Experimental studies on the effect of different metallic substrates on marine biofouling

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Abstract

In the wake of adoption of the resolution by the International Maritime Organization to control biofouling on vessels, which is recognized as a major vector for transfer of invasive species, this study attempts to create a baseline data on major hard-shelled biofouling organisms in the harbour waters. This study was primarily focused towards understanding the biofouling and corrosion pattern on various metals and their performance under immersed condition in a marine environment, at 0.3 and 3.0 m depths. Furthermore, the study attempts to understand the surface dependent characteristics of barnacle base plate and its adhesion strength. Barnacle, mussels and oysters were the major fouling organisms accounting for 72.33% of the variation. Stainless steel and Titanium panels showed the highest average biofouling load of 176.36 and 168.35 g/m²/cm², respectively. The variance in biofouling between metals and depths was highly significant at p < 0.001 and p < 0.01, respectively. Morphology of barnacle base plate interfacial surface varied between metals. Barnacles with 8-9 mm base diameter showed the maximum adhesion strength in shear of 0.86 ± 0.95 kPa.

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1. Introduction

Biofouling causes increased loading to structures and surfaces of vessels leading to their poor performance, resulting in considerable economic losses for centuries. Recently, biofouling has also been indicated as the major mechanism for transfer of species which might result in bioinvasion. Bioinvasion, pose threats to human, animal and plant life, economic and cultural activities and the aquatic environment itself. This has been recognized by the International Maritime Organization (IMO) leading to the adoption of the resolution in July 2011, for the control and management of biofouling on ships to minimize the transfer of invasive aquatic species [1].

The biofouling problems have received scientific attention for more than 60 years [2]. The worldwide marine industry incurs annual expenditure of several billion US dollars to combat the problems associated with biofouling [3], especially on ships which are a major cause of bioinvasion globally [4]. In India, about US$ 8 million are being spent annually to keep the ships and oil & gas platforms free from fouling apart from the loss incurred due to shutting down of the power plants and other industries [5].

Studies carried out in some of the major naval ports of India have shown the quantum of biofouling debris is 2- to 10-folds higher than temperate and semi-tropical water [6]. Nevertheless, the seasonal and site-specific variation in marine biofouling and corrosion needs scientific attention [3]. Often, the practical difficulties in field testing lead to the usage of laboratory test results, which may lead to catastrophic failure of the system in marine applications. Hence, performance studies of various metal panels were conducted in the field at two different depths. This comparative study is one among the very few attempts to characterize the surface features of the interfacial region of barnacle base plates and its adhesive strengths on various metals. The study attempts to understand the seasonal variation and the effect of fouling organisms on the test material in terms of corrosion. Furthermore, the study aims at creation of a baseline data on biofouling at Ennore Port.

2. Materials and methods

2.1. Description of the experimental site

The study was carried out in the Ennore Port waters, situated on the Coromandel Coast of India, about 24 km north of Chennai Port (location map shown in Fig. 1 of supporting information) along the...

2.2. Test panel preparation

Metallic panels, measuring 100 mm × 150 mm × 1 mm (with total area of 300 cm²), such as low carbon steel (LCS), AISI type 316L stainless steel (SS), copper (Cu), cupronickel (90:10 CuNi), titanium grade 1 (Ti) and hot dip galvanized steel (GS) were selected for this study. All panels (except GS) were prepared as per the ASTM Standard G1-03 [7]. The panels were polished using mechanized polisher to achieve a uniform surface finish, with a surface roughness ($R_s$) of <0.10 µm, cleaned with sterile water, sterilized using 70% (v/v) ethyl alcohol and immersed in acetone until use. Initial weight of the panels was recorded and the panels were secured to the polypropylene frame using a nylon rope.

The frames with the test panels suspended from a floating test rig (illustration shown in Fig. 2 of supporting information) were positioned at surface (submerged below the surface at 0.3 m) and at the bottom (submerged at 3 m depth) as designated, allowing sufficient space between the frames.

2.3. Experimental design

The study was conducted for a period of 390 days (starting from March 2007 to April 2008). Two sets of panels, each comprising of all six metal panels, were studied in replicates of four and were immersed in the seawater at two different depths (one at 0.3 m depth and the other at 3 m depth). The panels were brought to the surface every month, observed and returned to the environment immediately. One set of panel from each depth was retrieved for investigation after 185 days and the other set was retrieved on completion of 390 days. The following were the parameters studied during the experiment.

