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Rate of Micro fouling on panel assembled by hardwood of commercially important four different Sps.

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Abstract: Biofouling particularly micro-fouling is a very complex phenomenon to understand. This present work is carried out to understand the influence of wood type on the development of microfoulers. The panels were constructed by using commercially important hard wood such as *Tectona grandis, Pterocarpus Sp, Thespesia populnea* and *Mangifera indica*. The assembled panels were immersed and studied for the period of 21 days in velar estuary, Porto-Novo. The result shows that the settlement of microfoulers are high on the panel constructed by *Mangifera indica* (89 X 10^{-5} CFU/cm^2) and low on the panel constructed by *Pterocarpus Sp* (71 X 10^{-5} CFU/cm^2). Thus, the selection of materials for the marine structures must be heedful and well established to prevent the loss due to the fouling process.

Keywords: Biofouling, microfoulers, panels, wood, *Tectona grandis, Pterocarpus Sp, Thespesia populnea* and *Mangifera indica* etc.

INTRODUCTION

Marine fouling is an undesirable accumulation of biotic and abiotic depositions on a submerged engineered surface in sea water [1]. Deposits of abiotic materials such as organic and inorganic substances called molecular fouling and deposits of biotic molecules such as micro and macro organisms known as bio fouling [2]. The fouling process occurred by three phases i.e. (a) Molecular, (b) Microfouling and (c) Macrofouling. The first phase-molecular fouling or abiotic fouling is the accretion of organic and inorganic molecules from solution onto immersed structures [3]. The structures may have several characteristics that are important in the addition process [4, 5]. The establishment of microbial colonization raise as the surface irregularity develops [6]. Adhesion of abiotic materials alters the property of any substratum making all surface becomes wet [7, 8]. Many researchers reported that microorganisms fasten more rapidly to hydrophobic, non polar surface such as teflon and other plastic than to hydrophilic materials such as glass or metals [9, 10, 11]. Molecular fouling or abiotic fouling is hasty, reversible and is synchronized by the laws of mass action and the chemistry of molecular bonding relations [12]. The second stage- microfouling is the establishment of microorganisms such as bacteria, fungi, algae, etc on the immersed structures [5]. Adsorptions of abiotic molecules, the microorganisms started colonizing on the surface of substratum [13]. Microfouling is a dynamic process and also reversible [14]. Substrate appears to influence microfouling at all stages of development (15]. In any aquatic environments, microbial cells attached to the submerged substratum, including metals, immobilized cells grow, reproduce and produce extracellular polymers forming a biofilm [16]. Microbial colonization of a solid-liquid interface may occur in sequence. First in transport of cells to a surface by three different modes i.e., by diffusive transport, connective transport of cells and by active movement. The next occurrence is initial adhesion. After the bacterium deposited special structures fibrils form strong link between cell and solid surface [17, 18]. After attachment, microorganisms initiate production of slimy adhesive substances termed extracellular polymeric substances (EPs) which assist in the formation of micro colonies and microbial films [19]. Last in the sequence is surface colonization. Followed by micro fouling the macro organisms such as plants and animals started colonizing on the immersed surfaces [20]. This is highly irreversible process [21]. These macrofouling secretes a proteinaceous adhesive [22]. Macrofouling further divided into soft fouling (algae, ascidians, bryozoans etc.) and hard fouling such as calcium corbonate secreters of barnacle and oysters [23]. These macrofoulers react to physical and chemical natures of the substratum [24, 25, 26 27, 28, 29, 30]. The mussel forms many threads by secreting an adhesive protein from the foot and attaches with more than 50 byssal threads which makes most mussels clump together. But

in benthic diatoms, it attach by mucus secretion [31, 32]. After the major settlement steps the fouling community evolves continuously by mechanisms such as disturbances, facilitation inhibition, tolerance etc. [33, 34].

Effects of biofouling

Marine fouling phenomenon causes considerable economic losses in various industries throughout the world. These fouling organisms cause immense mechanical troubles through attaching on hulls of ship, power plants, cooling structures, aquaculture, fishing nets, pipelines and other marine infrastructures [35]. Once if the ship hull is fouled it increases drag and surface corrosion, followed to reduced velocity and thereby causing higher fuel utilization [36]. This in turn has economic and environmental issues, as increased fuel consumption leads to increased output of green house gases [37] and maintenance cost [38]. Parts of a ship other than the hull are also affected due to biofouling. The other structures with frequent contact in sea water such as heat exchangers, water cooling pipes, propellers etc. are also affected with biofouling [39]. Build up of matter inside cooling system pipes extends decreased performance due to biofouling [38]. A third important category of maritime industry plagued by biofouling problems are coastal power stations [40]. They draw vast quantities of seawater for cooling purposes [40]. Sessile organisms colonizing in cooling system components can reduce water flow and block condenser tubes affecting power generation and under certain circumstances may even cause safety problems [41].

