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Settlement and growth of copper-tolerant *Ectocarpus siliculosus* (Dillw.) Lyngbye on different copper-based antifouling surfaces under laboratory conditions

Part 2 *A comparison of the early stages of fouling using light and scanning electron microscopy*

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The second paper of this series reports the results of comparative studies on the marine antifouling properties of a range of six copper and copper-alloy materials, using settlement and growth of a copper-tolerant strain of the marine-fouling alga *Ectocarpus siliculosus* as criteria. Samples of ordinary copper, arsenical copper, copper–nickel 90/10, copper–nickel 70/30, aluminium brass and aluminum bronze were preincubated in sterilized seawater medium under laboratory conditions and then used to follow the early stages of spore settlement and subsequent development of colonies of the alga in the culture system by both light and scanning electron microscopy. The results of this bio-assay are compared with other published information relating to the long-term antifouling properties of these materials in trials at sea.

1. Introduction

The first paper of this series [1] described the development of an algal culture system employing a copper-tolerant strain of the marine-fouling alga *Ectocarpus siliculosus* (Dillw.) Lyngbye as a bio-assay organism for assessing antifouling potential of copper-based materials under standardized laboratory conditions. Using immersed sample plates of copper–nickel 90/10 as a test material, the conditions necessary for the formation of passive corrosion films typical of those formed at sea were investigated. Up to 140 days pre-incubation in nutrient-enriched, sterilized seawater medium were required before the rate of copper loss from the surfaces was constant and sufficiently low to enable algal growth and settlement to occur.

This paper reports the results of screening trials on the antifouling potential of a range of six copper and copper-alloy materials all of which have or may have marine application. The bio-assay system described in Part 1 was used to follow the early stages of algal settlement and colonization of prepared and pre-incubated sample plates, using both conventional incident light and scanning electron microscopy (SEM). As the early stages of spore settlement and subsequent development of *Ectocarpus* filaments to produce a "brown mat" on copper-based surfaces have not been studied before, it was thought that comparison of surfaces at these early stages could reveal potential differences in long-term algicidal properties.

2. Materials and methods

2.1. Range of materials screened and initial preparation of samples

Preliminary tests suggested that a selection of six copper-based materials: ordinary copper, arsenical copper, copper–nickel 90/10, copper–nickel 70/30, aluminium brass and aluminium bronze, would include a suitable range of freely-available and widely used materials. Table I details the specification and full elemental analysis of these materials.

Three replicate plates (50 mm × 50 mm × 1.3 mm) of each of the copper materials were cut and the surfaces prepared by IMI Yorkshire Imperial Ltd by deburring and abrading. A hole was drilled in one corner of each plate for suspension and then all surfaces degreased with acetone. All edges, one side and around this hole were "stopped-off" with "Lacomit" lacquer. A further three replicates of aluminium bronze were prepared in this way but the original hot-rolled (oxidized) surface as supplied by the manufacturer was retained. A similar number of replicates of three control surfaces were also prepared. Fibreglass and Perspex were included to enable a comparison of settlement and growth of *Ectocarpus* on a rough and a smooth non-toxic surface respectively, and "Lacomit"-painted fibreglass was used to assess any toxicity of this lacquer.

2.2. Preincubation of sample plates

The rationale for preincubation of samples in sea-

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TABLE I Elemental composition (%) of copper surfaces employed in the fouling trials

Alloy	Cu (+Ag)	P	As	Ni	Fe	Mn	Al	Zn	Impurities	Total impurities
Ordinary copper C106 DONA*	99.86	0.032	0.05						Zn	< 0.06
Arsenical copper C106*	99.56	0.025	0.34						Zn, Ni, Fe, Pb, Ag	< 0.06
Copper-nickel 90/10 CN102 Kunifer 10*	86.97			10.28	1.65	0.80			Ti, Co, S, Mg	< 0.30
Copper-nickel 70/30 CN107 Kunifer 30*	67.64			30.32	0.85	0.89			Zn, C, S, Co, Mg	< 0.30
Aluminium brass CZ110 Yorcalbro*	76.31		0.037				1.99	21.36	Ni, Fe, Si, Pb	< 0.30
Aluminium bronze B169 Alloy No. 61400†	89.22				3.20		7.20		Ni, Mn, Si, Pb, Sn, Zn, P, Mg	< 0.27

*Supplied by IMI Yorkshire Imperial Ltd.
†Supplied by N. C. Ashton Ltd.

water has been discussed in detail in Part 1. In summary, preincubation allows the formation of protective corrosion films with low and constant rates of copper loss, compatible with the growth and development of the alga in the bio-assay system. The prepared sample plates were freely suspended by nylon-covered drive cord from the Perspex lids of plastic containers (three replicates per container). They were totally submerged in 6000 cm^3 sterilized seawater medium and incubated at room temperature (20° C). The medium was aerated continuously and changed at 14 day intervals, when the samples were agitated to disperse loose corrosion product. The volume of spent medium was made up to 6000 cm^3 with distilled water and concentrated nitric acid added to produce a final molarity of 0.014 M. Any deposit on the sides of the containers was brought into solution by mixing the acidified medium with compressed air. Aliquots of 30 cm^3 were removed for copper analysis by atomic absorption spectrophotometery (AAS). When copper concentrations had reached a constant and relatively low level, indicating that a protective corrosion film had formed, preincubation was considered complete. A final sample of the incubating medium was taken for analysis of copper and other likely contaminant and potentially toxic elements (nickel, manganese, zinc, iron, lead, silver, cobalt, tin and aluminium) by either AAS or inductively-coupled plasma emission spectrometry (ICP).

Two replicate plates of each of the ten sample materials were used without further treatment for incubation in the presence of *Ectocarpus* and assessment of colonization and growth by light microscopy. The remaining replicate from each group was removed from the seawater medium and air-dried. Each plate was then sawn into seven small replicates (8 mm × 8 mm), handling minimally to cause as little damage as possible to the corrosion film. The edges, one side and the outer rim of the experimental surfaces were "stopped-off" with three coats of "Lacomit". The seven small replicates of each sample were then attached to nylon thread with "Aquarium" glue and suspended in 2000 cm^3 aerated, sterilized seawater medium for further preincubation at room temperature. The medium was again replaced regularly at 14 day intervals for 12 weeks and copper concentrations measured at each change by AAS to ascertain if the surfaces were still protective. The samples remained under these conditions until required for experiments on spore settlement by SEM.

2.3. Incubation of samples in the presence of *Ectocarpus*

2.3.1. Short-term spore settlement and growth up to 14 days

The early stages in spore settlement and subsequent development were investigated by SEM on small samples suitable for the equipment. Full details of the source and laboratory culture of the copper-tolerant *Ectocarpus* used in these and subsequent studies are given in Part 1. Spores were released from small samples of the alga in the reproductive state in the following manner. The alga was partially dried by squeezing between filter paper and immediately immersed in a small amount (10 cm^3) of seawater medium in a petri dish. This was incubated on a cool, north-facing window sill for 2 h. The presence of a brown meniscus indicated that a large number of motile spores had been released. A drop of seawater drawn from near the meniscus was placed on the surface of the small, preincubated samples and incubated for 2 h in the conditions described previously for algal culture. During this time spores settled on the surfaces. The plastic containers were then flooded with seawater medium and incubated for a further period to make a total incubation of 6 h, 24 h, 4 d, 7 d or 14 d. The volume of the medium was controlled so that the copper concentration was maintained below 0.2 $mg\,l^{-1}$ during incubation.