2.4. Environmental parameters

Environmental parameters, such as, temperature, conductivity, dissolved oxygen, pH, salinity and turbidity were measured using a water quality monitoring system (Hydrolab, Quanta Instruments, USA). Total suspended solids (TSS) on the test panels were estimated according to Parsons et al. [8]. The biofilm formed on the surface of the metal panels was collected using a sterile nylon brush into 500 ml of pre-sterilized seawater. From this, 10 ml of sample was filtered through a pre-weighed 0.45-µm (47 mm) nylon filter membrane using a vacuum filtration assembly. The filter paper with the retentate was dried at 60°C overnight and weighed repeatedly until a stable reading was obtained. The dry weight of the retentate gives the TSS in mg cm⁻².

2.5. Total viable bacterial count (TVC)

TVC was estimated from the biofilm sample (100 µl), after serial dilution. Twenty microlitre of the sample was plated on Zobell Marine Agar (ZMA) (Himedia, India) and incubated at 28°C for 24 h. Viable colony counts were obtained by visually counting them.

2.6. Macro fouling analysis

To study the macrofouling pattern, the panels in replicates of four were taken out at an interval of 30 days from the sea for observation and one set of test coupon was retrieved and transported to the laboratory after 185 days and the other set was taken out and studied after 390 days. Macrotomicroorganism were observed and enumerated by visual analysis. Macrofouling count was expressed as the numbers/plate (each plate measuring 300 cm² in total area).

2.7. Biofouling load

Biofouling load accumulated on the panels as a result of fouling was estimated by subtracting the initial weight of the panels from the final wet weight of the panels along with fouled organisms after 185 and 390 days.

2.8. Percentage coverage

Percentage of area covered by biofouling was estimated by superimposing transparent grid of 1 cm² with minor divisions of 1 mm² on to a digital image of the fouled panel and counting the squares under the area covered. The results were recorded as percentage of area covered on the test panel.

2.9. Weight loss and corrosion rate

Weight of the panels was recorded after cleaning as per the ASTM Standard G1-03 [7]. Weight loss in grams was calculated from their initial weight. Corrosion rate of the experimental panels was calculated using the formula [7,9] as given below in Eq. (1):

$$\text{Corrosion rate} = \frac{W \times 87,600}{A \times T \times D} \text{mpy}$$

where $W$ = weight loss in grams; $A$ = area in square centimetres; $T$ = time in hours; $D$ = density in g cm⁻³; mpy = corrosion rate in mm per year.

2.10. Surface profile analysis of test panels

Surface profile images of the samples (1 cm²) were obtained in contact mode using atomic force microscope (Model: SPM-9500, Shimadzu Corporation, Kyoto, Japan) equipped with a phase imaging system and a data processing unit. Surface profile of an area and line profile were obtained using a pre-calibrated piezoelectric scanner and the scanned images and the roughness curve of the samples were analyzed using the Shimadzu off-line SPM software version 2.32 (SPM manager version 2.11). Arithmetic mean roughness ($R_a$), maximum height ($R_h$) - a measure of the distance between the crest line and trough line, mean interval between irregularities ($S_m$) - the arithmetic mean value of many irregularities, and root mean square roughness ($R_{rms}$) of the surfaces were determined.

2.11. Surface profile of barnacle base plate

Surface profile of the interfacial surface of barnacle base plates, sliced from the barnacle of size 5-11 mm that was originally in contact with the metal surface was studied. The surface profile data were collected using atomic force microscope as performed for the metal surfaces.

2.12. Barnacle adhesion strength in shear

Barnacle adhesion strength in shear was measured as per ASTM Standard D5618-94 (Reapproved 2000); Standard Test Method for Measurement of barnacle Adhesion Strength in Shear [10] following the procedure standardized earlier [11]. Adult barnacles of 5-11 mm base diameter were selected for measuring the shear strength. The force required for detaching individual barnacles were noted and grouped according to the barnacle base diameter.
Table 1
Environmental data at Ennore Port.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>pH</th>
<th>DO (mg/l)</th>
<th>Turbidity (NTU)</th>
<th>Temp (°C)</th>
<th>Salinity (PSU)</th>
<th>ORP</th>
<th>Sp C (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>7.99 ± 0.21</td>
<td>5.71 ± 1.56</td>
<td>5.74 ± 5.38</td>
<td>26.18 ± 1.39</td>
<td>34.34 ± 0.27</td>
<td>246.22 ± 31.34</td>
<td>51.94 ± 0.34</td>
</tr>
<tr>
<td>1.0</td>
<td>7.88 ± 0.34</td>
<td>5.39 ± 1.40</td>
<td>7.99 ± 4.52</td>
<td>27.62 ± 1.12</td>
<td>34.30 ± 0.32</td>
<td>227.22 ± 31.84</td>
<td>51.94 ± 0.38</td>
</tr>
</tbody>
</table>

