



ASSESSMENT OF BIO GROWTH ON TITANIUM AND STAINLESS-STEEL COUPONS IMMERSED AT DIFFERENT CHLORINATION ENVIRONMENT SEAWATER

S. Ganesh^{1*}, A. Malar Retna², S. Godwin Wesley³

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Abstract

This paper describes the efficiency of chlorination in the seawater cooling system of a power plant located in the Southern coastal area of India. Stainless Steel (SS) and Titanium (Ti) coupons were immersed for a period of 4 x 6 months. Test coupons were collected and wet weight was determined at monthly intervals from intake dyke and pump house regions for biofouling biomass. Species abundance and composition were analysed and the mean value was noted. Low-dose chlorination (0.2 ± 0.1 ppm) and shock-dose chlorination for 3 x 20 min/day (0.4 ± 0.1 ppm) were given in the pump house region during entire study period. Biofouling biomass at the seawater intake station ranged from 98 to 4767 g/m² and 102 to 4831 g/m² on SS and Ti coupons respectively whereas in the pump house (chlorinated) the settlement was negligible and this proved the efficiency of chlorination in mitigating the biofouling.

^{1,2}Department of Chemistry, Scott Christian College (Ph.D. Reg No.11838, Manonmaniam Sundaranar University, Tirunelveli), Nagercoil, Tamil Nadu, India-629003.

³Department of Zoology, Scott Christian College, Nagercoil, Tamil Nadu, India-629003.

***Corresponding Author:** S. GANESH (Ph.D. Reg No.11838)

Department of Chemistry, Scott Christian College Nagercoil-629003, India.

Corresponding email: ^{1*}cnpsganesh71@gmail.com

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1. Introduction

In most aquatic habitats, biofouling is a common occurrence. Microbial biofilms have been linked to the settlement and metamorphosis of marine invertebrate larvae, and are thus thought to be the precursors of the biofouling phenomenon. Biofouling is an issue in industrial cooling circuits because it reduces heat transfer efficiency, causing large pressure drops in condensers; affecting the operation of connected ancillary systems. Condenser fouling and corrosion causes operational failures and hence they are not economically feasible (Rao, 2015) and so, antifouling techniques are very important. It is necessary to find an efficient biocide to limit the biofilm development. A review of the literature shows that chlorine is widely employed (70–80%) to prevent biofouling in power plant cooling systems around the world. Chlorination has become the most extensively used biocide for water treatment over the years. The following are the three most important factors that favour its use: (1) It is a highly effective biocide; (2) it is widely available; and (3) it is inexpensive. To prevent fouling of the condenser and pre-condenser parts of a cooling circuit, chlorination is usually done either intermittently or continuously. Other halogen-based formulations can be used as well, but they are frequently supplemental and expensive (Rajagopal *et al.*, 2012). The organisms invading the cooling circuit determine the chlorination schedule for controlling macrofouling in an industrial cooling system. Intermittent chlorination is favoured, if the foulants are soft-bodied, such as bryozoans and hydroids. Low-level continuous chlorination is commonly employed to combat barnacle and mussel macrofouling, and this method is also useful in preventing slime build-up inside the condenser tubes. The amount of chlorine dose used in exomotive chlorination is insufficient to kill fouling organisms but enough to produce an environment that prevents them from settling in the cooling system. This method of chlorination is said to be both cost-effective and environment friendly (Rajagopal *et al.*, 1996). Numerous studies have been done on the application of chlorine as biocide (Rajagopal *et al.*, 1995; 2002; 2003; 2012; Verween *et al.*, 2009; Venugopalan *et al.*, 2012; Venkatnarayanan *et al.*, 2016). Because of its established effectiveness, ease of availability and low cost, chlorination has remained as the most efficient biofouling method to date. However, as environmental concerns have grown, the discharge limit for residual chlorine levels at industrial outfalls or in effluents has been lowered (Rao, 2015).

