THE IMMOBILIZATION OF COPPER BY MARINE FOULING MICROORGANISMS

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A. EXECUTIVE SUMMARY

Copper and copper alloys are used in the marine environment because of their excellent resistance to corrosion and biofouling. Typical applications are in condenser tubing and as sheathing for ships and offshore structures. Despite this initial biofouling resistance, microfouling and macrofouling (attachment of barnacles, etc.) may occur and then later slough off. This study was undertaken to determine the mechanism involved and give assurance of continuing biofouling resistance in the long life (e.g. forty years) service required for offshore structures. Also, a better understanding of the relative biofouling resistance of the alloys would allow more efficient selection for different marine applications.

In this work it was discovered for the first time that alternating layers of corrosion products and bacteria develop on 90/10 CuNi surfaces. The subsequent decay of the bacteria film may trigger the sloughing off mechanism. This layering effect was not observed with copper or other copper alloys.

Measurements on the first microfouling layers, however, showed that unexpectedly they were firmly attached to the various alloys.

B. TECHNICAL SUMMARY

This study has demonstrated that copper and copper alloy surfaces (90/10 copper-nickel, aluminium brass) are able to prevent fouling for periods of up to 22 weeks. Bacteria and algae settle on these toxic surfaces after this time but slime films never seem to become established. On the non-toxic surfaces of titanium fouling organisms settle soon after exposure and slime films became thicker than those on copper, 90/10 copper nickel and aluminium brass. The antifouling nature of these toxic surfaces can in part be attributed to the leaching of copper ions but it has been noted that corrosion products and any macroorganism settlement on these surfaces intermittently peels or sloughs off revealing clean, non-fouled areas. This means that slime films, although present, are never able to become very thick. This was the situation after approximately 3 years. In this respect copper and copper alloys compare favourably with an "average" anti-fouling paint and the sloughing off of films and corrosion products may continue over long periods (Efird and Anderson, 1975). This has lead to the use of these alloys as antifouling surfaces as a viable economic proposition for physically demanding marine applications.

This study has documented the development of slime films on titanium, copper and copper alloy surfaces and the results indicate a) that bacteria are closely associated with corrosion products, b) that these bacteria can withstand the toxic ions which are released from surfaces by:

i) immobilizing copper extracellularly in bacterial mucilage,

ii) immobilizing copper in bacterial cell walls, and

iii) immobilizing copper intracellularly.

The close association of bacteria and corrosion products on these surfaces suggests they play a role in corrosion of copper and copper alloys. On copper, bacteria are incorporated in the corrosion layer. On 90/10 copper-nickel there are alternating layers of bacteria and corrosion products. It is these parallel layers on 90/10 copper-nickel which have been shown to break away exposing a relatively clean new surface.

The parallel corrosion layers which were found on 90/10 copper-nickel exposed in the sea after 22 weeks did not develop on this alloy in estuarine conditions until 70 weeks. Similarly the corrosion products on aluminium brass exposed in these environments for approximately 20 weeks also have a different structure. These corrosion products have been analysed and the differences for each alloy studied in these environments have been recorded.

The bonding between bacterial mucilage and corrosion products appears to be weak and insufficient to support the additional weight of hard shell fouling and the drag forces created by seasonal storms and tidal flows. Attempts to measure the bond strength of the layered slime film on 90/10 copper-nickel have been unsuccessful. The tests suggest there may be additional factors creating periodic variations in bond strength between the individual layers, which contribute to the 'sloughing off' mechanisms.

Comparative studies on the antifouling properties of paints containing copper powder and copper flakes showed that they were heavily fouled within weeks. Reasons for this poor performance are discussed.

C. INTRODUCTION

Copper alloys particularly 90/10 copper-nickel and aluminium brass are used extensively in situations where high resistance to corrosion is required. These alloys also have pronounced biofouling resistance and are therefore useful in controlled sea water environments such as condenser tubes where both of these characteristics are valuable. In addition to this, copper alloys, particularly 90/10 copper-nickel, are being increasingly used in open marine environments for intake piping, for sheathing boat hulls an off-shore platform structures, and in the form of mesh for fish cages and diversion screenings. For example fishing vessels and fire tugs constructed from 90/10 copper-nickel have given good results (Moreton and Glover, 1980). This alloy has now been MIG welded to larger steel structures such as offshore platforms in order to prevent corrosion. Observations of these struct from the time of fabrication should give information on the behaviour of large welded areas of copper-nickel in the sea.

Elucidation of the underlying anti-fouling mechanism of these alloys is of considerable technical and economic importance. The present study was therefore initiated to contribute towards a better understanding of the fouling process on copper and copper alloys compared to that on non-toxic surfaces. In particular, more information was required on the structure and composition of the fouling slime community and its interaction with the metal/alloy surface. It was also necessary to determine if these alloys have surface properties other than copper ion release which prevent biofouling and to which extent these toxic ions are immobilized by the fouling micro-organisms.

D. PROJECT OBJECTIVES

The broad aims of this project are threefold:

a) to measure the accumulation of bacteria, diatoms and other algae on different metal surfaces in marine and estuarine environments,

b) to study the interaction of the surface of these metals and fouling organisms. Copper ions leaching from anti-fouling paint coatings has previously been shown to be immobilised intracellularly in some diatoms and the possibility exists that other organisms immobilize copper ions intracellularly (Daniel and Chamberlain, 1981). As well as intracellular immobilization copper may also be bound to extracellular carbohydrates. Using X-ray microanalysis on transmission electron microscopy (T.E.M.) sections through slime films and biochemical techniques on isolated mucilage it should be possible to elucidate the mechanisms of copper resistance.

c) to measure the adherence of slime films during their development.

E. MATERIALS AND METHODS

I. Exposure conditions: Discs for characterisation of biofilm settlement with time

In this study 13 mm discs of pure copper, 90/10 copper nickel, aluminium brass and titanium were exposed in:

a) Coastal marine conditions in Langstone Harbour, Hampshire. Discs were attached to frames which were submerged to a depth of 1 meter. Some of the discs were exposed to three consecutive fouling sessions in 1983, 1984 and 1985.

b) The Thames Estuary near Dartford in Kent where the discs were totally submerged for periods of over two years from November, 1982. Discs were also exposed in indoor marine tanks with flowing sea water, bacterial cultures and in activated sewage sludge at Budds Farm sewage works, Portsmouth. In these environments seasonal fluctuations were absent or were more limited than those in the open sea or in the Thames Estuary.
Further treatment of the discs is outlined in section E.II.

I. Exposure conditions: Plates for biofouling adhesion tests

In this study large 10cm diameter plates, machine cut to size by Dr. Wise, University of Birmingham, were prepared from a range of metals/alloys. These were then immersed in an outside tank condition at Hayling Marine Laboratory. Corrosion was monitored using the light section microscope and the adhesive strength of the corrosium products investigated using the Radial Flow Growth Chamber (see below). Altogether 8 metals/alloys were used. These were copper, titanium, 90/10 copper-nickel, cunifer 30, aluminium brass, 70/30 copper-nickel, cunifer 10 and stainless steel.

I. Exposure conditions: Copper antifouling paints

Paints containing crystic copper flakes were used to compare the antifoul behaviour of copper and copper alloys with various proprietory copper antifouli coatings. Strips 3 cm x 7 cm of 3 paint samples containing 1) copper powder in a polyester gel-coat system, 1) copper powder in a polyester flow coat system, and 3) copper flakes in an epoxy matrix , were exposed in the sea at Langstone Harbour.

Samples were taken regularly and colonization by marine fouling organisms monitored by scanning electron microscopy (S.E.M.). The fixation

procedure was similar to that for T.E.M. (see section E.II.) but after dehydration in ethanol, paint samples were critical point dried and coated with gold in a sputter coater.

Examination was also made by light microscopy and observations made of the fouling community present. Measurements of biofilm thickness were also obtained using the light section microscope.

II. Electron microscope studies: Transmission electron microscope.

After exposure for varying lengths of time discs were removed and fixed for electron microscopy in 4% gluteraldehyde, buffered with 0.1M sodium cacodylate, pH 7.2. 3% sodium chloride was added to the fixative if the material was exposed in the sea.

After washing, discs were post fixed in 1% osmium tetroxide again buffered with sodium cacodylate, before being dehydrated in an acetone series and embedded in expoxy resin mixture.

After polymerisation, the resin with embedded biological material was removed from the alloy surface by immersing in liquid nitrogen and then in water at 40°C. Approximately 50 nm thick sections were cut on an L.KB3 or ultramicrotone using a diamond knife. They were stained in uranyl acetate and lead citrate and examined in a JEOL 100S electron transmission microscope.

Slime film thickness was determined by:

a) measuring the thickness of film in the T.E.M.,

b) measuring the thickness of films in thick 1µm sections: which gave a measurement for the width of both the corrosion layer and slime film, and,

c) measuring film thickness perpendicularly to the surface of the metal in a polymerised E.M. block.

II. Electron microscope studies: Energy dispersive X-ray microanalysis (EDAX).

Energy dispersive X-ray microanalysis (EDAX) was carried out using a Philips E.M. 400 analytical electron microscope equipped with a Link 860 X-spectrophotometer. Microanalysis was performed on thick sections (140 nm) were unstained and which had not been post-osmicated.

III. Measurement of film thickness: light section microscope

Particular interest has recently focussed on the measurement of slime thickness in view of its effect on fluid frictional resistance (Characklis, Trulear, Bryers and Zelver, 1982; Tokunaga and Baba, 1980; Mitchell and Kir 1981). Although various methods have been proposed, the use of light microscopy and in particular the light section microscope, has gained increasing support (Loeb, 1980, 1981; Loeb and Smith, 1981). With the recent acquisition of such a microscope at the Marine Laboratory an investigation was carried out of corrosion product thickness using the metals/alloys.

To determine film thickness, the discs were placed on the bench and a long cut made in the corrosion film. The corrosion film was then carefully removed from one half of this cut (Figure A). Measurements were taken at 0.25 mm intervals along the cut and slightly to either side of it in order to avoid any distortion of the corrosion products and/or metal/all surface caused by the insertion of the razor blade. Approximately 30 separate measurements of film thickness were then taken along the length of each cut. Usually two cuts were made on each of the eight discs in representative areas of corrosion and the average measurement of corrosion thick is presented.

FIGURE A.

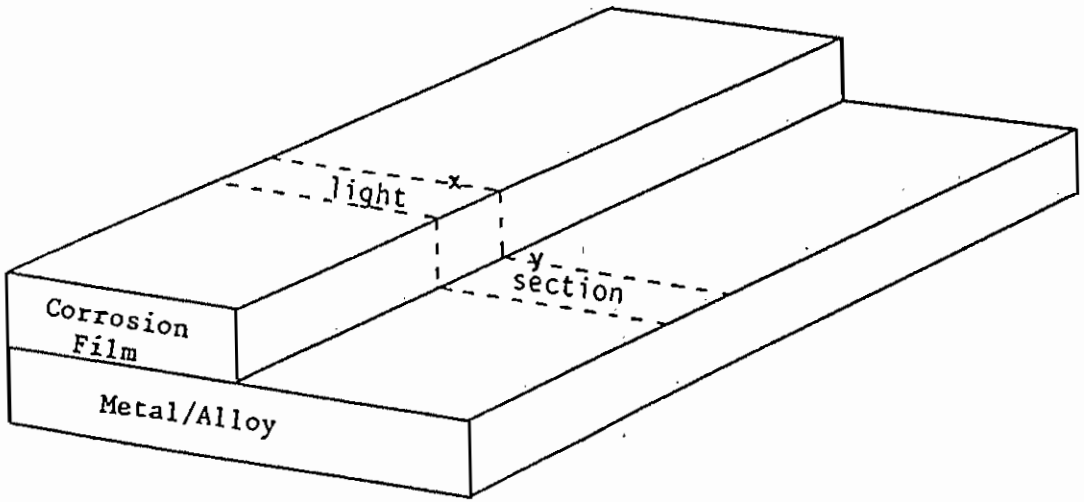
FILM THICKNESS MEASUREMENT

$$X - Y = \text{Film thickness (arbitrary units)} + \frac{1000 \times C \times S(m)}{2 \times B} = \text{Film thickness } (\mu m)$$

Where:- B = No.of graduation intervals of the eye piece micrometer which corresponds to the number C

C = No.of graduation intervals of object measuring plate which corresponds to number B

S(m) = Metric value of graduation of the object measuring plate (in this case 0.01 mm)



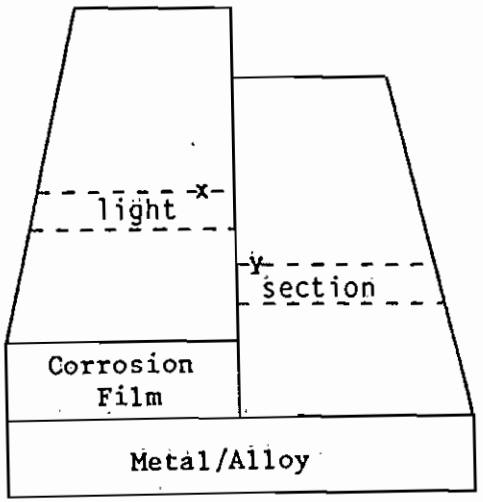
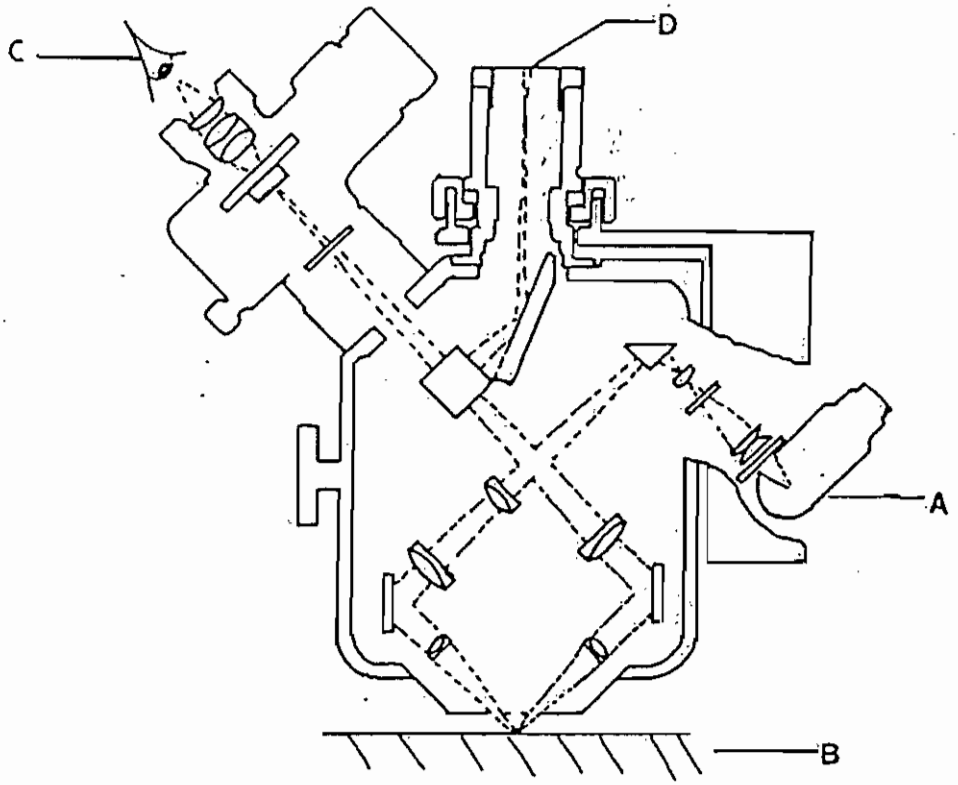