3.3. Macrofauna on substrates

The barnacles and polychaetes (hydrobids and nereids) were the initial macrofauna settlers, whereas the recruitment of ascidians and green mussels occurred during the latter part of the fouling process. Few incidences of recruitment of seaweeds, obelia colonies and algal mat were observed after a period of 185 days. A thin film of micro-algal mat was also observed on copper and cupronickel panels after 390 days of exposure. The non-sedentary organisms such as crabs and polychaetes (neries) were also observed during the later part of the study period occupying the dead shells and crevices.

The observation (mean ± S.D.) of biofouling on metal panels after 185 and 390 days is shown in Tables 2 and 3 and presented in Fig. 3 of supporting information. The predominant and persistent biofouls observed during the study were barnacles (dominated by Amphibalanus reticulatus Utinomi 1967) while a few representational counts of A. amphirhite Darwin 1854, A. varigatus Darwin 1854 and A. cirratus Darwin 1854 were recorded), polychaetes (Hydroides elegans Haswell 1883), green mussels (Perna viridis Linnaeus 1758), oysters (Crassostrea mauraensis Preston 1914) and colonial ascidians. Among the metals exposed, the highest barnacle recruitment was recorded on titanium followed by SS. There was no recruitment of barnacles on Cu panels, at 3 m depth, throughout the 390-day period. CuNi panels recorded the lowest recruitment.

Numerically, barnacles formed the major fouling organisms and contributed considerably to fouling load with a Pearson's correlation coefficient of 0.95 (at 0.3 m) and 0.95 (at 3 m), respectively. The recruitment pattern of barnacles between metals was significant both at 0.3 m (p < 0.01, ANOVA) and at 3 m (p < 0.05) after 390 days. Barnacle recruitment also varied significantly (p < 0.001, ANOVA) between times of exposure. The recruitment of barnacles between different metals and between two different depths also varied with high significance (p < 0.001, two-way ANOVA).

The highest average polychaete recruitment was observed on Ti panels after 122 days coinciding with the onset of the south-west monsoon. Cu panels showed the lowest polychaete recruitment of 2 per plate after 390 days at 0.3 m. There was no polychaete recruit- ment on copper at 3 m. Density of polychaetes recorded at various time points during the 390-day study period varied significantly, for example, between metals at 0.3 m depth (p < 0.001, two-way ANOVA), at various time points/season at 0.3 m depth (p < 0.05), between metals at 3 m depth (p < 0.001, two-way ANOVA) and between seasons/time point at 3.0 m depth (p < 0.05).

The recruitment of green mussels during the latter part of the study had a strong impact on the fouling load on the substrates showing a Pearson's correlation coefficient of 0.98 at 0.3 m and 0.95 at 3 m, respectively. Oyster and ascidian recruitment was completely absent on copper. Maximum oyster recruitment was observed on SS panels after 185 days at 3 m depth. The maximum ascidian recruitment was observed on Ti panels after 390 days. The recruitment pattern of oyster varied significantly between metals (p < 0.001) and between different depths (p < 0.001).

The variation in the various fouling organisms (barnacles, polychaetes, oysters, mussels and ascidians) was highly significant (p < 0.001 at surface, p < 0.05 at 3.0 m depth), whereas the variation in recruitment between various non-toxic/less toxic metal panels (SS, Ti and GS) was not significant (p < 0.05).

2.13. Scanning electron microscopy of barnacle base plate

Scanning electron microscopy (SEM) of the barnacle base plates was carried out using a $S-3400N$ electron microscope (Make: Hitachi) operated between 0.3 and 30 kV and equipped for a magnification of 300,000×. The barnacle base plates were prepared as mentioned under AFM and the features on their interfacial surface were studied.

