IMPACTS OF WOOD PRESERVATIVES (CCA, CCB, CDDC, ACZA, ACQ AND ACC) ON THE SETTLEMENT AND GROWTH OF FOULING ORGANISMS

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ABSTRACT

A study was conducted to examine the effects of wood preservatives on settlement, abundance, growth and biomass development of fouling organisms (Non-target organisms) on treated panels exposed at an Indian harbour, Krishnapatnam (Lat.13^{0}28' to13^{0}59' N; Long: 80^0 10' to 80^0 16'E) on the east coast during January 1998. Observations made till January, 2000 are reported in this paper and compared with fouling communities on control panels. Wooden panels of Bombax ceiba were treated with various wood preservatives like Copper chrome arsenic (CCA), Copper chrome boric acid (CCB), Ammonical copper zinc arsenate (ACZA), Ammonical copper quaternary (ACQ) and Ammonical copper citrate (ACC). Similarly, panels of Hem fir treated with copper dimethyldithio carbamate (CDDC) were exposed in the same harbour during May 1999 and observations were made up to May, 2001. (The preservatives ACZA, ACQ, ACC and CDDC are being tested for the first time in the present study under marine conditions in India). Results indicated considerable variations in abundance, growth and biomass of foulers among the preservatives. Even though, algal and bryozoan settlement was found to be common on all the treated and untreated panels at initial stages (up to one month), later, these communities were replaced due to heavy settlement of calcareous organisms such as barnacles, oysters and serpulids. A greater variety of fouling assemblages were recorded on control, CCB and CCA treated panels compared to CDDC, ACZA, ACC and ACQ treated panels. CCA treated panels had heavier settlement of barnacles followed by oysters and bryozoans, while CCB treated panels had heavier settlement of oysters followed by few barnacles and bryozoans. Serpulid settlement was found to be negligible on these panels, however, their settlement was heavy on control panels. This indicates the above preservatives have positive impact on settlement of barnacles, oysters and bryozoans. Whereas, on CDDC panels bryozoan settlement was found to be more followed by barnacles, oysters and serpulids. The fouling assemblages that developed on ACZA, ACQ and ACC panels consisted of fewer species and were in small numbers. It indicates that these preservatives have negative impact on settlement of fouling organisms. Biomass levels were found to be higher on CCB

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treated panels compared to other treatments, while the least value was recorded on ACQ panels. Similarly, slight variations were noticed in growth pattern of different fouling species on treated panels of various preservatives. The results of the study on the fouling organisms settlement on untreated and treated panels are discussed in this paper.

Keywords: Impact of wood preservatives, Fouling settlement (Non target organisms), Marine environment and *Bombax ceiba*.

INTRODUCTION:

In recent years due to extensive usage of organic and inorganic preservatives (containing toxic metals) in the marine environment for protection of timber and other structural materials, there is a considerable problem of marine pollution. So, there is a need to assess the impact of these toxicants on marine flora and fauna. Among the various preservatives, CCA is extensively used throughout the world due to its wide range of effects on target (bacteria, fungi, termites, marine borers, etc.,) organisms. Although, it is very effective, its usage is under increasing public and regulatory pressure to move away from chromium and arsenic based preservatives. This is due to the fact that individual elements of CCA have been found toxic to non target organisms (foulers and other edible organisms). The earlier studies have shown that metals of copper, chromium and arsenic release from CCA treated timber upon submergence in sea water even when the metals are fixed properly within the wood [1,2,3]. The metals lost from treated wood enter into the surrounding water. Hence, the highest concentration of metals in the water are found closest to the treated wood. Since these metals are toxic to aquatic organisms in above trace levels [4,5,6], it may have both chronic and acute toxicological effects [7,8]. In well flushed areas, these toxic substances may get diluted immediately after release due to tidal action but in stagnated waters, the metals can buildup to high concentrations in immediate environment [9,10]. If the metals leached from the CCA treated wood are sufficient to induce adverse biological effects, then these are likely to be seen closest to treated wood [10]. Among the various aquatic species, marine benthic organisms are well placed for monitoring acute and sub lethal effects of potential pollutants released from the surfaces of submerged substrata in the sea as they settle directly on treated structures, where they will be exposed to higher levels of preservative leacheate.