Experimental study area

The study was carried out at the Southern coast of India (8°9' N and 77°39' E) for a period of 4 x 6 months. The first and the second locations are intake dyke region and pump house respectively. SS and Ti coupons were immersed at both locations concurrently. There is no chlorine addition in the first location where as in the second location continuous and periodic chlorine addition was carried out. The data were collected at 6-monthly intervals from December 2015. After one cycle of 6-month period, a new set of coupons were immersed for another cycle of 6 months. The coupons were retrieved at monthly intervals for analysis.

Cooling water system of coastal power plant

Sea water is drawn through a caisson structure to intake dyke. There is a Fish Protection system (FPF) between the intake dyke and concrete pipeline to expel the zooplankton and send back to the sea, which is achieved by continuous supply of compressed air. Seawater from the forebay passes through 40mm and 4mm screens, pump house and finally reaches out through titanium condenser tubes. In the pump house, the water was chlorinated. Low-dose chlorination (continuous) of about 0.2 ± 0.1 ppm and shock-dose chlorination of about 0.4 ± 0.1 ppm was provided. Chlorination process was executed in an electro-chlorination plant.

Coupon exposure and assessment

The test coupons were of similar size measuring 1 mm thickness and 100 cm² area. For obtaining a uniform surface, 80, 200 and 480 grit sand papers were used. The samples were triplicated and the mean was considered. The triplicate samples were fixed in a fibre-reinforced plastic metal frame and immersed in the seawater at a depth of 1 m. Due to high velocity of water in the pump house region; the test coupons were deployed in a 2000 litres polypropylene tank which was fed continuously with chlorinated seawater from a condenser pump. The flow through system was maintained continuously for a period of 4 x 6 months. The submersion period (December 2015 to November 2017) and the initial submersion time of both the coupons were same and the samples collected from the intake dyke region and the pump house region were considered as untreated control and test respectively.

Periodically, the samples were collected at monthly intervals from both the intake dyke and the pump house region and the wet weight was determined initially. Loading of biofouling biomass, its species abundance and composition were analysed and the mean value was noted. Subtracting the initial weight from the final weight of the coupons gives biomass loading (g/m²). Species identification, its composition and density were calculated using the methods mentioned by Nandakumar (1996). A Nikon digital camera was used for documentation

and abundance was estimated by taking into account the total count of the biofouling organisms. ImageJ software was used to estimate the percentage area cover. The residual chlorine level in the two stations was estimated colourimetrically (HACH pocket Colorimeter II(R) 5870000). The measurement range of the colorimeter was 0.1–1 ppm and had a resolution of 0.01 ppm. It was measured in terms of total residual oxidant and the sampling was done each day and averaged monthly.

Statistical analysis:

All exposure studies were conducted with triplicate coupons and the results expressed as mean and standard deviation. Triplicate seawater samples were collected for hydro-biological measurements and the results were expressed as mean with

standard deviation. Statistical analysis was done using the software Graphpad Instat. Pearson's correlation was carried out for water temperature, salinity on biofouling load and species density. One-way ANOVA was done on biofouling loading between the two tests substrates and between the two different sites of exposure.

2. Results and Discussion

Residual chlorine

The residual chlorine level in the intake dyke and the pump house region ranged from 0.30 to 0.39 mg/L and 0.20 to 0.40 mg/L, respectively, with the biannual mean level of chlorine being 0.34 ± 0.02 mg/L and 0.30 ± 0.05 mg/L at the intake and pump house region respectively (Fig. 1).