2.14. Statistical analysis

Replicates of data from four experimental panels for each metal and time point were collected and the results reported as mean ± S.D. Data were tested for normality and ANOVA was carried out to study whether the groups differed and if the ANOVA-calculated p-value was significant (p < 0.05). Factor analysis with 'varimax rotation' was used for extracting and deriving principal components. Cluster analysis was performed to assess the inter-relationships among the parameters, and Pearson's correlation coefficient was used in the correlation matrix. Differences were taken as statistically significant when p < 0.05 and 0.01, respectively.

3. Results

Because of the loss of LCS panels as a result of excessive corrosion, the data pertaining to biofouling and other parameters on LCS panels were limited to panels studied at surface (0.3 m) for 185 days and insufficient in some cases for statistical analysis. In such cases, to avoid statistical bias and error, the data on LCS have been excluded from the statistical analysis.

3.1. Environmental parameters

The environmental parameters measured during the study were within the normal levels (Table 1) throughout the period with the exception of turbidity values, which were highly unstable at the surface compared with the turbidity levels at the bottom, although the turbidity levels at the bottom were moderately higher than those recorded at the surface. Moderate to heavy rains were witnessed during July to September with the onset of monsoon.

Total suspended solids (TSS) observed on various metals, exposed to different water depths, during the period of the study are given in Table 2. Their difference in variation on various metal panels was highly significant (p < 0.01).

3.2. Total viable count

TVC on metal panels is presented in Table 2. Analysis of data on TVC showed that Pearson's correlation coefficient between CuNi and Ti (0.880), and SS and GS (0.978) are significant at the 0.05 level. TVC, after 185 days, between panels at 0.3 and 3 m depths showed a strong negative Pearson's correlation coefficient (-0.902) significant at 0.05 level. The difference in variation in the total viable counts between depths and different time points was observed to be nonsignificant (p > 0.05).
Table 2

Evoking and concomitant sex at Emmeram Port.

<table>
<thead>
<tr>
<th>Metric</th>
<th>TSS (g/1000 cm²)</th>
<th>TSS (g/1000 cm²)</th>
<th>Bedrock load (g/1000 cm²)</th>
<th>Area tested X</th>
<th>Concomitant sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 days</td>
<td>300 days</td>
<td>100 days</td>
<td>100 days</td>
<td>100 days</td>
</tr>
<tr>
<td>Surface (10 mm depth)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>0.50 ± 0.53</td>
<td>0.50 ± 0.53</td>
<td>0.50 ± 0.53</td>
<td>0.50 ± 0.53</td>
<td>0.50 ± 0.53</td>
</tr>
<tr>
<td>LO</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
</tr>
<tr>
<td>SS</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
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<tr>
<td>G</td>
<td>0.40 ± 0.40</td>
<td>0.40 ± 0.40</td>
<td>0.40 ± 0.40</td>
<td>0.40 ± 0.40</td>
<td>0.40 ± 0.40</td>
</tr>
<tr>
<td>MS</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>Bacteria (100 mm depth)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
</tr>
<tr>
<td>LO</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
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<tr>
<td>SS</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
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<tr>
<td>G</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
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<tr>
<td>MS</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
</tr>
</tbody>
</table>

* No data available.

Table 3

Major evoking organs in emerald ground at Emmeram Port.

<table>
<thead>
<tr>
<th>Metric</th>
<th>No. of counts (100 cm²)</th>
<th>No. of counts (100 cm²)</th>
<th>No. of counts (100 cm²)</th>
<th>No. of counts (100 cm²)</th>
<th>No. of counts (100 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue (10 mm depth)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>0.50 ± 0.53</td>
<td>0.50 ± 0.53</td>
<td>0.50 ± 0.53</td>
<td>0.50 ± 0.53</td>
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<tr>
<td>LO</td>
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<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
<td>0.30 ± 0.30</td>
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<tr>
<td>SS</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>G</td>
<td>0.40 ± 0.40</td>
<td>0.40 ± 0.40</td>
<td>0.40 ± 0.40</td>
<td>0.40 ± 0.40</td>
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<tr>
<td>MS</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
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<tr>
<td>Bacteria (100 mm depth)</td>
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<td></td>
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<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>SS</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
</tr>
<tr>
<td>G</td>
<td>0.20 ± 0.20</td>
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<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>MS</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
</tr>
</tbody>
</table>

* No data available.
indicating that chemically inert substrates have less influence on fouling recruitment.