The samples were then prepared for SEM studies. They were pre-fixed in cold 4% glutaraldehyde with 0.25 M sucrose buffered with 0.1 M sodium cacodylate buffer for 2 h. They were then washed for 30 min sequentially in cacodylate buffer containing (1) 0.25 M sucrose, (2) 0.125 M sucrose, and (3) no sucrose. They were post-fixed in 1% cacodylate buffered osmium

TABLE II Copper concentration of the seawater medium during preincubation of copper surfaces for 18 weeks and during incubation with *Ectocarpus siliculosus* for 16 weeks ($mg\,l^{-1}$). Estimated rates of copper loss from the surface during preincubation are also given ($\mu g\,cm^{-2}\,d^{-1}$)

Copper surface	Preincubation time (weeks)									Incubation time with *Ectocarpus siliculosus* (weeks)						
	2	4	6	8	10	12	14	16	18	2	4	8	10	12	14	16
Ordinary copper																
$mg\,l^{-1}$	24.8	19.0	22.8	13.8	13.6	13.6	11.8	8.5	11.1	0.23	0.23	0.44	0.28	0.23	0.21	0.21
$\mu g\,cm^{-2}\,d^{-1}$	160	123	147	89	88	88	76	55	72							
Arsenical copper																
$mg\,l^{-1}$	20.6	9.2	12.6	7.7	8.4	7.3	5.8	3.8	4.0	0.10	0.09	0.23	0.13	0.16	0.16	0.13
$\mu g\,cm^{-2}\,d^{-1}$	133	60	82	50	54	47	38	25	26							
Copper–nickel 90/10																
$mg\,l^{-1}$	2.5	1.22	0.52	0.27	0.32	0.93	0.80	0.54	0.57	0.04	0.05	0.11	0.05	0.06	0.09	0.02
$\mu g\,cm^{-2}\,d^{-1}$	16	8	3	2	2	6	5	3	4							
Copper–nickel 70/30																
$mg\,l^{-1}$	1.2	0.87	0.50	0.31	0.35	0.51	0.22	0.19	0.18	0.01	0.01	0.03	<0.01	0.01	0.01	<0.01
$\mu g\,cm^{-2}\,d^{-1}$	8	6	4	2	2	3	1	1	1							
Aluminium brass																
$mg\,l^{-1}$	0.5	0.28	0.13	0.19	0.23	0.20	0.15	0.13	0.12	0.01	0.01	0.02	<0.01	<0.01	0.01	0.01
$\mu g\,cm^{-2}\,d^{-1}$	3	2	1	1	1	1	1	1	1							
Aluminium bronze (prepared)																
$mg\,l^{-1}$	0.5	0.18	0.11	0.10	0.09	0.14	0.13	0.11	0.11	0.02	0.02	0.01	0.02	0.04	0.02	<0.01
$\mu g\,cm^{-2}\,d^{-1}$	3	1	1	1	1	1	1	1	1							
Aluminium bronze (as supplied)																
$mg\,l^{-1}$	1.7	2.51	1.50	1.22	1.36	1.29	1.13	0.95	1.08	0.08	0.08	0.17	0.13	0.12	0.11	0.11
$\mu g\,cm^{-2}\,d^{-1}$	10	16	10	8	9	8	7	6	7							

tetroxide for 2 h and dehydrated in an acetone series (30%, 50%, 70%, 85%, 95%, 100% twice). The samples were critical point dried and coated with gold–palladium.

It was found that acetone destroyed the surface of the Perspex and "Lacomit" control samples. SEM studies were therefore made only on fibreglass. Both preincubated and unincubated (clean) surfaces of fibreglass were included in the investigation.

Because the inoculation of copper surfaces with spores occurred only once (i.e. at time 0) these studies examined algal material of known age. They did not however, reveal interactions between material at different developmental stages which would result from continuous spore settlement as would take place in the normal fouling environment.

2.3.2. Long-term settlement and growth up to 16 weeks

Plastic containers holding 2000 cm^3 seawater medium were inoculated with approximately 25 mg wet weight of copper-tolerant *Ectocarpus*. The cultures were incubated for 20 days in a controlled-environment room at 15° C/18 h day and 12° C/6 h night. At the end of this period, when the sides of containers were densely colonized by reproductive algal filaments, the medium was replaced. The surface of each preincubated large sample plate was examined under a low-power ($\times 20$) microscope and a map made of areas of corrosion products and irregularities. The samples were then suspended from the lids and re-immersed in seawater, one per container and incubated for 16 weeks in the controlled-environment room. At 14 day intervals each sample plate was removed and examined microscopically and estimates made of the extent of algal colonization on the surface. The incubation medium was replaced at 14 day intervals as before and filtered samples of the spent medium used for copper analysis.

3. Results

3.1. Copper losses from samples during preincubation and incubation with *Ectocarpus*

The copper concentrations in the preincubating medium and estimated rates of copper loss at 14 day intervals up to 18 weeks are presented in Table II. During preincubation, copper losses from all materials were reduced and in three cases (copper–nickel 70/30, aluminium brass and aluminium bronze – prepared) were low and constant at this stage, resulting in copper concentrations in the incubating medium below $0.2\,mg\,l^{-1}$, compatible with uninhibited growth and spore release of copper-tolerant *Ectocarpus*. Losses from the remaining four samples (ordinary copper, arsenical copper, copper–nickel 90/10 and aluminium bronze – as supplied) showed evidence of becoming constant. However, these losses resulted in copper concentrations in the incubating medium of above $0.5\,mg\,l^{-1}$. Such concentrations will limit both growth and spore release even in copper-tolerant *Ectocarpus*. However, it was known that the actively growing alga can absorb a large proportion of the copper present in the incubating medium thus lowering its effective concentration [1]. It was not known whether this algal absorption would be great enough to detoxify the medium incubating all samples.

Table II also presents the copper concentrations in the medium after introduction of *Ectocarpus*. As can be seen, the copper concentration did not exceed

TABLE III Time sequence of colonization by *Ectocarpus siliculosus* on copper and fibreglass surfaces during a 14 day incubation observed by scanning electron microscopy. Figures are average filament length (μm)

Surface	State of development	Time after spore inoculation				
		6 h	24 h	4 d	7 d	14 d
Ordinary copper	spores	+	–	–	–	–
	filaments	–	–	–	–	–
Arsenical copper	spores	+	–	–	–	–
	filaments	–	–	–	–	–
Copper–nickel 90/10	spores	+	+	+	+	–
	filaments: prostrate	–	10	7	25	50
	erect	–	–	–	10	30
Copper–nickel 70/30	spores		+	+	+	–
	filaments: prostrate		–	–	30	+
	erect		–	–	15	> 300
Aluminium brass	spores	+	+	+	–	–
	filaments: prostrate	–	7	25	25	< 30
	erect	–	–	10	–	> 300
Aluminium bronze (prepared)	spores	+	+	+	+	+
	filaments: prostrate	–	3	30	25	< 30
	erect	–	–	20	40	> 300
Aluminium bronze (as-supplied)	spores	+	+	+	–	–
	filaments: prostrate	2	2	2	60	40
	erect	–	–	–	40	100
Fibreglass (preincubated)	spores	+	+	–		–
	filaments: prostrate	–	–	–		–
	erect	–	–	150		> 300
Fibreglass (clean)	spores	+	+		+	–
	filaments: prostrate	3	6		60	60
	erect	–	–		15	> 300

– This stage not recorded.
+ This stage observed.

$0.45\,mg\,l^{-1}$ in any container and for all the copper-alloy samples was substantially lower at all stages of incubation, indicating that growth and spore release would not be inhibited.

A full elemental analysis of the spent preincubating medium at 18 weeks did not reveal excessive concentrations of any other elements thought to be potentially inhibitory to the growth of *Ectocarpus*. Nickel concentrations never exceeded $0.7\,mg\,l^{-1}$, lead $0.4\,mg\,l^{-1}$, zinc $0.1\,mg\,l^{-1}$, cobalt $0.35\,mg\,l^{-1}$ and tin $1.6\,mg\,l^{-1}$.

For the second preincubation of the small sample plates after preparation from the larger preincubated plates, all materials initially lost greater amounts of copper than at the final stage of the first preincubation. Copper losses gradually became smaller, however, so that after 12 weeks those from all samples except one (arsenical copper) were lower than or very similar to the steady-state losses from the parent plate.

3.2. Settlement of *Ectocarpus* on sample surfaces in the first 14 days of incubation

Stages of spore settlement and subsequent development of algal filaments on the small samples plates were studied by SEM. Photographic records readily supplied details of the external morphology of *Ectocarpus* spores (*c.* 3 μm diameter), developing sporelings and surface features of the incubated materials and also revealed the presence of any microbial contaminants. Figs 1 to 32 illustrate the full range of the various stages of settlement and subsequent development of the alga on the materials tested. Table III provides more quantitative information on the presence or absence of settled algal spores and filament growth (length) at various times during the 14 day incubation period.