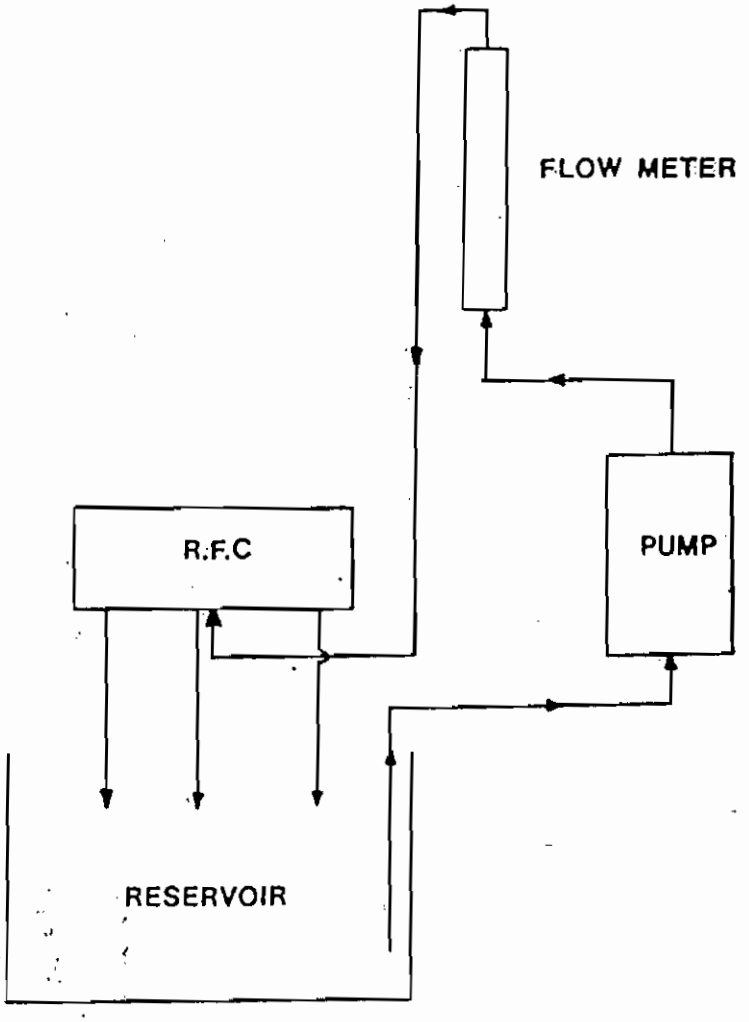
FIGURE B. Diagramatic representation of the light section microscope.



A = illumination light.
B = Substrate.
C = Visual observer.
D = Camera attachment site.

FIGURE C.

FLOW DIAGRAM OF RADIAL FLOW APPARATUS



KEY TO FIGURE C.

A = Base plate with legs d, and fastening screws e, extending from it.

B = Upper plate with central viewing port.

C = Test disc.

D = Silicon rubber "O ring".

a = Central entrance port for test fluid.

b_1 = Sectioned exit port for test fluid.

b_2 = Exit port for test fluid.

d_1 = Sectioned leg attached to base plate A.

d_2 = Leg attached to base plate A.

e_1 = Sectioned fastening screw.

e_2 = Fastening screw.

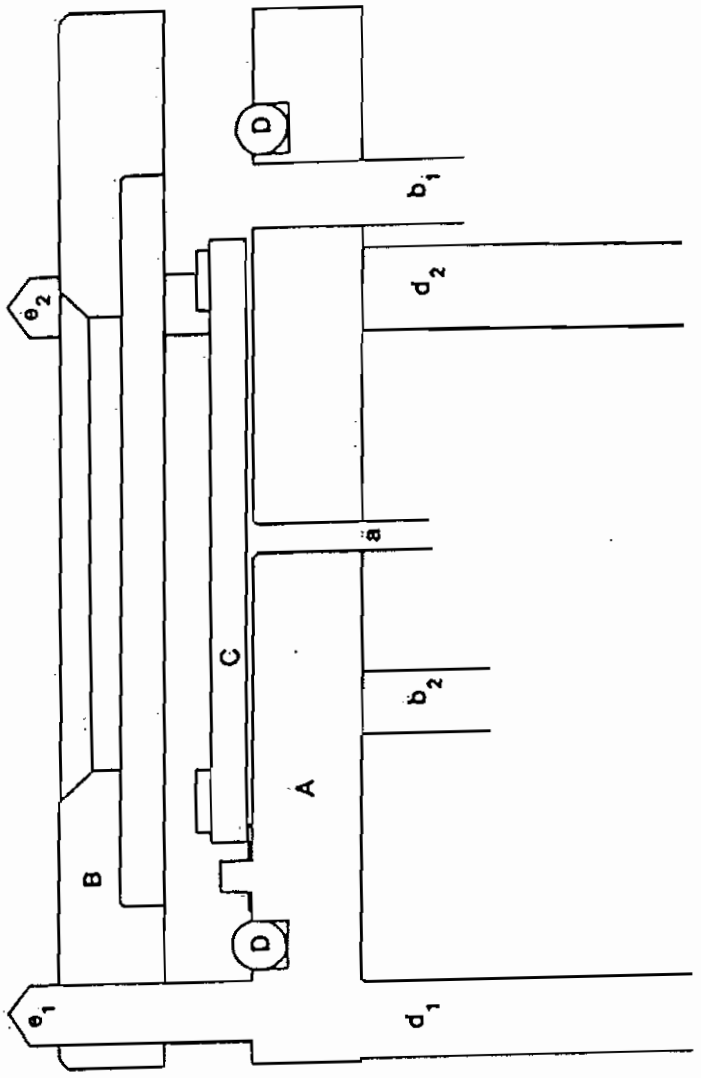


FIGURE D. SECTION THROUGH RADIAL FLOW CHAMBER

When using the Light Section Microscope a narrow slit of light (a light section) is projected onto the surface of the specimen at an angle of 45°. Light reflected from the surface at 45° to that surface and 90° from the projected light beam, is collected by the objective lens (Figure B). Any aberration in an otherwise uniform surface causes a discontinuity in the reflected light section which can be measured (Figure A). These measurements were taken from one edge of the light section at the top and bottom of the cut step and were relative to that point's position in the field of view. The difference between the two measurements represents film thickness and can be converted to micrometers.

IV. Extraction of copper containing proteins from diatoms grown in culture.

Amphiprora hyalina cells were isolated from aluminium brass surfaces exposed in marine tanks. Cells were grown on 2/3rds strength F_2 medium (Guillard and Ryther, 1962) with the omission of both Tris buffer and EDTA. Copper was added to give a final concentration of 15 ppm (15 ug/ml) as $Cu\ Cl_2$. Cells after growth in this medium for periods of over 4 weeks were harvested by centrifugation, washed in media not containing copper and resuspended in cold Tris buffer, 0.5M, pH 7.8, containing 0.5% mercaptoethanol. Cells were sonicated (using an M.S.E. soni-prep) and the soluble proteins collected in the supernatant after centrifugation. Native proteins were separated using electrophoresis on a polyacrylmide tube gel syste After running, cells were stained in a mixture of caramine blue, acetic acid and methanol. Gels were de-stained in acetic acid and methanol and separate bands from eight similar gels were excised and similar bands pooled. The concentration of copper in these bands was determined using atomic absorbance spectrophotometry.

V. Radial flow chamber studies

The adhesive strength of the corrosion products were investigated using the Radial Flow Chamber (R.F.C.) of Fowler and McKay. Details of the R.F.C. and its operation are illustrated in Figures C and D. It is based on a re-circulating system with water passing from a reservoir into the pump, the flow meter and then the R.F.C. (Figure C). The water enters the chamber through the inlet port (see Figure D), flows radially out across the base plate, A. and test surface, C, to the exit ports, b_1 and b_2 and then back to the reservoir. Leakage of water and ingress of air is prevented by the presence of a silicon rubber O ring, D. As the pumped water passes across the test disc it decreases in speed and there is a resultant decrease in shear stress exerted on attached cells or spores. By regulating the flow rate it is possible to determine the shear stress operating at any point along the radius of the disc. Table 2 gives details of the test run using the discs. It can be seen that over the radius of the disc a considerable range of shear stress was achieved (1414 to 8484 Reynolds number).

Table 1. Experimental details of test runs using the Radial Flow Growth Chamber.

N.B.1) 0.75 mm spacers

2) 8 l $min.^{-1}$ water cirulation

3) Entry velocity = 1.33 $x10^{-4}$ m/sec^{-1}

mm from centre	Shear	Linear flow $m - sec^{-1}$	Reynolds number
5	50.9	5.66	8484.8
10	43.3	2.82	4242.4
15	20.9	1.89	2828.3
20	12.4	1.41	2121.21
25	9.1	1.13	1696.97
30	7.54	0.94	1414.14

VI. Water jet system

A water jet system was used to remove slime films and corrosion products from alloy surfaces. Sample discs were placed in a circular holder 200 cm bel the jet nozzle which had a bore of 1 mm. Pressure was generated by mains wate pressure and measured using a 'U' tube containing mercury.

F. RESULTS AND DISCUSSION

I. Colonization of surfaces in the sea

Fouling of the different surfaces tested indicate that titanium was more rapidly fouled and developed thicker slime films than copper or copper alloys. For instance, in the sea the colonization of titanium by bacteria takes place after about one week. After the fourth week a bacterial slime film had developed on the surface (Figure 10). This was proceeded by the settlement of brown algal spores which germinate and develop into creeping prostrate filaments producing erect growths (Figure 1). Diatoms also settled at this time (11 weeks) and included species of Cocconeis (Figure 2) and Synedra. Cocconeis cells produced mucilage from their adpressed surface, which cemented the cells into the slime film. Silt particles in suspension stuck to the mucilage which was broken down and utilized by bacteria (Figure 2).

In this study 90/10 copper-nickel surfaces became fouled after eleven weeks indicating that it was more toxic than titanium. At this time bacteria were observed attached along cracks in the alloy surface (Figure 4). Little fouling had occurred on pure copper surfaces at this time and this may be due to the peeling or sloughing off of the corrosion products which was observed at this stage (Figure 3).

Fouling by rod-shaped and coccoid bacteria was however seen on copper after thirty weeks. A thick slime film measuring over 200 μm comprising red and brown algae was present on titanium at this time, whilst on copper-nickel surfaces large numbers of protozoa, copper-tolerant diatoms and bacteria were present (Figures 6,7). Two species of the diatom Amphora were predominantly found on copper-nickel surfaces. These diatoms have

previously been shown to colonize copper antifouling paints and are able to immobilize copper ions intracellularly. From 25 weeks onwards at least four types of stalked protozoa occurred on copper-nickel surfaces, these were: Vorticella sp. Zoothamnium sp., Vaginicola sp. and a suctorian sp. The attachment of these unicellular and colonial protozoa has been described elswhere (Brown, Blunn and Jones, 1984).

After 70 weeks the corrosion products on copper-nickel, although comparatively free from fouling when compared to the titanium surfaces were seen to be colonised by protozoa. This has also been observed with diatoms and other algae on this surface and on copper.

Although scanning microscopy revealed interactions of the surface of the corrosion products with colonising cells and the settlement of organisms to form slime films, it yields little of the actual thickness of these films or of the interaction of cells within the corrosion products.

TEM of aluminium brass surfaces after 19 weeks of exposure showed a slime measuring 6-10 μm thick comprising cells of the diatom Amphora, bacteria and silt particles. Corrosion products had a feather-like appearance and enclosed bacteria. A fine electron-dense layer was found above this feathery layer (Figure 9).

Corrosion products were not detected on titanium by these methods. After only four weeks however, a slime film measuring 2-5 μm thick comprised of four different species of bacteria was observed on titanium (Figure 10). Generally bacteria in slime films on copper and copper alloy surfaces were predominantly of one type, being gram-negative and rod-shaped. This type

of bacteria were found in the corrosion products of aluminium brass even after 102 weeks. The thickness of slime films and corrosion products for this alloy appeared to be variable. For example, after 30 weeks films measuring over 90 μm were found (see Figure 9). Figure 11 shows shows that after 102 weeks slime films measured considerably less than those found at 30 weeks (40 μm). This slime film was composed of diatoms, brown algae and dead, empty, algal cells. Even at this time on a single disc some areas may have developed a thick slime film, whilst others remained relatively clean. After 130 weeks the slime film on aluminium brass was composed of red and brown algae and was considerably thicker than that observed previously (over 150 μm). The corrosion products at this time were much thicker and were found intracellularly, in empty algal cells and also between algal cell walls in the slime film (Figure 12).

The presence of bacteria and algae within the corrosion products on 90/10 copper-nickel was also observed. Fouling was detected in TEM sections taken through the corrosion products after 15 weeks. Gram-negative bacteria were found attached to the corrosion product surface and single bacteria were buried within these corrosion products (Figure 14). After 22 weeks these corrosion products were multilayered and measured 10-20 μm thick. Bacteria were not found on the surface of the corrosion products but were found in sheets between these layers (Figure 15). These sheets of bacteria were in continuous layers of secreted mucilage. These bacteria were motile and in some areas between corrosion layers cells were absent, although their mucilage lining was still evident (Figure 16). This lining appeared to be formed in the corrosion layers adjacent to the surface. Bacteria in this

layer were tightly packed and were actively secreting mucilage which surrounde the cells. In the lower layers of the corrosion product sheets of mucilage li these layers were the result of the movement of bacteria through the secret polysaccharide. (Figure 17).

The corrosion products in the uppermost layer was less well compacted tha those in lower layers (Figure 18). This was also evident in sections taken through the corrosion layer and slime film after 34 weeks (Figure 18). In this instance brown algal cells were found between the uppermost corrosion layers. The corrosion product was loosely organised and particles of corrosion were attached to the algal cell wall. The slime film at this stage measured up to 100 um and was composed predominantly of the diatom Amphora, bacteria and brown algal cells (Figure 11).

Ijsseling et al. (1980) suggested three different mechanisms for the formation of these corrosion products on copper-nickel alloys. There were eit by a) direct oxidation at the alloy surface, b) by selective dissolution of elements from the surface, or c) by dissolution followed by precipitation back onto the alloy surface.

In this study bacteria may invade the existing parallel corrosion layers or they may be incorporated into these layers as they form on top of corrosion products.

On pure copper bacteria appeared to be buried in the corrosion products as they formed on the surface of the metal. The corrosion products and the bacteria which were scattered throughout these are shown in Figure 20. This was the appearence of the corrosion products after 22 weeks of exposure. Bacteria were not found on the surface of the corrosion products

at this stage. The bacterial cells in the corrosion products were evenly distributed and corrosion products were deposited intracellularly. It was not until the 27th week that bacteria were found on the surface of the corrosion products. At this stage they formed a slime film 2-3 cells thick measuring 2-3 μm. Generally the cells towards the outer surface of the slime film appeared to be healthy and produced mucilage whilst those adjacent to the surface of the corrosion products were often lysed and their appearance under TEM suggested that they are affected by the copper leaching from the underlying metal (Figure 21).

