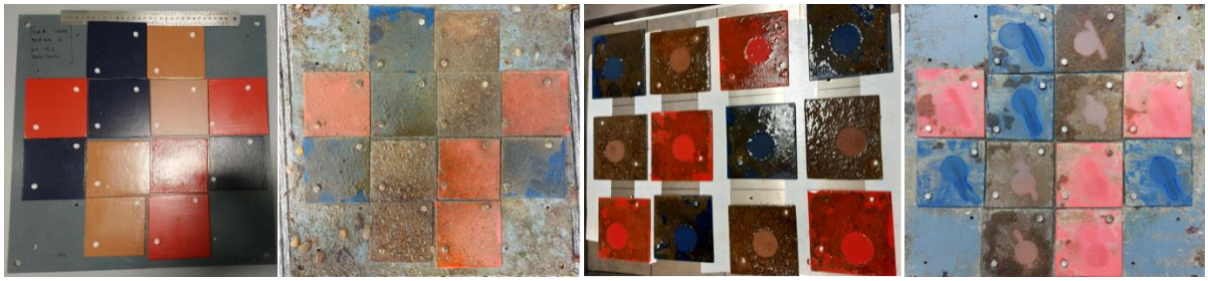
PML | Applications Ltd

Comparing weight loss, metal leaching and leachate toxicity for Coppercoat and two conventional Self-Polishing coatings under Standardised Scrubbing and Pressure-washing.

Final Report: Version 1.2



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Through our parent company Plymouth Marine Laboratory (a world-renowned marine research organisation with over 500 global partners) PML Applications has access to cutting edge marine technology and knowledge, enabling our experts to provide you with independent and impartial advice, assessments, surveys and testing.

Operating in a complex, and often extreme, environment presents numerous challenges, the implications of which can be costly to operators and to the environment itself. We provide services, expertise and guidance to a diverse range of commercial customers and through a greater understanding of the marine environment both time and money can be saved.

Specialities

Biofouling, Ballast Water, Ballast Water Treatment Systems, Marine Surveys, Marine Environment Assessment, Remote Sensing, Earth Observation, Harmful Algal Blooms

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2 Executive summary

The effect of simulated hull cleaning was tested on experimental panels coated with Coppercoat and two conventional self-polishing coatings (SPCs), namely Cruiser 250 and Micron 350. Standardised scrubbing (30 seconds) and standardised pressure washing (3 sec) were used to simulate cleaning.

Eight panels were used for each coating. On four panels, simulated cleaning was conducted immediately after the panels were removed from the water. On the other four panels, cleaning was conducted after the panels had been removed from the water and left to dry under ambient conditions for four hours.

The concentration of leached metals in the wash-off water and its toxicity were tested at three timepoints; at 2, 4.5 and 6.5 months after immersion. Total weight loss was tested at the end of the trial (6.5 months).

Coppercoat showed a lower copper content in the wash-off water (59 – 88 % reduction) compared to the other coatings. Coppercoat showed no zinc release above background levels in the majority of the trial.

Coppercoat showed no acute toxicity to the microalga *Phaeodactylum tricornutum* after 48 hours, while both SPCs tested showed significant toxicity expressed as a reduction in growth rate.

Finally, Coppercoat lost significantly less weight (82-86 %) compared to the two SPCs tested.

The small losses in both copper and overall weight during simulated cleaning of Coppercoat compared to the two SPCs tested, reflects the fundamental difference in the nature of these coatings. Coppercoat is a hard anti-foul coating that is not meant to lose much material, while Cruiser 250 and Micron 350 are self-polishing coatings, which are meant to slowly erode in the water.

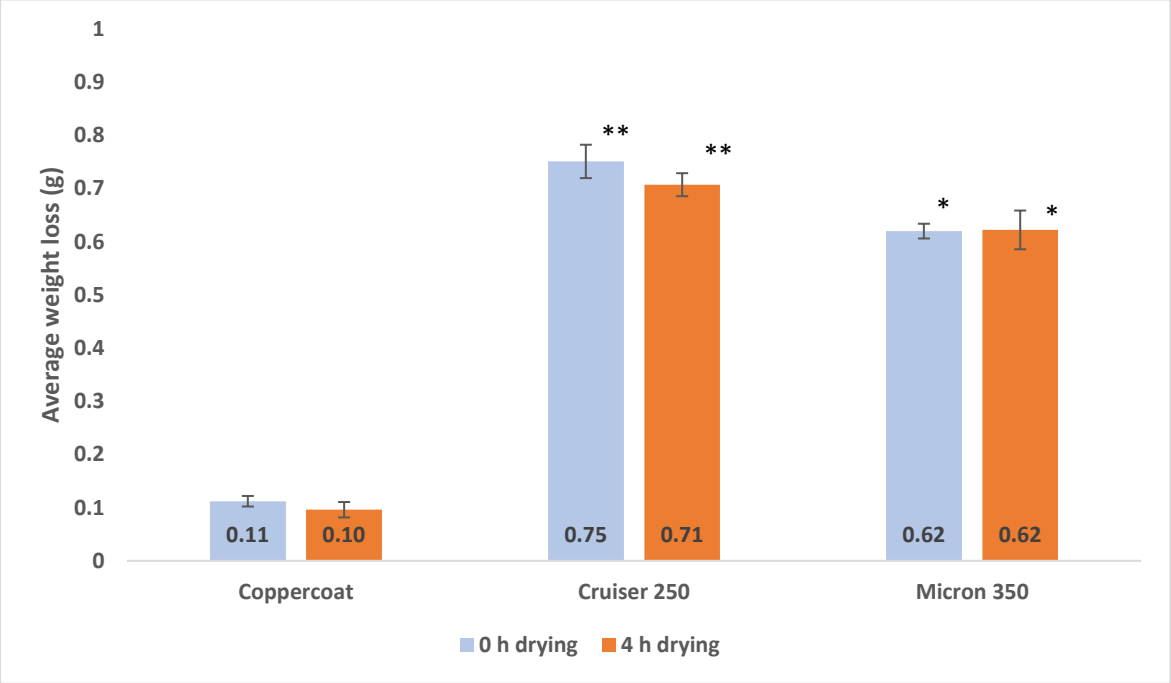

Figure 1: Panel weight loss from different coatings after three rounds of standardized scrubbing and pressure-washing. Bar height represents mean average (n=4) and error bars represent ± 1 standard deviation. Asterisks denote significance groups, i.e. bars with different number of asterisks denote averages which are significantly different from each other ($p<0.05$).

3 Background and Scope

- The client, Aquarius Marine Coatings Ltd., is the manufacturer of Coppercoat, a hard, epoxy-based antifouling coating impregnated with pure copper powder which acts as a biocide, giving the coating its antifouling properties.

- The client contracted PML Applications Ltd. to carry out trials to compare the metal leaching of Coppercoat with two self-polishing (soft) antifouling coatings under a simulated cleaning regime.

- The client supplied painted panels of all three coatings, which were immersed at PML Applications' test site at Millbay Marina (Plymouth, UK) for periods of two and three months, before being subjected to various tests.

- Standardised scrubbing and standardised pressure-washing were selected as the simulated cleaning treatments.

- Wash-off water was collected for metal analysis (copper and zinc) and toxicity tests.

4 Summary of Methodology

4.1 Coatings Tested

The main coating tested in this trial is Coppercoat, a hard, epoxy-based coating containing 82 % w/w of fine Cu powder when cured. In contact with seawater, Cu reacts to form Cu_2O, which in turn, provides the coating with antifouling properties.

To provide valid comparisons, two commercial self-polishing coatings were tested alongside Coppercoat, to assess metal leach rates; Cruiser 250 (blue colour panels), and Micron 350 (red panels). Both of these coatings are manufactured by Akzo Nobel (International Paint) and both are self-polishing copolymer (SPC) coatings. Their active ingredients are dicopper oxide (Cu_2O) and zinc oxide (ZnO).

All coatings were selected, procured, and applied by the Client. Coated panels were supplied by the Client to PML Applications Ltd. for testing.

4.2 Testing Overview

The experimental procedure's main steps were as follows:

Upon receipt:

- Receive panels
- Drill panels; remove any loosely attached flakes
- Weigh panels; record weights (0.001 g sensitivity scales)

Simulated cleaning procedure (at three 2 month intervals):

- Attach panels to a backing sheet
- Immerse panels at Millbay marina for 2 months
- Remove panels from water
- “No drying” panels removed from backing sheet and placed in individual, zip-lock bags with 20 mL of filtered seawater
- “4-hour drying” panels were left on the backing sheet and allowed to air dry at ambient temperature for four hours before the next step
- Panels were subjected to standardised scrubbing treatment
- Panels were subjected to standardised pressure washing treatment, wash-off water was collected
- Panels re-attached to the backing sheet and re-submerged at Millbay marina
- Wash-off water was split into two parts; 50 mL for toxicity testing and the rest (~250 mL) for metals analysis
- One part of wash-off water was sent for metals analysis (Cu & Zn)
- One part of wash-off water was used for toxicity testing

Final weight loss calculation:

- Gently clean panels with water and sponge, to remove all biofouling and silt
- Dry for 48 h at 50 °C.
- Weigh post-drying
- Allow panels to re-absorb atmospheric moisture
- Re-weigh (final weight)
- Store

4.3 Panel Preparation and Immersion at Millbay Marina

Aquarius Marine Coatings Ltd. supplied coated plastic panels of approximately 100 x 100 mm dimensions (range 90-120 mm). Ten replicate panels were provided of each of the three coatings to be compared (see section 4.1). Eight of these panels were prepared for testing and two were kept as spares. Each panel was given a unique, identifying code written on the back with a waterproof, permanent marker pen. Panels were then drilled, using a 5 mm drill bit in two corners to attach to the backing sheet. Loosely adhered particles of paint and plastic were carefully removed and the drill holes were smoothed using a round file. Each panel was then weighed on an Ohaus PR series fine balance (1 mg sensitivity) and initial weights were recorded.

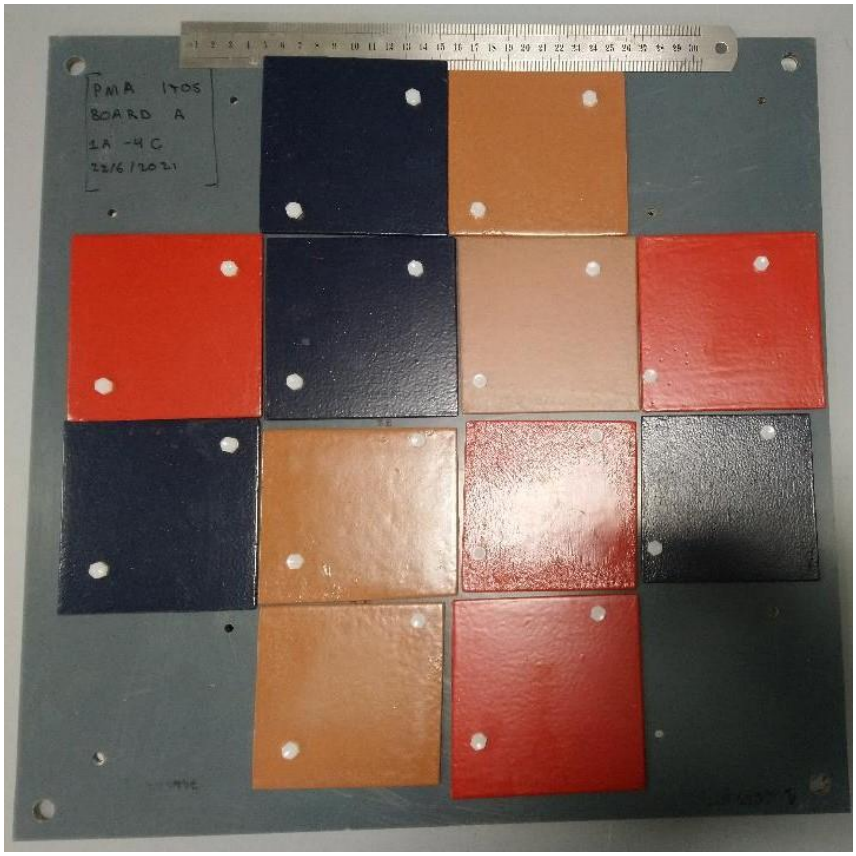

Figure 2: Backing sheet holding four replicate panels of each of three test coatings: Cruiser 250 (blue), Coppercoat (copper/brown) and Micron 350 (red).

Following weighing, the panels were attached to a backing sheet made of PVC with pre-drilled holes. Panels were attached using plastic nuts and bolts (5 mm). Two backing sheets were populated with 12 panels each, i.e. four replicates of each test coating (n=4) (Figure 2). These two backing sheets would serve as different treatments, with different drying times allowed before testing (see section 4.5).

The backing sheets were then immersed at PML Applications' test site at Millbay marina on the 23rd of June 2021. Backing sheets were submerged at a horizontal position with the test panels facing down, at a depth of 1.5 m.