3.2.1. Development of Ectocarpus spores on non-toxic (control) surfaces

It was found that the fibreglass samples which had been preincubated for 9 weeks in enriched seawater medium open to the air had extensive microbial colonization. The thickness and heterogeneity of this layer made observation of early spore development within it difficult. In less heavily colonized areas, zoospores and settled spores were observed. No stages of germination were seen but after 14 days a thick mat of erect filaments was noted growing above the microbial layer.

Because of these limited results, settlement was also studied on fibreglass samples which had not been preincubated (fibreglass – clean). Stages in the first 14 days of spore development are illustrated in Figs 1 to 8. The flagellated (motile) zoospore (Fig. 1) settled on the surface. During settlement it lost its flagellum and formed a pitted cell wall, its shape becoming spherical (Fig. 2). Within 6 h it was capable of germinating (Fig. 3) to produce the initial development of a prostrate (anchoring, surface-spreading) filament

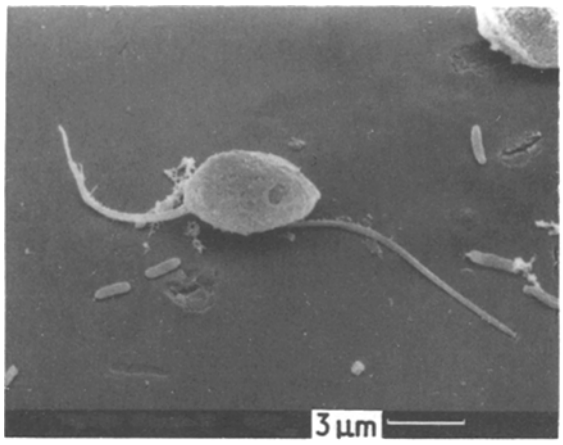

Figure 1 Clean fibreglass, 24 h; zoospore*. × 5500.

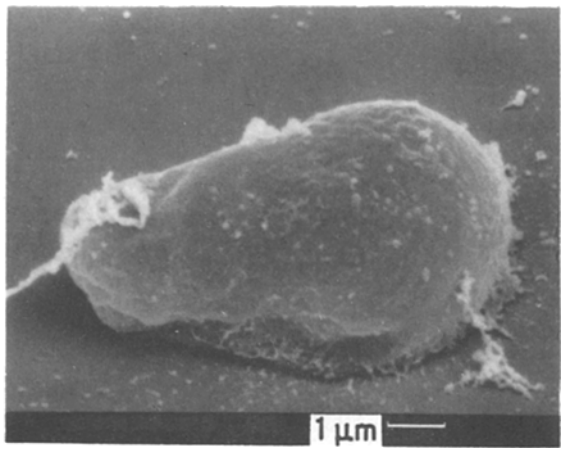

Figure 3 Clean fibreglass, 24 h; germling. × 12000.

(Figs 4 and 5). Within 7 d an erect (aerial) filament was formed (Fig. 6). After 14 d a mat of long, mainly unbranched erect filaments (> 300 µm in length) was formed (Fig. 7), one or two erect filaments arising from the shorter (30 µm long), thinner prostrate filaments (Fig. 8). In most cases, there was little extra cellular adhesive material observed around the edges of the developing filaments. However, some adhesive was noted around spores in the earlier stages of germination (Figs 3, 4 and 5).

3.2.2. The development of *Ectocarpus* spores on copper and copper-alloy surfaces

3.2.2.1. Ordinary copper. After 6 h, very few zoospores and settled spores with atypical morphology were recorded on the surface. The surfaces and flagella of the zoospores appeared to be granular possibly due to the presence of corrosion product (Fig. 9). The cell walls of the settled spores were incompletely formed. At no other time was algal material recorded on the surface, suggesting toxicity of the surface after 6 h was great enough to inhibit germination of any spores which had settled initially. In a few small areas, usually in clefts in the corrosion film, a small number of bacteria were observed (Fig. 10).

3.2.2.2. Arsenical copper. Algal colonization was found to be very similar to that observed on ordinary copper, in that very few spores were noted after 6 h but not subsequently. Bacteria were observed within small pockets in the rough corrosion film (Fig. 11).

3.2.2.3. Copper–nickel 90/10. Algal material was recorded at all observation times. Development was a described on a non-toxic surface (Figs 1 to 8) but proceeded more slowly and fewer spores were apparent. No germination was observed by 6 h and filaments were slightly shorter than those on fibreglass after 7 d growth (Fig. 12). After 14 d, growth of the filaments was arrested and they were in poor condition and covered by a dense microbial growth, suggesting that a build-up of copper toxicity occurred over the 7 to 14 day period (Fig. 13). Earlier evidence of possible toxicity was also observed. In some zoospores the loss of flagella appeared to be arrested (Fig. 14) and the production of extracellular material may have been increased in some germlings (Fig. 15). The generally smooth surface was heavily colonized by other microorganisms (Fig. 14).

3.2.2.4. Copper–nickel 70/30. Settled spores were observed after 24 h (Fig. 16). The presence of heavy

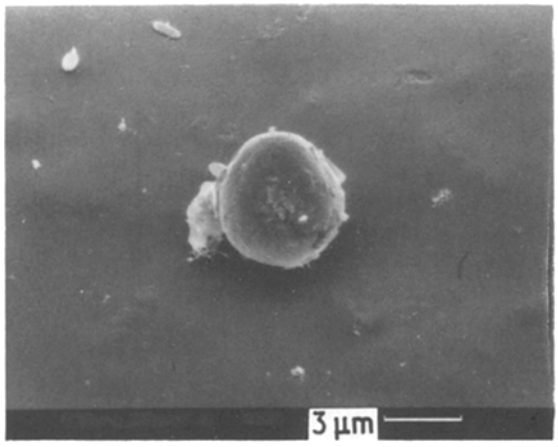

Figure 2 Clean fibreglass, 24 h; settled spore. × 5500.

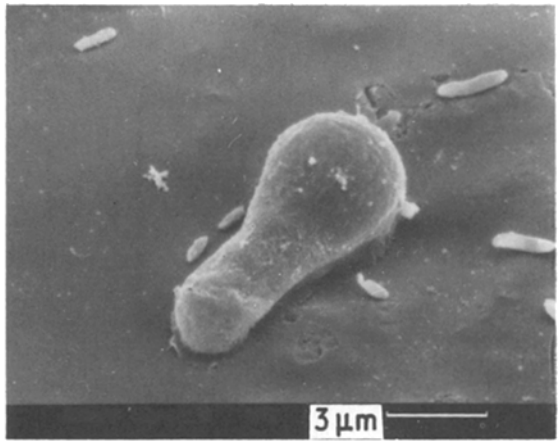

Figure 4 Clean fibreglass, 24 h; germling. × 7000.

**Figures 1 to 32* show the early stages of spore settlement and development of *Ectocarpus* filaments on the range of surfaces studied as revealed by scanning electron microscopy.

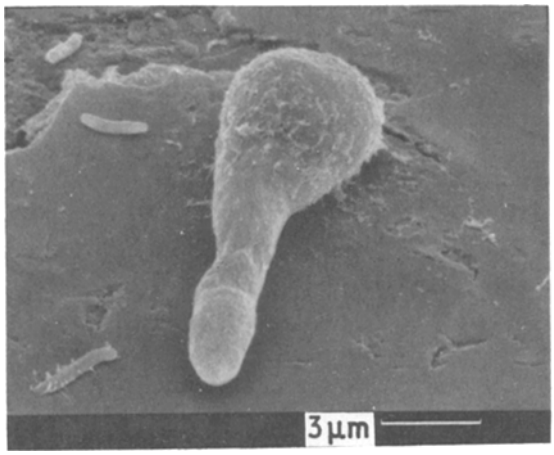

Figure 5 Clean fibreglass, 24 h; germling. × 7000.