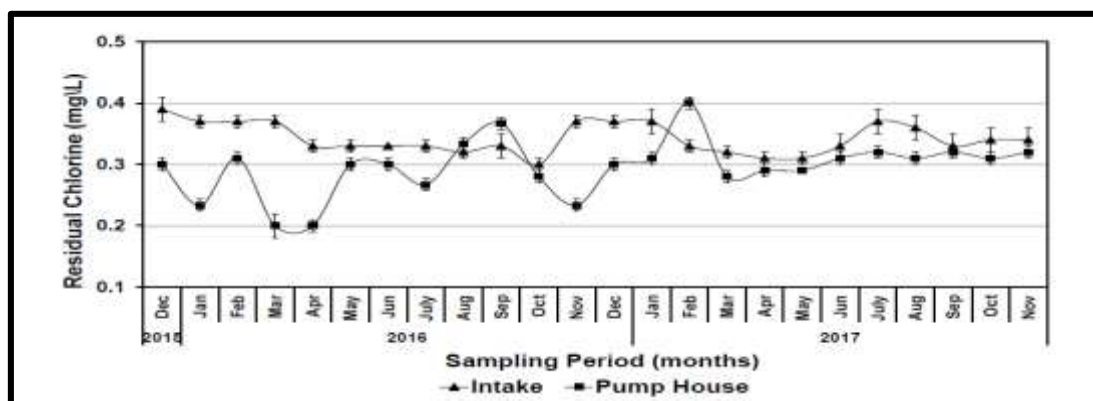


Fig. 1: Seasonal variation of residual chlorine levels in the cooling water measured at the intake and at the pump house station

Bioload on coupons in two different stations

Figs. 2 and 3 depicts the bioload on SS and Ti coupons immersed at intake station during the experimental study and showed high bioload ranged from 98 to 4767.66 g/m² and 75 to 4479 g/m² on SS and Ti coupons respectively. The maximum bioload was observed during November 2017 and October

2017 on SS and Ti coupons, respectively and the minimum bioload was observed during December 2016 and January 2017 on SS and Ti coupons respectively. There is no significant bioload observed on the coupons immersed in the pump house region.

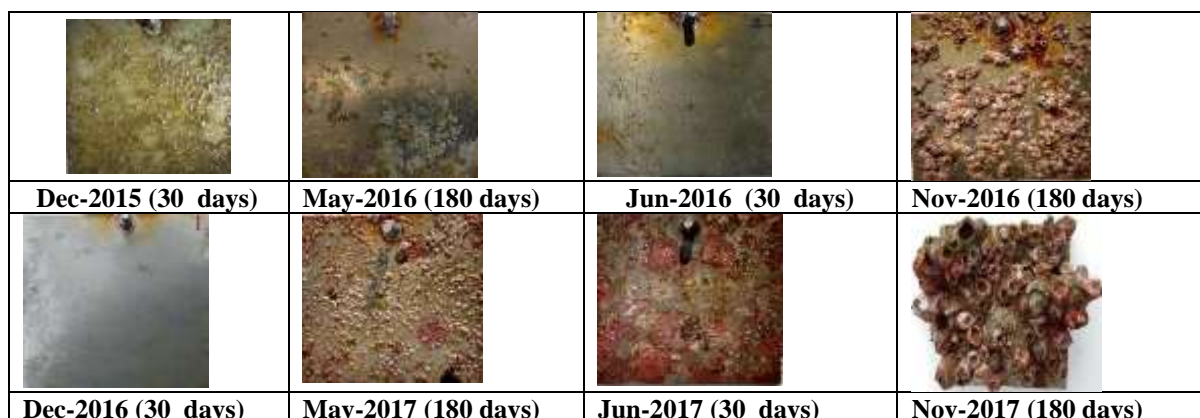


Fig. 2: Biogrowth on SS coupons deployed at the intake region (1st, 2nd, 3rd and 4th- 6 month study)