Earlier studies indicated that communities which develop on CCA treated wood have low species diversity, abundance of certain species and biomass levels compared to untreated panels [9,11]. An adverse impact has also been found on the physiology of some organisms in close proximity to CCA treated surfaces [5]. These studies quantitatively examined the effects of CCA treated wood on the early successional stages of epi-biotic development. Generally, differences in community structure between fouling on untreated and CCA treated wood were reported during the initial stages of submersion.

Extensive information was collected on the effect of wood preservatives on settlement pattern, growth and biomass development of epi-biota in European countries as well as from United States and Australian waters [6, 7, 12,13,14,15,16,17,18]. While considerable data exists on the effects of wood preservatives on non target organisms from temperate waters [3,7,12,14,19,20] such information is lacking from Indian waters.

In response to above, a number of conventional as well as alternative new eco-friendly preservatives have been evaluated for the first time in Indian waters in the present study. These include conventional preservative such as Copper chrome arsenic (CCA), Copper chrome boric acid (CCB) and new novel preservative- Ammonical copper zinc arsenate (ACZA) and non arsenical preservatives like Ammonical copper citrate (ACC), Ammonical copper quaternary (ACQ), and Copper dimethyldithio carbamate (CDDC), which appears to be less lethal to non target organisms (foulers, fish, etc.). Due to paucity of information on these preservatives an attempt was made in this study to ascertain the impact of different wood preservatives on the settlement pattern, growth and biomass accumulation of macro foulers at Krishnapatnam harbour.

MATERIAL AND METHODS:

Bombax ceiba panels were treated with different wood preservatives such as Copper chrome arsenic (CCA), Copper chrome boric acid (CCB), Ammonical copper zinc arsenate (ACZA), Ammonical copper quaternary (ACQ) and Ammonical copper citrate (ACC) with retentions mentioned below. Similarly, *Hem fir* panels were treated with Copper dimethyldithio carbamate (CDDC). The panels were labeled and holes were made at both ends for tying in marine environment. Then the panels were end coated with an epoxy resin to retard longitudinal penetration of preservative and then weighed. Panels were treated with preservatives according to American Wood Preserver's Association Standard (AWPA 1996a).

- CCA – 32.4 kg/m^3
- CCB – 32 kg/m^3
- ACZA –32.0 kg/m^3
- ACQ – 35 kg/m^3
- ACC – 28 kg/m^3
- CDDC- 10.9 Kg/m^3

The test panels were kept in plastic containers and then appropriate solution was added to cover the blocks. The containers were placed in treatment cylinder and vaccum created for 30 min. at 80 Kpa followed by a 5 hr pressure period (880 Kpa). Then, pressure was released and the blocks were removed from the tanks. Each block was blotted dry and weighed to determine gross solution uptake. The samples were then stored in polythene bags for 2 to 3 days to allow any fixation reaction to proceed. Then the panels were air dried for 3 weeks in laboratory. Treated panels along with controls were exposed at Krishnapatnam harbour one meter below the low tide level from a jetty during January, 1998 and observations were taken till January, 2000 incase of *Bombax ceiba* panels. Similarly, *Hem fir* panels were also exposed during May 1999 and observations were taken till May, 2001.

Panels were examined for barnacles, serpulids, bryozoans and oysters (dominant fouling organisms on test panels) after 1, 3, 6, 12, 18 and 24 months. For barnacles, serpulids and oysters, individual counts were taken and for encrusting bryozoans total colonies were counted and colony spread (diameter) was measured on entire panels. Fouling species were identified up to species level. Growth and biomass values were recorded at 1, 3, 6, 12, 18 and 24 months. At each observation, fouling settlement on each preservative treated panel was scraped and brought to the laboratory in plastic covers and weighed in digital balance. After that, the epi-biota was kept in hot air oven, dried at 60^0C to constant weight. Dry weight biomass on each preservative treated panels was recorded.