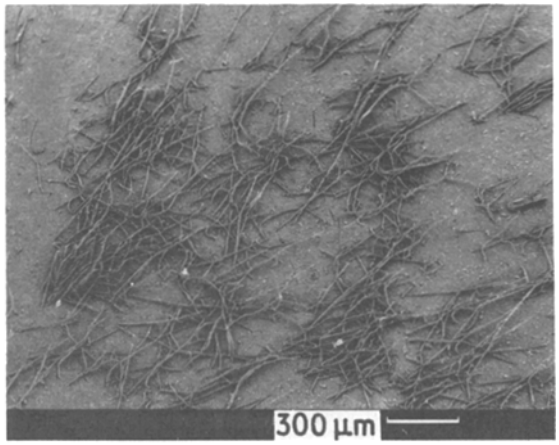

Figure 8 Clean fibreglass, 14 d; mat of filaments. × 50.

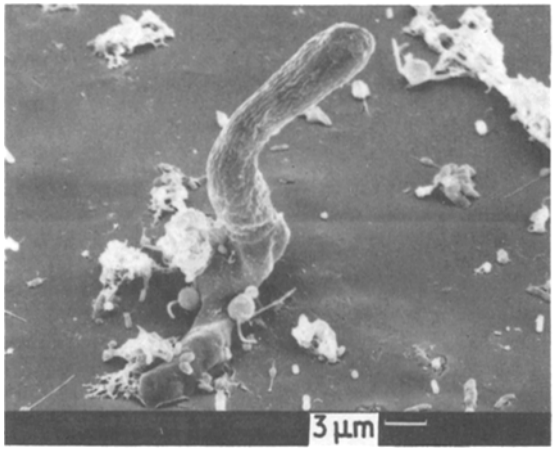

Figure 6 Clean fibreglass, 7 d; prostrate and erect filaments. × 3000.

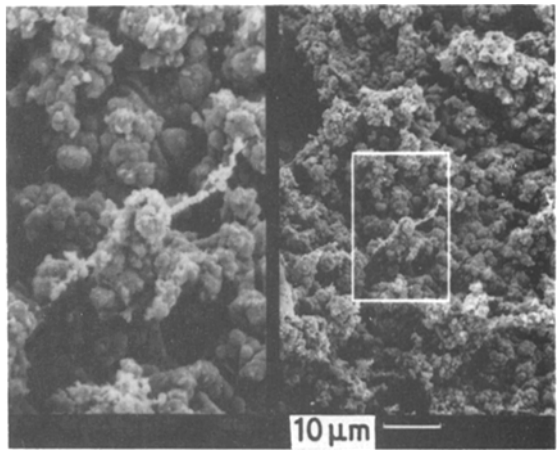

Figure 9 Ordinary copper, 6 h; zoospore. × 1200, inset × 3600.

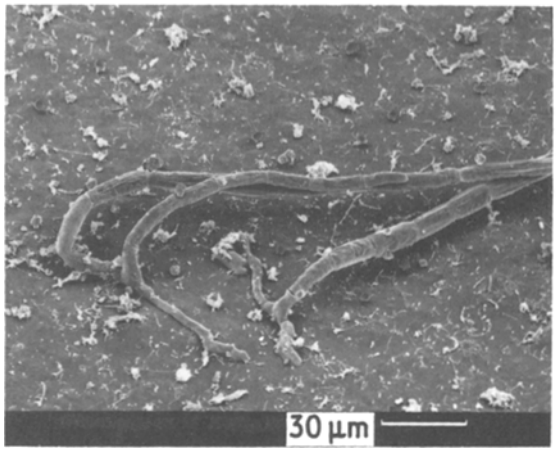

Figure 7 Clean fibreglass, 14 d; prostrate and erect filaments. × 600.

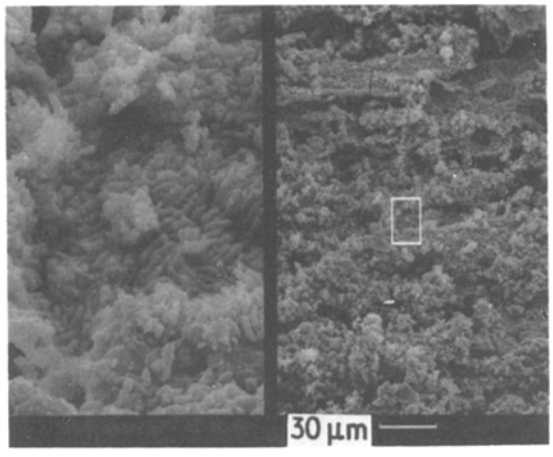

Figure 10 Ordinary copper, 7 d; bacterial contaminants. × 400, inset × 4000.

microbial colonization restricted study of later stages in germination, but erect filaments were present after 7 d. A thick mat of long, mainly unbranched, erect filaments was formed above the microbial layer after 14 d (Fig. 17). Some unhealthy filaments were apparent.

3.2.2.5. Aluminium brass. Algal development proceeded as described on the non-toxic surfaces but germination was not seen until 24 h. A spore (Fig. 18) and a germling (Fig. 19) are shown. After 14 days a thick mat of long, healthy, unbranched, erect filaments was formed (Fig. 20). Microbial colonization was not heavy.

3.2.2.6. Aluminium bronze (prepared). The progress of germination on this surface from settled spore to germling to formation of an erect filament is shown in Figs 21 to 24. There was no difference observed between the structural changes during this development and that on the non-toxic surfaces. However, spore density was lower and after 14 d only a few long

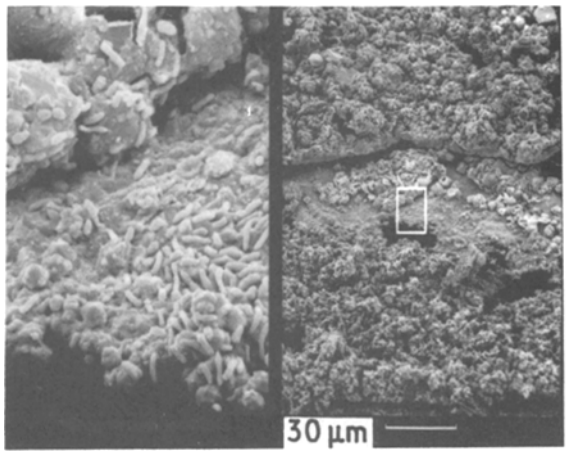

Figure 11 Arsenical copper, 24 h; bacterial contaminants. × 400, inset × 4000.

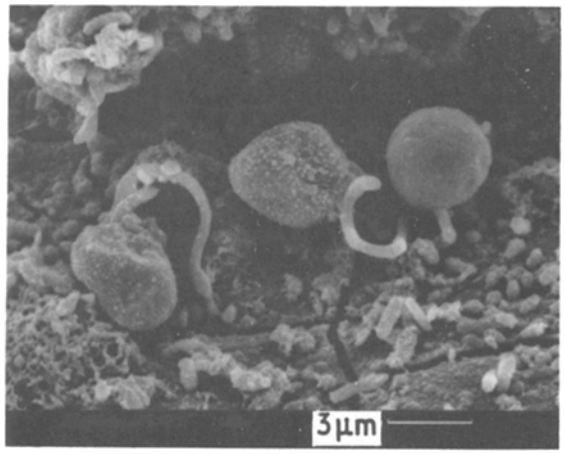

Figure 14 Copper–nickel 90/10, 6 h; settling zoospores. × 6000.

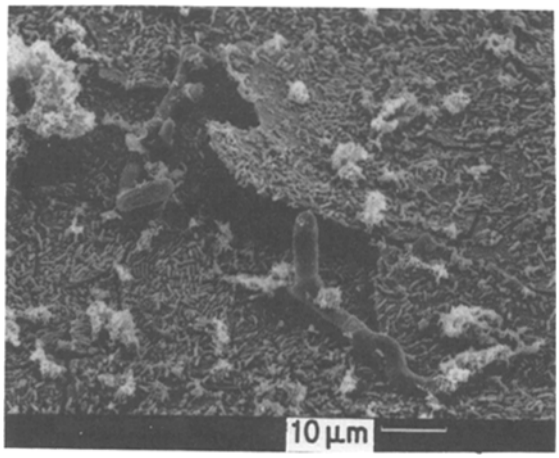

Figure 12 Copper–nickel 90/10, 7 d; prostrate and erect filament. × 1500.