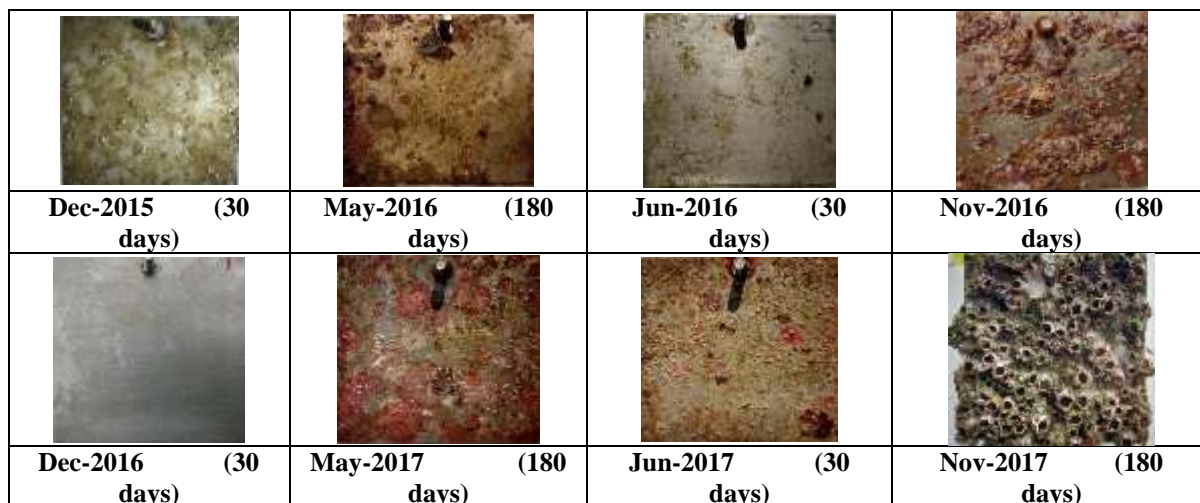


Fig. 3: Biogrowth on Ti coupons deployed at the intake region (1st, 2nd, 3rd and 4th- 6 month study)

The settlement of *Balanus amphitrite* on the surface of coupons increased the bioload. While comparing both the immersed coupons in the intake dyke region, the bioload was high on SS coupons (Fig. 4). The bioload on SS coupon during the first 6-month study (December 2015 to May 2016) showed an increase in bioload from 297.25 g/m² (December 2015) to 756.25 g/m² (May 2016). Similar observations were found on the surface of Ti coupons, where the bioload increased to 560.25 g/m² from 234.75 g/m² and in the second 6-month study (June 2016 to November 2016), there was an

increase in bioload till October 2016 on SS (1836.5 g/m²) and Ti (1960.5 g/m²) coupons, and a slight decrease in bioload of 1639.66 g/m² (on SS) and 1823.75 g/m² (on Ti) in November 2016. The third set of the 6-month study (December 2016 to May 2017) revealed maximum bioload on both the coupons (ranged from 98 to 625.83 g/m² on SS and 123.33 to 742.16 g/m² on Ti). In the fourth set of the 6-month study, consecutive decrease and increase on bioload were observed on both the coupons. The maximum bioload was observed on SS during the tenure of experimental investigation.

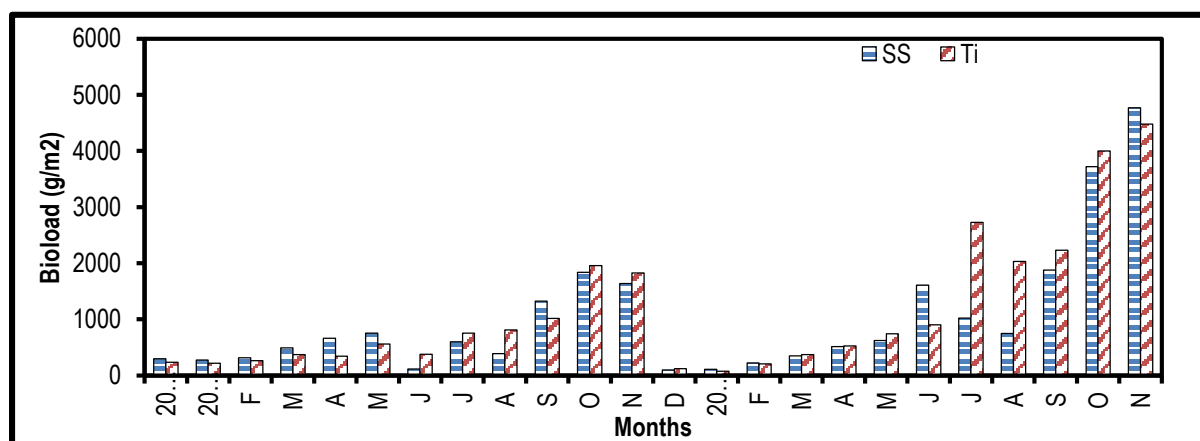


Fig. 4: Bioload of fouling organisms on SS and Ti coupons

The materials used in the test substratum such as SS or Ti can be considered as a factor responsible for increase or decrease in biomass loading (Chase *et al.*, 2016). The bioload was higher on SS surface than Ti surface and this indicated the influence of SS material over the settlement of biofouling organisms. Also, the minimisation of bioload on Ti surface in comparison with the SS surface at the intake dyke region indicated the resistance of surface for species settlement. Similar type of minimised bioload was observed in a study