RESULTS:

The Observations made in the present study generally indicate that settlement of foulers was not inhibited on CCA treated panels compared to other preservatives. Actually CCA, CCB and CDDC treated panels had higher number of barnacles, bryozoans and oysters compared to ammonical preservatives. The biomass values and growth rates also exhibited a similar trend. A greater variety of fouling organisms were recorded on control, CCA and CCB treated panels. The organisms that developed on ACZA, ACQ and ACC panels consisted of fewer species in small numbers (Table:1). The abundance of fouling organisms, growth and biomass accumulation on treated and untreated panels are presented in table:2 . By the end of first month, fouling settlement was found moderate on control, CCA and CCB treated panels, while CDDC, ACZA, ACQ and ACC treated panels had least fouling. However, by the end of 3 months, fouling settlement was found to be heavy on control, CCA, CCB and CDDC treated panels, while ACZA, ACQ and ACC treated panels continued to have lesser fouling. By the end of 3 months on CDDC treated panels barnacles and bryozoans settlement was found to be heavy and covered maximum space compared to ammonical preservatives. After 3 months, control panels were rejected from the test due to heavy attack of borers. By the end of one year, oysters, barnacles and bryozoans dominated all the treated panels and exhibited maximum growth on CCA, CCB and CDDC treated panels, while, ACZA, ACC and ACQ treated panels were covered only up to 50% of the area. The fouling organisms that settled on treated and untreated panels were: algae (*Enteromorpha intestinalis, E. compressa, Ulva lactuca);* serpulids *(Serpula vermicularis, Hydroides elegans, Mercierella enigmatica, Pomatoceros triquetor);* oysters *(Crassostrea madrasensis, Saccostrea cucullata);* barnacles *(Balanus amphitrite, Megabalanus tintinnabulum);* bryozoans *(Membranipora amoyensis, Hippoporina americana, Alderina arabianensis)* and bivalves *(Modiolus striatulus, Perna indica* and *P. viridis).*

Considerable variations were observed in abundance of foulers on timber panels treated with various preservatives. Algal and bryozoan settlement was common on all the treated and untreated panels at initial stages. Later, these communities were replaced due to heavy settlement of calcareous organisms such as barnacles, oysters and serpulids. CCA, CCB and CDDC treated panels had heavier settlement of barnacles, bryozoans and oysters and lesser settlement of serpulids throughout the study while, ACZA, ACQ and ACC treated panels had lesser fouling settlement. However, on control panels heavy settlement of algae, serpulids, bryozoans and sparse settlement of oysters and barnacles were observed.

Biomass buildup by fouling organisms was more on CCB, CCA and CDDC treated panels than on the ACZA, ACQ and ACC treated panels. However, on control panels, biomass values were found to be higher than CCA, CDDC, ACZA, ACQ and ACC but lower than CCB treated panels by the end of first month. By the end of 3 months, control panels were rejected due to heavy borer attack and lower biomass values were recorded than on CCA, CCB and CDDC treated panels.

On control panels, maximum growth of 2.68 cm was recorded in case of serpulids (tube length) followed by barnacles 1.2 cm (shell height), oysters 1.23 cm (basal diameter of shell) and bryozoans 1.78 sq.cm (colony width). Algae showed a maximum growth of 5 cm on control panels by the end of 3 months, while on treated panels its growth was found to be only 1.2 cm. The biomass values varied from 1.5 kg/m^2 (one month) to 11.6 kg/m^2 (3 months).