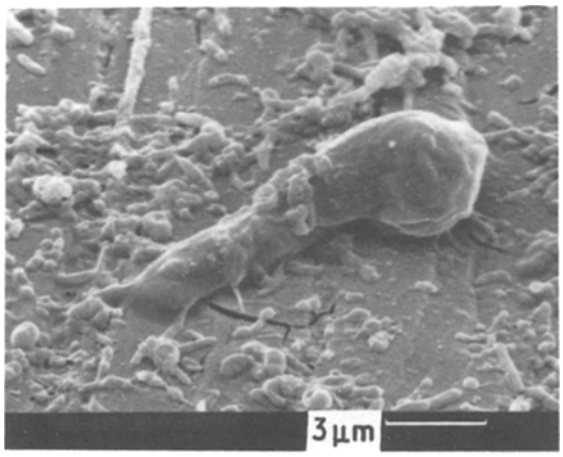

Figure 15 Copper–nickel 90/10, 24 h; germling. × 7000.

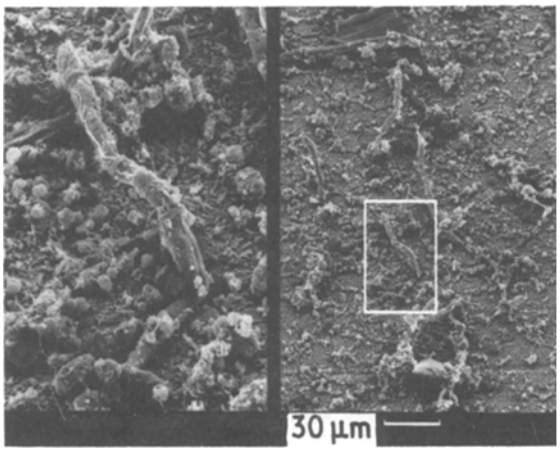

Figure 13 Copper–nickel 90/10, 14 d; unhealthy plants. × 400, inset × 1600.

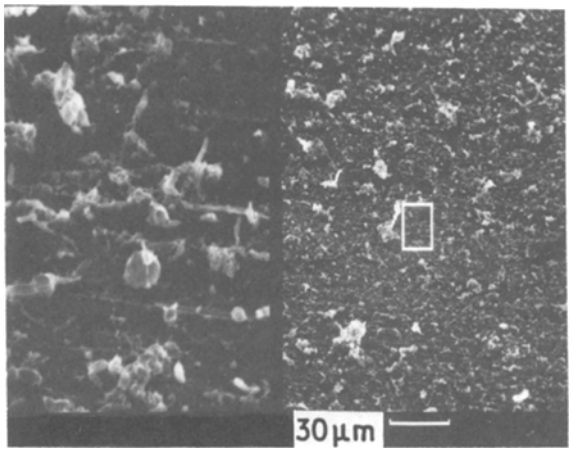

Figure 16 Copper–nickel 70/30, 24 h; settled spores. × 400, inset × 4000.

filaments were recorded (Fig. 25). The smooth, well-striated surface had a covering of micro-organisms. In some areas, small depressions in the surface were seen which contained rough corrosion product and were presumably incipient pits (Fig. 26).

3.2.2.7. Aluminium bronze (as supplied). The surface was made up of two distinct areas. Most of the surface was smooth with small holes (Fig. 27). In a few areas there were corrosion pits, which were circular in outline (approximately 300 μm diameter) and covered with mounds of corrosion product. The immediate surroundings were also covered in corrosion product (Fig. 28). The pit mounds had many cracks in the surface. Algal growth occurred to a small extent on the general surface (Fig. 29) and on parts of the corrosion pits (Figs 30, 31 and 32), being quantitatively greater on the periphery of the corrosion pits (Fig. 31)

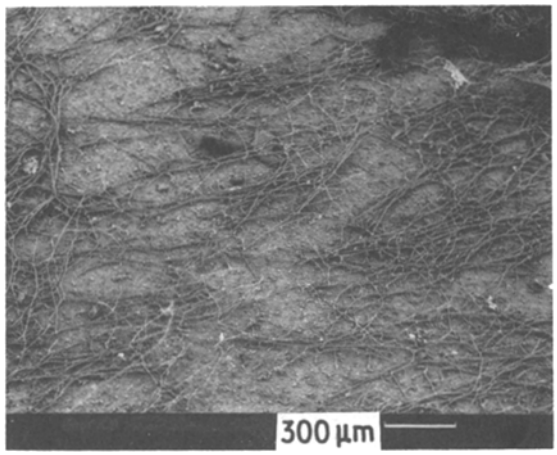

Figure 17 Copper–nickel 70/30, 14 d; mat of filaments. × 50.

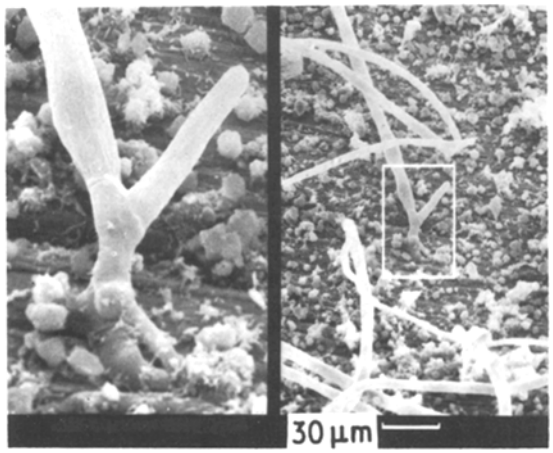

Figure 20 Aluminium brass, 14 d; erect filaments. × 400, inset × 1600.

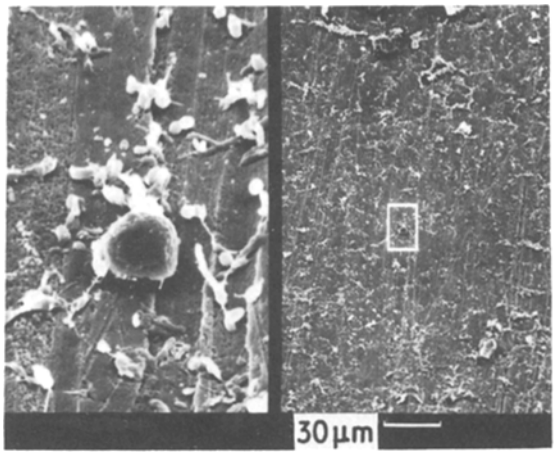

Figure 18 Aluminium brass, 6 h; settled spore. × 400, inset × 4000.

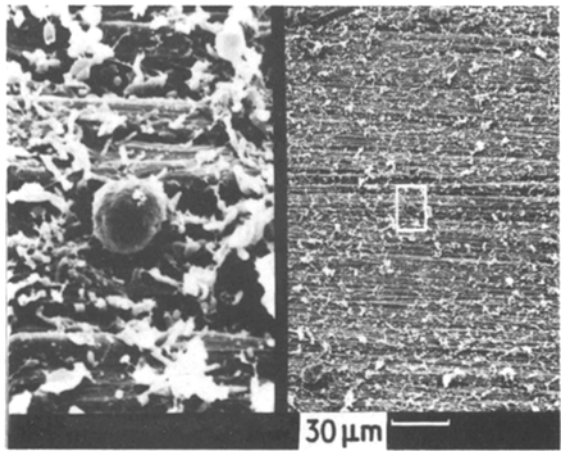

Figure 21 Aluminium bronze, prepared, 6 h; settled spore. × 400, inset × 4000.

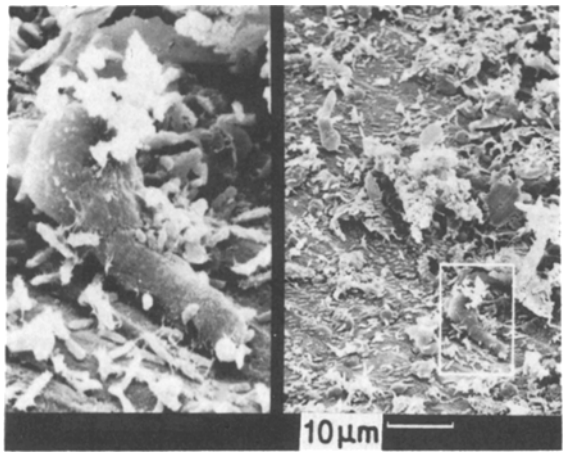

Figure 19 Aluminium brass, 24 h; germling. × 1500, inset × 6000.