4.4 Testing Timepoints

Three testing timepoints were set:

- 2 months submersion (23 Aug 2021),
- 4.5 months submersion (8 November 2021), and
- 6.5 months submersion (11 January 2022)

4.5 Panel Recovery and Drying Times

After the pre-determined immersion times, the backing sheets were recovered from the marina for testing. The panels on each of the two backing sheets served as different treatment. The panels on backing sheet A (n=4) were not allowed to dry before testing. This was achieved by removing the panels from their backing sheet immediately upon recovery and placing each panel in labelled zip-lock bags containing 20 mL of filtered seawater.

The panels on backing sheet B (n=4), were allowed to dry for 4 hours at room temperature (18 ±2 °C), in a temperature-controlled room. The rationale behind this treatment was to simulate a scenario where a vessel cannot be immediately treated to remove fouling, but instead is left to dry for a few hours in-between tides before pressure-washing or scrubbing.

4.6 Standardised Scrubbing

Following recovery, panels were first subjected to a standardised scrubbing test.

4.6.1 Rig Description

The standardised scrubbing rig was constructed from an orbital shaker fitted with an external frame and a rigid aluminium top-plate (Figure 3).

Specifically, the orbital shaker, shaking plate (the bottom-plate) housed the panels, which were kept in place by double-sided tape. An external frame was constructed to fit around the shaker and bottom plate, with four upright rods where the top-plate was slotted in place.

The top plate (Figure 3B) was constructed out of a rigid aluminium sheet with 12 pockets (20 mm diameter, 5 mm depth) drilled in to house the scrubbing pads. Velcro attachments were glued in each of these pockets to ensure the scrubbing pads remained in place during testing. The weight of the top-plate was 3.71 kg. As the top plate rested on the bottom plate under its own weight, this weight provided the scrubbing pressure and could be adjusted by adding more weights on the top-plate. No extra weight was added in this instance, however.

The scrubbing pads were cut into 20 mm diameter discs (7 mm thickness), from a larger sheet (RS Components, UK, Stock No.: 898-8286) using a sharpened corer.

The rotation diameter of the shaker was 20 mm. In combination with the 20 mm scrubbing pads, this resulted in a 40 mm disc-shaped scrubbing area (Figure 3C).

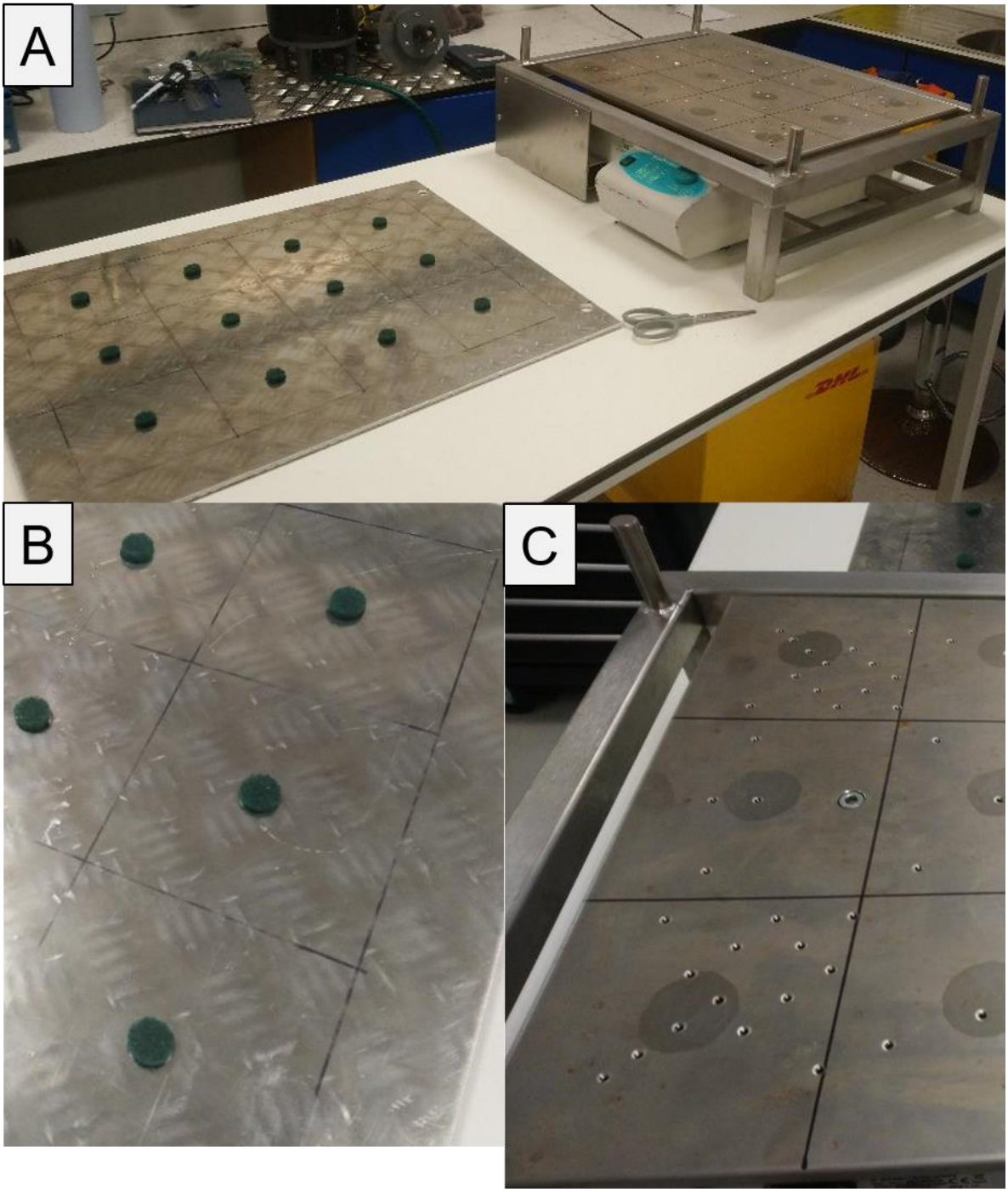

Figure 3: Scrubbing test rig: A, shaker, frame and top-plate; B, detail of the top-plate with scrubbing pads attached in place; C, detail of the bottom plate showing the scrubbing area after a test-scrub.

4.6.2 Testing Procedure

- Panels were placed on the bottom plate and 1 mL of filtered seawater was added to the centre of the panel.
- Fresh scrubbing pads were placed in the Velcro pockets of the top-plate before it was placed on top of the panels.
- Scrubbing was carried out at a speed of 100 rpm, for 30 seconds.
- Following scrubbing, all scrubbing pads were removed and placed in the labelled zip-lock bags with 20 mL of filtered seawater, previously used to transport the panels from the marina to the laboratory.
- Once in the zip-lock bags, the scrubbing pads were squeezed 10 times to release any coating and metals absorbed during scrubbing (Figure 4), removed from the bags and discarded.

Figure 4: Zip-lock bags and scrubbing pads after squeezing coatings absorbed during the scrubbing test.

4.7 Standardised Pressure Wash

Following scrubbing, panels were tested by pressure-washing.

4.7.1 Rig Description

4.7.1.1 Pressure washer

- The pressure washer used for this test was a Kärcher K4 Full Control.
- The pressure washer was used at its maximum pressure (130 Bar nominal) with the full control power jet adapter. This created a fan-shaped water spray, resulting in a line of pressure washed area on the panel (as shown in Figure 5).
- The pressure-washer was connected to a mains fresh water supply, and the flow rate of the pressure washer at the settings used was 90 mL s^{-1} (±10 mL).

Figure 5: Trace of pressure-wash on a test panel coated with Micron 350. Contrast of the image has been enhanced for clarity.

4.7.1.2 Housing

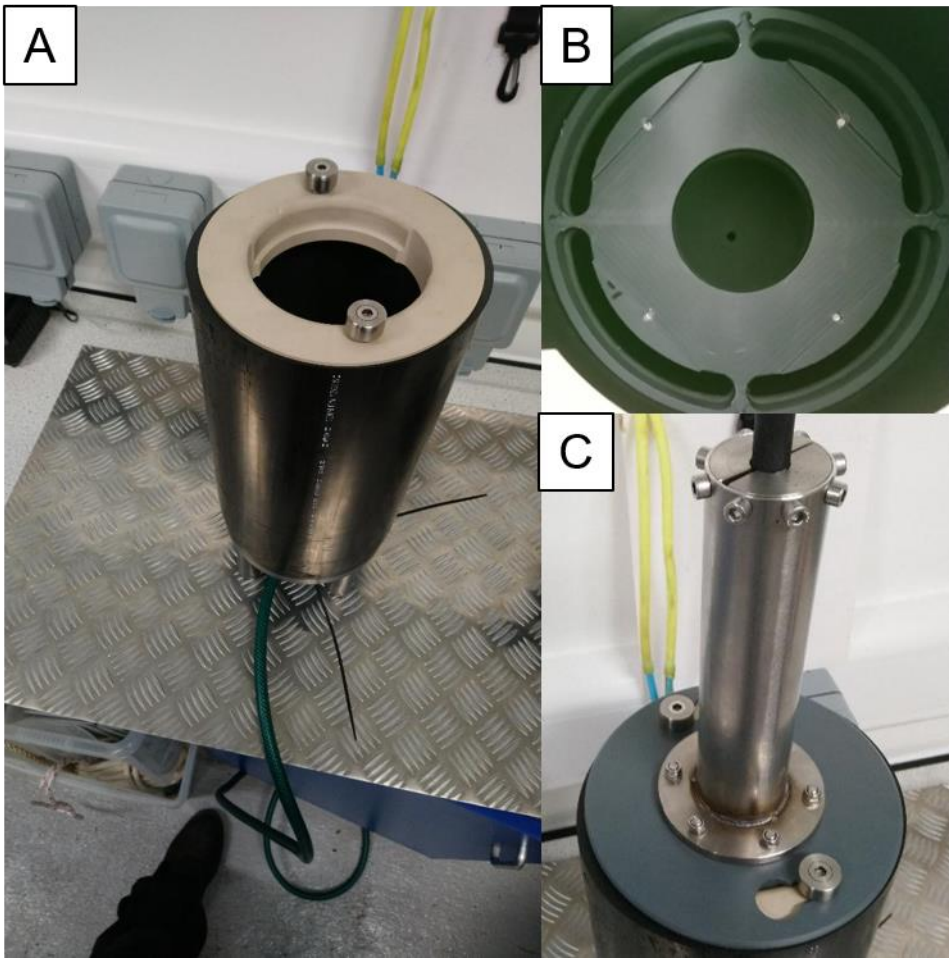

Figure 6: Standardised pressure-washing housing. A, Main body and outlet hose; B, Panel holder; C, twist-locking lid housing the pressure washer nozzle.

The pressure washer housing consisted of three main parts shown in Figure 6. These were the main body (Figure 6A), which included the housing cylinder, aluminium bottom plate and outlet hose, the panel holder (Figure 6B) which consisted of a 120x120 mm indent square to hold test panels in place, and the lid (Figure 6), which housed the pressure washer nozzle and a twist-locking mechanism.

Both the panel holder and lid ensured that pressure washing occurred at a fixed impact angle (90°) and distance (150 mm), while the main body and twist locking mechanism ensured that all wash-off water could be collected and analysed with no sample loss.

4.7.2 Testing Procedure

- After standardised scrubbing, panels were placed in the pressure washer panel holder and the lid was twist locked.
- Each panel was pressure-washed for 3 seconds, measured using a timer, and the wash-off water was collected in pre-labelled, 1 L, PET bottles.
- Subsequently, panels were removed and re-attached to the backing sheet.

Between samples, the housing was pressure-washed “empty” for 5 seconds and the water was collected in a waste bucket to ensure the independence between samples. After all of the samples had been processed, six process controls were performed, where a wash was performed without panels present in the housing.

4.8 Post-sampling Handling and Storage

After pressure-washing was complete, the 20 mL of filtered seawater and the residue from the scrubbing pads (see section 4.6.2), was added to the sample bottles (Figure 7). The sample bottles were then thoroughly shaken, and 50 mL was removed for toxicity tests and stored at 4 °C. The remaining sample volume (~270 mL) was sent for ICP-MS analysis for copper (Cu) and zinc (Zn).