Figure 22 shows how bacteria are gradually incorporated into the corrosion products. Gram-negative bacteria actively producing mucilage were observed towards the outer surface of the slime film. Bacteria nearer the corrosion products surface were gradually surrounded by electron-dense material adjacent and in contact with the corrosion products surface. The electron-dense material was deposited within cells on the surface of the corrosion products. In Figure 22 the only evidence of bacteria in the corrosion products was the remains of electron-translucent cell walls.

At 40 weeks protozoa had settled on top of the bacterial slime film and it was not until about the 70th week that the diatom Amphora appeared on this surface. At this time slime films measuring only 9-12 μm were found on this surface.

At seventy weeks on some parts of the copper discs, the corrosion products appeared to be thin with little biological fouling evident. A few bacteria were observed but these were enclosed in loosely organised corrosion products (Figure 23). A compacted corrosion film was however present adjacent to the

metal surface but was much thinner than observed in previous specimens (cf Figure 19). This may have been the result of the loss of a corrosion product/slime film (sloughing off). Figure 23 may represent the newly formed corrosion film.

The corrosion and slime film after 102 weeks of exposure appeared to be more compacted with bacteria found within the corrosion products. This slime film measured 15-20 um thick and was composed of bacteria, diatoms and silt particles. A layer of bacteria was found adjacent to the surface of the corrosion products, with diatoms and other bacteria above this layer. The bacteria adjacent to the surface are probably more resistant than the diatoms and are likely to provide a degree of protection from copper by: a) forming a barrier to the transport of toxic ions, or b) reduce the level of toxic ions by immobilizing copper in their mucilage and within the cell.

II. Colonisation of surfaces in the Thames

Titanium surfaces behaved similarly to those exposed in the sea. Bacteri were first observed on surfaces after one weeks exposure. Non-toxic surfaces exposed at the same site were previously fouled by bacteria after eighteen hours (Brown and Jones 1982). Figure 25 shows rod-shaped bacteria found on this surface after two weeks exposure. These bacteria were attached to the surface by short mucilagenous threads. After eleven weeks, films on titanium were composed of protozoa, actinomycete hyphae and bacteria (Figure 26). Thes protozoa were composed of two separate morphological groups which affected the thickness of slime films differently. Carchesium, Zoothamnion, Vorticella are protozoa which when mature are stalked and stand considerable distances from the titanium surface. Choanoflagellates and the Protozoan Vagincola (which has a short stalk) remain adpressed to the surface of titanium. These

two groups would affect the drag and heat exchange capacity of these surfaces differently and together would produce a two tiered fouling layer. Figure 29 shows the surface of titanium after 11 weeks of exposure. This lower fouling tier is composed of silt particles, at least three different types of bacteria and protozoa. The maximum thickness of this layer was 12 μm, whilst the second tier comprised Zoothamnium and Vorticella and was over 200 μm thick. This two tiered organisation was present on this surface after 19 weeks although the lower layer had increased in thickness to 20 μm. After one year titanium surfaces were covered by a thick layer (100 μm) of silt bound together by bacteria and bacterial mucilage (Figure 30). Fouling protozoa were not evident on this surface at this time.

After 19 weeks, copper (Figure 31) aluminium brass (Figure 32) and 90/10 copper-nickel (Figure 33) surfaces were all covered by a bacterial slime film. Copper surfaces were covered by a thin slime film only 1-2 cells deep and bacteria were also found within the corrosion products which covered the metal surface. Corrosion products were being deposited on the bacterial mucilage and intracellularly (Figure 31).

Bacterial slime films on aluminium brass measured 2 μm thick after 15 weeks exposure in the Thames. These formed above well structured corrosion products which had a reticulate lower layer and an upper dense layer which contained bacteria. Figure 32 shows the appearance of these after 19 weeks of exposure. Film structure remained unchanged and even after 70 weeks of exposure corrosion products composed of these two layers and bacterial slime films of only 5-10 μm were found on this alloy.

A film of bacteria, silt particles and bacterial polysaccharides was found above the corrosion products on 90/10 copper-nickel after 15 weeks of exposure. This slime film measured only 10 μm thick and was composed of gram-negative

rod-shaped cells producing short mucilage fibres (Figure 23). The outer cell wall of the bacteria was not smooth, but appeared to be producing cell wall protrusions (or blebs). These have been reported for bacteria in cultures treated with other heavy metals.

A thin slime film composed of bacterial mucilage and silt particles persisted on the surface of the corrosion products of 90/10 copper-nickel after 70 weeks and measured 10 µm thick. These corrosion products were composed of more or less parallel layers, and large numbers of bacteria were found between these layers. (Figure 34).

Some sections taken through these layers at this time showed evidence of the loss or sloughing off of the upper most corrosion layer. Figure 35 shows a broken outer layer of corrosion product; beneath this can be seen the newly exposed corrosion layer.

IV. Analysis of corrosion products on copper and copper alloys

Sections taken from the surface of discs were ideal specimens for the elemental analysis of the corrosion products by energy dispersive X-ray microanalysis. Table 2 summarises the observations made and lists the elements found within the corrosion products from discs exposed in the Thames Estuary and in the sea. The characteristic layers found on copper-nickel alloys exposed in the sea are predominantly rich in iron and nickel with lesser amounts of copper. Iron was also the predominant element in the corrosion products on this alloy in the Thames but little nickel was found in these samples.

The feathery layers of corrosion found on aluminium brass in the sea are composed largely of copper and iron. The thin, more electron-dense layer which was found above this layer (Fig.9) is less iron rich but has a high proportion of zinc. The layers of corrosion products found on this alloy in the Thames

Estuary were composed of similar elements to those found in the sea, with the upper electron-dense layer resembling the thinner upper layer in the sea. The lower reticulate layer was similar in composition to the feather-like layer in the sea. The presence of high phosphorous levels in these layers may be due to the presence of phosphates in the water.

IV. Immobilization of copper in fouling organisms

It was evident from this study that some fouling micro-organisms were able to withstand copper concentrations leaching from discs exposed in different environments. This was shown by the specificity of the organisms settling on these surfaces when compared with titanium. For example, at least three or four different species of bacteria were found on the surface of titanium after four weeks of exposure (Figure 10), however, only one morphological type was observed on copper (e.g. Figures 17,33).

Amphora has previously been shown to be copper tolerant and was the first alga settling on antifouling paints containing copper. In the present study, Amphora was the first diatom observed on copper and copper alloys, but cells settled after bacteria had first become established, often within the corrosion film.

Amphora cells have been shown to accumulate copper in electron-dense vesicles which protrude into the cell vacuole and in electron-dense inclusions in the chloroplast. Both types of deposits have been seen in this study.

In addition, another diatom, Amphiprora hyalina was found to be the dominant diatom on discs exposed in marine tanks. Members of this genus have previously been shown to be copper tolerant, these being found also on copper antifouling paints (Hendey, 1951). This diatom formed slime films several micrometers in thickness on aluminium brass (Figure 63). The

predominance of Amphora over Amphiprora on copper surfaces in the open sea is explained by the former's habit; Amphora cells adhered closely to surfaces, had a more streamlined shape and produced larger amounts of mucilage. This enables Amphora cells to adhere to surfaces in turbulent environments (Danel, Chamberlain and Jones, 1980). Tests in laboratory culture suggested that Amphiprora could withstand higher copper concentrations and grow in concentrations as high as 15 ppm copper (as $CuCl_2$).

Isolation of soluble proteins from Amphiprora cultured at various copper concentrations, and from normal cells, showed that diatoms grown in the presence of copper produced two extra proteins (Figures 36,37). Analysis of the copper concentration within isolated bands using AAS has shown that copper was associated with one of these bands (Figure 37, see a). Gel filtration using G75 has also demonstrated the presence of a protein containing bound copper from Amphiprora.

A metal-binding protein (metalothionein) has tentatively been identified by gel filtration from a soluble fraction obtained from the diatom Skeletonema costatum (Cloutier-Mantha and Brown, 1980) after treatment with mercury, copper and zinc. This mechanism of producing metalothioneins in response to toxic levels of heavy metals has been well documented for animals but little work has been carried out on algae and bacteria.

Bacteria are able to tolerate considerable levels of copper and have been shown to be present within the slime film. In this study bacteria have been shown to immobilize copper ions in their extracellular mucilage. For example, X-ray microanalysis of the bacterial mucilage surrounding the corrosion layers on copper-nickel alloys has shown these to be high in copper with lesser amounts of iron (Blunn and Jones, 1986). Similarly, the mucilage surrounding the bacteria found in slime films on copper exposed in the sea also contained relatively high levels of copper.

Particles containing copper were attached to the cell walls of bacteria found on the corrosion products of aluminium brass exposed in marine tanks. It appears from the micrographs, which show electron-dense particles of various sizes attached and moulded to the shape of the bacteria, that these particles were formed on the cell wall. The cell wall in bacteria seems to be an important site for the precipitation of heavy metals, for example, silver ions form sulphides an the cell walls of some bacteria and appear as numerous electron-dense spheres.

It has also been shown that the isolated peptidoglycan sacculus (part of the cell wall) of Escherichia coli could bind heavy metals. The involvement of the cell wall in the precipitation of copper is seen clearly in micrographs of sections taken through corrosion products on copper exposed in the sea (Figure 20).

Finally, bacteria have been shown in this study to immobilize copper intracellularly (Blunn and Jones, 1987: Presented paper). Work is currently in progress (not sponsored by INCRA) investigating uptake of heavy metals, includ copper into resistant bacteria, the effects of heavy metals on bacterial metabolism, and the immobilization of these toxic agents in cells.

V. "Sloughing off" of the corrosion products on copper and copper alloy

In this study it appeared that copper alloys are able to combat fouling by two mechanisms. These are: a) they are able to retard and alter the colonization sequence (due to the release of toxic ions), and b) the fouling and corrosion layers appear to "slough off".

Figures 39-41 show the surface of copper and alloy discs after exposure in the sea for 121-130 weeks. These photographs highlight the proble of measuring slime film thickness on such discs. For example, on the copper discs (Figure 41) four stages in the fouling-corrosion of its surfaces can be seen. There are a) corrosion products and fouling layers have sloughed off from the centre of the disc, b) this area is surrounded by corrosion products which may have a bacterial slime film (similar to that shown in Figure 21), c) areas of diatom fouling can be distinguished, and d) areas of brown algal settlement.

The situation on 90/10 copper nickel discs may be even more confusing as small areas seem to have lost their corrosion products and slime film (Figure 40). On the aluminium brass disc shown in Figure 39, the central area appeared to be fouled. All of these discs when compared with the titanium surface (Figure 38) have a much thinner slime film and showed areas where the slime had "sloughed off".

Quantification of the forces required to remove these corrosion-fouling layers has proved difficult. Alloys removed from the sea at a similar time of exposure to those shown in Figures 39-41 and also showing signs of surface sloughing were subjected to water jets of varying pressure. Pressure was measured as millimeters of mercury generated. The maximum pressure generated by this system failed to remove any fouling or corrosion layers on these discs. A more accurate and powerful device (Radial Flow Growth Chamber) is now being used to try and measure the forces required to slough of naturally generated corrosion (see section VI).

Sloughing off of the corrosion films on discs incubated in culture media for periods of over six months suggest that the corrosion products differ from those formed in the sea. Large areas of the corrosion products on copper and copper-nickel sloughed off, using very low water jet pressures. Similar results were obtained by Pyne, Fletcher and Jones (1984) for diatoms in laboratory culture.

VI. Film thickness measurements and degree of adhesion

I. Thickness of corrosion layers

Figure 42 shows the corrosion thickness measurements obtained after 10 weeks exposure for the 8 metals/alloys under investigation.

It can be seen that measurable corrosion layers were only obtained for 5 metals/alloys: these were copper (36 μm), cunifer 10 (34 μm), 90/10 copper/nickel (22 μm), cunifer 30 (19 μm) and 70/30 copper/nickel (18 μm). No obvious corrosion layers were observed for the Titanium, Aluminium brass and stainless steel.

1. Radial Flow Chamber Measurements

Detailed examination of the plates, following their removal from the R.F.C. revealed no obvious removal of the corrosion products.

VII. Colonization of antifouling paints containing copper powder and copper flakes.

Examination was made of the crystic copper particle coatings after 2 and 3 year immersion periods. Both coatings were well fouled by a specialis community of algae which formed a discontinuous layer with intermittant bare copper areas. Both the gel and flow coat systems supported a similar fouling community although this did appear to be more pronounced on the flow coat. Table 3 lists the macroalgae indentified. It can be seen that

both surfaces comprised a low lying, almost encrusting community of algae. These comprised both microscopic algae, such as diatoms and blue-green algae, and macroscopic green, brown and red algae. The most obvious animal present was the tube dwelling amphipod, Jassa fulcata. The most frequently identified diatom genera were Amphora, Cocconeis, Licmophora and Navicula. These either formed a thin biofilm layer directly on the coatings or contributed to the more extensive mixed algal community. Usually associated with the diatoms were blue-green algae, particularly species of the filamentus genera Lyngbya, Oscillatoria and Spirulina although palmelloid colonies of genera such as Pleurocapsa were also not uncommon.

The most commonly recorded macroalgal representations included both a) reduced growth forms of algae as well as, b) some reduced algae.

a. Reduced Growth Forms. Examples of these include species of the green algal genus Ulothrix (particularly U.flacca, U.pseudoflacca and U.subflaccida) and the brown alga Ectocarpus siliculosus. With respect to Ulothrix the species usually occur as short, narrow prostrate filaments scattered over the coating surface. Ectocarpus, however, characteristically forms very short, discrete, sometimes confluent, dark brown, pile-like tufts of branched, often fertile, filaments, usually less than 1 mm in height. In this small form it is often collectively termed 'brown mats'. Both Ulothrix and especially Ectocarpus, usually form macroscopic erect filamentous growths several cm in length, and these reduced growth forms can be attributed directly to the toxic environmental conditions present on the coatings surface. A number of isolates of these algae have been obtained and grown in toxin free conditions in the laboratory and in all cases the characteristic macroscopic form is produced. There is increasing evidence

from laboratory toxicity trials that both Ulothrix and Ectocarpus are particularly resistant to a wide range of metallic biocides. With respect to Ectocarpus siliculosus it is further interesting to note that genetically based copper-tolerant populations of this species have now been reported.

2. Reduced species

a) Chlorophyta Two regularly observed green algae on the toxic surfaces were the unicellular Chlorococcum submarinum and the polystromatic crust forming Pseuodendoclonium submarinum.

b) Fucophyceae A number of reduced brown algal genera were observed contributing to the fouling community. These included small, branched, filamentous genera such as Streblonema and closely encrusting discoid/pseudodiscoid genera such as Chilionema, Hecatonema, Microspongium and Myrionema. The presence of these genera is particularly interesting as these have become known almost exclusively as epiphytes or epi-endophytes on various large green, brown and red macroalgae. They are very opportunistic in their mode of existence and are characterised by a fast growth rate and early fertility with a very short and 'direct' type of life history. This ensures a ready availability of spores for colonising surfaces and a rapid build up in a local population by self inoculation. The ability to colonise antifouling paints is in general agreement with their failure to compete with other organisms for space on 'normal' non-toxic substrata and their corresponding adoption of an epi-endophytic life style. However, almost certainly they must also possess pronounced tolerance to the antifouling toxins and this aspect is currently under investigation at Portsmouth Polytechnic.