There are very few works about the accumulation pattern of marine fouling bacteria on different woods. Thus, this present work aims to observe the bacteria fouled on different panels immersed in the Vellar estuary. With the objectives to study quantitatively the bacteria fouled on four different types of wooden panels such as *T. grandis*, *P. indicus*, *M. indica*, and *T. populnea* , which immersed in the velar estuary.

MATERIALS AND METHODS

Study area

A study on the rate of biofilm formation of different types of wooden panels was carried out at Vellar estuary during January/February, 2015. The study area of Vellar estuary is located along Lat. 11°29'N and Long. 79°46'E near Parangipettai, Cuddalore District, Tamil Nadu. It has permanent connections with Bay of Bengal and near its mouth it gets connected with a complex system of backwaters extending southwards to Coleroon estuary, about 15 km away.

Assembly and Deployment of different test panels

Test panels consisting of different wood

Triplicates of 10 x 20 x 1.5 cm of the pieces of *T.grandis*, *M. indica* , *T. populnea* and *P.indicus* woods were collected from timber depot. They were fastened with nylon rope of about 10 m and used them as a test panel.

Deployment of panels

Before using, panels consists of different woods were sterilized with 10% HC1, washed with water and dried in an oven and kept in a dry place until being used. The primed panels were immersed about 1 meter depth in surface waters (~l m) of the Vellar Estuary at low tide mark.

Collection and estimation of fouling bacteria

The samples were periodically (three days once) scraped randomly 1cm^2 area and cultured [42, 43]. The samples were serially diluted and cultured using Zobell marine agar medium by pour plate method [44]. The plates were incubated at (28°C ± 2°C) for 24 hours in the incubator. After 24 hrs the CFU were counted.

STATISTICAL METHODS

Duncan's Multiple Range Test was performed using spss16 packages for all the data to know the significance.

RESULTS

Rate of bacteria fouled on different wood

Panels constructed using 1. *T.grandis* 2. *P.indicus* 3. *M. indica* and 4. *T. populnea* influenced significantly on the rate of fouling of bacteria on the panels (Table 1 and 2).The variation on total viable count of biofilm bacteria during 3,6,9,12,15,18 and 21days exposed in the Vellar estuary in Parangipettai is given in the tables 1,2,3,4,5,6 and 7. The scraped materials were serially diluted and CFU were calculated. Though the total viable count of CFU assessed by different dilutions (10^{-1} to 10^{-6}) the 10^{-5} dilution only considered for the statistical interpretation. The determined CFU of 3, 6, 9,12,15,18 and 21 days are on the *T.grandis* wood (20, 25, 33, 40, 46, 64, and 84 X 10^{-5} CFU/cm^2), *P.indicus* wood (19, 22, 29, 35, 49, 58 and 71 X 10^{-5} CFU/cm^2) *M. indica* wood (24, 28, 45, 53, 71, 76 and 89 X 10^{-5} CFU/cm^2) *T. populnea* wood (22, 25, 38, 40, 56, 59 and 80 X 10^{-5} CFU/cm^2) respectively.

These observations clearly show that the rate of fouling is directly proportional to the time of exposures. If the duration of exposure increases the rate of fouling also increased.The type of wood shows significant variation on the rate of fouling of bacteria, the *M. indica* wood showed the higher accumulation of bacteria i.e. 89 X 10^{-5} CFU/cm^2 and the *P.indicus* wood showed lower accumulation i.e.71X 10^{-5} CFU/cm^2 on the 21st day of the study.

Table-1: Bacterial densities on the wooden panel exposed to estuary of study area during different time intervals in different woods.

S.NO	Wood type	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
1.	*T.grandis*	20.00±0.70	26.00±0.50	40.00±0.45	46.00±0.50	60.00±0.50	64.00±0.50	84.00±0.50
2.	*P.indicus*	19.00±0.50	22.00±0.45	29.00±0.40	35.00±0.46	49.00±0.40	58.00±0.46	80.00±0.40
3.	*M. indica*	24.00±0.50	28.00±0.45	45.00±0.50	53.00±0.50	71.00±0.45	76.00±0.50	89.00±0.45
4.	*T.populnea*	22.00±0.50	25.00±0.50	38.00±0.50	40.00±0.48	56.00±0.50	59.00±0.48	71.00±0.50

Table-2: Statistical observations for bacterial densities on the wooden panel exposed to estuary of study area during different time intervals in different woods.