conducted at the Madras Atomic Power Station, Kalpakkam (Rajagopal *et al.*, 1991; 2012). 43.9 kg/m²/8m and 50.2 kg/m²/8m for Ti and SS respectively. The low-dose continuous and shock-dose chlorination regimes were shown to be particularly successful in preventing fouling organisms from settling in the power plant's condenser area. On the coupons used in the pump house, the larval settlement was completely blocked, and the biomass loading was found to be very low.

Abundance of biofouling organisms on coupons immersed in intake and pumphouse region

The current investigation reported the control of biofouling species by using chlorine as a biocide. The recruited organisms on the surface of coupons immersed in the intake dyke region were *B. amphitríte*, *Hydroides norvegica*, cnidarian hydroids, ascidians, *Crassostrea* sp., *Chaetomorpha linum* and *Membranipora* sp. Variation exists in the

recruitment and colonisation of biofouling species and it was dependent on the test substratum and the environmental conditions. The coupons submerged in the chlorinated environment were free from invertebrates. Fig. 5 and 6 shows the total count of barnacle, tubeworm and oyster during 6-month study at intake region on SS and Ti coupons respectively.

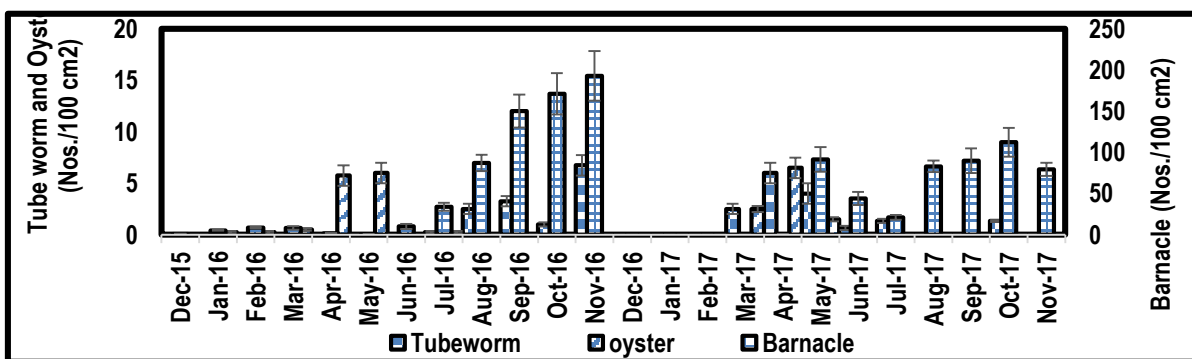


Fig. 5: Total number of barnacle, tubeworm and oyster in 4 sets of 6 month duration at the intake region on SS coupons

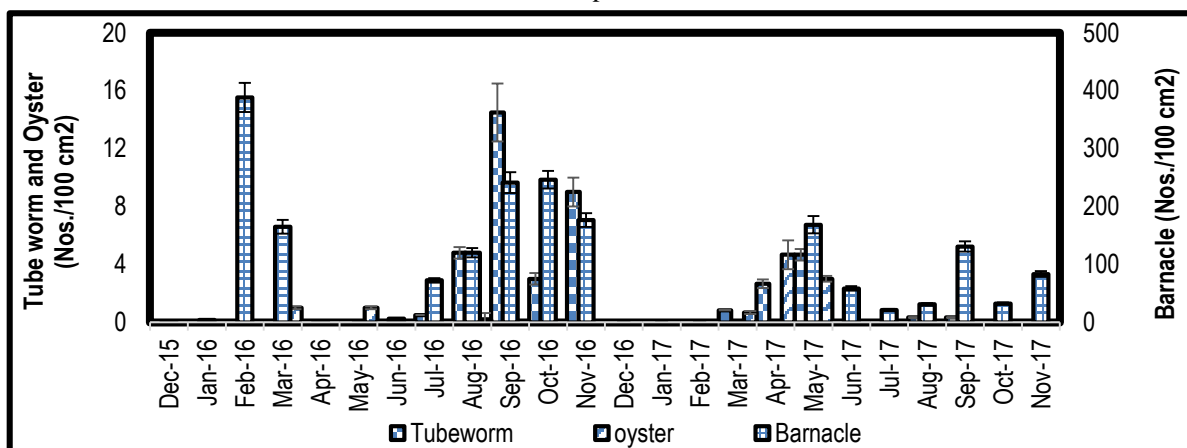


Fig. 6: Total number of barnacle, tubeworm and oyster in 4 sets of 6 month duration at the intake dyke region on Ti coupons