On CCA treated panels, barnacles showed a maximum growth of 2.7 cm (shell height); oysters 3.9 cm (basal diameter); serpulids 1.81 cm (tube length) and bryozoans 2.96 sq.cm (colony diameter) in a period of 2 years, respectively. The biomass values varied from 1.32 kg/m^2 (one month) to 30.5 kg/m^2 by the end of 24 months. On CCB treated panels oysters showed a maximum growth of 4.7 cm; bryozoans- 3.22 sq. cm; serpulids- 2.01 cm and barnacles - 2.1 cm. Biomass values varied from 1.91 kg/m^2 (one month) to 43.6 kg/m^2 by the end of 24 months. On CDDC treated panels bryozoans showed a maximum growth of 6.7 sq. cm; barnacles- 1.41 cm (shell height); serpulids- 1.8 cm and oysters 1.9 cm. Biomass values varied from 1.17 Kg/m^2 (one month) to 21.6 kg/m^2 by the end of 24 months.

Fouling settlement on ACZA panels was found to be lesser which consists of more barnacles followed by few oysters, bryozoans and serpulids. Oysters showed a maximum growth of 3.99 cm followed by bryozoans- 2.5 sq. cm; barnacles- 1.91cm and serpulids- 1.76 cm. The biomass values varied from 1.1 kg/m^2 (one month) to 14.2 kg/m^2 (24 months). Panels treated with ACQ and ACC preservatives showed least fouling settlement compared to other preservatives, which consists of oysters, barnacles, serpulids and bryozoans. Barnacles showed a maximum growth of 1.88 cm; oysters- 2.18 cm; serpulids- 1.84 cm and bryozoans- 2.7 sq.cm. on ACC treated panels. While on ACQ treated panels, barnacles showed 1.9 cm; oysters- 3 cm; serpulids- 1.6 cm and bryozoans- 2.5 sq.cm. The biomass values varied from 0.4 kg/m^2 (one month) to 7.1 kg/m^2 (24 months) in the case of ACQ treated panels and 0.44 kg/m^2 to 7.9 kg/m^2 in case of ACC treated panels for the same period.

DISCUSSION:

The results of the study indicate that the impact of CCA preservative on epi-biotic community in the sea is negligible. The total number of individuals, biomass and growth were actually higher on CCA, CCB and CDDC treated panels compared to control ones. This suggests that leached components of the preservative had no adverse effect on fouling organisms. This is in agreement with earlier studies conducted in temperate regions [3,7,12,17,18,19,21,22].

Generally, the rates of metal loss from treated structures were reported to be highest soon after submersion in the sea water, which gradually decreases as time progressing. The impact of leacheate was found to be higher on fouling organisms in confined conditions. But, in actual field conditions, the leached metals may get diluted immediately due to water mixing. Brooks [23] reported that the leaching of metals is most rapid during the first 5 or 6 days after installation in aquatic environment and that leaching rates halved on each successive day after immersion in water. Lebow et al. [2,3,24] have reported that levels of chromium and arsenic emitted in all of these studies were very low and were always below the toxic levels quoted in a review of the environment risk of CCA treated timber. On the other hand, Weis and Weis [9, 11, 25] suggested that leached metals from treated wood are a source of both chronic and acute pollution to marine biota which resulted in significantly lower number of species, diversity index and biomass. The experiments conducted by Weis and Weis [9, 11] however, were found to have been done in poorly flushed, relatively stagnant waters and performed in the laboratory created by artificial conditions, where concentrations of leachate could buildup to high levels. Also, the results reported by them are based on relatively short exposure of one month period. However, after 3 months differences were no longer significant between the communities, which settled on treated and untreated panels, which was attributed to the decreased toxicity of the wood due to lower rates of CCA loss. This suggests that any biological effect of leachate from CCA treated wood are short term relative to the service life of timber [22].

In the long term experiments (up to 2 years) as in the present study, it has been observed that wood treatment does not affect the settlement and abundance of fouling organisms. In fact, higher numbers and maximum growth rates were observed on panels treated with CCA, CCB and CDDC preservatives. This is in agreement with results of [15, 22]. There was only one case where a significant reduction in serpulid abundance on CCA treated panels compared to control panels was observed. Bryozoan settlement was found to be even higher on CCA treated panels with increasing CCA retention level [26]. In actual field conditions, CCA treated wood does not appear to present a gross source of pollution to epi-biota settled on treated wood and is thus unlikely to affect communities at further distances from the wood.