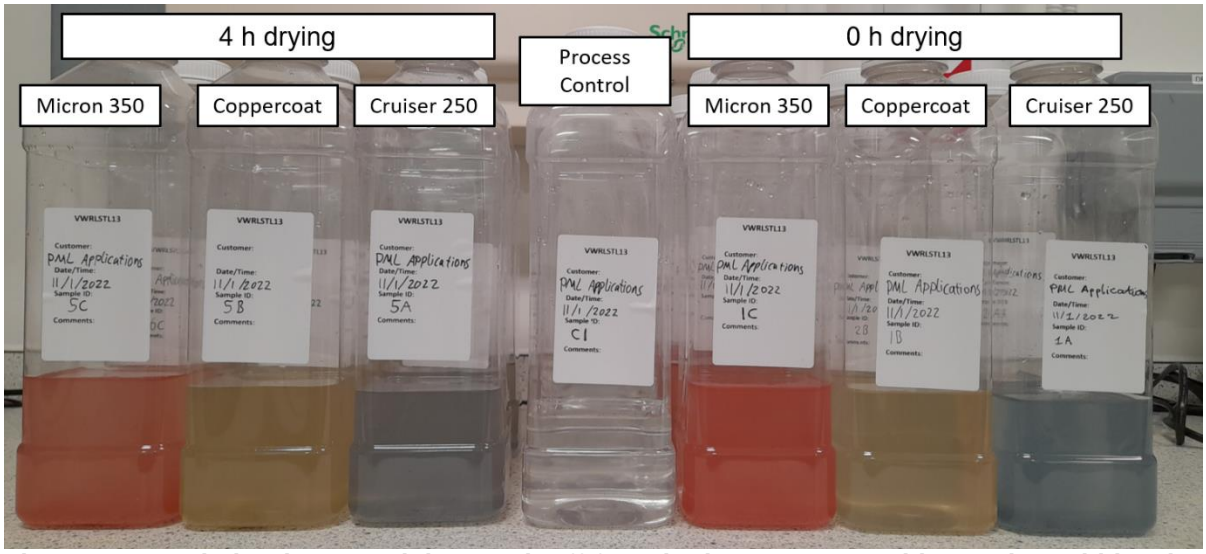

Figure 7: Sample bottles containing wash-off from both pressure-washing and scrubbing (T3 sampling point – January 2022).

4.9 ICP-MS for Metal Detection

ICP-MS was carried out by a subcontractor, ALS Environmental Ltd. The matrix used was Process water and the method used was WAS076. According to the method principle: “Metals are determined by ICP-MS after heated dissolution in the presence of nitric acid. The digestion pre-treatment ensures that any suspended or colloidal forms are converted to soluble forms.” (https://www.alsenvironmental.co.uk/media-uk/method_statements/coventry/waste-water-inorganics/method-statement-was076.pdf).

4.10 Toxicity Test

4.10.1 Test organisms and growth conditions

The test organism was the ubiquitous microalga *Phaeodactylum tricornutum* strain CCAP1055/15, isolated from Blackpool, UK. This species was chosen because of its worldwide distribution, robust growth and because it is commonly used in these types of assays (Cid et al., 1995; International Organisation for Standardisation, 2016; Tovar-Sanchez et al., 2019; Wei et al., 2014) .

Stock cultures were maintained in filtered seawater f/2 media (FSW-f/2) (Guillard and Ryther, 1962). Culture maintenance and toxicity tests were carried out in a temperature-controlled growth room with a temperature of 21 °C and a light:dark cycle of 16:8 hours. Light levels were 20 (±5) µmol PAR photons m^{-2} s^{-1}.

4.10.2 Media preparation from wash-off water

“Washoff f/2” was prepared by adding 20 g/L of tropical aquarium salts to the 50 mL washoff samples kept for toxicity tests and dissolving by vortexing before adding 50 µL of a 1000x concentrated, sterile filtered, f/2 nutrient, trace metal and vitamin mix. Wash-off f/2 was stored in a fridge at 4 °C.

4.10.3 Toxicity test setup

Toxicity tests were set up in 24 well plates (Figure 8). Four well plates were set up in total. Each well plate was inoculated with wash-off media from one panel of each coating and each drying time in triplicate giving six columns for the different panels and three rows for the technical replicates for each panel. The bottom row was inoculated with the three process control wash-off media in duplicate.

The functional volume of each well was 2 mL. 1.8 mL of wash-off media and 0.2 mL of algal culture were added to each well.

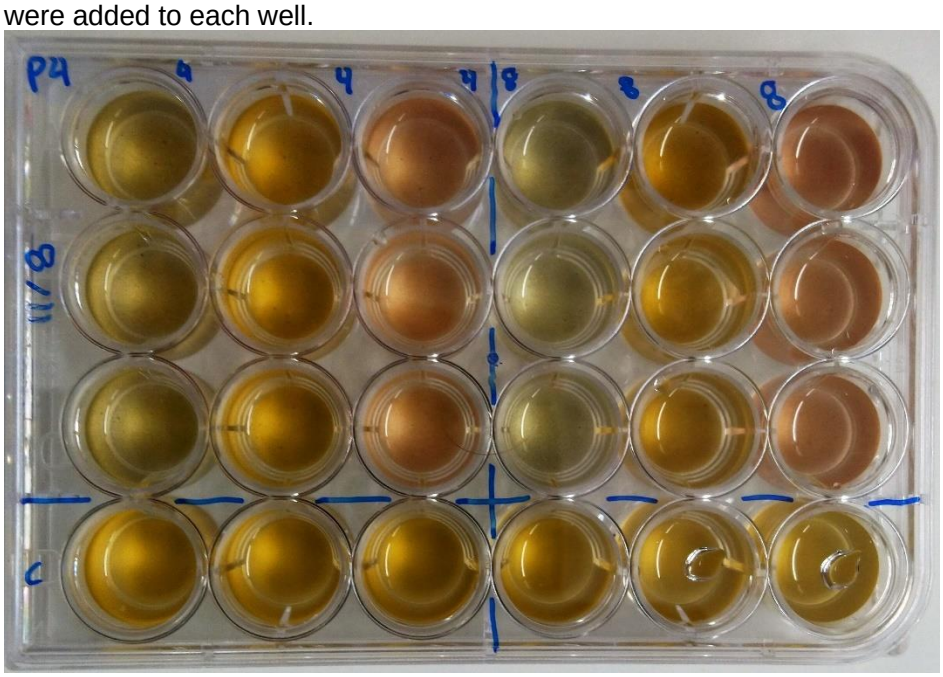

Figure 8: Example microwell plate for the toxicity trial after 48 hours of incubation. The plate is split into four compartments; top left – 0 hours drying, top right – 4 hours drying, bottom left – controls 1-3, bottom left – controls 1-3 replicate. Columns from left to right represent Cruiser 250, Coppercoat and Micron 350 respectively for each of the two drying times. The three top rows represent different technical replicates for each coating. The bottom row represents process control replicates.

All microwell plates were incubated in a temperature-controlled growth room (see section 4.10.1) for 48 hours with chlorophyll fluorescence being recorded at times 0, 24 and 48 hours. Chlorophyll fluorescence was chosen as a toxicity indicator because chlorophyll fluorescence changes both with growth and with the relative health of the microalgae. Furthermore, due to its unique fluorescence spectra, chlorophyll fluorescence data can be recorded with no interference from other materials in the wash-off such as paint and metal particles.

4.10.4 Plate reader setup and data acquisition

Chlorophyll fluorescence was recorded using a ClarioStar plate reader. The excitation wavelength was 480 nm and the emission wavelength was 680 nm. Gain was adjusted to 1740 and focal depth was set to 9.6 mm.

Chlorophyll fluorescence was read at 0, 24 and 48 hours. Before readings were taken, algae were re-suspended by mixing each well with a pipette.

4.11 Total weight loss measurement

After all three analysis timepoints had been performed, all panels were gently cleaned to remove any biofouling and silt, before being dried at 50 °C for 48 hours. Following drying, panels were weighed immediately and again after being allowed to equilibriate with ambient moisture levels for several weeks.

The moisture equilibriation was necessary because the panels were not dried before the initial weighing, therefore atmospheric moisture was included in the initial measurements. Allowing an equilibrium with atmospheric air moisture before the final weighing ensured that the weigh loss estimates were as accurate as possible.

Final weights were recorded on an Ohaus PR series fine balance (1 mg sensitivity).

4.12 Statistical Analysis

4.12.1 Metal Content of Wash-off Water

Metal concentrations derived from ICP-MS analysis were averaged from the four replicate panels for each coating and presented as bar charts. Standard deviation was used for error bars. Significant differences were assessed at the 95 % confidence level (α=0.05). Two-tailed t-tests assuming unequal variances were used to assess significant differences and derive p-values.

4.12.2 Algal growth data processing

Algal growth rates were calculated from chlorophyll fluorescence data between t=24 hours and t=48 hours. Growth rate and doubling time was calculated using equation 1 and 2 below:

Growth rate equation (1):

$$\mu = \frac{\ln\left(\frac{X_2}{X_1}\right)}{t_2 - t_1}$$

with μ denoting growth rate, X_2 and X_1 denoting the chlorophyll fluorescence at 48 h and 24 h respectively, and t_2 and t_1 denoting the respective times in hours.

Doubling time equation (2):

$$T_d = \frac{\ln(2)}{\mu}$$

T_d denotes doubling time while μ denotes growth rate.

5 Results

5.1 Metal Leaching and Effects of Drying Time

5.1.1 First Time Point (2-months submersion)

Panels coated with two self-polishing coatings (Cruiser 250, Micron 350 by Akzo Nobel) were compared to the Client's epoxy-based, hard AF coating, Coppercoat (Aquarius Marine Coatings). Four replicate panels for each coating were subjected to a standardised scrubbing followed by pressure washing with the wash-off water being collected for metal analysis (Cu and Zn). Figure 9 shows panels after testing, while the metal contents of wash-off waters are summarised in Table 1, Figure 10 and Figure 11.

Figure 9: Representative image of a backing sheet and panels after standardised scrubbing, pressure-washing and post-testing re-assembly. Blue panels represent Cruiser 250, brown/copper panels represent Coppercoat and red panels represent Micron 350. Note the circular scrubbing marks and the linear pressure-washing marks. Also note that in all cases, the treatments were powerful enough to remove all fouling from the treated areas.

Table 1: Summary of ICP-MS results for copper and zinc concentration for different coatings and process control.

	Copper (mg/L)		Zinc (mg/L)	
	No drying	4 h drying	No drying	4 h drying
Process Control	0.037	0.037	0.140	0.140
Coppercoat	3.175	1.600	0.350	0.215
Cruiser 250	7.775	5.650	3.950	4.325
Micron 350	12.150	11.050	6.375	8.875

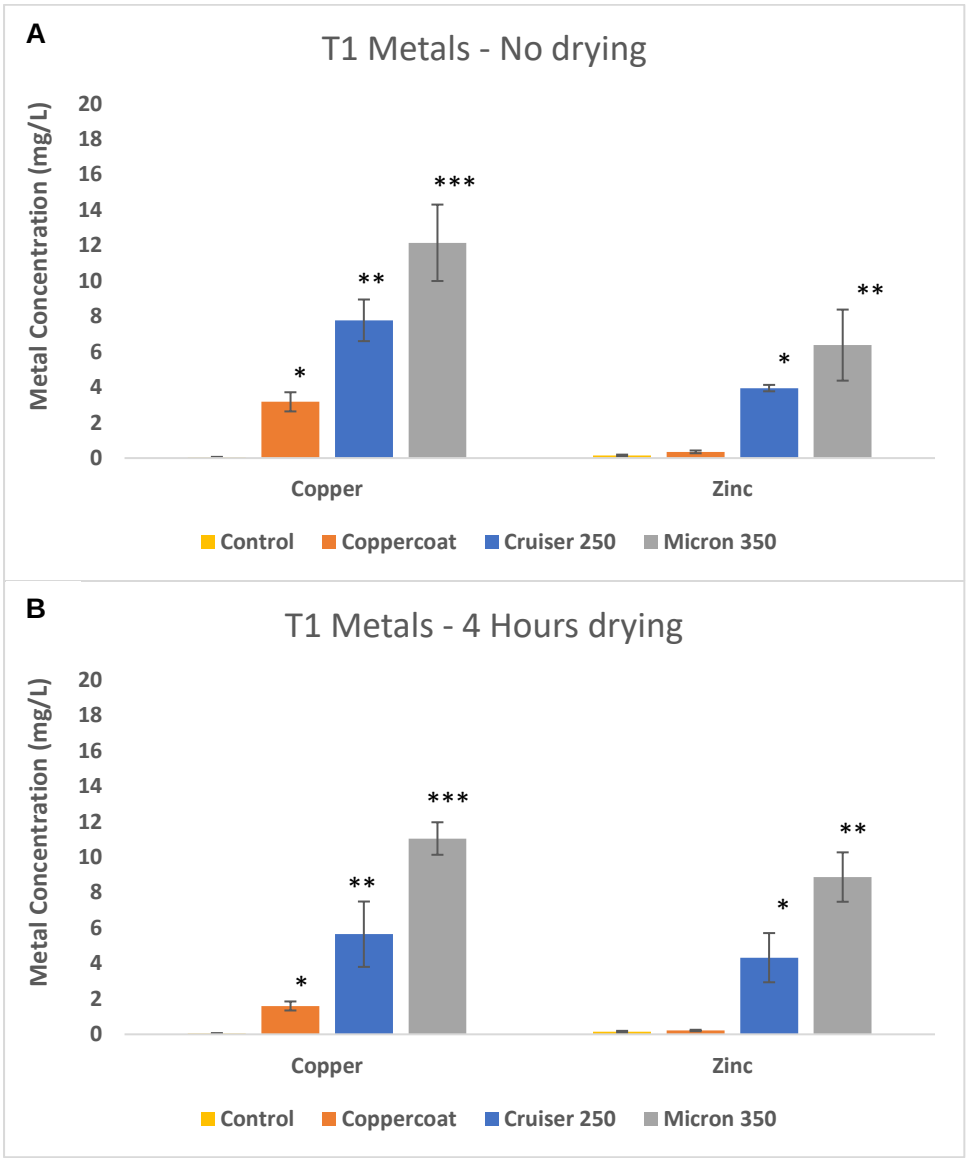

Figure 10: Copper and Zinc content of wash-off water after standardised scrubbing and pressure-washing. A, panels processed immediately without being allowed to dry, and, B, panels allowed to dry for 4 hours. Error bars represent ±1 standard deviation of four replicate panels (n=4). Asterisks represent groups of significant difference in metal concentration between coatings for a particular metal and drying time ($p>0.05$).