Figure 43 presents film thickness measurements obtained for the Gel coat and Flow coat systems. It can be seen that the fouling communities reacted up to 164 um in height, which mainly comprised the encrusting green alga Pseudoendoclonium submarinum.

Table 2. Marine Algae identified from the Gel and Flow crystic copper coat systems

Chlorophyta	Fucophyta	Rhodophyta
Chloroccotum submarinum	Chilionema sp.	Audouninella sp
Enteromorpha intestinalis	Ectocarpus siliculosus	Goniotrichum alsidii
Enteromorpha prolifera	Giffordia sandriana	Polysiphonia urceolata
Pseudoendoclonium submarinum	Hecatonema maculans	Polysiphonia sp.
Ulothrix flacca	Microspongium globosum	
Ulothrix pseudoflacca	Myrionema sp.	
Ulothrix subflaccida	Punctaria latifolia	
	Streblonema oligosporum	

G. CONCLUSIONS

Five major conclusion can be drawn from these studies: a) that copper alloys and pure copper leach toxic ions which retard and alter the colonisation and settlement of fouling organisms, b) that although these surfaces do eventually foul, slime films on these surfaces are much thinner than those found on non-toxic surfaces, c) that this fouling layer may be lost along with the corrosion products when it "sloughs off", d) that alternating layers of corrosion products and bacteria develop on 90/10 copper-nickel surfaces, and, e) that these bacteria and their mucilage immobilize copper ions.

These five properties depend upon the complex inter-relationship between rate of release of copper ions, the colonisation of tolerant organisms and the environmental conditions determining

a) rate of corrosion;

b) species present able to colonise such surfaces and

c) physical forces such as tides and storms.

The above conclusions are of considerable economic importance. Modern antifouling paints for ships are able to retard settlement of organisms for 2-3 years. Tests of antifouling paints in environments similar to these in which samples in the present study were exposed indicate that the life of such paints may be only 18 months.

Copper and copper alloys are effective beyond this period of time. 90/10 copper-nickel is of considerable significance because, a) it is more resistant than copper to erosion-corrosion by sea water b) although appearing to be less biofouling resistant than copper it is more so than aluminium brass and c) is able to slough off its corrosion product at frequent intervals, sufficient to prevent any major marine settlement (macro or hard shell) building up over a period of some years.

The unsuccessful attempts to determine the attachment strength of the layered slime film on 90/10 copper-nickel indicates that these are firmly attached to the alloy. It is obvious, however, from the present study that these do become detached from time to time by forces in the sea at the site where the specimens were exposed. Those forces would also undoubtedly be less than those experienced by a ship's hull underway or the support leg of an offshore platform. It would be very valuable to determine the environmental forces and inherent factors responsible for this sloughing

off mechanism. In particular more information is required on the role of the bacterial layers in this process, and whether they represent planes of weakness.

Although this study was carried out in the sea, the Thames Estuary and other locations, the effect of the copper and copper alloys would apply equally in a condenser tube environment where fouling is of great concern and of economic importance. The leaching of toxic ions and the "sloughing off" of the corrosion products would be expected to occur in a condenser tube environment.

This work also shows that bacteria are able to immobilize copper ions extracellulary in bacterial mucilage, in their cell walls and intracellularly. Bacteria are found within the corrosion products on these surfaces, but it is not clear whether these cells play a role in the corrosion of these surfaces. Certainly sulphate reducing bacteria found in anaerobic environments on oil rigs and deep within slime films do enhance corrosion of the steel. This aspect has not been investigated in this study. These bacteria would also effectively remove toxic copper ions and a layer of bacteria has been seen between the corrosion products and the settling algae. In both antifouling paints, and on these metal and alloy surfaces, the effect of bacteria on subsequent coloniziation by algae has never been determined.

Information from this and other studies support the case for the continued development of 90/10 copper-nickel alloys as

anti - fouling surfaces. Legislation limiting the application of tributyltin antifouling paints has been passed in Japan and other countries. In Britain there is now a strong case against using these paints on small craft in shallow harbours and small estuaries because of their known toxic effects on the fecundity and growth of oysters. With these organotin based antifouling paints coming under increased scrutiny it is likely that greater attention will once again be given to copper-based antifouling systems.

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Two others in press.

K. SUGGESTIONS FOR FUTURE WORK

A numbers of factors remain to be investigated and include the following:

a) Can the sloughing off process reported in this investigation be enhanced or speeded up without unduly affecting the corrosion rate of the metal or alloy?

b) Little is known about the bacteria that are present in slime films. Are sulphate reducing bacteria present?

c) The role of mucilage in the immobilization of toxic ions warrants further investigation. In particular, does the presence of copper stimulate secretion of exopolysaccharide?

d) Further work on the incorporation of copper into different matrices should be investigated.

e) Determine whether the corrosion products release toxic ions and play a direct antifouling role.

f) Determine the role of reduced macroalgae in the development of the biofouling film. In particular identify the range of organisms present, the extent of their contribution to the film, their developmental and life history characteristics as well as their mechanisms of resistance to the toxic ions.

g) Determine the role of blue-green algae in slime formation on the copper and copper alloys. In particular 1) ascertain the extent of occurrence of B.G. algae on toxic surfaces 2) identify the range of genera/species occurring 3) investigate their mode of adhesion to the surfaces. 4) experimentally determine (using S.E.M. and T.E.M. techniques) the effect of B.G. algae on surface corrosion and 5) determine the extent and mechanisms of resistance of selected B.G. algae to toxic ions.

L. FIGURES

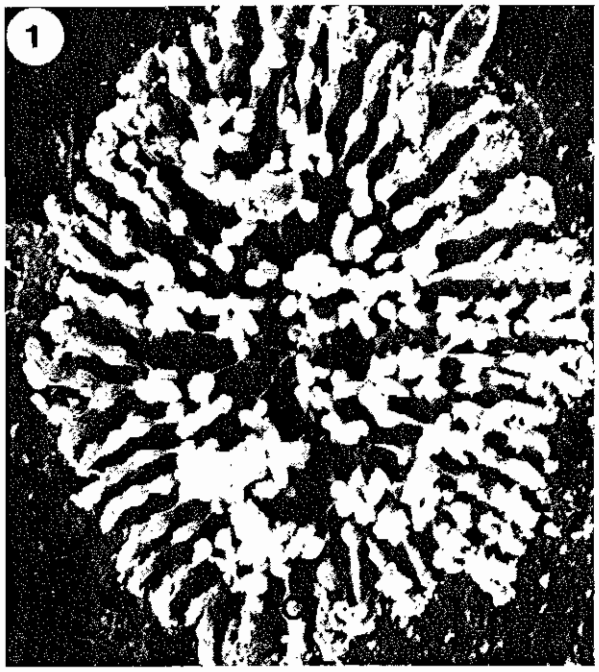
Plate 1.

Figure 1. Surface of titanium exposed in the sea for 11 weeks, showing the colonization of the surface by brown algae producing a rosette of creeping filaments (c) which produce the erect growths (S.E.M. x 2,500).

Figure 2. Scanning electron micrograph of titanium surface exposed in the sea for 11 weeks showing an area which has been colonised by the diatom Cocconeis (D) which produces large amounts of mucilage (M). Bacteria (B) are found amongst this mucilage (S.E.M. x 800).

Figure 3. Surface of copper after 11 weeks exposure in the sea which shows the peeling or sloughing off (P) of the corrosion layers (L) exposing a smooth non-fouled surface (S) (S.E.M. x 120).

Figure 4. Scanning electron micrograph of bacteria (B) attached to the surface of 90/10 copper-nickel which has been exposed in the sea for 11 weeks. Bacteria are mostly attached along cracks in the alloy surface (S.E.M. x 12,500).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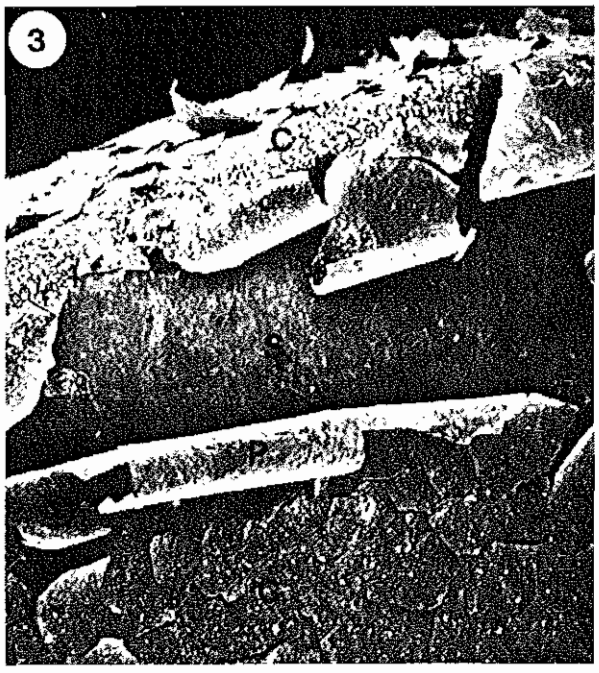
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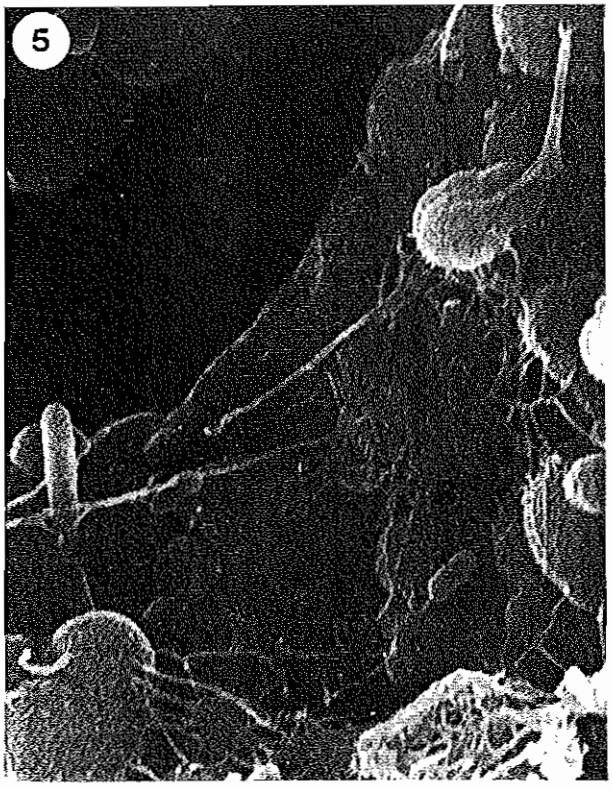
Plate 2.

Figure 5. Micrograph showing rod-shaped (R) and coccoid (C) bacteria on copper exposed in the sea for 40 weeks (S.E.M. x 10,000).

Figure 6. Surface of 90/10 copper-nickel after exposure in the sea for 30 weeks showing the attachment of protozoan Vorticella sp. (S.E.M. x 200).

Figure 7. Micrograph showing the diatom Amphora forming slime films on copper surfaces after 45 weeks exposure in the sea (S.E.M. x 3,000).

Figure 8. Surface of 90/10 copper-nickel after 70 weeks exposure. Note that the corrosion products (C) appeared to have buried the protozoa (P) (S.E.M. x 250).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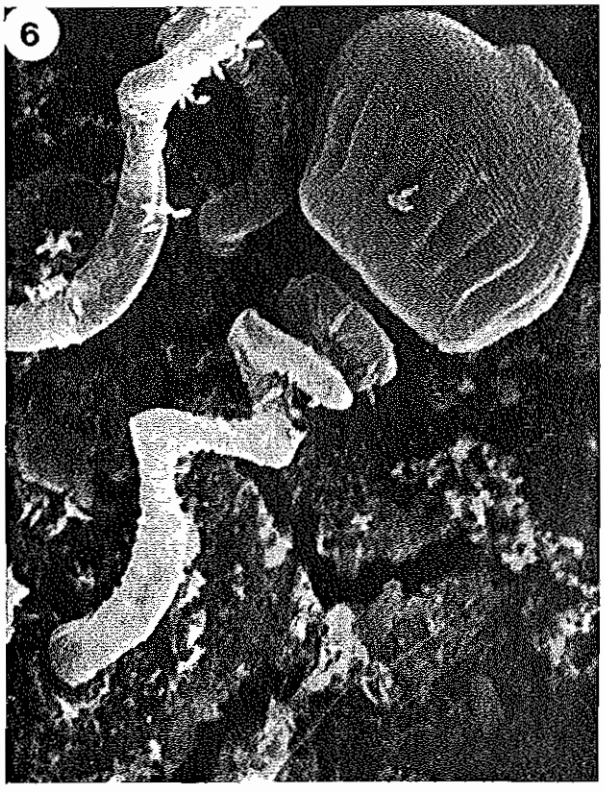
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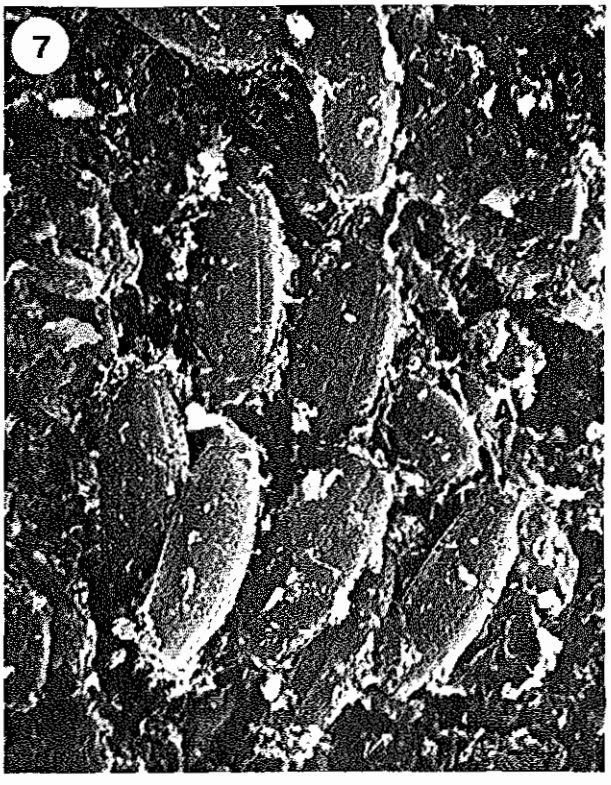
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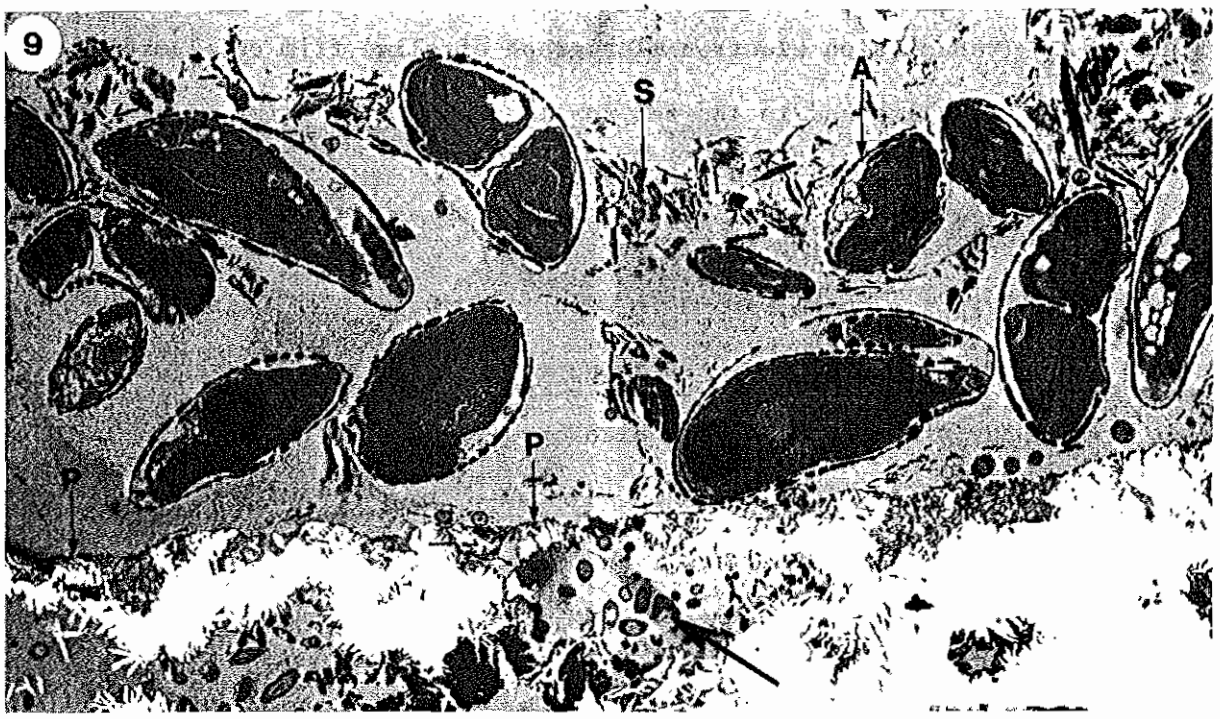
Plate 3.