Table 1 represents the comparison of recruitment of biofouling organisms on both SS and Ti coupons immersed at the intake site and the pump house site. Initially, *C. linum* settled on both the coupons during the study period. Colonisation of *C. linum* was high on SS coupons than on Ti coupons. The percentage cover was 65% and 44% on SS and Ti coupons, respectively, during first set of the 6-month study (December 2015 to May 2016), and showed a gradual decrease (up to 1%) on the coupons. The decrease in percentage cover was due to the recruitment of *B. amphitríte* during February 2016

(388 nos/cm² on SS and 9 nos/cm² on Ti). This showed the resistance of algae due to the newly settled species (Souza, 1979). The barnacle population was about 6 nos/m² and 10 nos/m² on SS and Ti coupons, respectively, during the second set of the 6-month study (June 2016 to November 2016) with a gradual increase in population till October 2016 (246 and 171 nos/m² on SS and Ti respectively) and a sudden decrease during November 2016 (60 and 68 nos/m² on SS and Ti respectively). It may be due to the onset of Northeast monsoon.

Table 2: Abundance of biofouling organisms in two different stations

Biofouling organisms-Recruitment in intake dyke region (Mean)	

Experimental pattern	Month and year	Barnacles (no./100 cm ²)		Tubeworms (no./100 cm ²)		Oysters (no./100 cm ²)		Algae (% cover)		Ascidi ans (% cover)		Hydroids (% cover)		Bryozoans (% cover)		Recruitment in pump house region (Mean)	
		SS	Ti	SS	Ti	SS	Ti	SS	Ti	SS	Ti	SS	Ti	SS	Ti	SS	Ti
1st 6 month study 2015-16	Dec	0	1	0	0	0	0	65	44	0	0	1	3	0	0	0	0
	Jan	4	5	0	0	0	0	2	13	1	0	3	3	0	0	0	0
	Feb	38	9	0	0	0	0	0	9	2	12	10	3	5	9	0	0
	Mar	16	8	0	0	1	1	0	1	12	12	6	9	11	12	0	0
	Apr	1	2	0	0	0	6	0	0	8	12	28	9	2	7	0	0
	May	0	0	0	0	1	6	0	0	7	7	0	0	0	1	0	0
2nd 6 month study 2016	Jun	6	10	0	0	0	0	0	20	0	1	17	1	0	0	0	0
	Jul	73	34	1	0	0	0	0	2	5	3	2	2	0	0	0	0
	Aug	12	87	5	3	0	0	0	2	1	4	3	10	1	4	0	0
	Sep	24	15	15	3	0	0	0	4	3	2	4	3	4	4	0	0
	Oct	24	17	3	1	0	0	0	1	1	3	3	0	3	7	0	0
	Nov	60	68	9	7	0	0	0	3	9	12	0	3	0	4	0	0
Experimental pattern	Month and year	Biofouling organisms-Recruitment in intake dyke region (Mean)														Recruitment in pump house region (Mean)	
		Barnacles (no./100 cm ²)		Tubeworms (no./100 cm ²)		Oysters (no./100 cm ²)		Algae (% cover)		Ascidi ans (% cover)		Hydroids (% cover)		Bryozoans (% cover)			
		SS	Ti	SS	Ti	SS	Ti	SS	Ti	SS	Ti	SS	Ti	SS	Ti	SS	Ti
3rd 6-month study 2016-17	Dec	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
	Jan	0	0	0	0	0	0	0	1	5	4	1	1	0	0	0	0
	Feb	0	0	0	0	0	0	0	0	13	9	0	0	0	0	0	0
	Mar	0	0	1	3	1	3	0	3	17	20	1	0	0	0	0	0
	Apr	0	0	3	6	5	7	0	0	21	24	2	3	0	0	0	0
	May	16	9	92	5	4	3	2	0	0	16	38	1	4	3	2	0
4th 6-month study 2017	Jun	59	44	0	1	0	0	0	4	0	3	0	6	0	3	0	0
	Jul	21	21	0	1	0	0	0	1	1	0	0	0	5	0	0	
	Aug	31	83	0	0	0	0	0	8	4	13	0	0	5	6	0	0
	Sep	13	1	90	0	0	0	0	7	11	0	5	0	11	2	0	0
	Oct	32	11	2	0	1	0	0	2	0	0	0	0	15	1	0	0