In both treatments (zero hours drying and four hours drying), and both metals (Cu and Zn), Coppercoat always showed a significantly lower ($p<0.05$) metal content than either Cruiser 250 or Micron 350 (Figure 10).

Following identical treatment, Coppecoat showed 59.2 % to 73.9 % less copper leaching in the wash-off water than Cruiser 250 and Micron 350 respectively (0 h drying) and 71.7 % to 85.5 % less copper leaching in the wash-off water than Cruiser 250 and Micron 350 respectively (4 h drying).

In terms of zinc, Coppecoat showed no zinc release above the levels of the process control ($p>0.05$). This is mainly because Coppecoat does not contain zinc as an active ingredient in its formulation, in contrast to the two coatings it was compared to.

The effects of drying time on metal leaching after standardised scrubbing and pressure-washing, are summarised in Figure 11.

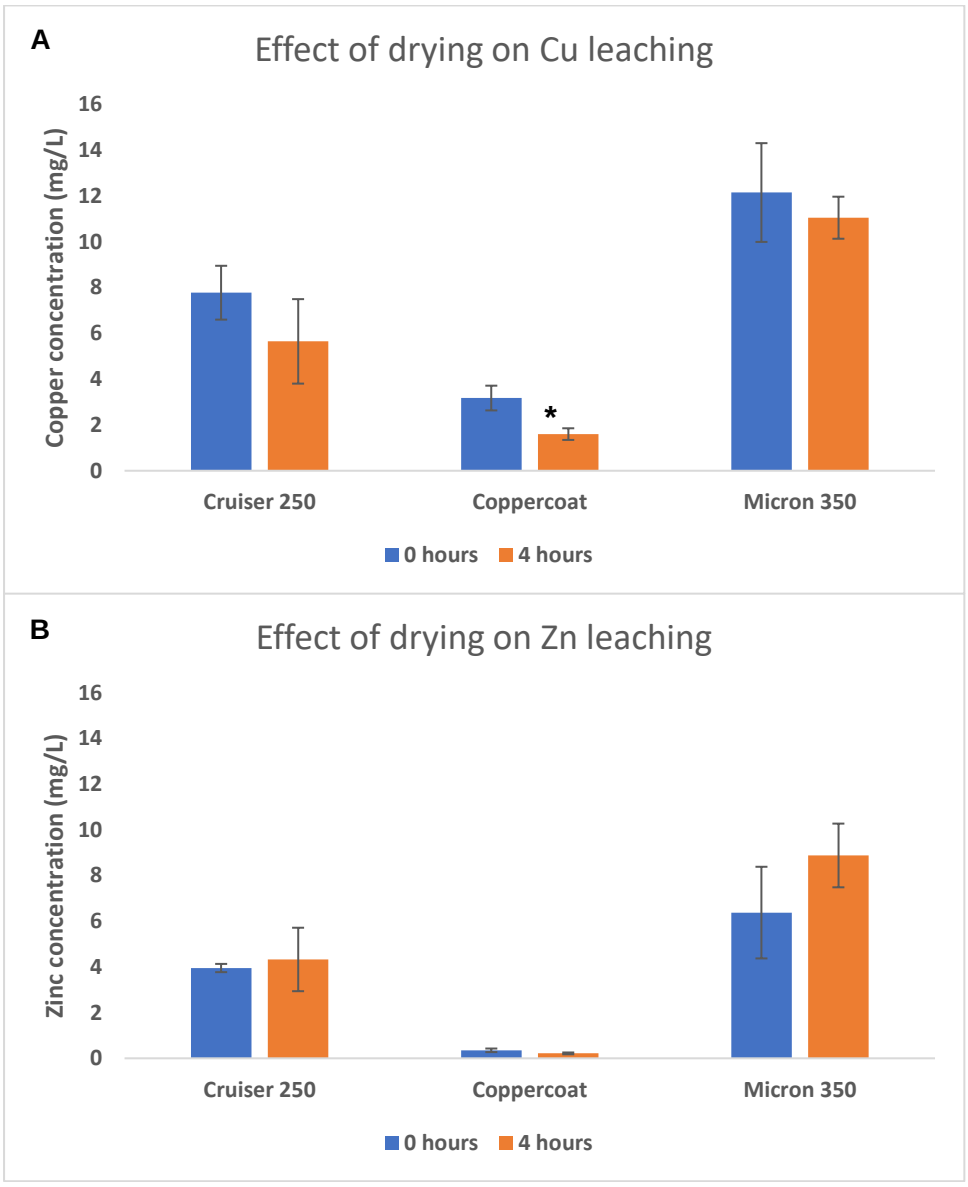

Figure 11: Comparison of drying effect on Copper (A) and Zinc (B) content of wash-off water after standardised scrubbing and pressure-washing on Coppercoat and two self-polishing AF coatings. Error bars represent ±1 standard deviation of four replicate panels (n=4). Asterisks represent groups of significant difference in metal concentration between drying times for a particular metal and coating ($p>0.05$).

All three coatings showed a reduction in average copper concentration of wash-off water after being allowed to dry for four hours (Figure 11). However, only Coppercoat showed a statistically significant reduction ($p<0.011$) in copper content after drying, while the others were within the margin of error of the trial.

Conversely, zinc levels showed a non-significant increase on average after four hours of drying for the two self-polishing coatings and a small decrease for Coppercoat. Small uncertainties on zinc concentration measurements are possible due to its common use in laboratory settings and shipping (Kim et al., 2015). However, all these changes were within the margin of experimental error and thus, no claims about a real effect of drying time can be made in this case.

5.1.2 Second-time point (4.5-month submersion)

The second timepoint testing and analysis took place after 4.5 months of total immersion of the coated panels. At the customer's request, during this round of testing, the scrubbing area was moved to the top left corner of each panel to avoid overlapping with the pressure washing area (see Figure 12). The results from this analysis closely matched the patterns seen in the first timepoint tests (summarized in Table 2).

Specifically:

- Coppercoat released significantly less copper than both SPCs tested (Figure 13)
- The magnitude of the difference was considerable, with Coppercoat releasing 79% - 88% less copper than the SPCs tested
- Coppercoat did not release more zinc than the process control while both SPCs did release significant amounts of zinc (Figure 13)
- Drying time did not have a statistically significant effect on metal release in most cases (Figure 14)

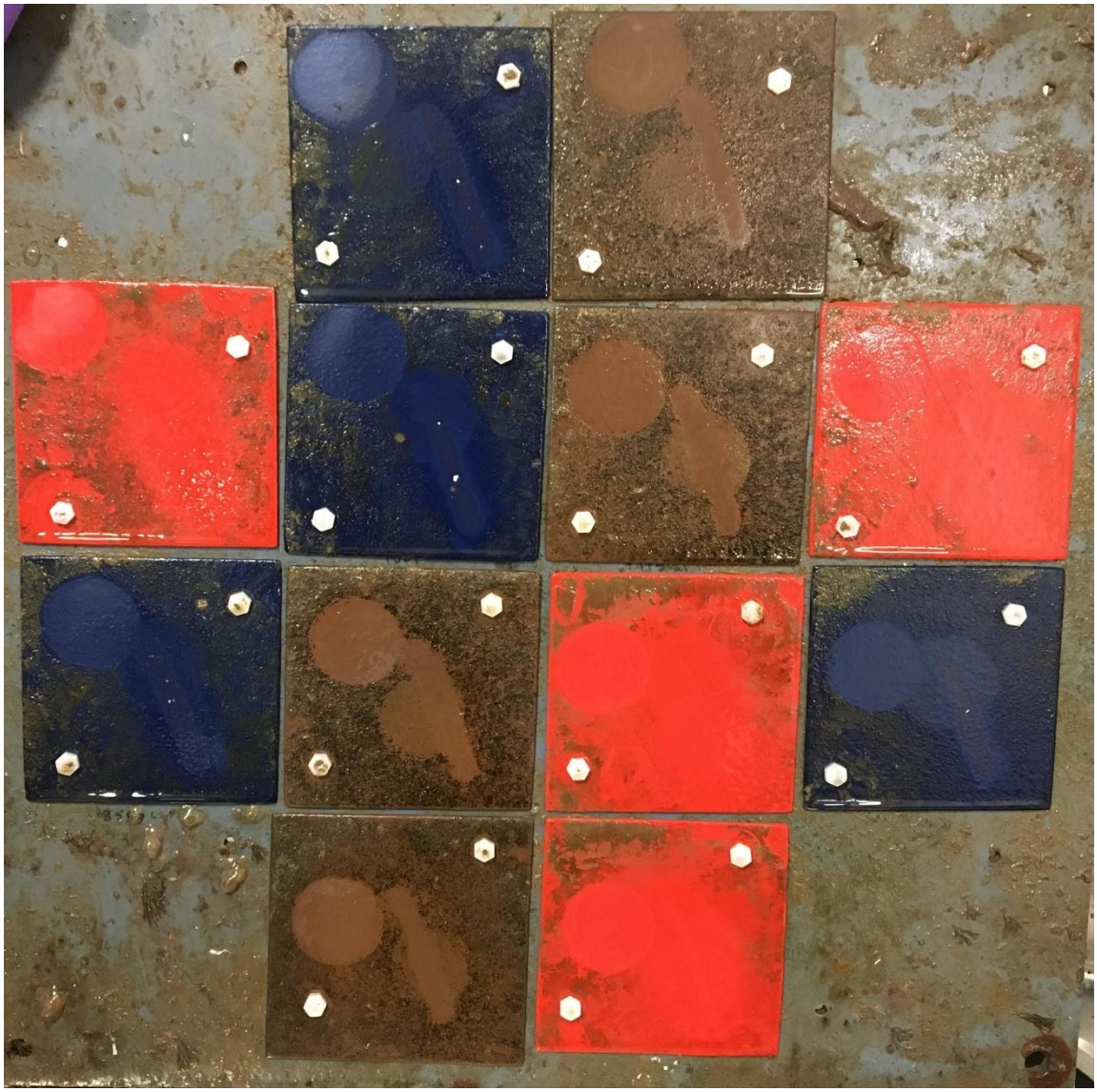

Figure 12: Representative image of backing sheet and panels after standardised scrubbing, pressure-washing and post-testing re-assembly, after the second testing timepoint. Blue panels represent Cruiser 250, brown/copper panels represent Coppercoat and red panels represent Micron 350. Note the scrubbing area, which was moved on the second timepoint to not overlap with the pressure washing area.

Table 2: Summary of timepoint 2 (T2), ICP-MS results for copper and zinc concentration for different coatings and process control.