Factor analysis showed that, component 1 comprising of barnacle polychaete and oyster counts, accounted for 72.33% of variation, while component 2 comprising mussel and ascidian accounted for 23.62% of the variation (Table 4). The dendrogram derived from the cluster analysis of macrofouling on various metals, showing variation between the metal panels exposed to surface waters (0.3 m) for 185 days, is illustrated in vertical icicle plots in Fig. 1.

3.4. Biofouling load on metal panels

The maximum fouling load at 0.3 m depth was observed on SS panels after 185 days. Furthermore, fouling load on Cu, SS and Ti panels after 390 days was low compared with the values observed after 185 days. Conversely, the fouling load on GS panels at 3 m after 390 days increased significantly compared to 185 days (Table 2), and the enormous growth of recruited oysters on GS panels was conspicuous (Fig. 3.5c of supporting information). Fouling load between depths after 390 days showed a high positive Pearson’s correlation coefficient of 0.99. On the whole, the fouling load varied significantly between metals (p < 0.001) and between depths (p > 0.01).

3.5. Percentage area coverage by biofouling

The highest average surface area coverage by macrofouling organisms was observed on LCS panels, and Cu remained almost 100% fouling free (~0.001 coverage) after 185 days (6 months). One of the distinct observations was the complete coverage of SS panels by oysters after 390 days of exposure, which significantly increased the fouling load.

3.6. Corrosion rate of metals

Corrosion rates of metal at different depths are presented in Table 2 and presented in Fig. 4 of supporting information. Among the materials used in this study, LCS showed the uniform and highest corrosion rate. This was followed by Cu, CuNi and SS. In LCS, the corrosion rate was higher at 0.3 m depth compared with the coupons immersed at 3 m depth. The corrosion in SS was found to be more localized forming corrosion pits, which were prominent on surfaces beneath barnacle settlement (shown in Fig. 3.3d of supporting information). Corrosion was absent in Ti up to 5,000 days. Fig. 3 of supporting information shows the appearance of the metal panels before and after cleaning. The differences in the corrosion rates between various metal panels and between panels immersed at different depths were highly significant (p < 0.001). The results derived from the cluster analysis on the corrosion behaviour of various metals are illustrated in the vertical icicle plot (Fig. 5 of supporting information).

3.7. Surface profile of metals and barnacle base plate

Atomic force microscopy of surface profiles of metals and barnacle interfacial surfaces are shown in Table 5. Surface profile analysis of the experimental coupons indicates that Ti had the highest root mean square roughness (Rms), arithmetic mean roughness (Rz) and maximum height (Rmax) and had the shortest mean distance between irregularities, given as Sm. Whereas the CuNi metal surfaces had the lowest Rz, Rmax and Rms, the interfacial surface of the barnacle base plate formed on CuNi surfaces showed the highest Rz and Rms values among the samples studied. A positive correlation was observed between roughness of surface (Rz) exposed at 0.3 m depth and TSS with a Pearson’s correlation coefficient of 0.89. TSS correlated positively with occurrences of fouling organisms, area covered by fouling organisms and fouling load. Although the same trend is observed at 3 m depth, the relationship between roughness and TSS was weak (Pearson’s correlation coefficient = 0.62). A comparative chart of 10 μm line profile of barnacle base plates is shown in Fig. 6 of supporting information. All the surface profile features (Rz, Rmax and Rms) of metal panels seem to have a positive correlation with the profile of the interfacial surface of barnacle base plate.

3.8. Barnacle adhesion strength in shear (BASS) on metals

The adhesion strength in shear of barnacles attached to various metal substrates and according to their size distribution is presented in Table 6. The highest strength in shear of barnacles was exhibited by barnacles of size 8–9mm diameter attached to all the five metal substrates studied. Among the metals tested, the strength in shear of barnacles was exhibited in the following order: LCS > Ti > SS > CuNi > Cu.

The average adhesion strength in shear of barnacles of 5–11 mm base diameter on various metals showed a weak positive correlation with Rz (maximum height) component of the surface profile of barnacle base plate. The difference in BASS between different metal substrates and between barnacles of various base diameters was highly significant (p < 0.001, two-way ANOVA). The BASS showed greater positive Pearson’s correlation coefficient of 0.73 with Sm (mean interval between irregularities) of the metal surfaces. Positive Pearson’s correlation coefficient of 0.59, 0.40 and 0.58 was observed between BASS and Rmax, Rz and Rms, respectively.