	Nov	83	79	0	0	0	0	1	1	10	0	0	0	9	0	0	0
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In the third set of the 6-month study (December 2016 to May 2017), the recruitment of *B. amphitrite* was negligible till April 2017 and during May 2017, the recruitment was found on both the coupons (169 nos/m² and 92 nos/m² on SS and Ti respectively). In contrast, in the same period, the recruitment of ascidians was found to be high on both the coupons. The coverage ranges were 5–21% and 4–38% on SS and Ti coupons respectively. Ascidians were found to minimise the recruitment of barnacles mechanically by scraping the substrate surface and removing the larvae at the time of settlement (Menge, 1976; Leonard, 1999) and this validated the poor presence of *B. amphitrite* on both the coupons in the third set of the 6-month study. In the fourth set of the 6-month study (June 2017 to November 2017), the recruitment of *B. amphitrite* was high and the *C. linum* was low on both the coupons. At the same time *Membranipora* settled frequently on both SS and on Ti coupons. The recruitment of *Crassostrea* sp. was not regular during the entire study period with maximum recruitment during April 2017 (5 nos/m²) and April 2016 (7 nos/m²) on SS and Ti surfaces. The fourth set of the 6-month study showed least presence of *Crassostrea* sp. Predation pressure could be considered as a reason for a similar type of settlement pattern (Pineda, 1994). Settlement of *Cnidarian hydroides* was high on both the coupons during the first, second and third set of the 6-month study with peak settlements during April 2016 (28% on SS) and August 2016 (10% on Ti) but the recruitment was low during the fourth set of the 6-month study. *C. hydroides*, in general, colonised bare substrates (Hughes *et al.*, 1991) and played a vital part in the connection of pelagic and benthic populations. The irregular settling on coupons seen throughout the course of the 4 x 6-month study period could be related to the larvae's settlement behaviour (Withers and Thorpe, 1977). It is extremely likely that some species settled on the coupon's vacant spots, which were free of other foulants. The recruitment of ascidians was too irregular with the peak settlement during April 2017 (21% on Ti) and May 2017 (38% on SS) and it ranged from 1% to 12% on SS, 7% to 12% on Ti during the first set of the 6-month study, 1% to 9% on SS, 1% to 12% on Ti during the second set of the 6-month study, 5–21% on SS, 4–38% on Ti during the third set of the 6-month study and from 1–10% on SS, 3–13% on Ti during the fourth set of the 6-month study. The recruitment of *Membranipora* sp. occurred in a discontinuous manner during the sampling period with a range from 2% to 11% on SS, and 1% to 12% on Ti during the first set of the 6-month study, from 1% to 4% on SS, 4% to 7% on Ti during the second set of the 6-month study, and from 5–15% on SS, 1–6% on Ti during the fourth

set of the 6-month study. The third set of the 6-month study showed poor recruitment of *Membranipora* sp.