	Copper (mg/L)		Zinc (mg/L)	
	No drying	**4 h drying**	**No drying**	**4 h drying**
Process Control	0.157	0.157	0.277	0.28
Coppercoat	1.88	2.20	0.31	0.38
Cruiser 250	11.05	10.53	9.38	7.93
Micron 350	14.88	19	14.33	15.82

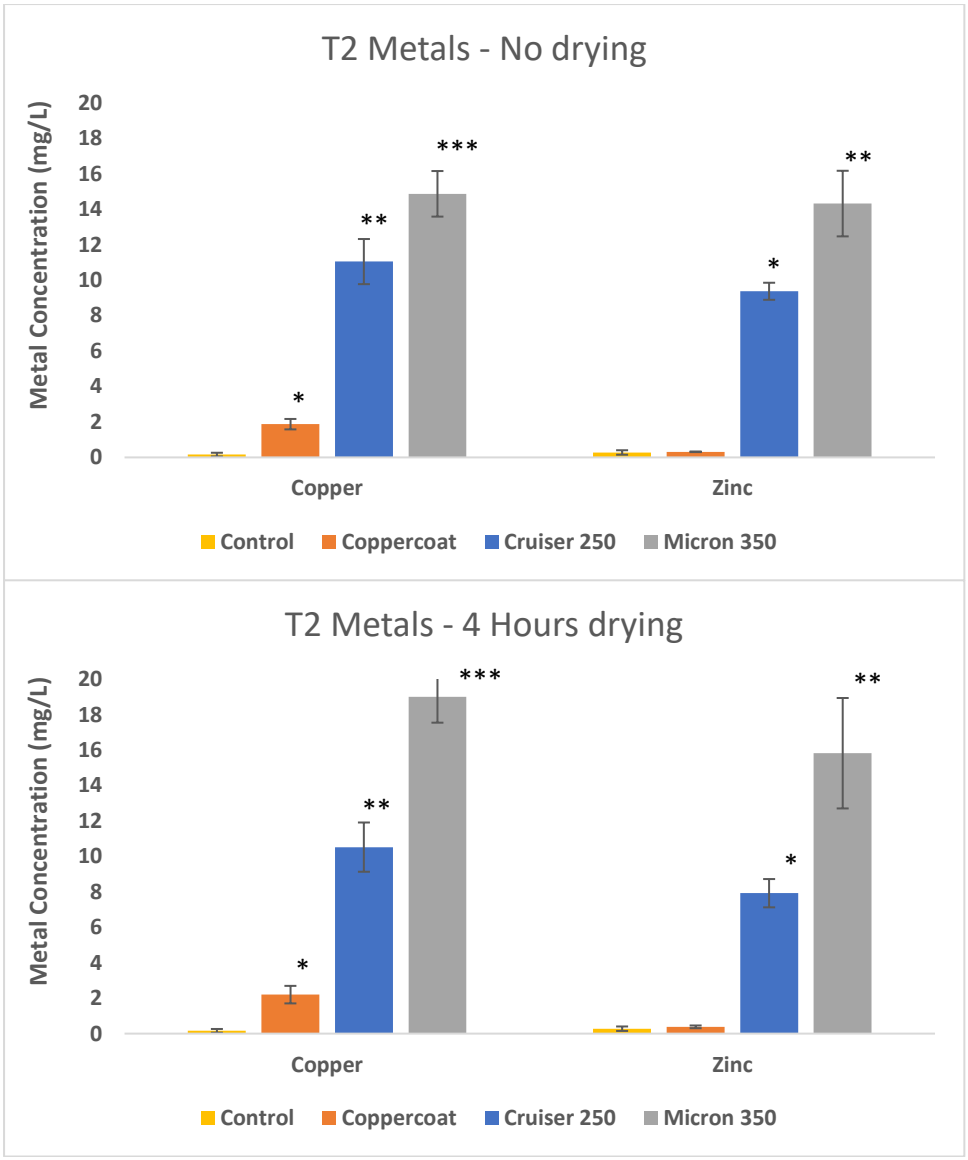

Figure 13: Copper and Zinc content of wash-off water after standardised scrubbing and pressure-washing. A, panels processed immediately without being allowed to dry, and, B, panels allowed to dry for 4 hours. Error bars represent ±1 standard deviation of four replicate panels (n=4). Asterisks represent groups of significant difference in metal concentration between coatings for a particular metal and drying time ($p>0.05$).

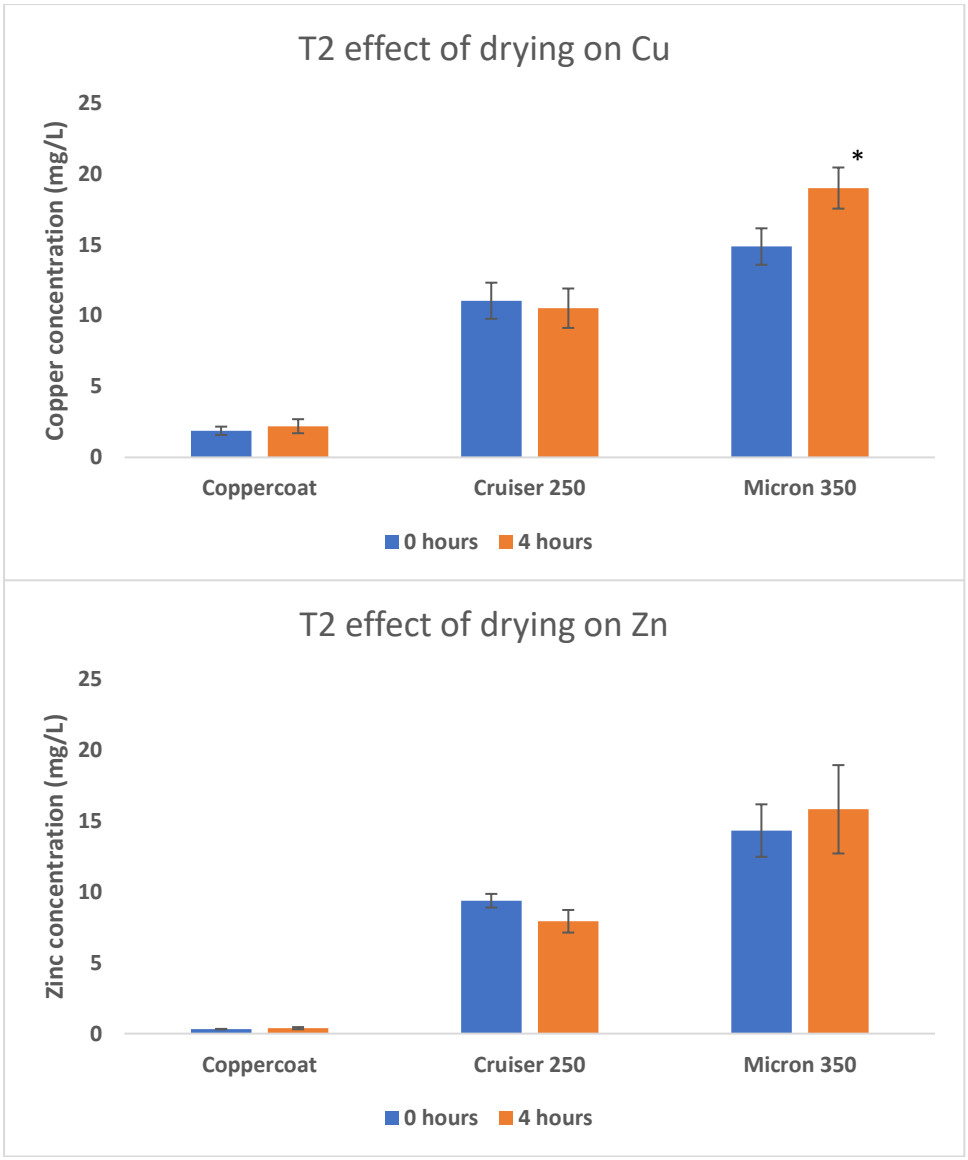

Figure 14: T2 comparison of drying effect on Copper (A) and Zinc (B) content of wash-off water after standardised scrubbing and pressure-washing on Coppercoat and two self-polishing AF coatings. Error bars represent ±1 standard deviation of four replicate panels (n=4). Asterisks represent groups of significant difference in metal concentration between drying times for a particular metal and coating ($p>0.05$).

5.1.3 Third timepoint (6.5-month submersion)

The third and final testing timepoint took place after 6.5 months of total immersion of the coated panels. The results from this analysis somewhat matched the patterns seen in the first two timepoint tests, however, some notable differences were seen in this final timepoint.

Similar patterns of coatings behaviour were seen in terms of:

- Coppercoat released significantly less copper than both SPCs tested (Figure 19) in all but one instance (due to a low-metal sample of Micron 350, discussed later).

- The magnitude of the difference was considerable, with Coppercoat releasing 66% - 70% less copper than the SPCs tested.

- Coppercoat did not release more zinc than the process control while both SPCs did release significant amounts of zinc (Figure 19).

- Drying time did not have a statistically significant effect on metal release in most cases (Figure 20).

However, some marked differences were also seen, compared to the other two timepoints. Specifically:

- Three out of four Cruiser 250 panels showed areas of coating depletion after the final testing timepoint, where the white-coloured substrate under the blue-coloured coating could be seen (see arrows, Figure 15).

- Metal leaching averages (Table 3) were generally lower than during the first two timepoints (Table 1 & Table 2). This might indicate depletion of the antifouling performance in both SPCs after repeated testing, however, this was not specifically tested for.

- One replicate of Micron 350 (8C – 4 hours drying), generated unexpected outlying data with metal contents similar to the control. This might also indicate coating depletion.

Table 3: Summary of timepoint 3 (T3), ICP-MS results for copper and zinc concentration for different coatings and process control.

	Copper (mg/L)		Zinc (mg/L)	
	No drying	**4 h drying**	**No drying**	**4 h drying**
Process Control	0.079	0.079	0.383	0.383
Cruiser 250	3.975	3.450	6.125	4.900
Coppercoat	1.250	1.055	0.495	0.428
Micron 350	4.225	3.140	9.175	4.075

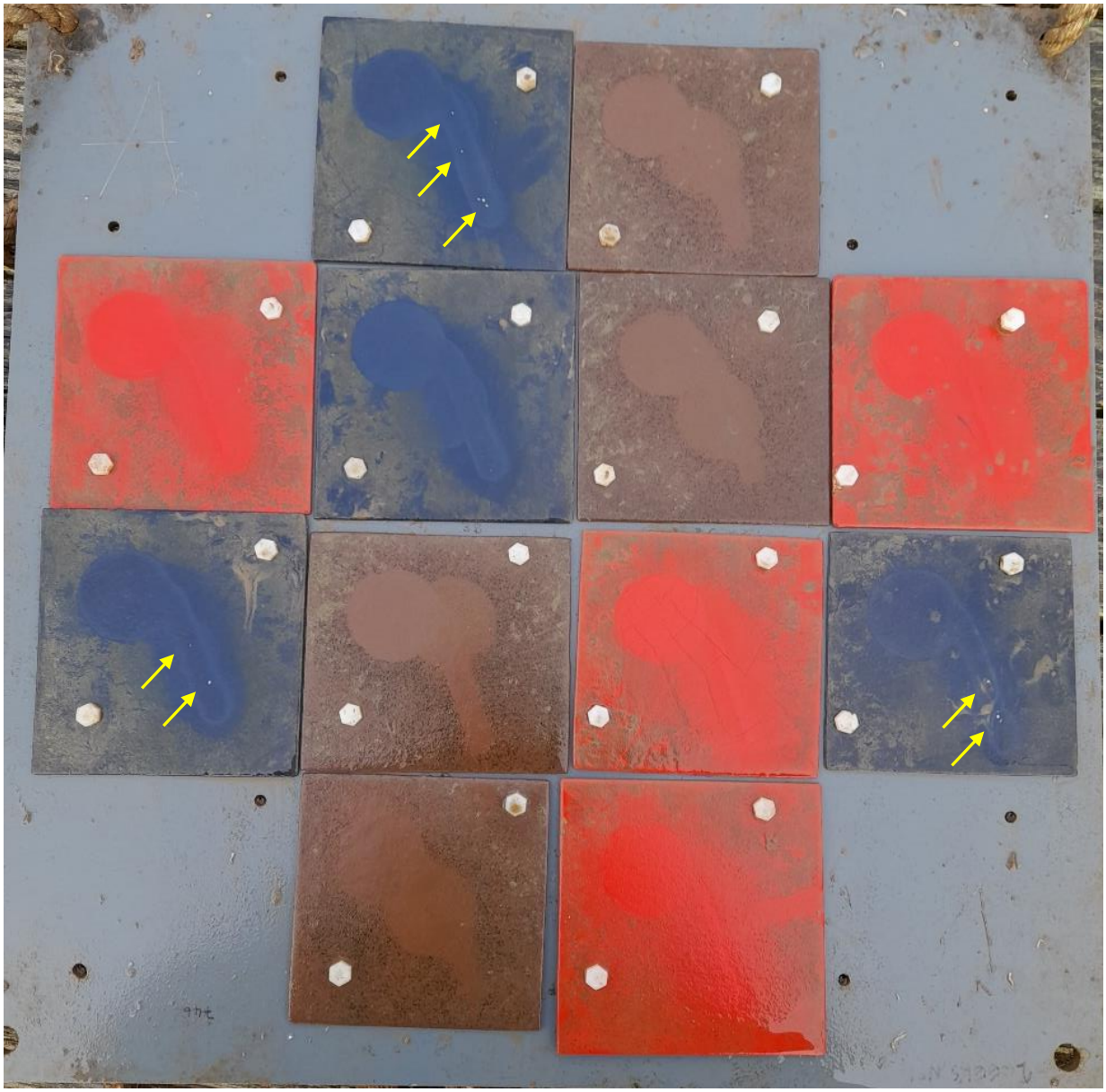

Figure 15: Representative image of backing sheet and panels after standardised scrubbing, pressure-washing and post-testing re-assembly, after the third testing timepoint. Blue panels represent Cruiser 250, brown/copper panels represent Coppercoat and red panels represent Micron 350. Yellow arrows mark areas of coating depletion on Cruiser 250 panels, i.e. areas where the substrate could be seen.