Heavy settlement of barnacles, bryozoans, oysters and moderate settlement of serpulids on CCA and CCB treated panels indicates that these preservatives may have less impact on fouling organisms or the organisms may be tolerating the toxicants. Moreover, these two preservatives contain copper which may act as a nutrient enrichment on the surface of panel. Copper being one of the essential elements for bivalves and for other organisms, available metal from leaching might have given more scope for recruitment, metamorphosis and subsequent growth especially for the bryozoans, barnacles and oysters. Boron compounds are known to have low toxicity and are considered as environmental friendly preservatives [27]. Hence, heavy settlement of foulers, higher biomass levels and maximum growth were observed on CCB treated panels. A study by Marchall and Martin [28,29] reported differences between the effects of different preservative types such as CCA, Copper chrome (CC) Copper organ (CO) and Creosote used in comparative toxicity tests with aquatic species *Daphnia magna* and *Acartia tonsa.* These studies revealed that CCA treated wooden panels showed a better environment profile than the CC, CO and Creosote treated wood, especially for acute and semi chronic toxicity tests with weaker toxic response. Even , the genotoxic results also confirmed these results.

The heavy settlement of bryozoans and barnacles on CDDC treated panels implies that the material has little effect in the way of repellency against fouling organisms. Moreover, Bryozoans are known to tolerate wide range of toxicants and in present study also, these are the major organisms which settled initially on all the treated panels. In case of oysters, even though large quantity of metals, especially copper accumulated in body tissues no adverse effects have been observed on settlement, growth, biomass, shell thinning, etc., on treated panels [26].

The lower intensity, biomass and growth on ACZA, ACQ and ACC treated panels compared to CCA and CCB suggests that panels treated with ammonical preservatives probably release higher quantities of metals, that may deter or repel fouling larvae. Sources of moderate pollution can lead to such reduction in species diversity [30], which is apparent with these ammonical preservative treated panels. The consistently lower levels of fouling on the ACZA treated panels may be either due to higher levels of metal loadings in these panels or greater bio availability of metals on the surface of panels, which might prevent settlement and subsequent colonization [31]. Otherwise, surface of these treated panels might have modified physicochemical properties of the wood resulting in a substratum which was relatively unattractive to species settlement during larval period. Alternatively, greater release of metals from treated panels might have discouraged initial larval settlement leading to the development of a community containing fewer species with low population abundance. ACQ formulation contains more copper than CCA formulation, thus more copper will be available for its leaching. Alternatively, copper released from ACQ treated panels might be in more bio-available forms than copper in leacheate from CCA. It is also possible that the presence of chromium and arsenic in CCA leacheate may have lead to metal-metal complexation at uptake sites and hence net lower levels of copper from CCA treated wood [12]. Also, citrate compounds in the treated wood are found to be highly susceptible to leaching and the presence of citrate is believed to alter copper distribution in treated panels, thereby leading to heavy metal loss from treated panels [32]. This could be one of the reasons for lower intensity and biomass level on ammonical treated panels.

Albuquerque [22] reported variations in mussel settlement and biomass on panels treated with CCA, ACQ and Creosote preservatives and lowest values were observed on ACQ treated panels. But, growth was found to be similar for all chemical types in case of oysters. This indicates that mussel settlement is affected by the type of preservative used, but that growth of successional settlers showed no evidence of growth suppression by proximity to treated surfaces.

A lower abundance of barnacles on control panels compared to CCA treated panels indicates that, the surface of control panels may be unattractive to barnacle larvae or reduced recruitment might have occurred in the early stages of surface colonisation. Studies by Cragg *et al.* [21] showed that after 3 weeks of exposure, recruitment of barnacles larvae was much higher on CCA treated panels then on untreated panels. This indicates that high levels of recruitment could ultimately result in greater abundance of foulers. This also suggests that they select the surface of treated panels rather than untreated panels. This may be due to changes in the physico chemical properties of the wood surface associated with metal leaching/movement [15].