S.NO	ANOVA		
	Period	F	Sig.
1.	Day 3	47.581	.000
2.	Day 6	75.000	.000
3.	Day 9	536.000	.000
4.	Day12	696.000	.000
5.	Day 15	724.000	.000
6.	Day 18	819.000	.000
7.	Day 21	1001.000	.000
S.NO	ANOVA		
	Wood	F	Sig.
1.	*T.grandis*	3.987E3	.000
2.	*P.indicus*	5.791E3	.000
3.	*M. indica*	7.294E3	.000
4.	*T. populnea*	3.987E3	.000

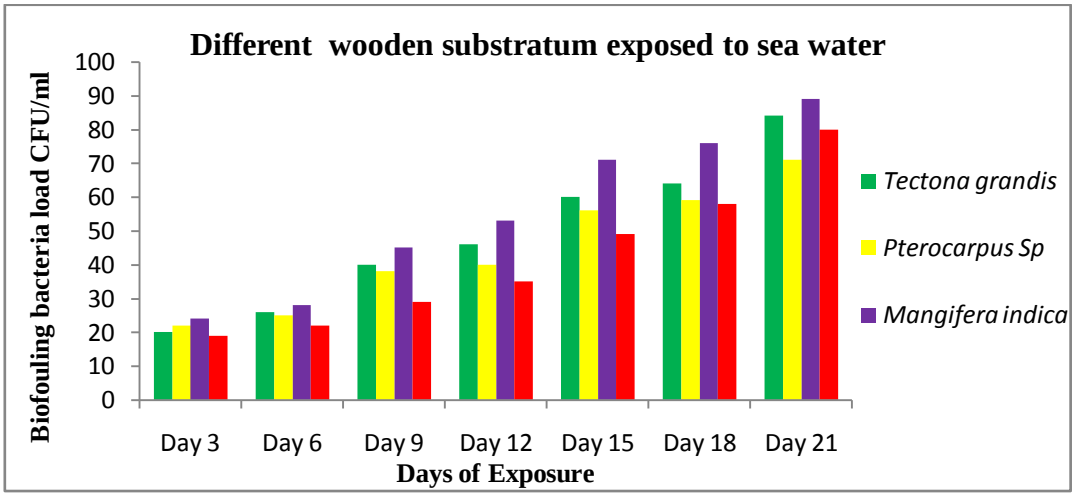

Fig-9: Bacterial densities on the wooden panel exposed to estuary of study area during different time intervals in different woods.

DISCUSSIONS

Deposition of molecules and microorganisms then macro organisms is the general phenomenon of marine fouling [45]. Biofilms occur spontaneously on both inert and living systems, being of concern to a wide range of scientific disciplines [46]. In industry, biofilms can have a detrimental impact on account of the undesirable effect of bio corrosion promoted by microbial cell accumulation at interfaces [2]. Competition for living space is more intense in marine environment; hence all submerged surfaces in the marine environment are rapidly colonized by bacteria and they form the important component in the development of a fouling community [47]. Biofilms are formed by microbial cells embedded in an exopolymeric matrix. The extracellular matrix is mainly composed of polysaccharides and proteins, although

other compounds such as DNA and humic substances [48, 49].

In the present study, four different woods (substrata) were exposed to seawater in order to determine their influence on the rate of fouling of bacteria along velar estuary, Southeast coast of India. During the experimental period, CFU is gradually increased according to the increase of exposure time. Thus the developments of colonies are directly proportional to the time of exposures in all the study panels. [50, 51] also observed similarly that the increase in the microfouling biomass was due to enhanced settlement and growth of already colonized microbes on the panel surface.

Surfaces in contact with seawater medium are rapidly colonized by bacteria due to ease access to nutrients, protection against antibiotics, maintenance of extracellular enzyme activities and shelter for predation [52]. The materials which possess high wet ability also attract more foulant [7]. If the substratum has such properties the rate of fouling will be increased. In the present study, out of four substrata tested, *M. indica* show that the highest bacterial load; whereas, the lowest bacterial population is observed on the *P. indicus*. The highest population on the *M. indica* may be due to its surface nature and wetability, etc... It has been widely reported that rough surfaces are more favorable for settlement than the smooth surfaces [53].

In agreement with the present result, [54] stated that the pattern of colonization of the submerged surfaces by bacteria is influenced by the physical and chemical characteristics of the surface. Also [7] reported that absorbed molecules alter the property of the substratum making all surface wettable and influence the rate of attachment of bacteria to a variety of substrates. [55] Have reported that the rate of initial bacterial colonization is substratum dependent, i.e., not all surfaces are colonized at the same rate or to the same extent and hence the observed differences in total viable count with different substratum are obvious.

CONCLUSIONS

Despite the relationship between the fouling of bacteria on the substratum is highly complex, the following conclusion can be drawn from the present study executed to comprehend the influence of different wood on the rate of fouling of marine bacteria. The *M. indica* fascinated higher bacterial foulant than the other woods. The *P. indicus* attracted less bacterial colonies. Rate of settling and the exposure time of the substratum are directly proportional.

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