Figure 16: Representative close-up image of a Cruiser 250 panel after three simulated cleaning (scrubbing and pressure washing) events, final cleaning of biofouling and drying. Note the round mark of standardised scrubbing towards the top left of the panel and the linear mark of standardised pressure washing on the middle to bottom right side of the panel. Also note that this coating was sufficiently removed by pressure washing that the substrate started to become visible after the third pressure washing cycle (white patches on pressure-washing area).

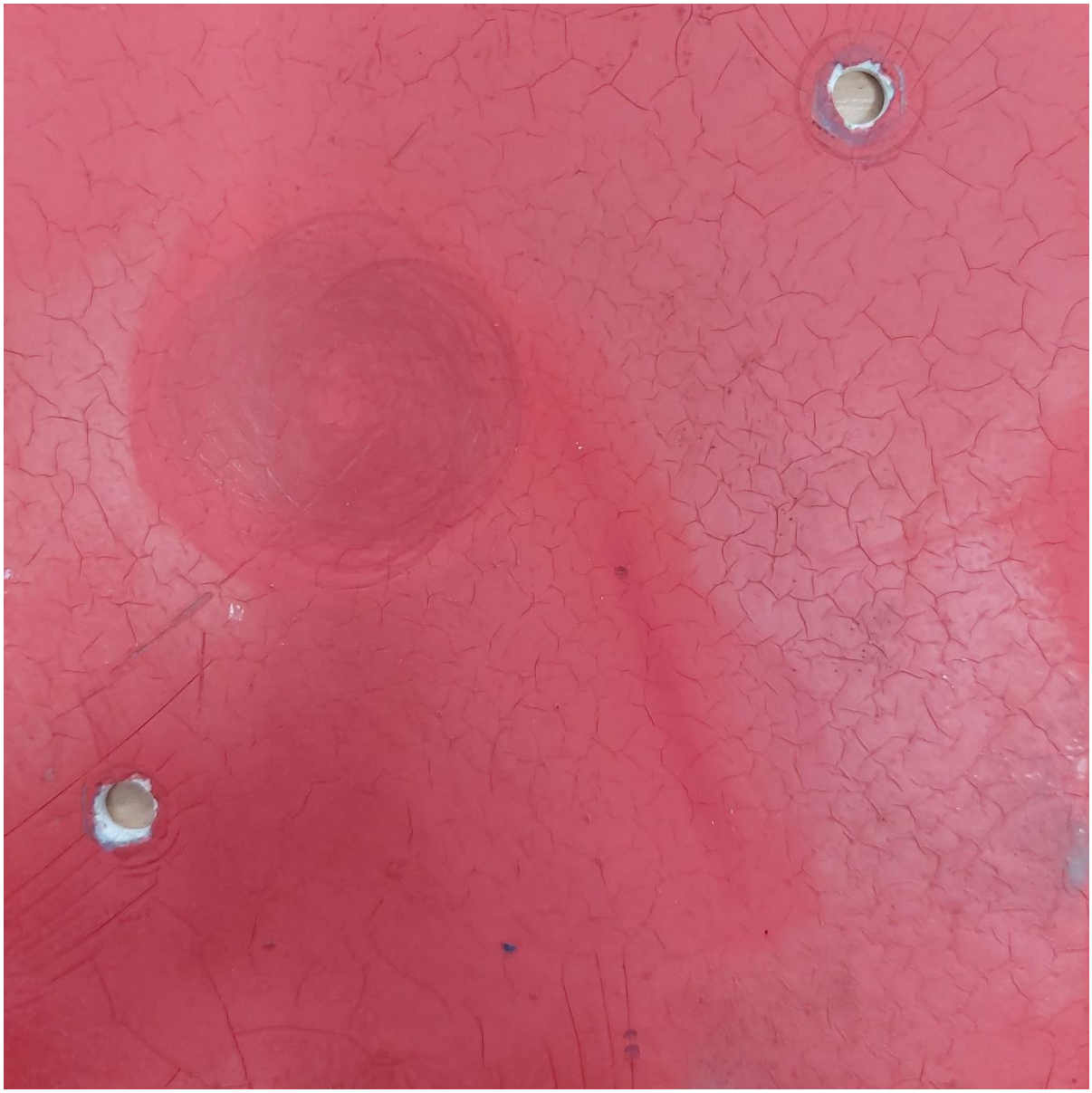

Figure 17: Representative close-up image of a Micron 350 panel after three simulated cleaning (scrubbing and pressure washing) events, final cleaning of biofouling and drying. Note the round mark of standardised scrubbing towards the top left of the panel and the linear mark of standardised pressure washing on the middle to bottom right side of the panel.

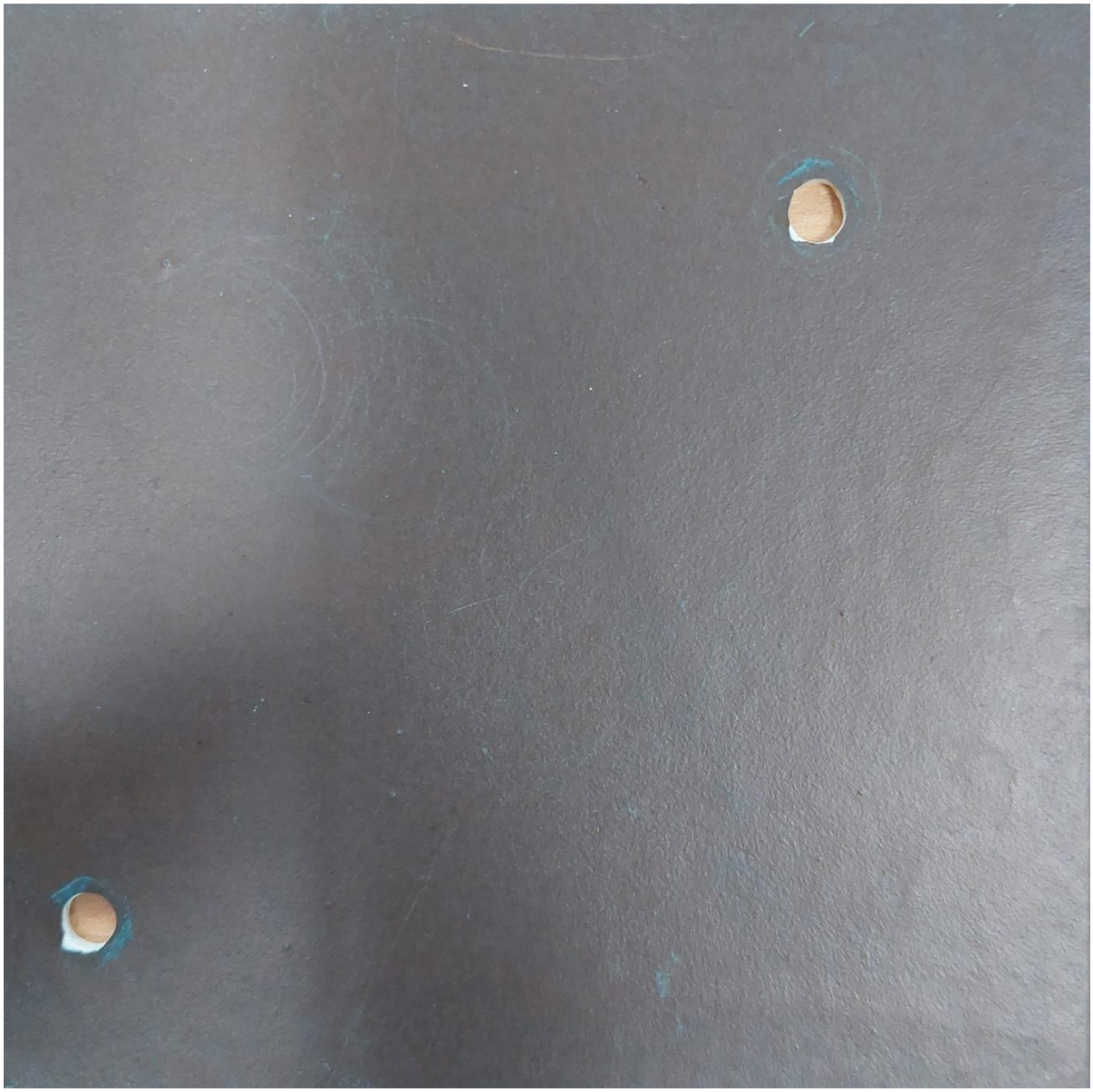

Figure 18: Representative close-up image of a Coppercoat panel after three simulated cleaning (scrubbing and pressure washing) events, final cleaning of biofouling and drying. Note the very faint round mark of standardised scrubbing towards the top left of the panel and complete absence of a pressure washing mark, contrary to the SPC coatings tested.

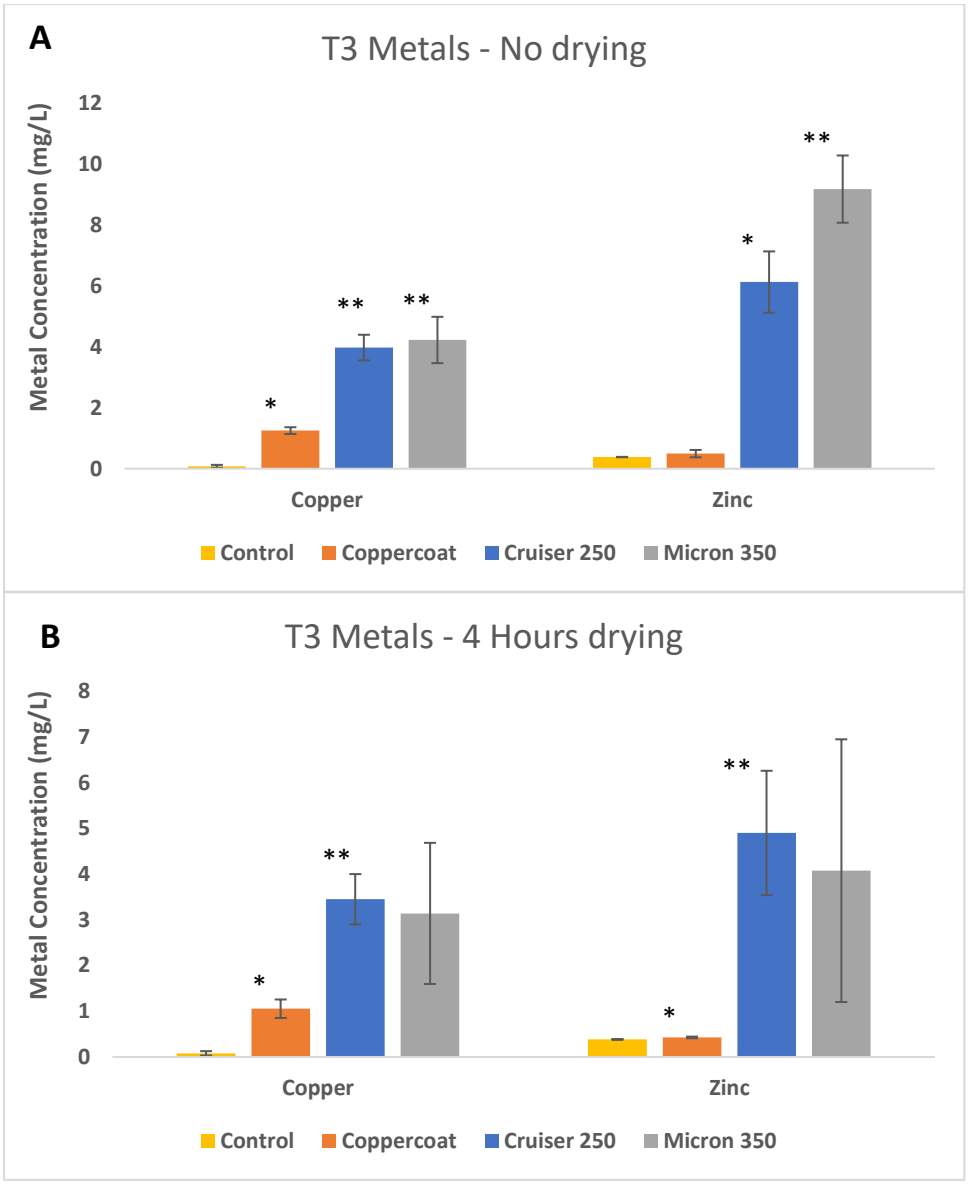

Figure 19: Copper and Zinc content of wash-off water after standardised scrubbing and pressure-washing during the third testing timepoint. A, panels processed immediately without being allowed to dry, and, B, panels allowed to dry for 4 hours. Error bars represent ±1 standard deviation of four replicate panels (n=4). Asterisks represent groups of significant difference in metal concentration between coatings for a particular metal and drying time ($p>0.05$). Note that after 4 hours of drying (B), Micron 350 was not significantly different to any of the other treatments due to one of the four replicate samples showing very low metal content.