Figure 9. Transmission electron micrograph of slime film on aluminium brass exposed in the sea for 19 weeks. The slime film above the corrosion products surface (P) is composed of *Amphora* cells (A), silt particles (S) and bacteria (B). Bacteria are also found within the corrosion products (arrowed). Corrosion products are composed of a thin, electron-dense layer at the surface, which occurs above a feather-like layer. (T.E.M. x 10,000).

Figure 10. Surface of titanium after 4 weeks exposure in the sea, showing a bacterial slime film composed of at least three different species of bacteria producing threads of mucilage (B_1), mucilage capsules (B_2) and mucilagenous sheaths (B_3)(T.E.M. x 16,000).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P
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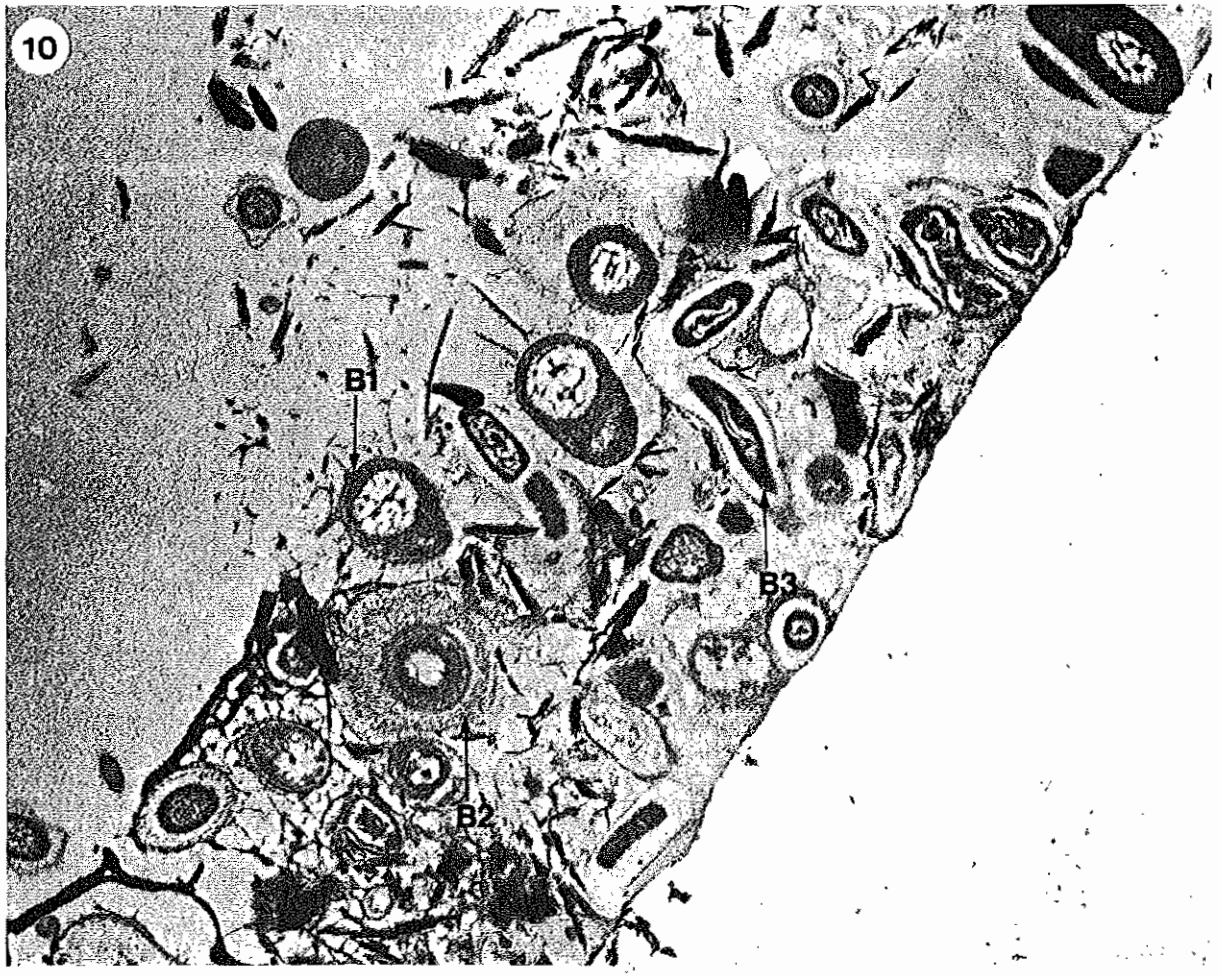
10
B1
B3
B2
A

Plate 4.

Figure 11. T.E.M. section through slime film and corrosion products on aluminium brass after 102 weeks. Note the layered slime film with diatom frustules (D) above dead brown algal cells (A) (T.E.M. x 2,500).

Figure 12. Section through part of a slime film and corrosion products on aluminium brass after 130 weeks in the sea. This shows thick corrosion products (C) which extends between the algal cell walls (arrowed), and electron-dense deposits (D) are seen within the cells (T.E.M. x 2,500).


11

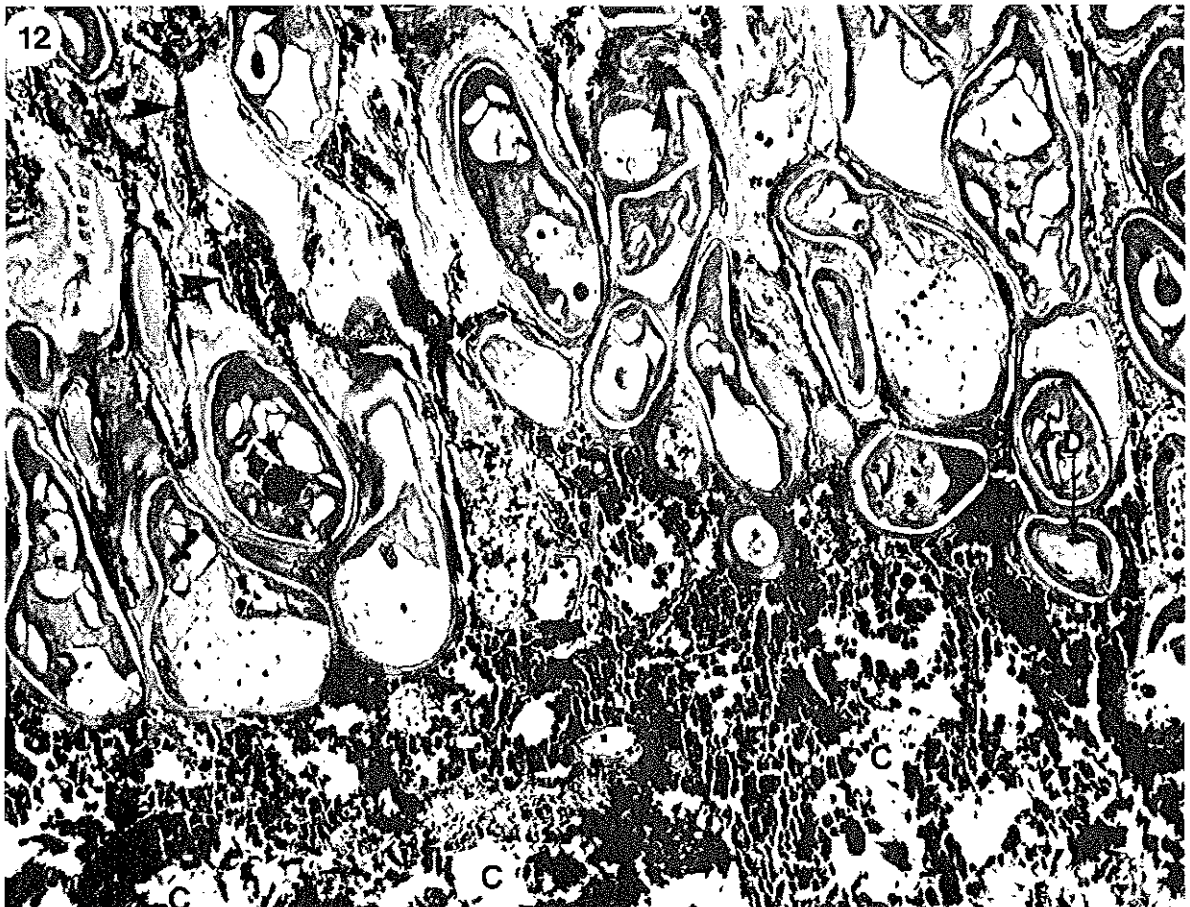
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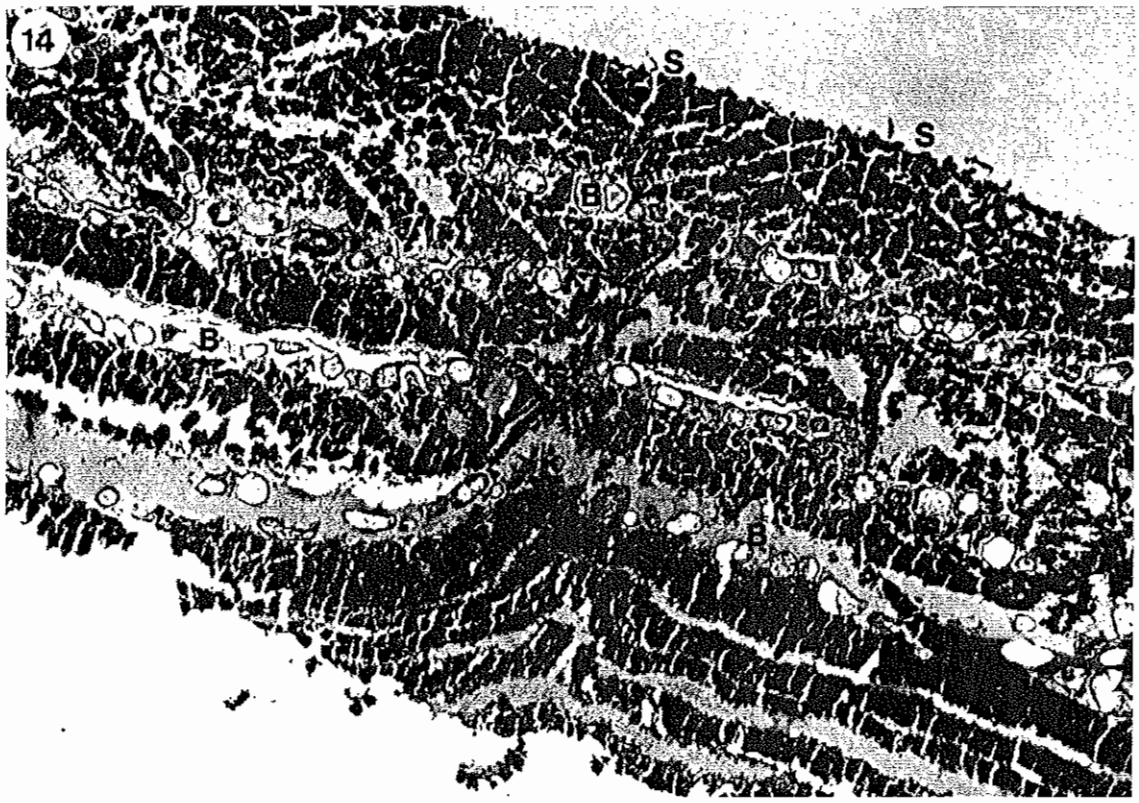
Plate 5.

Figure 13. T.E.M. micrographs of corrosion products of 90/10 copper-nickel exposed in the sea for 15 weeks. Note bacteria (B) on the outer surface of the corrosion products and bacteria enclosed within these products (arrowed) (T.E.M. x 50,000).

Figure 14. Corrosion products of 90/10 copper-nickel after 22 weeks exposure. Bacteria (B) are found between layers of corrosion products but are not found on the surface(s) of the corrosion products (T.E.M. x 10,000).

Figure 15. Section of bacteria (B) between layers of corrosion products showing the continuous layer of bacterial polysaccharide (P) lining the layers (T.E.M. x 31,000).