Physico-chemical parameters are known to influence the settlement of biofoulers. Seasonal settlement of ascidians was observed in Kudankulam waters (Satheesh and Wesley, 2008; Sasikumar *et al.*, 1989). The settlement could also be related to larval presence and breeding activity. Ascidian settlement prevented barnacle recruitment in Kudankulam (Satheesh and Wesley, 2008). Replacement of species was a natural phenomenon, that was greatly influenced by different modes of organismic properties (Valiela, 1984). The diversity of biofoulers will be low on man-made substratum than on natural habitats (Vaselliet *et al.*, 2008). This mode of temporal settlement may be based on the availability of larvae, other than environmental parameters. The ecosystem of cooling water system was different with difference in hydrodynamic parameters and different substrata. In the present study, *B. amphitrite*, *C. linum*, *C. hydroides* and ascidians formed a stable community on the experimental coupon surfaces with other groups such as *H. norvegica*, oysters and bryozoans exhibiting a seasonal occurrence. Also, the recruitment was high on SS than on Ti and it could be due to the presence of bacterial films and settlement behaviour of larvae on the surface of coupons (Olivieret *et al.*, 2000). Chlorination is one of the remedial measures for biofouling control in power plant systems (Venkatnarayanan, 2016). Numerous studies have been conducted in relation to the chemistry and eco-toxicity of chlorine (White, 1972; Mattice and Zittel, 1976; Jolley and Carpenter, 1983; Allonier *et al.*, 1999; Verween *et al.*, 2009; Venkatnarayanan, 2016) and relatively few studies have focused on chlorination and its by-products in marine waters (Lopez-Galindo *et al.*, 2014). Seawater electrolysis results in the formation of hypochlorite ion and hypochlorous acid, and its equilibrium is based on the pH of the seawater (White, 1972). The reaction between bromide ion and hypochlorous acid resulted in the formation of hypobromite ion and hypobromous acid (White, 1972; Jolley and Carpenter, 1983). Reaction of hypochlorous acid with ammonia resulted in the formation of organohalogen compounds and haloamines (Lewis, 1966). Haloacetic acids, halophenols, trihalomethanes and haloacetonitriles are some of the chlorination by-products formed from the reaction of chlorine with seawater (Jenner *et al.*, 1997). All by-products have been proved to be effective as a biocide in inhibiting the activity of enzymes, disrupting the regulation of ions and causing damage to cellular membranes

(Jolley and Carpenter, 1983). The efficacy of chlorine was dependent on the abundance and species of biofoulers (Mattice and Zittel, 1976). In previous studies, low-dose chlorine (0.075 mg/L) exhibited 50% reduction in phytoplankton growth and a chlorine dose of 0.8 mg/L was required to inhibit the growth of microalgae (Lanza *et al.*, 1975). In this investigation in the pump house region, continuous low-dose followed by shock-dose chlorination process was followed. The population of *B. amphitrite* was greatly minimised in the chlorine exposure in pump house region and this evidence was in contrast with a study conducted by Venkatnarayanan *et al.* (2016), where chlorine (continuous low-dose) was not efficient in reducing the barnacle larval population. Similarly, the presence of other biofouling organisms found in the intake dyke region was not observed in the pump house region. Likely, the high-dose chlorination (1.4 mg/litre) followed by continuous low-dose chlorination (0.2 mg/litre) regimes practised in Kalpakkam showed minimal recruitment of mussels (Rajagopal *et al.*, 1996). The 6-month experimental study showed an unequal distribution of biofouling organisms on the surface of SS and Ti plates. However, the process of chlorination in the pump house region resulted in negligible presence of biofouling organisms on the coupons and hence continuous low-dose and shock-dose chlorination were effective to overcome the fouling issue in coastal power plant.

3. Conclusion

Biofouling is a well-known issue in industrial cooling systems. The development of biofouling and abundance on Ti and SS coupons exposed to ambient saltwater (intake) and chlorinated seawater (pump house) of the coastal nuclear power station was studied. During the 6-month study of this 2-year investigation, biofouling biomass at the seawater intake station ranged from 98 to 4767 g/m² and 75 to 4479 g/m² on SS and Ti coupons respectively. In the cooling water system, continuous chlorination of 0.2 ± 0.1 mg/L and shock-dose chlorination (3 x 20 min/day) of 0.4 ± 0.1 mg/L was extremely efficient in lowering the biofouling load. Between the intake and pump house stations, Ti coupons showed high reduction in fouling biomass. Thereby, it was proved that the chlorination regime practised in the pump house region will be efficient in mitigating the settlement and colonization of biofoulers in cooling water system of coastal power plant.

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