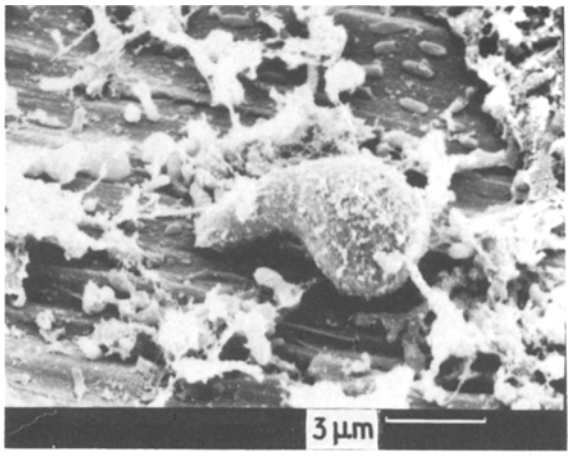

Figure 22 Aluminium bronze, prepared, 24 h; germling. × 7000.

and on the outer rim of the mounds themselves (Fig. 32). The prostrate (anchoring) filaments were not usually visible within the mass of corrosion product. After 14 d the filament growth was slower than on the non-toxic surfaces and on aluminium bronze (prepared) samples but structurally the filaments were in good condition. Microbial colonization was rarely observed in contrast to the aluminium bronze (prepared) surface.

3.3. Settlement and growth of *Ectocarpus* on surfaces during 16 weeks incubation

Observations of the later stages in the colonization of copper surfaces by copper-tolerant *Ectocarpus* were made by light microscopy (magnification × 20) and with the naked eye. After 14 days, the brown mat formed by the long erect filaments and the prostrate filaments is readily observed in this way. Table IV presents average scores for the extent of algal cover

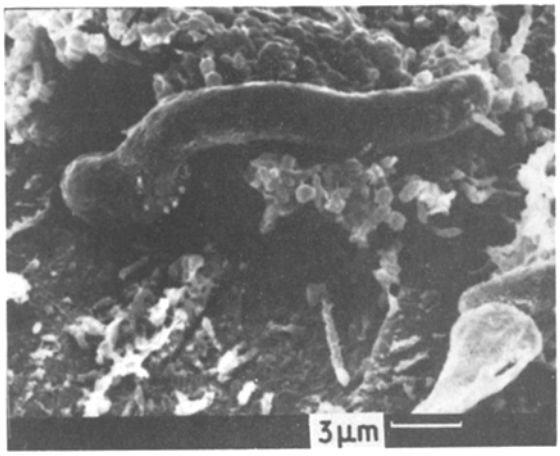

Figure 23 Aluminium bronze, prepared, 4 d; germling. × 5000.

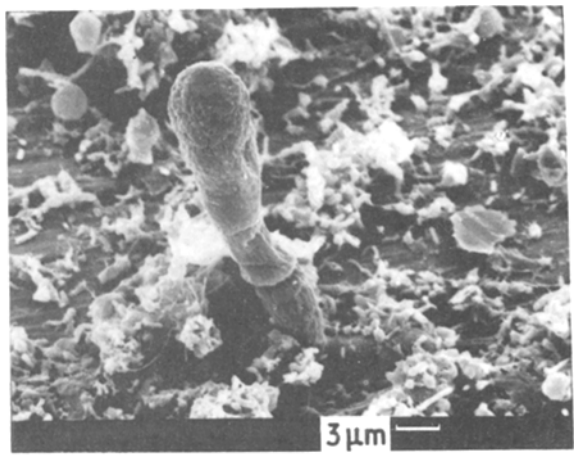

Figure 24 Aluminium bronze, prepared, 7 d; erect filament. × 3000.

observed on two replicates of each copper surface and controls over a period of 2 to 16 weeks. The information recorded for the period 10 to 16 weeks is given provisionally because after 8 weeks, *Ectocarpus* in the cultures incubating with the copper surfaces became contaminated by a single species of a photosynthetic micro-organism, as yet unidentified. The presence of this organism could affect both the growth and spore release of *Ectocarpus* so that observations of colonization on copper surfaces after 8 weeks should be interpreted with caution. However, the experiment was continued for a further 8 weeks to allow any hitherto unnoticed features to become apparent.

3.3.1. The development of Ectocarpus colonies on non-toxic surfaces

Settlement of the alga on non-toxic surfaces under our experimental conditions was apparent microscopically from 2 to 4 weeks and visible to the naked eye as a brown mat from 6 to 8 weeks. Colonization thus proceeded rapidly on all the non-toxic surfaces. The slightly lower scores for Perspex after 4 weeks probably are a result of the smoothness of this surface compared to the other controls, which could have resulted in less successful spore settlement, filament attachment and development of algal colonies. Successful colonization through to brown mat formation on the "Lacomit" surfaces suggested no problems of toxicity resulting from application of this protective lacquer.

TABLE IV Extent of colonization by *Ectocarpus siliculosus* on copper and other surfaces for up to 16 weeks incubation, as observed by light microscopy. Figures are estimates of cover-abundance (see key below)

Sample	Time of observation (weeks)							
	2	4	6	8	10	12	14	16
Ordinary copper	0	0	0	0	0	0	0	0
Arsenical copper	0	0	0	0	0	0	0	0
Copper–nickel 90/10	0	0	0	0	1	1	1	2
Copper–nickel 70/30	0	2	8	8	8	9*	9*	9*
Aluminium brass	0	2	5	6	7	7*	7*	7*
Aluminium bronze (prepared)	0	4	4	4	6	6*	6*	7*
Aluminium bronze (as-supplied)	0	0	1	1	1	2	2	3
Fibreglass	1	1	6	7	7*	7*	7*	7*
Perspex	0	4	5	5	5*	5*	5*	5*
"Lacomit"	0	2	5	7	7	8*	8*	8*

Key:
0, absent
1, filaments present in very small numbers
2, 25% cover – mainly microscopic
3, 50% cover – mainly microscopic
4, 75% cover – mainly microscopic
5, 100% cover – mainly microscopic
6, 25% cover – visible brown mat
7, 50% cover – visible brown mat
8, 75% cover – visible brown mat
9, 100% cover – visible brown mat
*Contaminating micro-organisms present on surface.

3.3.2. The development of Ectocarpus colonies on copper and copper-alloy surfaces

The extent of algal settlement on these surfaces was very variable. A brown mat was formed on three of the copper alloys (copper–nickel 70/30, aluminium brass and aluminium bronze – prepared), microscopic settlement only on two surfaces (copper–nickel 90/10 and aluminium bronze – as-supplied) and no settlement on the two copper surfaces. After 10 weeks incubation of copper–nickel 70/30, it became apparent that settlement was heaviest along straight lines suggesting that some features introduced by the original abrading may have produced areas of the surface more favourable for colonization. Subsquent mat for-

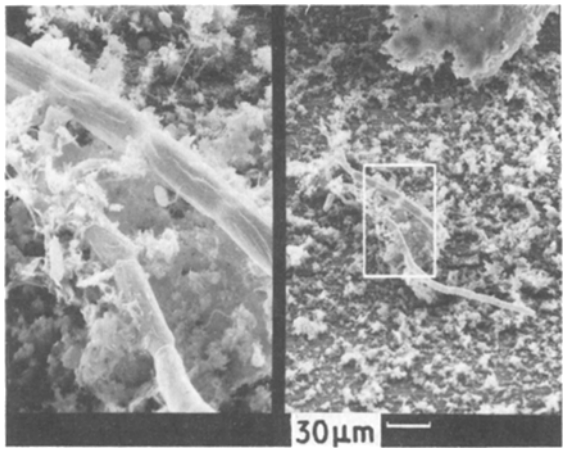

Figure 25 Aluminium bronze, prepared, 14 d; erect filaments. × 300, inset × 1200.