Figure 16. Section showing bacterial polysaccharide (P) lining corrosion layers (T.E.M. x 50,000).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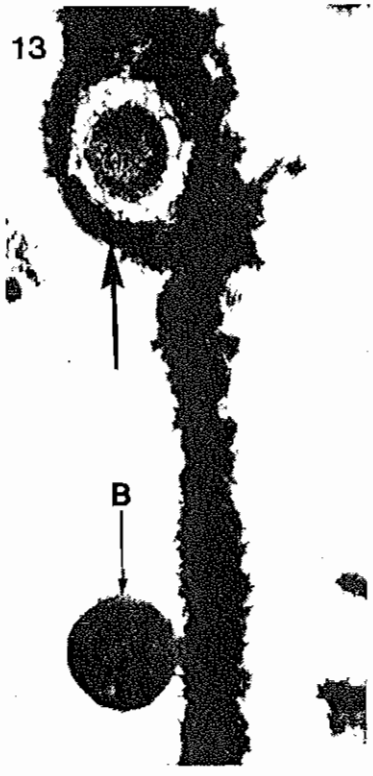
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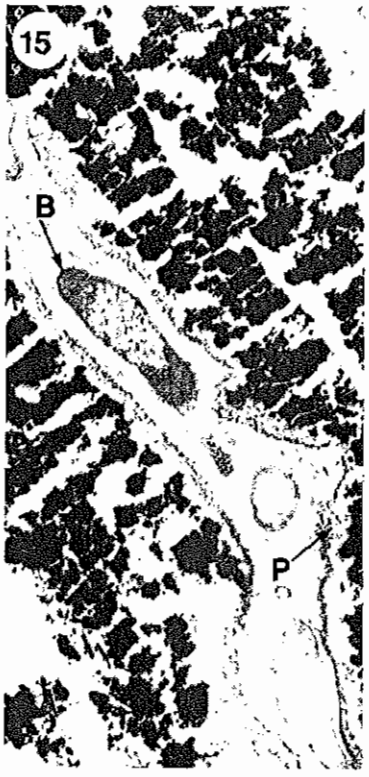
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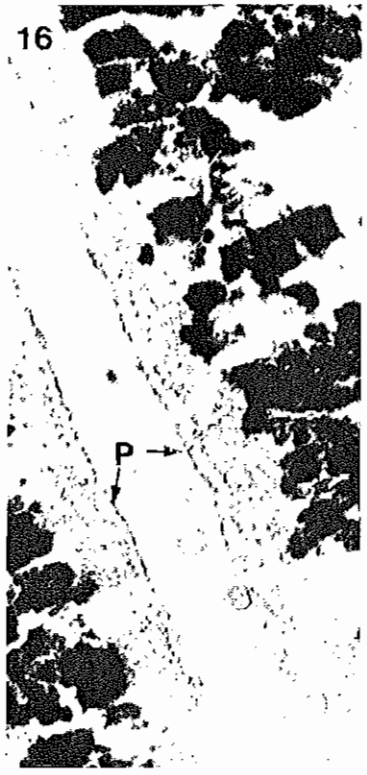
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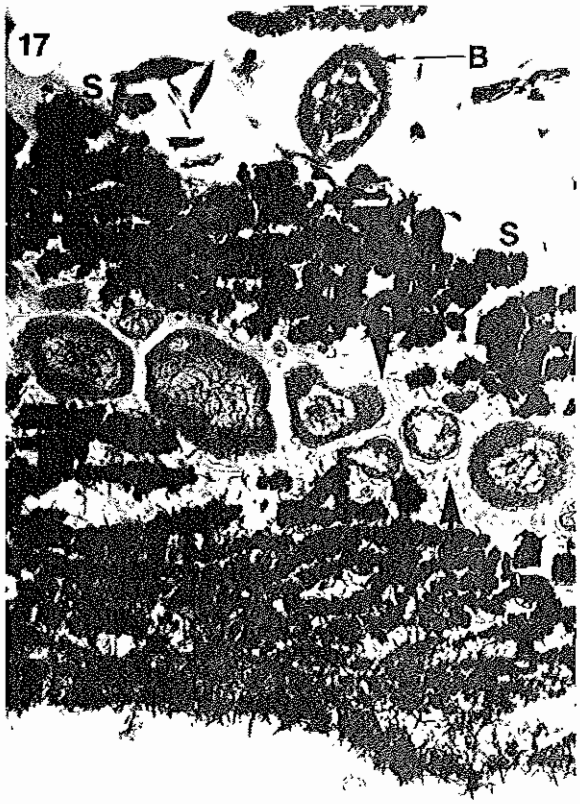
P

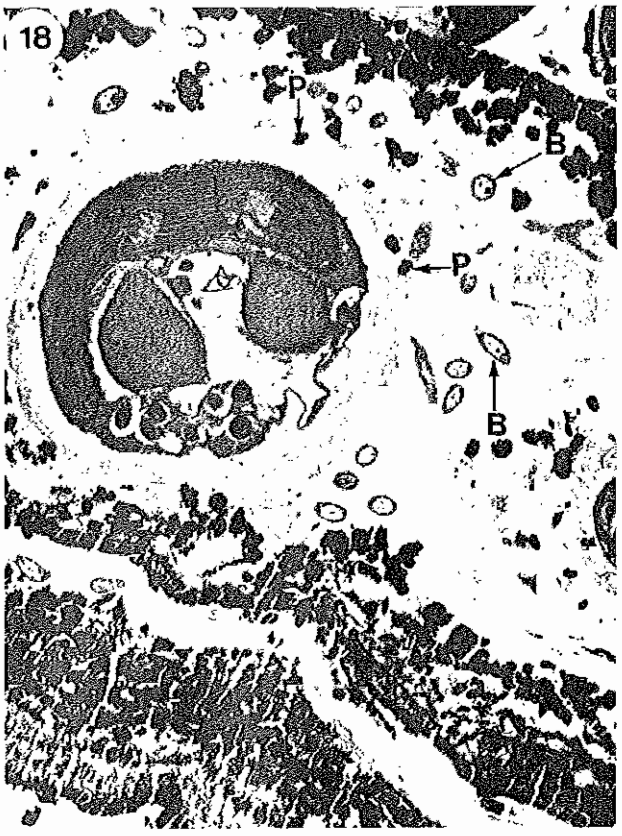
Plate 6.

Figure 17. Section showing bacterium producing mucilage (arrowed) in the space between corrosion layers on copper-nickel after 25 weeks exposure. A single bacteria (B) is attached to the surface (S) of the corrosion products. Note that in this surface layer the corrosion products are less compact than in other layers and that although mucilage is being produced by bacteria, a continuous lining is not evident (T.E.M. x 25,000).

Figure 18. Micrograph showing brown algal cells enclosed by the upper two layers of the corrosion products on copper-nickel after 34 weeks of exposure in the sea. Note the particles of corrosion products (P) attached to the algal cell wall, the electron-dense area in the chloroplast (arrowed) and some bacteria which also appear to have electron dense contents (arrowed B) (T.E.M. x 8,000).

Figure 19. Section through a slime film on 90/10 copper-nickel after 34 weeks exposure in the sea. The slime film is composed of the diatom Amphora (D), bacteria (B), silt particles (S) and brown algae (A) (T.E.M. x 5,000).


17
S
B
S
A


18
P
B
P
B

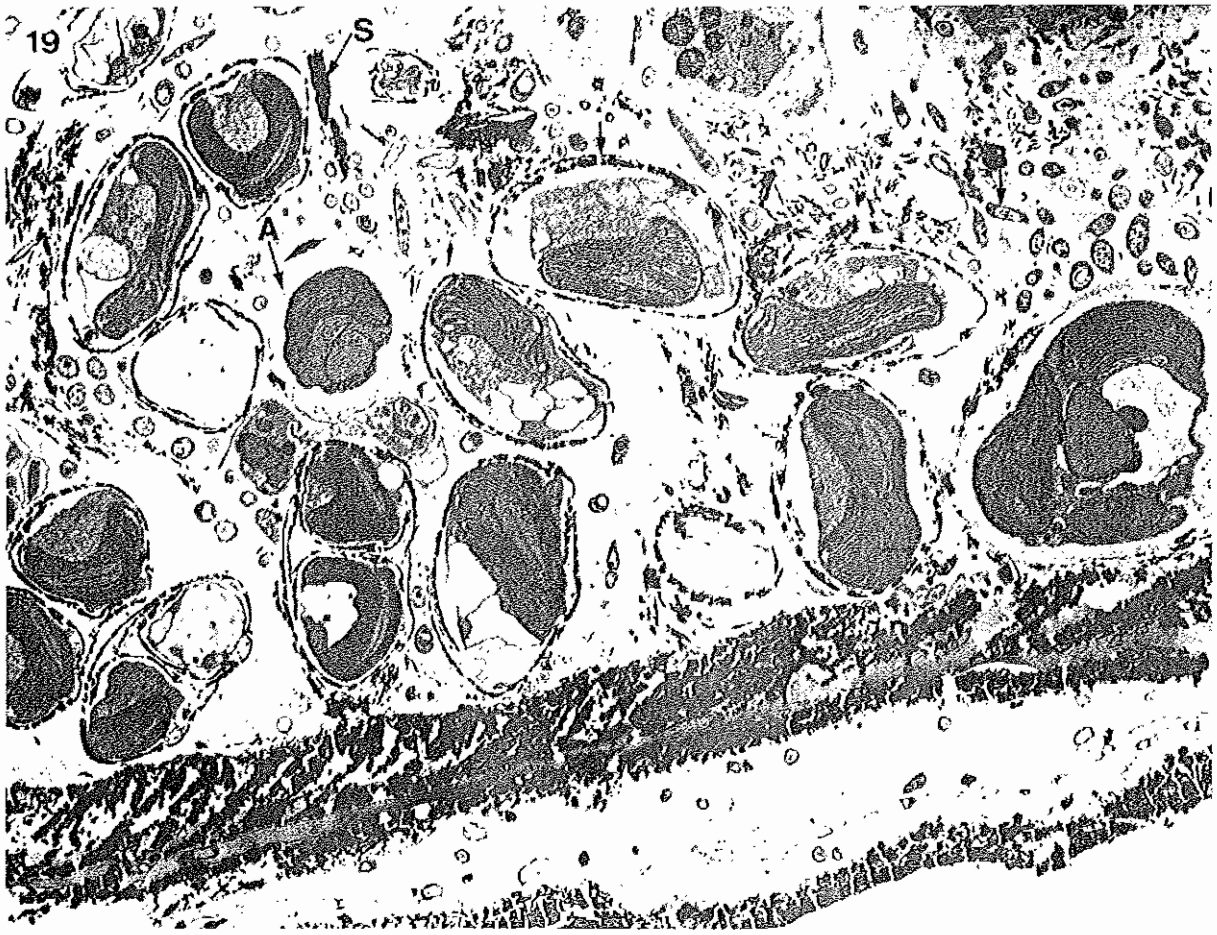
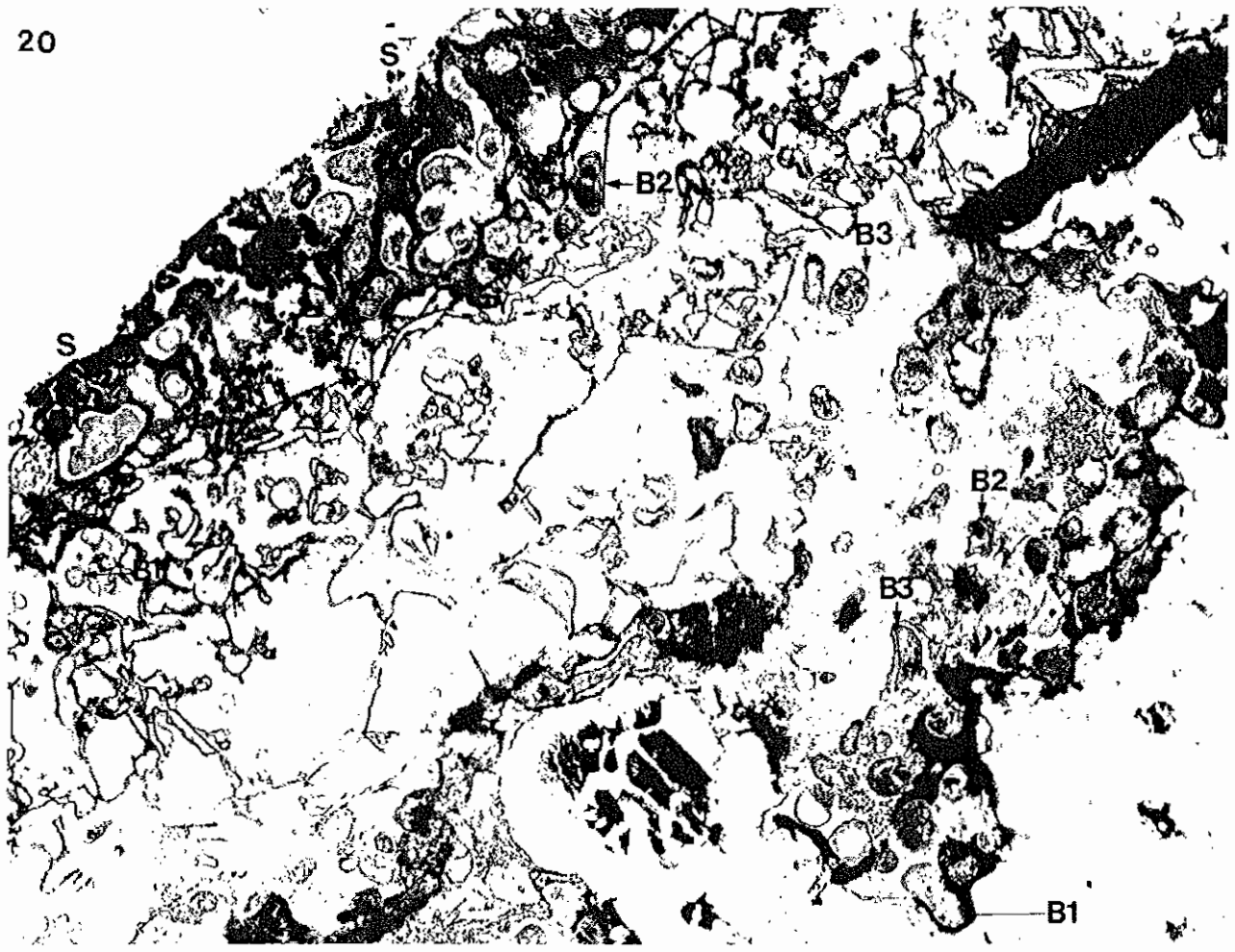

19
S
A

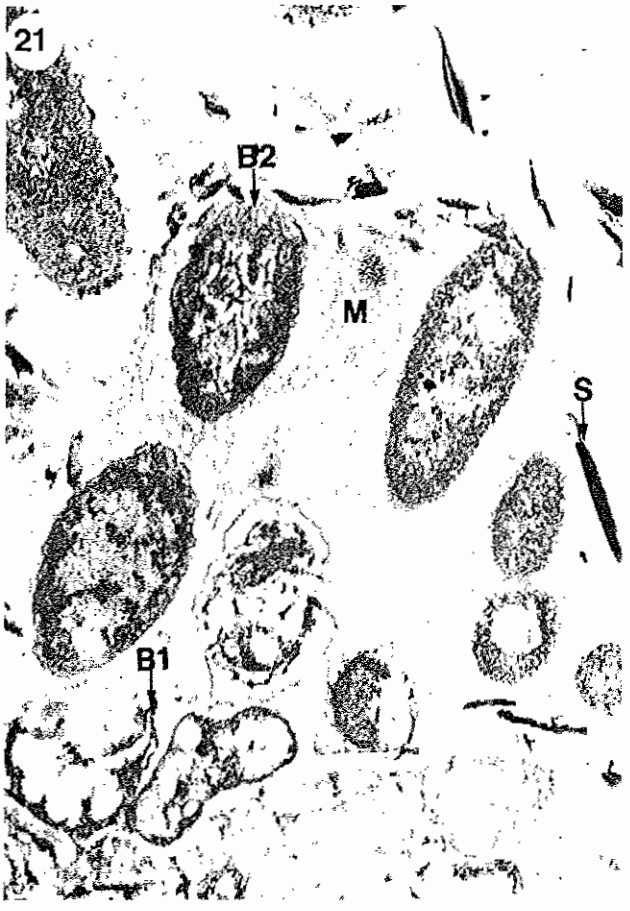
Plate 7.