Heavy settlement of barnacles on CCA treated panels indicates that this may be due to variation of physical, chemical and biological changes in the wood. CCA treatment gives wood a dark green colour compared to untreated wood. Barnacles prefer to settle on darker coloured substrata, hence this may add to the attraction of CCA treated wood. CCA also changes the physical properties of timber, giving it a different texture that may enhance barnacle settlement.

In case of serpulids, reduced settlement was noted on treated panels compared to control ones. Similarly, Albuquerque *et al*., [33] conducted a study at two harbours i.e. Sagres and Portugal and reported a significant reduction in *Pamatoceros triquetor* on CCA treated panels compared to control ones. Heavy settlement of serpulids on untreated panels indicates that, these tubeworms may prefer higher surface free energies. Also, earlier studies revealed that polychaete larvae showed species specific response in settlement indicating that the larvae settled in response to particular surface polysaccharide on glyco-protein in the bacterial films [34].

Oyster settlement was found to be abundant on CCB treated panels followed by CCA, CDDC, ACZA, ACC and ACQ treated panels. The lower number and biomass of oysters on ammonical treated panels indicates that the disturbance to the community is a chronic effect of ammonical preservative treated wood. Oyster larvae successfully colonised on CCB and CCA treated panels within a month of exposure. This may reflect the fact that oyster larvae are relatively tolerant to copper-based preservatives compared to ammonical preservatives.

Heavy settlement of bryozoans was found on CCA, CDDC and CCB treated panels compared to ammonical treated panels. Bryozoans are known to tolerate and accumulate high levels of toxicants and in the present study also, these are the major organisms which settled initially on treated panels. Similar results have been demonstrated in earlier studies from temperate waters [9]. They reported that while settlement of other fouling organisms was significantly affected by preservative treated panels, the bryozoan settlement was found to be enhanced on CCA treated panels especially in the case of *Bugula turrita*. Also, aggregation of the bryozoan *B. turrita* on CCA treated panels [9] and of *B. neritina* on copper treated panels [35] was observed. Aggregation was apparently initiated by copper ions progressively restricting the swimming movements of the larvae passing over the painted surfaces. Larvae that are unable to swim descend on the surfaces, thus greater number accumulated on these surfaces. Brown *et al*., [18] reported heavy settlement of bryozoans, *Electra pilosa* and *Bugula fulva* on CCA treated wood after 6 months of submergence, but on 12 months panels the bryozoan abundance was reduced.

Much variations have not been observed in growth of fouling organisms settling on treated panels of various preservatives. Maximum growth was found on CCA, CCB and CDDC treated panels compared to ACZA, ACC and ACQ treated panels. This indicates, ammonical preservatives may be slightly toxic to fouling organisms and may suppress the growth rate. Cragg *et al.*, [19] reported that there was no dose response relationship between CCA loading and growth and the size of the foulers was similar for all chemical types.

The biomass levels were found dissimilar between the wood treated with various preservatives. Higher levels were found on CCB, CCA and CDDC treated panels compared to ACZA, ACQ and ACC treated panels. Low levels of accumulation on ammonical treated panels may be due to gross source of pollution on substratum or treated surfaces may not be attractive to fouling larvae. Albuquerque and Cragg [12] reported lower species richness, abundance and biomass on ACQ treated panels compared to CCA and Creosote. The dissimilar biomass values on treated panels could be the result of either a modification of the physico-chemical properties of the wood surface or the exertion of a localised toxicity, either effect may have discouraged initial larval settlement leading to the development of a lower epi-biotic biomass on the treated panels [13]. Moreover, higher risks are found to be associated with alternative preservative treated wood products, which have higher levels of copper content used in aquatic environment. The use of alternative preservatives is not recommended in highly sensitive aquatic environment in areas characterized by limited flushing [8]. Even though, few alternative preservatives (ACQ and ACC) have the advantage from an environmental perspective, because they do not contain arsenic and chromium, moreover these preservatives do not leach arsenic and chromium into the environment, their effect on non target organisms was found to be significant in the present study. So, the present study indicate that, the effect of conventional preservatives on non target organisms are moderate or negligible compared to ammonical preservatives like ACZA, ACC and ACQ.