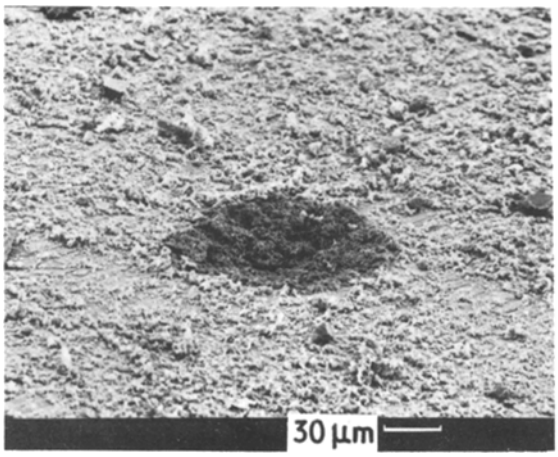

Figure 26 Aluminium bronze, prepared, 7 d; incipient pit. × 400.

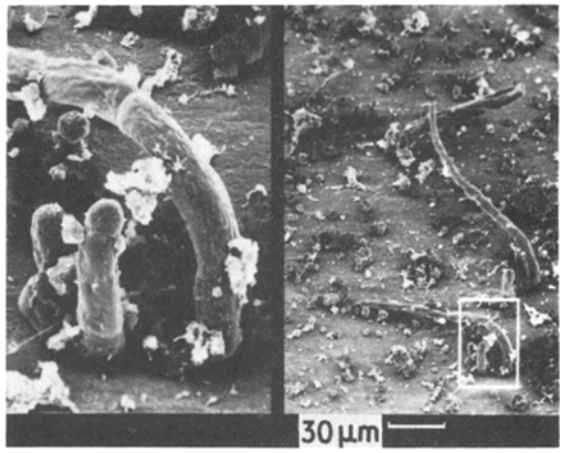

Figure 28 Aluminium bronze, as-supplied, 14 d; erect filaments. × 400, inset × 2000.

mation was rapid and complete by 12 weeks. Although other surfaces were abraded in the same way, linear colonization patterns were only also observed to a very limited extent on aluminium brass. Surface conditions also clearly affected the colonization of aluminium bronze, the as-supplied material having the greater resistance to settlement and mat formation. Unsuccessful attempts to remove filaments of the alga associated with corrosion pits on this surface suggested that they were indeed anchored filaments and not free-living material derived from the incubating alga in the seawater medium subsequently attached to the rough surface. The corrosion pits were found to be grouped on the surface in bands which led to some colonization of *Ectocarpus* in a regular pattern.

4. Discussion

4.1. Surface toxicity and algal settlement

The results of the SEM studies reported here suggest that zoospores of copper-tolerant *Ectocarpus* settled on all the copper surfaces tested since spherical spores with a cell wall but without flagella were observed on all surfaces. The further success of this initial settlement was variable. It appeared that the toxicity of ordinary copper and arsenical copper surfaces was great enough to cause even this initial development to be morphologically atypical and no further settlement was observed. In complete contrast, the corrosion films formed on copper–nickel 70/30 and aluminium brass had little detectable toxic effect on the alga. Growth on these surfaces proceeded almost as vigorously as on copper-free surfaces and only the very early stages of settlement within the first 24 h proceeded more slowly. The pattern of settlement on aluminium bronze (prepared) suggested that the corrosion film was toxic to some extent. The atypical morphology of much of the material observed by SEM on the surface, the small number of spores which developed successfully and the inceased time taken to form a brown mat were all evidence of this. The surface of copper–nickel 90/10 was sufficiently toxic to prevent algal colonization throughout most of the 16 week experimental period. It is interesting to note that early stages of settlement (up to the development of an erect filament) took place normally during this period and that inhibition did not occur until after 7 days growth. Structural disintegration was evident after this time suggesting that the gradual accumulation of copper absorbed by the filaments from the surface eventually reached a toxic level, making further growth impossible. The algal colonization which did take place at the end of the experimental period was not widespread and was slow-growing.

4.2. Surface toxicity and algal morphology

There were no obvious differences in the morphology

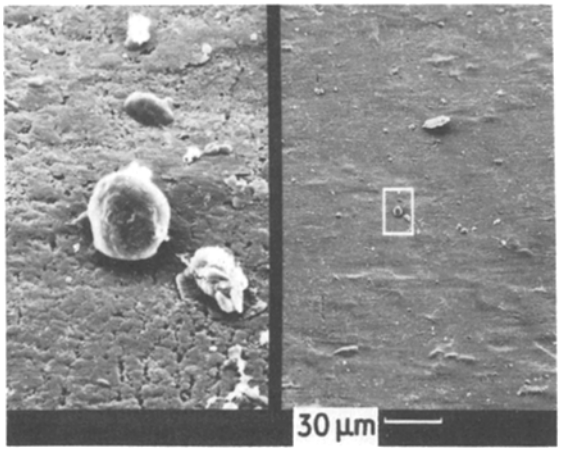

Figure 27 Aluminium bronze, as-supplied, 24 h; settled spore. × 400, inset × 4000.

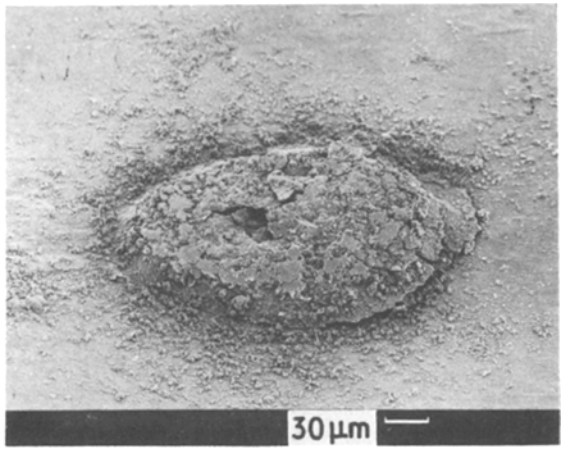

Figure 29 Aluminium bronze, as-supplied, 24 h; corrosion pit. × 300.

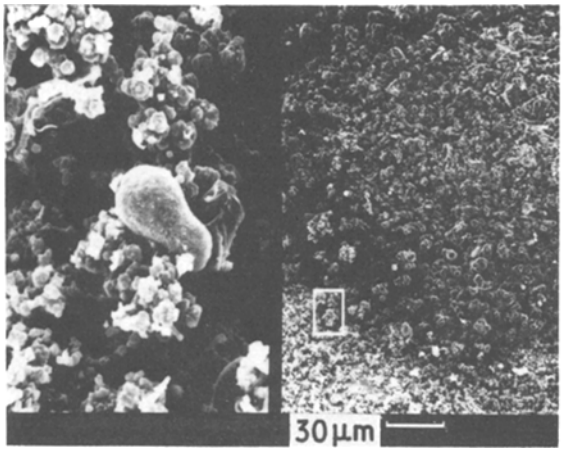

Figure 30 Aluminium bronze, as-supplied, 4 d; germling near corrosion pit. × 400, inset × 4000.

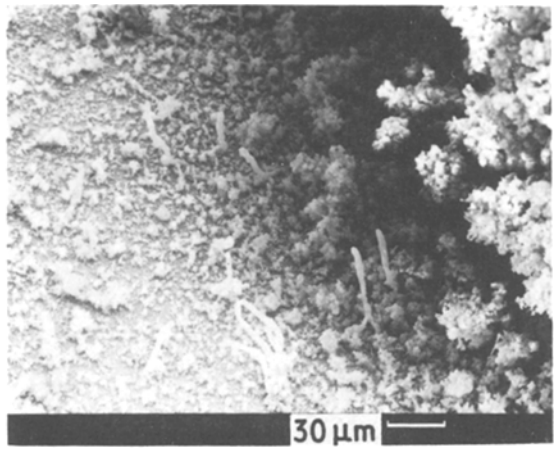

Figure 32 Aluminium bronze, as-supplied, 7 d; erect filaments near corrosion pit. × 600.

of the early developmental stages of copper-tolerant *Ectocarpus* settling on the copper surfaces and on the copper-free surfaces. Neither were there differences in the amounts of extracellular material observed around the settled spore and prostrate filaments. This suggests that even high copper concentrations do not result in structural modifications during this developmental phase. During later development some differences were, however, noted. Filaments were more easily removed from the copper-free surfaces but there was no obvious structural difference observed with SEM studies to explain why this should be the case. It may be that the smoothness of the control surfaces accounted for this difference as it is known that *Ectocarpus* spores will settle more readily on rougher surfaces which may provide better anchorage [2]. Differences were also noted in the extent of the erect filament system on the copper surfaces containing approximately 90% copper, the toxicity bringing about a reduction in filament length.