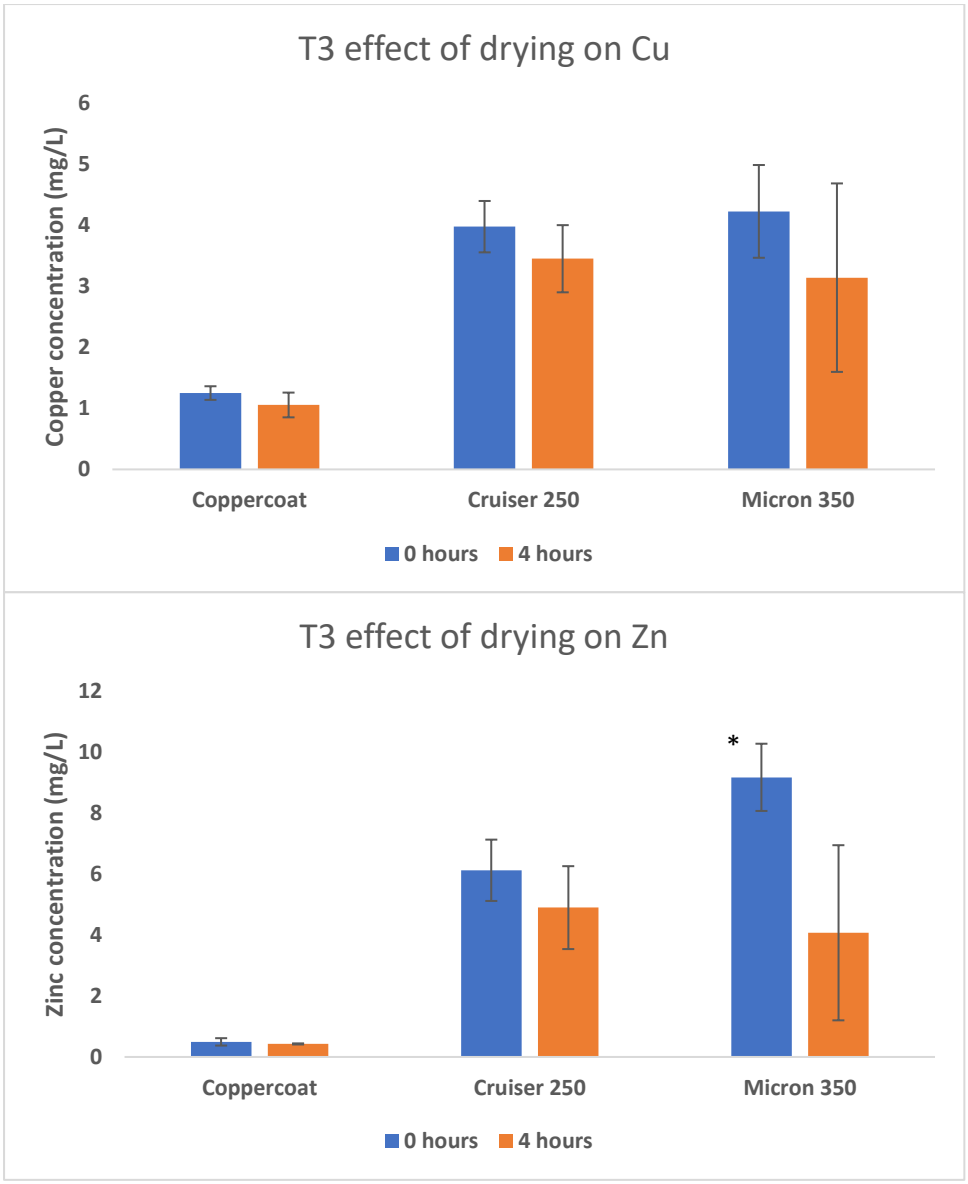

Figure 20: T3 comparison of drying effect on Copper (A) and Zinc (B) content of wash-off water after standardised scrubbing and pressure-washing on Coppercoat and two self-polishing AF coatings. Error bars represent ±1 standard deviation of four replicate panels (n=4). Asterisks represent groups of significant difference in metal concentration between drying times for a particular metal and coating ($p>0.05$).

5.2 Acute toxicity test

Acute toxicity tests were performed on the model microalgal species *Phaeodactylum tricornutum*. Chlorophyll fluorescence was used as a proxy for growth because the amount of chlorophyll fluorescence is an indicator of both growth and health. Toxicity can be manifested in terms of growth rate reduction (growth rate > 0 but significantly lower than control), growth inhibition (growth rate ~ 0) or organism mortality (growth rate < 0).

5.2.1 First timepoint (2-month submersion)

The results of the toxicity test resulting from simulated cleaning of panels after 2 months of submersion are summarized in Figure 21. In brief, wash-offs from Coppercoat panels showed no

measurable toxicity using this method, compared to process control, while the other two coatings tested, showed a significant reduction in growth rates (Figure 21). There was, however, a significant difference between the two coatings, with Micron 350 always resulting in lower growth rates than Cruiser 250. This is in agreement with the metal contents of the wash-off water (section 5.1.1), where Micron 350 in most cases showed higher metal leaching than Cruiser 250 panels (Figure 10).

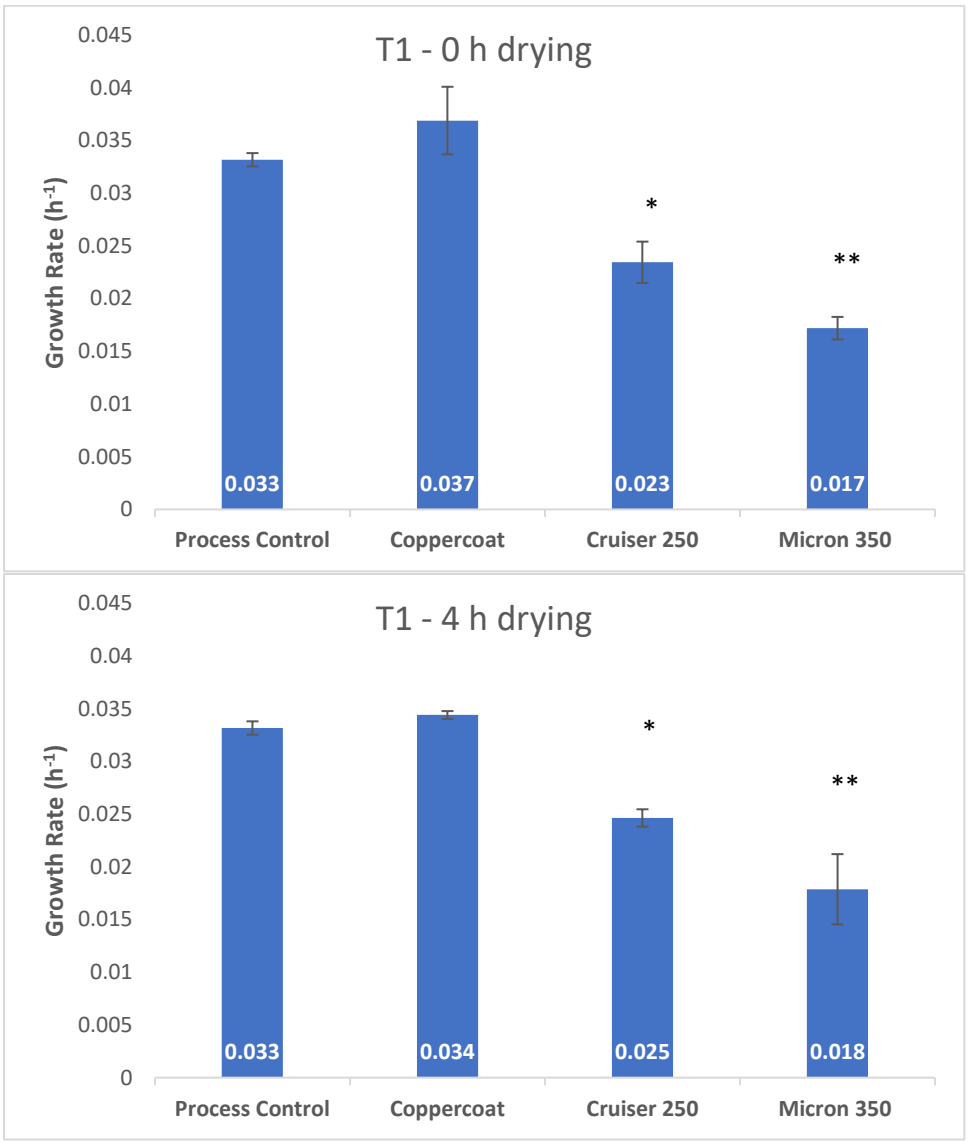

Figure 21: Growth rate of *Phaeodactylum tricornutum*, based on chlorophyll fluorescence, after 48 h incubation in f/2 ASW media from standardised scrubbing and pressure-washing wash-offs of panels coated in different coatings, after 2 months of submersion in the field. Top: panels processed immediately (0 hours of drying) and, bottom: panels allowed to dry for 4 hours before simulated cleaning. Bar height and number at the base of each bar represent average values. Error bars represent ± 1 standard deviation of four replicates (n=4) for the coating panels and three replicates (n=3) for the process controls. Asterisks denote groups of significant difference ($p<0.05$) between coatings for each drying treatment.

Drying time had no effect on the toxicity of wash-offs, for any of the coatings tested, as any differences in growth rates between no drying time and four hours drying were within the margin of error for this trial ($p>0.05$).

5.2.2 Second timepoint (4.5-month submersion)

Toxicity test results after 4.5 months of submersion very closely mirrored the 2-month results, both in terms of absolute growth rates as well as in the differences between coatings and drying times. The results of the second toxicity test are summarised in Figure 22.

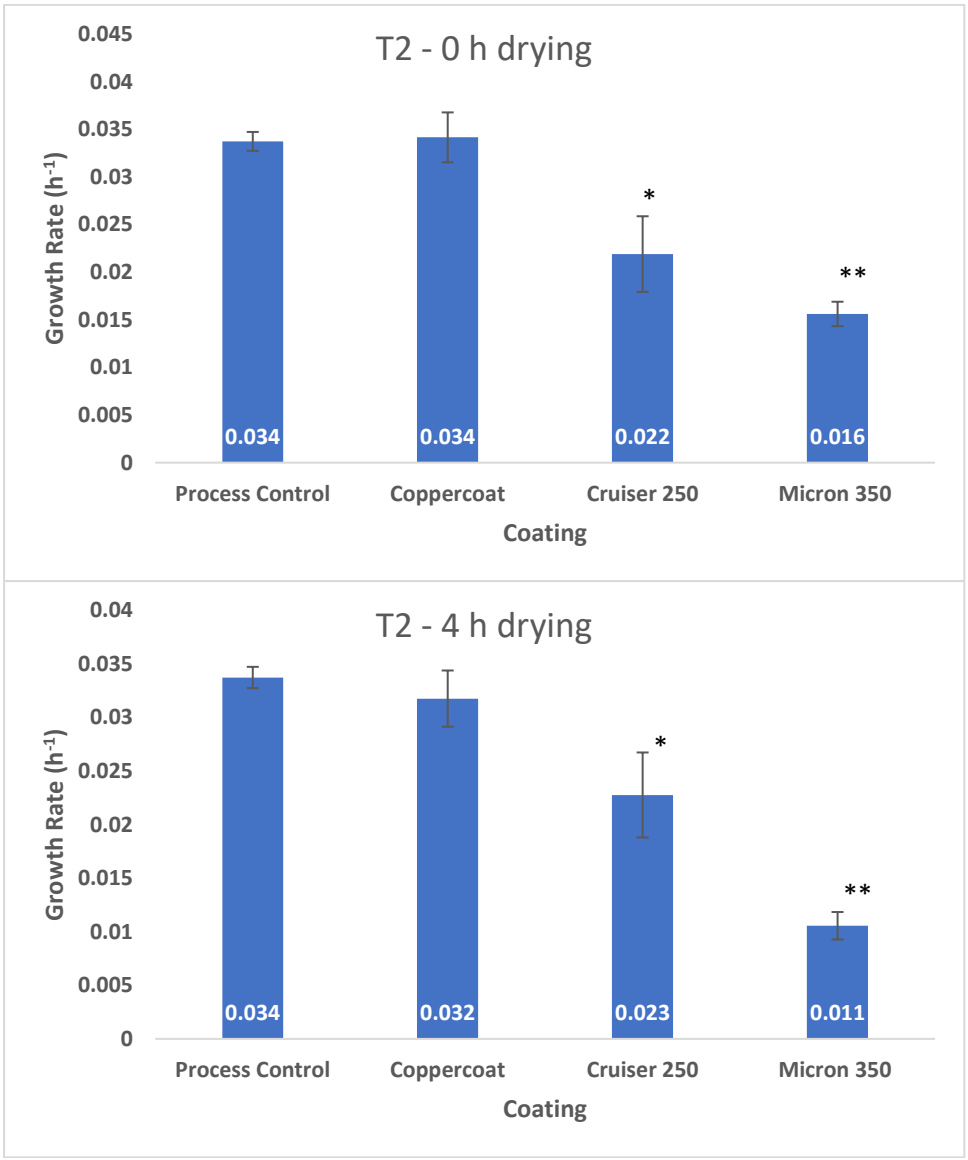

Figure 22: Growth rate of *Phaeodactylum tricornutum*, based on chlorophyll fluorescence, after 48 h incubation in f/2 ASW media from standardised scrubbing and pressure-washing wash-offs of panels coated in different coatings, after 4.5 months of submersion in the field. Top: panels processed immediately (0 hours of drying) and, bottom: panels allowed to dry for 4 hours before simulated cleaning. Bar height and number at the base of each bar represent average values. Error bars represent ± 1 standard deviation of four replicates (n=4) for the coating panels and three replicates (n=3) for the process controls. Asterisks denote groups of significant difference ($p<0.05$) between coatings for each drying treatment.