Figure 20. Section through the corrosion products found of copper after 22 weeks exposure in the sea. Although bacteria are not found on the surface (S) of the metal at this tiome they are found buried within the corrosion products. Some of these bacteria have a normal morphology and structure (B_1), whilst others have electron-dense contents (B_2), or cell walls associated with electron-dense material (B_3) (T.E.M. x 13,000).

Figure 21. Bacteria on the surface of copper after 90 weeks exposure. Silt particles (S) are attached to the bacterial polysaccharide (M). Cells close to the copper surface (B_1) are lysed and have an abnormal appearance, whilst those towards the outer surface of the slime film appear normal (B_2) (T.E.M. x 32,000).

Figure 22. Transmission electron micrograph of slime film on copper after weeks exposure in the sea. Healthy gram-negative bacteria (B) are found on the outer surface of the corrosion products. Electron-dense material is found adhering to the cell (arrowed), and is also deposited intracellularly (arrowheads). This electron-dense material merges with the corrosion products interface. Bacterial "ghosts" (G) are observed within these corrosion products (T.E.M. x 37,000).


20
S
S
B2
B3
S
B1
B2
B3
B1


21
B2
M
S
B1


22
B
G
G

Plate 8.

Figure 23. Surface of pure copper after 70 weeks exposure in the sea. Showing thin compacted corrosion products (C) and bacteria (B) enclosed by loosely arranged corrosion products (TEM x 5000).

Figure 24. Slime film on copper after 102 weeks of exposure in the sea, showing the corrosion products (C) a layer of bacteria (B) at the surface of the corrosion layer, diatoms (D) and silt particles (S). (TEM x 3000).


23
B
C
C
C

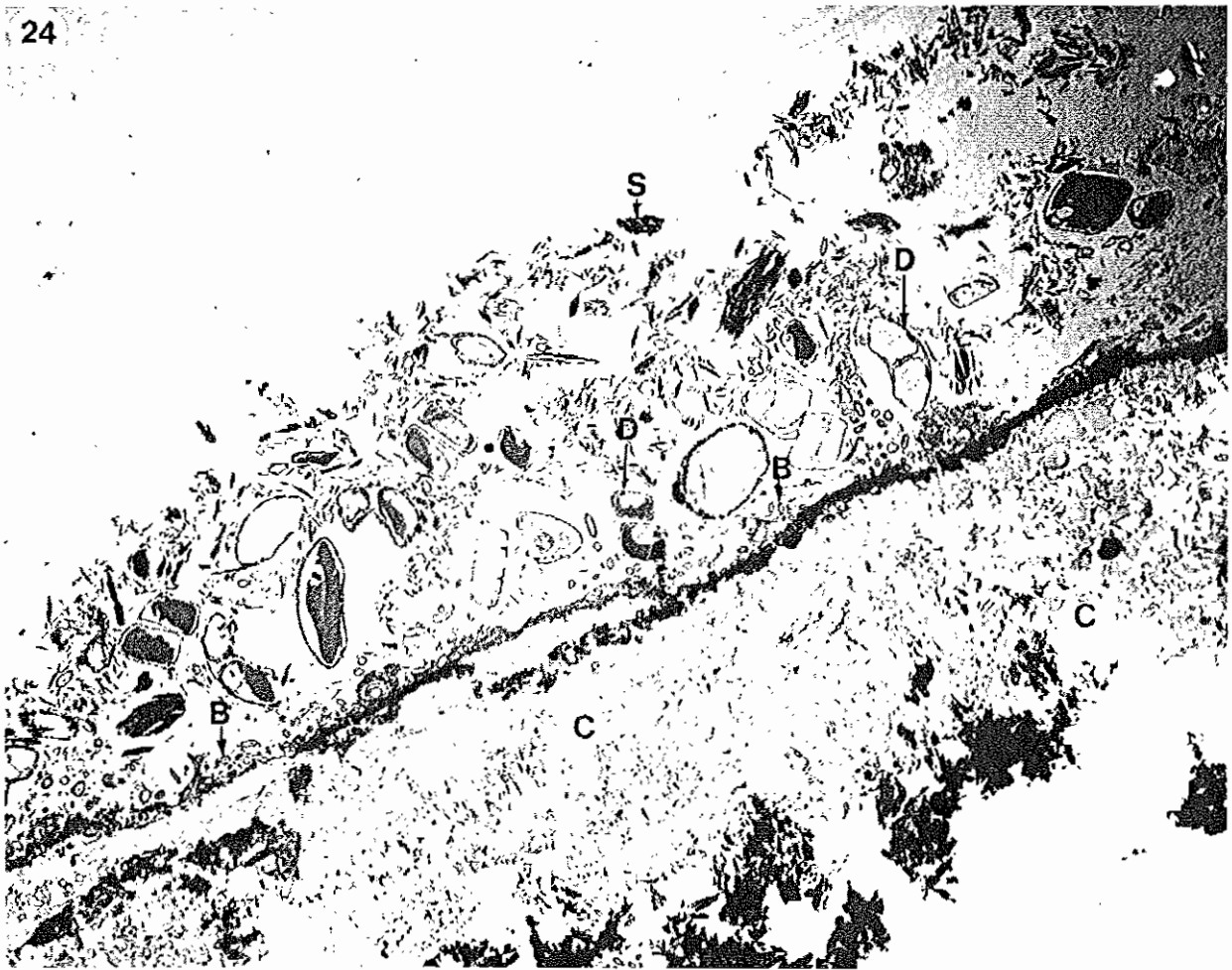

24
S
D
D
B
C
B
C

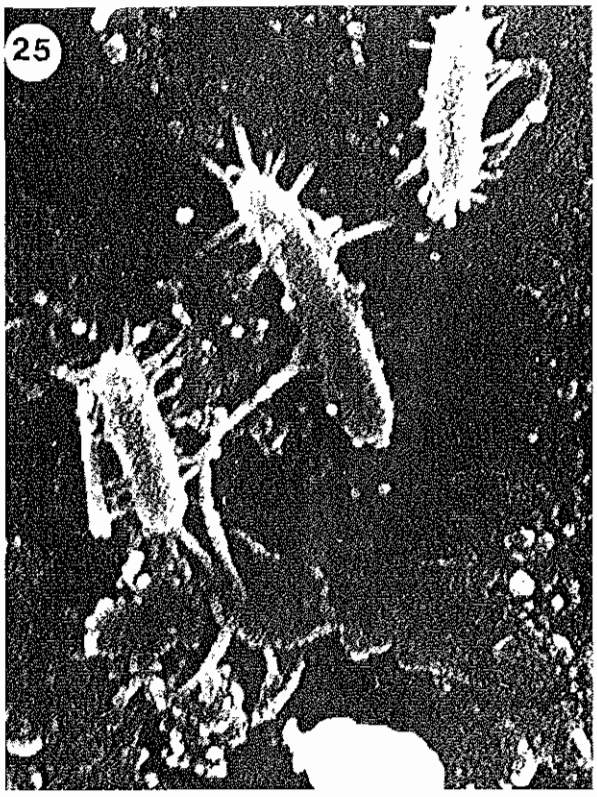
Plate 9.

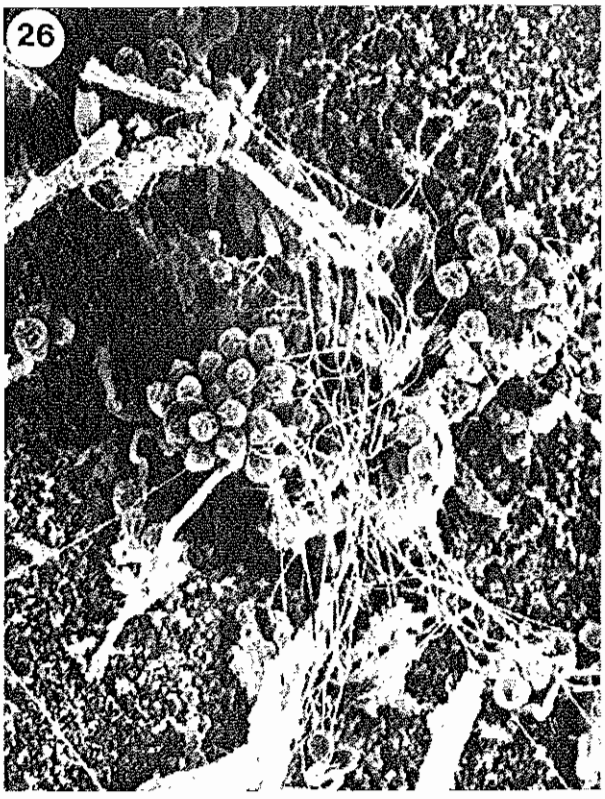
Figure 25. Scanning electron micrograph showing attachment of rod-shaped bacteria to the surface of titanium after 2 weeks exposure in the Thames Estuary (SEM x 48000).

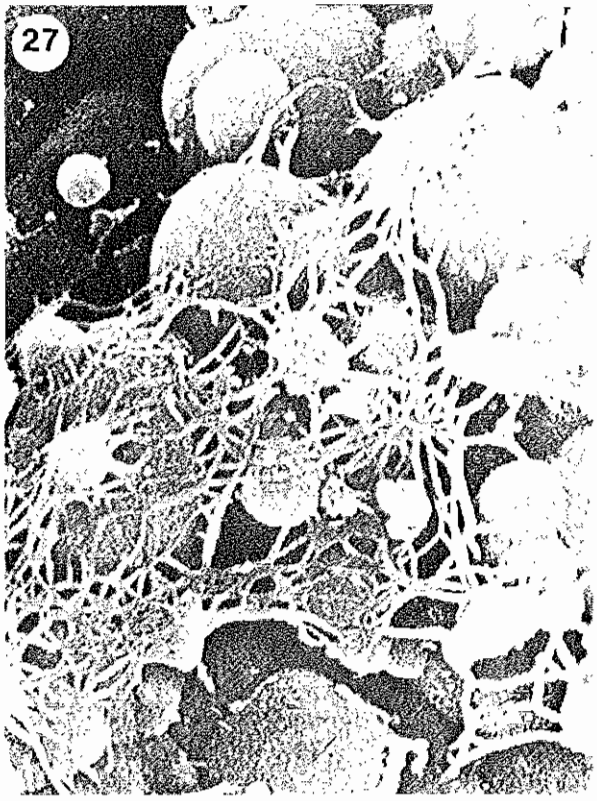
Figure 26. Micrograph showing slime film composed of protozoa and bacteria on titanium after exposure in the Thames for 11 weeks. (SEM x 200).

Figure 27. Rod-shaped and coccoid bacteria on the surface of copper after exposure in the Thames for 11 weeks (SEM x 13000).

Figure 28. Bacteria on the surface of copper. Note that these bacteria are becoming embedded within the corrosion layer with particulate material attached to the cell walls (SEM x 33000).


25


26


27

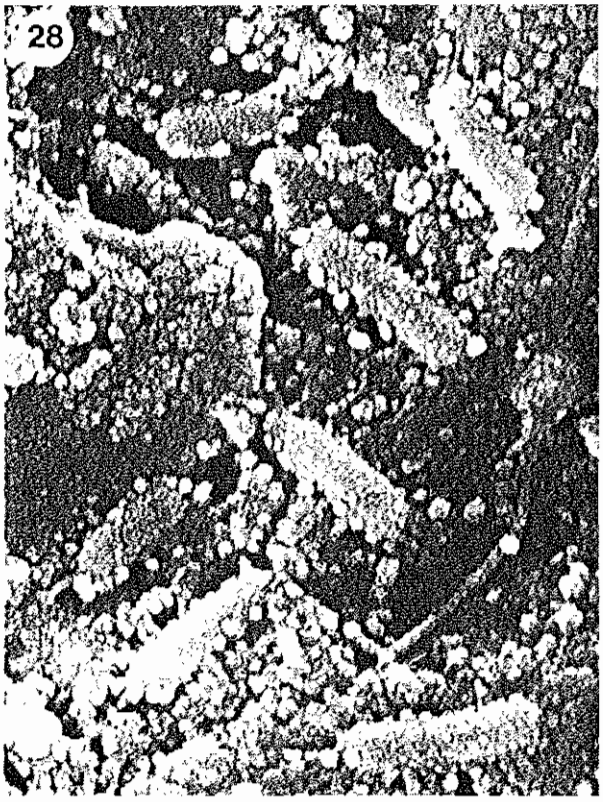

28

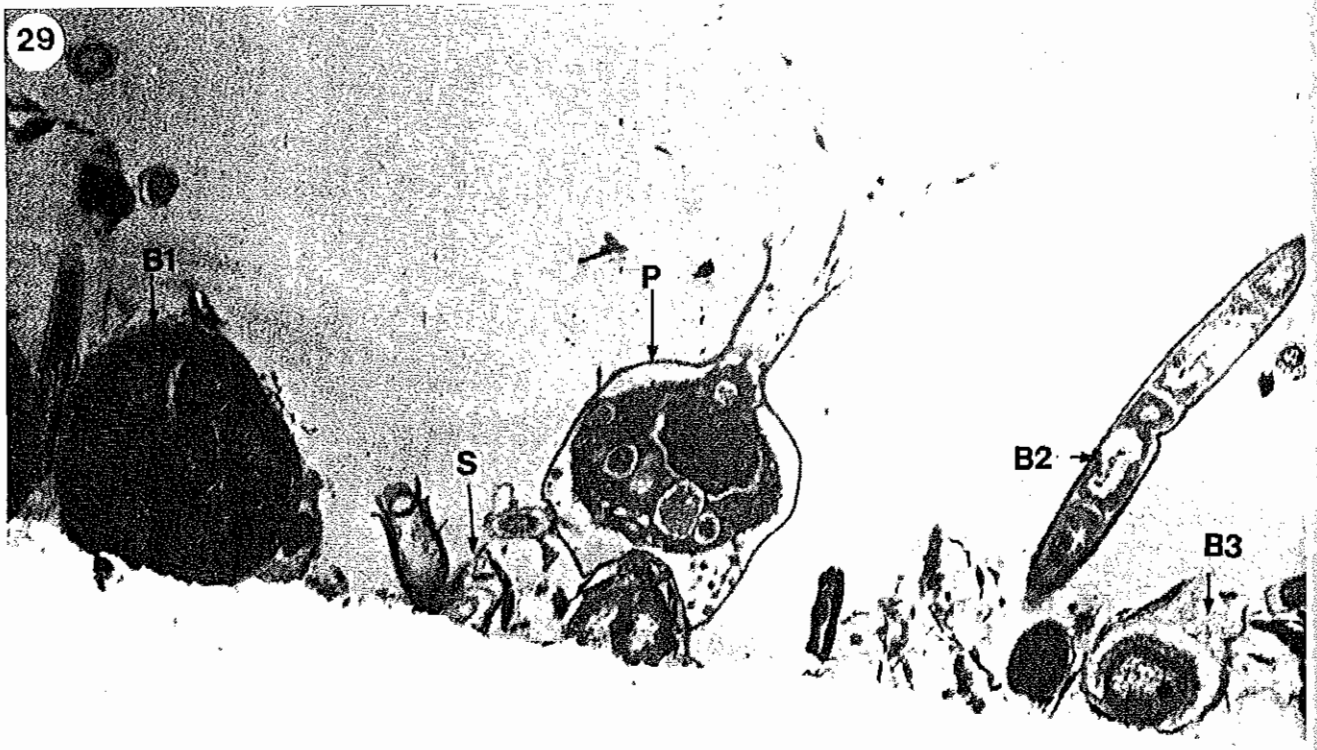
Plate 10.

