyse the accumulation of copper, chromium and arsenic in barnacles growing on

estuarine exposed panels. It was observed that barnacles take up metals from CCA treated wood in low levels that are non toxic/lethal to the organisms. But with increase in the ultimate retention of CCA in the wood, the accumulation in the tissues and shells also increase. The results suggest that 29 kg m^{-3} can be regarded as an optimum concentration with maximum protection and with minimum impacts on the environment.

Chapter IX explains the changes in the biodiversity of fouling organisms growing on estuarine exposed panels. Overall, results of 18-month study showed that percent coverage and richness of fouling organisms was least affected by preservative treatment while total biomass of the fouling organisms settled on the panels were significantly reduced especially in the case of CCA treated panels to 42 kg m^{-3} retention while relatively high abundance on FRP sheathed and pained panels

Chapter X

11. RECOMMENDATIONS

- Biodeterioration of the rubber wood under the estuarine tropical condition can be prevented by using CCA retention of about 29 kg m^{-3}
- Dual preservative treatment on rubber wood with an ultimate preservative retention of 150 kg m^{-3} found to be efficient in preventing biodeterioration
- Physical barriers like FRP and paint applied over CCA treated panels of 16 kg m^{-3} retention provide 100% protection against biodeterioration where CCA treatment (16 kg m^{-3} retention) alone failed against wood borers.
- CCA can be impregnated into the wood cells applying pressure upto 517.11k Pa without affecting the compression parallel to grain strength of rubber wood.
- Microstructural analysis, weight loss, changes in specific gravity, changes in compression parallel to strength, changes in the FTIR spectral bands can be effectively used to assess the extent of biodeterioration
- The epibiotic non-target organisms (barnacles) and organisms living in the vicinity (fishes) take up metals from CCA treated wood in low levels that are non toxic/lethal to the organisms
- Even though, percent coverage and the richness of fouling organisms was least affected by CCA and dual preservative treatment, total

biomass of the fouling organisms settled on the panels were significantly reduced recommending the use of lower retention of CCA under estuarine condition without disturbing the fouling community.

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