In brief, Coppercoat wash-offs resulted in growth rates that were not significantly different than the process control, and thus showed no toxicity for the test organism as determined using the methods described here. Cruiser 250 and Micron 350 wash-offs resulted in significantly lower growth rates than the control and Coppercoat. Furthermore, Micron 350 wash-off yielded lower growth rates than Cruiser 250.

As in the first toxicity test, there was no significant difference between 0 hours of drying and four hours of drying for any of the coatings tested in this trial.

5.2.3 Third timepoint (6.5-month submersion)

Toxicity tests performed on T3 wash-offs reflected the reduced metal levels of the two SPCs that were tested alongside Coppercoat (see Section 5.1.3). The results of this trial are summarised in Figure 23.

While growth rates for Cruiser 250 and Micron 350 were reduced compared to the process control and Coppercoat, these differences were not as marked as in the first two timepoints and the growth rate reduction was only statistically significant in one instance (Micron 350 – No drying).

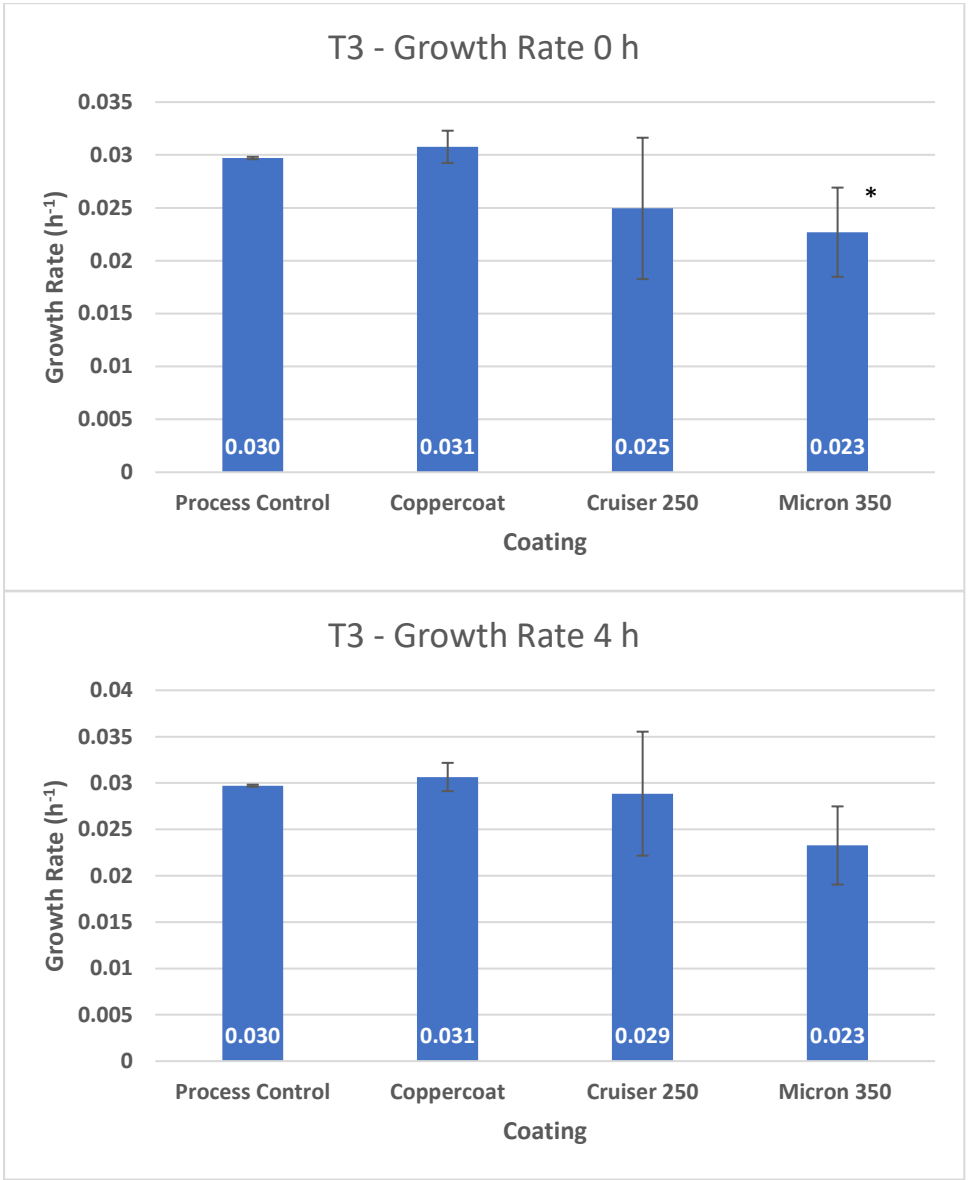

Figure 23: Growth rate of *Phaeodactylum tricornutum*, based on chlorophyll fluorescence, after 48 h incubation in f/2 ASW media from standardised scrubbing and pressure-washing wash-offs of panels coated in different coatings, after 6.5 months (T3) of submersion in the field. Top: panels processed immediately (0 hours of drying) and, bottom: panels allowed to dry for 4 hours before simulated cleaning. Bar height and number at the base of each bar represent average values. Error bars represent ± 1 standard deviation of four replicates (n=4) for the coating panels and three replicates (n=3) for the process controls. Asterisks denote groups of significant difference ($p<0.05$) between coatings for each drying treatment.

5.3 Panel Weight Loss

Panel weights were recorded at the start and the end of the trial. Weight loss for different coatings and drying times is shown in Figure 24. Weight loss is a good indication of the overall “softness” of the coating, with a smaller weight change indicating a harder coating, with less overall material (active compounds, biocides, paint particles etc.) being transferred from the coating to the wash-off water.

In summary, Coppercoat lost significantly less weight compared to the two SPCs tested. This lower weight loss was also of a large magnitude with Coppercoat showing a 82% - 86% less weight loss. This is analogous to the reduction in copper leaching seen during the trial (59% - 88%, see Section 5.1).

Drying did lead to lower weight loss in all cases, two of which were statistically significant (Coppercoat and Cruiser 250).

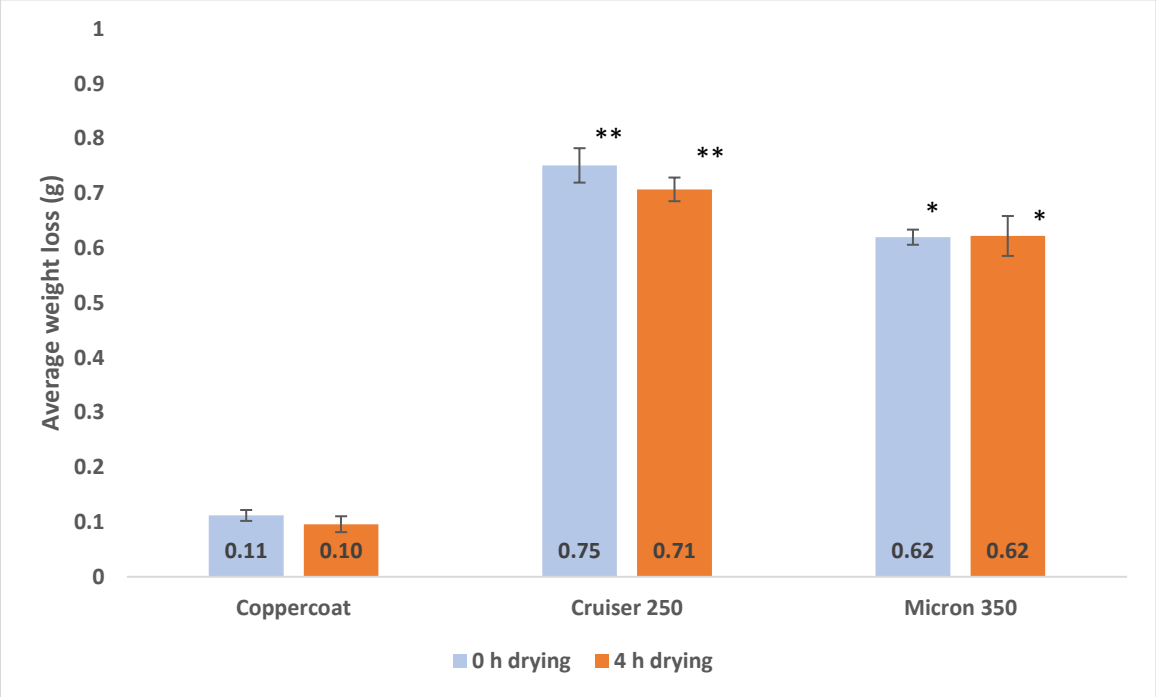

Figure 24: Panel weight loss from different coatings after three rounds of standardized scrubbing and pressure-washing. Bar height represents the average (n=4) and error bars represent ± 1 standard deviation. Asterisks denote significance groups, i.e. bars with different number of asterisks denote averages which are significantly different from each other ($p<0.05$).

6 Discussion and Concluding Remarks

6.1.1 General conclusions

It was evident in this study that Coppercoat is a hard coating and it responds very differently to simulated cleaning compared to self-polishing coatings.

Coppercoat yielded less Copper, less Zinc and showed an overall lower weight loss when subjected to the same simulated cleaning regime as two self-polishing coatings.

Coppercoat wash-off water also showed no acute toxicity to the microalga *Phaeodactylum tricornutum*, while the other two SPCs did show some signs of toxicity, evidenced by a significant reduction in growth rate.

The two SPCs tested did show some signs of coating integrity failure towards the end of the trial, manifesting as lower metal leaching and spots of coating "flaking". This trial, however, was not designed to test the overall durability or longevity of the tested coatings.

It is important to consider the results of this trial as a comparison between coatings subjected to the same standardised treatments rather than as stand-alone results.

As such, this report is not designed to make any claims regarding the overall environmental impact of any of the coatings tested.

6.1.2 Utility of *Phaeodactylum tricornutum* as a test organism and extrapolation of toxicity results to other organisms

Coppercoat wash-off was less toxic than the two SPCs tested. This result was expected, given the large differences in metal concentration between the wash-offs of these coatings.

However, Coppercoat wash-off also seemed to have no toxic effect on *Phaeodactylum* growth. This was unexpected as the copper levels seen in Coppercoat wash-off should have resulted in a measurable reduction in growth rate or even complete inhibition (Cid *et al*., 1995; Reiriz *et al*., 1994; Wei *et al*., 2014).

In fact, none of the wash-offs was toxic enough to induce mortality or total growth inhibition of the test organism. While these levels of toxicity are much lower than found in some of the literature (eg. Cid *et al*., 1995), similarly low copper toxicity has been reported before (Wei *et al*., 2014). This is likely to be because the wash-off water produced in this trial is naturally high in organic carbon from existing fouling on the panels as well as organic polymers present in the coatings. These can reduce the bioavailability of copper (Wei *et al*., 2014). While the relatively low toxic effects reported here for all three coatings are interesting, it was beyond the scope of the present study to investigate them further.

6.1.3 Effect of drying time on metal leaching and toxicity

In this study, drying time, in most instances, did not have a significant effect on either metal leaching or wash-off toxicity. This means, for the same effort applied to the coatings, approximately the same amount of metal leached into the wash-off. This was true for both Coppercoat and the two self-polishing coatings. The drying conditions were representative of the UK climate (18 °C), so cannot necessarily be extrapolated to tropical conditions, where four hours of drying might have a much more significant effect on coating behaviour. Furthermore, in a realistic scenario, because of fouling drying and thus being a little more difficult to remove, cleaning effort after a drying period might be increased compared to an immediate hull clean after removal of a vessel from the water. As such, applying exactly the same effort on the two treatments, while being a useful comparison to elucidate coating behaviour, does not constitute a fully realistic cleaning simulation.

7 References

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End of Report

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