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Table 1 Fouling species settlement on test panels treated with different wood preservatives

Fouling species	Control	CCA	CCB	CDDC	ACZA	ACQ	ACC
Enteromorpha intestinalis	+	+	+	+	+	+	+
E. compressa	+	+	+	+	-	+	+
Ulva lactuca	+	+	+	+	+	-	+
Serpula vermicularis	+	-	+	-	-	-	+
Hydroides elegans	+	+	+	+	+	-	-
Mercierella enigmatica	+	+	+	+	+	+	-
Pomatoceros triquetor	+	-	+	+	-	-	-
Crassostrea madrasensis	+	+	+	+	-	+	+
Saccostrea cucullata	+	+	+	-	-	-	-
Balanus amphitrite	+	+	+	+	+	+	+
Megabalanus tintinnabulum	+	+	+	+	+	-	-
Membranipora amoyensis	-	+	+	-	-	-	-
Hippoporina americana	+	+	+	+	+	+	-
Alderina arabianensis	-	+	+	+	+	-	+
Modiolus striatulus	+	+	+	-	-	-	-
Perna indica	-	+	+	-	-	-	-
P. viridis	+	+	-	-	-	-	-

- Absent + Present

Table 2 Dominant fouling organisms settlement, growth and biomass accumulation on control and treated panels