4.3. Surface preparation and algal settlment

Algal settlement was studied on the surface of aluminium bronze in two different surface conditions. The as-supplied samples retained the surface that had been formed during manufacture with a relatively thick, hot-formed oxide scale. The prepared samples had been abraded to remove the film and expose the unoxidized alloy surface. Evidence presented here shows that the two surfaces gave different antifouling performance. The corrosion films on these surfaces appeared different under SEM studies. The as-supplied surface was generally smoother with isolated corrosion pits and a corrosion rate approximately ten times greater than the prepared surface. In contrast, the prepared surface was rougher due to the presence of lines of abrasion and a few small depressions were also observed. Algal settlement on these films was different. There was much less settlement on the as-supplied samples and much of this occurred around the corrosion pits. On the prepared surfaces the greater amount of settlement present was not associated with particular structures on the surface. The protective film formed in seawater on the machined samples was evidently less toxic than that on the hot-formed film on the as-supplied samples. The increased roughness of the prepared surface may also have contributed to the additional settlement of *Ectocarpus* on this surface. Localized differences in surface characteristics also affected settlement on copper–nickel 70/30 and to a lesser extent on aluminium brass.

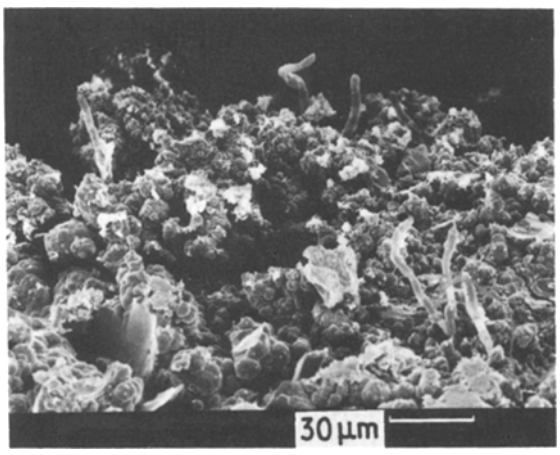

Figure 31 Aluminium bronze, as-supplied, 7 d; erect filaments near corrosion pit. × 400.

4.4. Corrosion pits and algal settlement

Corrosion pits developed only on the aluminium bronze as-supplied samples presumably at defects in the hot-formed oxide film. SEM studies revealed that the alga settled preferentially, but not exclusively, in close proximity to these pits. Algal filaments were observed within the outer rim of the pit itself and in a band immediately surrounding the pit, growing amongst the particles of corrosion product. LaQue [3] reports that on aluminium bronze subject to localized corrosion, fouling will occur on the uncorroded parts of the surfaces. Recent electro-chemical studies [4] showed that in cathodic regions copper does not come into solution. In the present case, SEM studies of algal settlement showed only a small unsettled region in the centre of the pit compared with a large outer area of settlement which would be consistent with these findings. These features are presumably a reflection of the morphology of pits and the cathodic activity of the hot-formed oxide film in the vicinity of the pits.

TABLE V A comparison of averaged rates of corrosion of the materials tested (estimated from measurements of copper concentration in the incubating medium) with published corrosion rates in trials at sea by other workers (measured by weight loss)

Material composition (%)					Duration of trial (d)	Corrosion rate ($\mu g\,cm^{-2}\,d^{-1}$)
Ordinary copper	Cu	As	P			
Bulow [6]	99.95	–	0.009		365	93
LaQue and Clapp [7]	~100				78	260
Hall and Baker (this study)	99.86	0.05	0.032		126	100
Arsenical copper	Cu	As	P			
Bulow [6]	99.63	0.33	0.023		365	107
Hall and Baker (this study)	99.56	0.34	0.025		126	57
Copper–nickel 90/10	Cu	Ni	Fe	Mn		
LaQue [8]	85.1	12.6	1.81	0.43	365	7
Efird and Anderson [5]	87.7	10.21	1.74	0.29	365	7–32
Hall and Baker (this study)	86.97	10.28	1.65	0.80	126	5
Copper–nickel 70/30	Cu	Ni	Fe	Mn		
Bulow [6]	69.95	28.94	0.42	0.63	365	21
Efird and Anderson [5]	68.2	30.68	0.61	0.45	365	5–27
Hall and Baker (this study)	67.64	30.32	0.85	0.89	126	3
Aluminium brass	Cu	As	Al	Zn		
Bulow [6]	76.49	0.014	1.99	21.48	365	45
Hall and Baker (this study)	76.31	0.037	1.99	21.36	126	1
Aluminium bronze	Cu	Fe	Al			
LaQue [8]	92.1	0.12	7.8		243	28
Bulow [6]	94.97	0.02	5.02		365	105
Hall and Baker (this study)	89.22	3.20	7.20		126	1

4.5. Corrosion rates of copper and copper-alloy surfaces

Average corrosion rates during preincubation of the materials tested have been calculated from Table II and are presented in Table V together with published corrosion rates for similar materials in sea trials. Copper losses from laboratory incubated samples of ordinary copper, arsenical copper, copper–nickel 90/10 and copper–nickel 70/30 were similar to losses from those materials exposed at sea, but much lower for aluminium bronze and aluminium brass. The development of much more protective films on the two latter materials in the present study could be due to differences in exposure conditions and/or composition and surface preparation. It is interesting to note that despite these large differences in corrosion rates for aluminium brass and aluminium bronze, the antifouling performance of the surfaces was not changed to any great extent. It may be that the nature of the corrosion product provides antifouling performance rather than the leaching rate of copper from the surface, in accordance with the findings of Efird and Anderson [5].

4.6. Overall assessment of antifouling performance

On the basis of the early observations on algal settlement and subsequent growth on sample plates of the range of materials examined, an assessment of overall antifouling performance is possible. A ranking of the six materials is presented in Table VI. The arbitrary scale of 0 to 4 combines both qualitative observations with quantitative estimates of algal cover at the end of the 16 week incubation period.

Previous studies on the antifouling nature of copper surfaces have taken place in the sea where settlement of many species of fouling organisms was observed, rather than one single species as was the case in this study. Ordinary copper, arsenical copper, copper–nickel 90/10, aluminium bronze and aluminium brass all had antifouling properties in seawater trials as in this study [5–7]. However, the behaviour of copper–nickel 70/30 is somewhat anomalous. The antifouling properties of this alloy are known to be influenced adversely when iron content is increased [3, 8] as in the sample used (see Table V). Efird and Anderson [5] showed that the 70/30 alloy was relatively free from hard shell fouling after 14 years. These results suggest that the surface of copper–nickel 70/30 has some antifouling properties and therefore might be expected to be toxic to *Ectocarpus*. In fact this organism did grow on this alloy in our study. It thus appears that *Ectocarpus* is one organism capable of growth on this alloy in some circumstances. It is doubtful that this result indicates a particular algal tolerance to the nickel component as the nickel content of the incubating

TABLE VI Overall assessment of the antifouling performance of the materials tested using copper-tolerant *Ectocarpus*

Material	Antifouling performance
Ordinary copper	4
Arsenical copper	4
Copper–nickel 90/10	3
Aluminium bronze	1
Copper–nickel 70/30	0
Aluminium brass	0

Key: 4, no growth, 3, inhibited growth on <25% of surface; 2, inhibited growth on 25% to 75% of surface; 1, inhibited growth over >75% of surface; 0, normal growth, cover almost complete.

medium was no greater than that for other surfaces. Comparison of the corrosion films formed in the laboratory with those formed on copper–nickel 70/30 during immersion at sea may indicate whether or not chemical differences account for these differences in toxicity.

The results of the relatively short-term trials reported in this paper thus suggest that settlement and growth of the copper-tolerant strain of *Ectocarpus siliculosus* employed can provide useful and rapid information on the long-term antifouling potential of copper and copper-alloy materials. The bio-assay system developed may therefore have wider application in routine screening of materials and surfaces under laboratory conditions.

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