Period (Months)	Treatment	Abundance of foulers settlement				Growth of foulers (in Cm.)				Biomass
		Barnacles	*Oysters*	*Serpulids*	*Bryozoans*	*Barnacles*	*Oysters*	*Serpulids*	*Bryozoans*	D.W (Kg/m^2)
1	Control	29±15	29±6.5	185±11	42.6±12	-	-	-	-	1.51
	CCA	128.5±39	31±10	12±4.2	45±12	-	-	-	-	1.32
	CCB	55.1±12	83.5±19	7.3±12	23.3±6.1	-	-	-	-	1.91
	CDDC	21.4±8	9.2±4.6	4.6±2.8	29.7±3.6	-	-	-	-	1.17
	ACZA	20.5±14	10.5±4.1	9±2	8.33±3.2	-	-	-	-	1.1
	ACQ	5.5±1.5	6.5±2.5	4.6±1.6	4±0	-	-	-	-	0.4
	ACC	6±1.4	6.5±2.6	3.5±.05	8.6±2.6	-	-	-	-	0.44
3	Control	48.6±17	84.3±30	343.5±79	22±7.5	1.2±0.2	1.23±0.1	2.68±5.9	1.78±0.4	11.6
	CCA	247.8±43	143.1±27	16.6±2.7	50.6±16	1.28±0.25	2.18±0.29	1.81±4.7	1.96±0.17	12.7
	CCB	35.1±16	186.1±23	13±4.6	16±6.5	1.31±0.15	2.21±0.30	2.01±6.4	1.73±0.24	14.2
	CDDC	26±12	34±19.2	21±5.8	36±6.1	1.12±0.27	1.38±0.12	1.24±4.2	2.7±0.38	5.2
	ACZA	23.8±6.1	27±7.5	7.66±2.4	8.4±3.07	0.98±0.26	1.66±0.26	1.76±2.05	1.53±0.25	2.9
	ACQ	13±2.4	20.6±6.4	6±1.4	7.5±2.6	1.15±0.25	1.78±0.4	1.46±3.29	2.05±0.16	1.71
	ACC	13.2±4.9	17±3.6	7.6±2.3	8±2.16	1.52±0.22	2.03±0.17	1.84±5.3	1.9±0.16	1.87
6	CCA	336.1±38.9	60.5±11.2	6.66±2.05	31.1±13.1	1.11±0.4	2.28±0.33	1.56±3.3	2.38±0.33	14.9
	CCB	90.5±28.1	196.6±7.1	16.5±2.6	10.8±5.3	1.51±0.36	2.41±0.45	1.71±4.25	2.26±0.33	17.8
	CDDC	123±32.2	46±5.8	28±3.4	24±3.6	1.28±0.26	1.8±0.32	1.78±3.22	3.4±0.46	8.8
	ACZA	29.6±15.8	41.8±1.37	9±2	13.1±3.38	1.46±0.36	2.9±0.52	1.5±4	2.13±0.2	4.76
	ACQ	14.1±4.4	24±6.1	12.3±3.3	6.5±1.8	1.26±0.22	2.11±0.3	1.6±3.5	1.82±0.42	2.93
	ACC	22.3±7.82	26±7.3	7.6±0.9	7.75±1.92	1.4±0.26	2.06±0.28	1.0±2.9	2.2±0.5	3.12
12	CCA	185.3±54.8	42.8±12.7	13.5±3.2	25.1±6.2	2.08±0.37	2.16±0.39	1.73±3	2.96±0.81	19.4
	CCB	69.8±19.5	129.5±16	18.5±2.92	10.3±1.8	1.85±0.34	3.5±0.5	1.78±2.5	2.13±0.4	23.1
	CDDC	37±13.2	56±6.8	21±2.65	48±3.2	1.32±0.26	1.2±0.34	1.62±2.4	4.12±0.43	13.6
	ACZA	25.3±15.3	48.8±9.5	11.2±2.6	11±2.4	1.58±0.19	2.15±.0.3	1.62±3.11	2.5±0.5	7.82
	ACQ	20±4.32	31.6±8.6	10.3±2.4	12.6±4.9	1.43±0.14	1.81±0.25	1.4±2.4	1.63±0.4	4.9
	ACC	19±4.7	22±5.77	10.3±4.4	9±1.63	1.73±0.39	2.05±0.41	1.5±4.86	2.36±0.24	5.17
18	CCA	231.3±38.9	27±5.4	15±2.2	13.1±2.9	2.4±0.12	3.3±0.53	1.75±3.77	2.23±0.49	22.4
	CCB	96.6±24	94.6±34.7	17.3±7.8	14.1±3.8	1.8±0.28	4.41±0.9	1.68±3.38	2.56±0.37	32.1
	CDDC	122±12	86±23.6	16±3.2	68±4.2	1.41±0.31	1.4±0.4	1.24±2.26	4.21±0.42	18.3
	ACZA	28.3±11.7	33.6±5.1	9±3.5	15.8±2.5	1.7±0.3	3.99±0.69	1.2±2.16	1.98±0.43	9.2
	ACQ	17.8±3.3	19.5±4.1	5.66±1.24	8.25±1.9	1.56±0.2	2.33±0.39	1.2±1	2.4±0.6	5.11
	ACC	31.1±8.6	18.5±4.6	12±3.6	11.7±1.92	1.88±0.22	2.18±0.29	1.37±2.7	2.05±0.2	5.7
24	CCA	298.6±55.2	38.5±8.4	9.8±4.11	18.5±4.68	2.7±0.35	3.9±1.03	1.78±3.81	2.76±0.76	30.5
	CCB	95±35.1	76.8±14.6	10.5±2.5	12±4.4	2.1±0.24	4.7±0.7	1.65±3.6	3.22±0.79	43.6
	CDDC	69±28.3	59±4.8	27±8.6	42±5.8	1.11±0.21	1.9±0.34	1.8±1.74	6.7±2.6	21.6
	ACZA	27.3±10.5	66.3±13.3	14.5±5.67	7.83±4.01	1.91±0.36	2.28±0.38	1.3±2.38	2.3±0.2	14.2
	ACQ	23.6±4.3	22.6±6.1	9±1.41	9.2±2.4	1.9±0.46	3±0.48	1.4±1.63	2.5±0.48	7.1
	ACC	19.5±6.26	20.8±7.55	10±4.24	5.25±1.29	1.3±0.12	2.7±0.5	1.15±2.8	2.7±0.48	7.9

Control panels were rejected after 3 months - Not recorded D.W – Dry Weight