Figure 29. Slime film on titanium after exposure in the Thames Estuary for 11 weeks showing three different species of bacteria attached to the surface (B_2, B_2, B_3), silt particles (S) and a protozoan (P) (thecate choanoflagellate) (TEM x 10000).

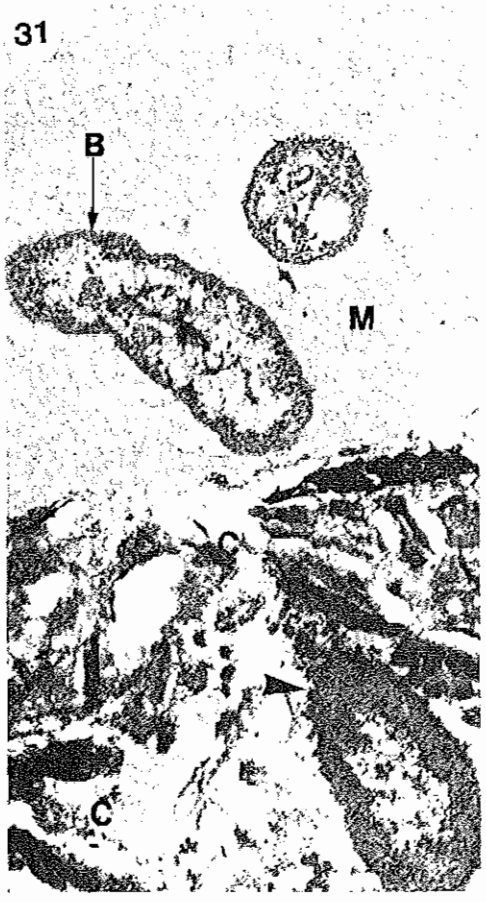
Figure 30. Surface of titanium after 70 weeks exposure in the Thames. Note the surface (arrowed) is covered by large amounts of silt (S). Bacteria (B) are found between silt particles. (TEM x 2000).

Figure 31. Surface of copper after 19 weeks exposure in the Thames, showing bacteria (B) on the corrosion product (C) and producing mucilage (M). Bacteria (arrowed) in the corrosion product have an electron dense particulate cell wall. (TEM x 33000).

Figure 32. Section through the corrosion layers and slime film on aluminium brass after 15 weeks. Note the slime film is composed of a single bacterial species (B), mucilage and silt particles (S). The multilayered corrosion products (C_1 and C_2) contain bacteria (arrowed) (TEM x 15000).


29
B1
P
S
B2
B3


30
S
S
B


31
B
M
C
C

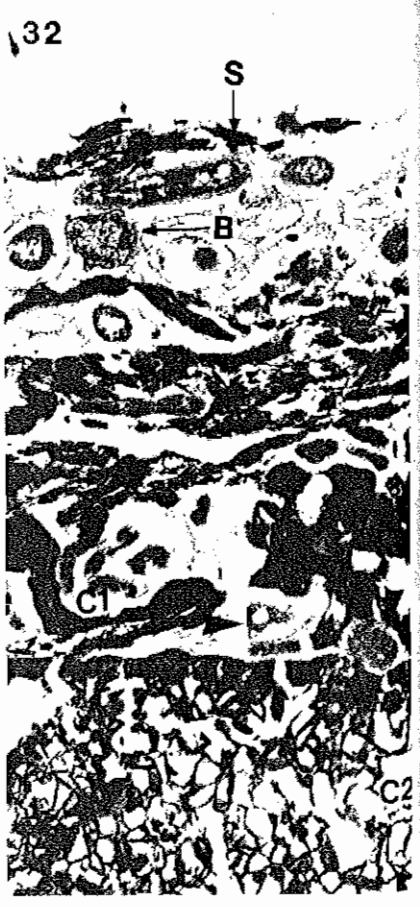
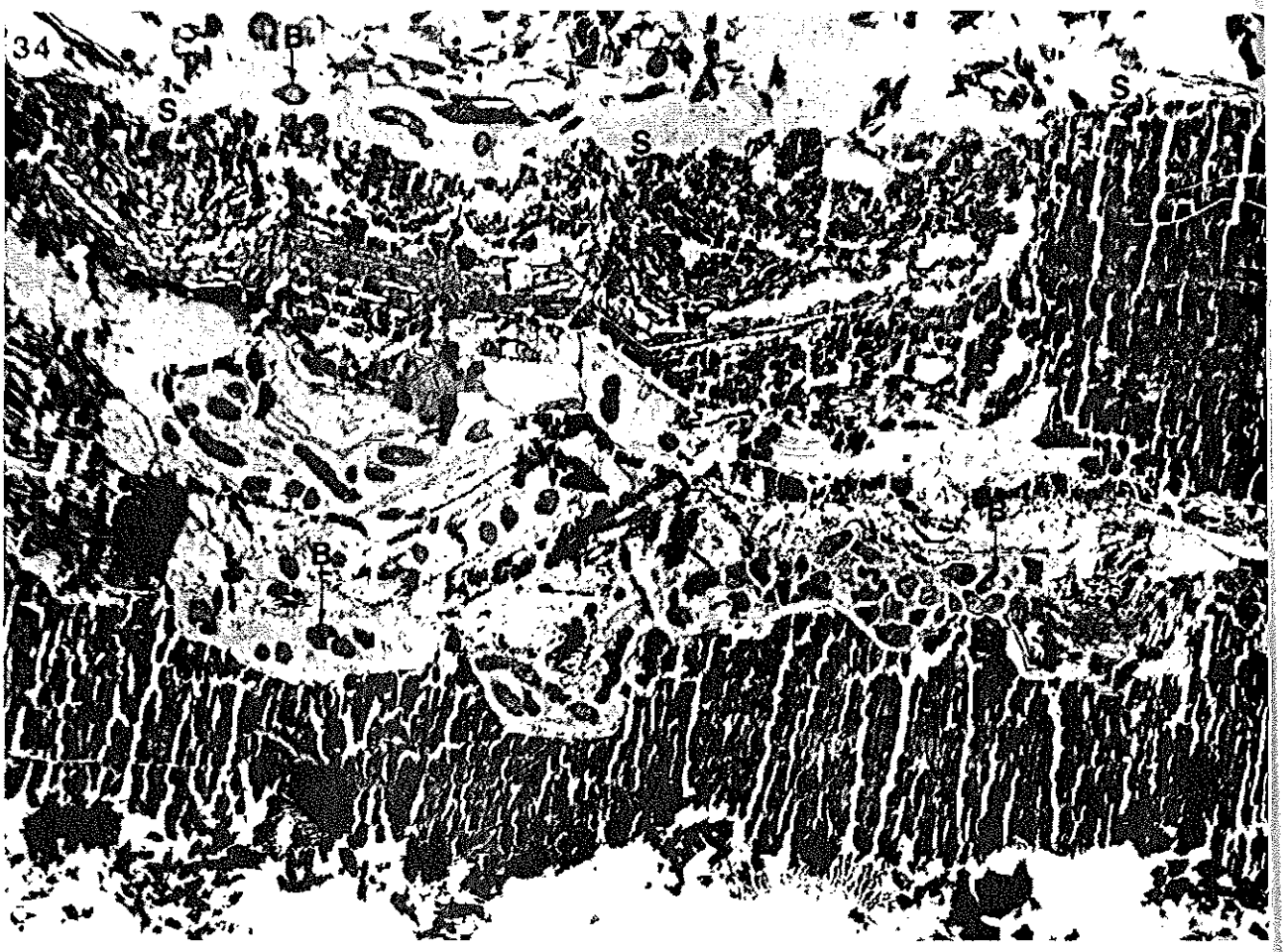

32
S
B
CT
C2

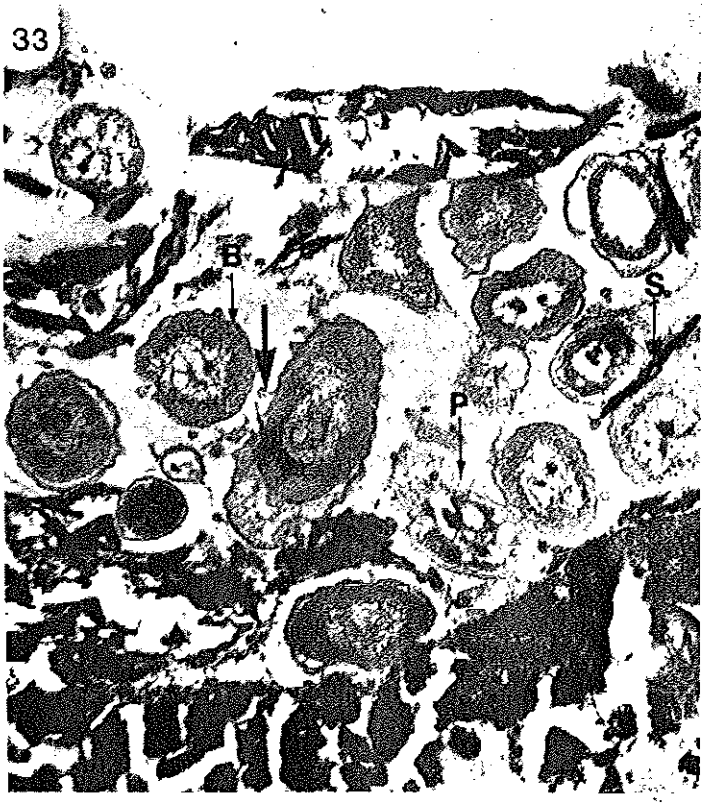
Plate 11.

Figure 33. Micrograph of slime film on copper-nickel exposed in the Thames for 15 weeks. Note gram-negative bacteria (B) producing fibrillar polysaccharide (P), cell wall protrusions (arrowed), silt particles (S) and corrosion product (C) (TEM x 8000).

Figure 34. Corrosion layers on copper-nickel exposed in the Thames for 70 weeks, showing bacteria (B) in slime film on surface of corrosion products (S) and between corrosion layers. (TEM x 3000).

Figure 35. Transmission electron micrograph of a discontinuity in the surface layer of corrosion products on 90/10 copper-nickel arrowed also showing bacteria (B) between corrosion layers, (TEM x 7000).


34
B
S
S
S
B
B


33
B
S
P

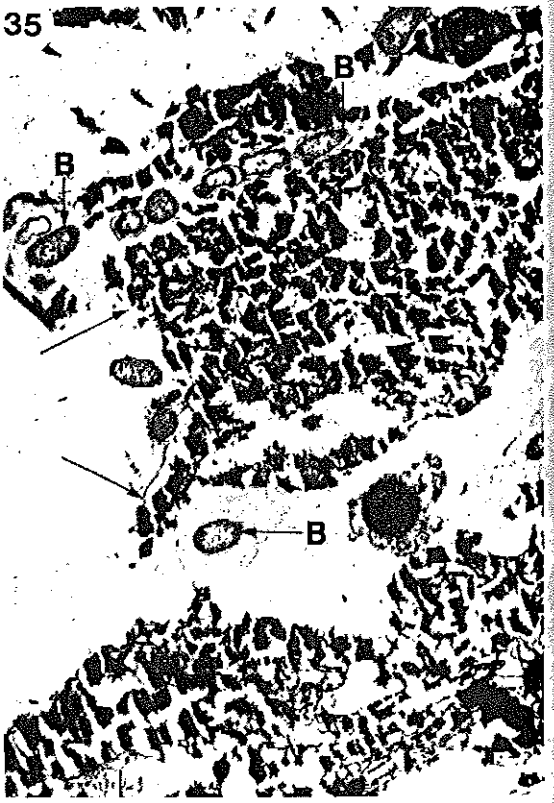

35
B
B
B

Plate 12.

Figure 36. Photograph showing separation of proteins from untreated *Amphiprora* cultures by gel electrophoresis.

Figure 37. Photograph showing separation of proteins from *Amphiprora* cultures treated with 20 ppm (g/ml) Cu as $CuCl_2$. Note the appearance in the gel of two extra protein bands (a, b) compared with the profile from untreated cells. Arrows indicate position of bromophenol blue marker.

36

37

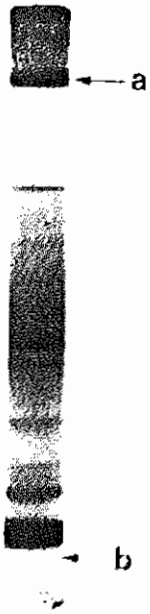


Plate 13.

Figure 38. Photograph showing the appearance of titanium disc after 121 weeks exposure in the sea. Note growth of algae and large amount of silt on surface of the disc.

Figure 39. Aluminium brass disc at 121 weeks. Note the growth of algae in the centre of the disc on yellow corrosion layer. However, parts of the disc appeared to be free from fouling.

Figure 40. Copper-nickel disc at 130 weeks. Note that only parts of the disc are fouled. Large areas of the disc appear to be bright and clean whilst fouling occurs only on patches of the discs which are yellow (arrowed).

Figure 41. Copper disc at 130 weeks. Most of the disc is covered in a green coloured corrosion layer. Parts of this corrosion layer has been colonized by diatoms (light brown area, arrowed) and by algae (A). Note that part of this corrosion layer has peeled/sloughed off revealing a bright clean surface in the centre of the disc Areas on disc which are eliptical in shape are areas where the disc has been clamped to frame.

38

39

40

41

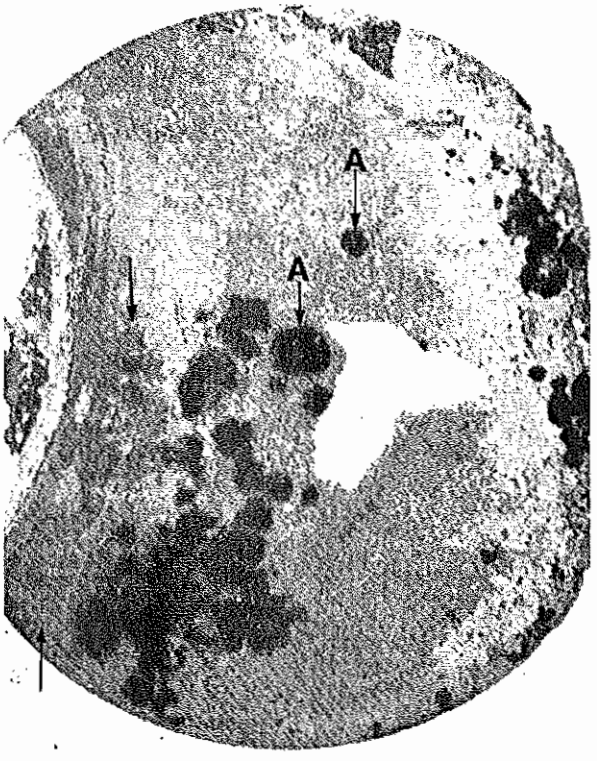
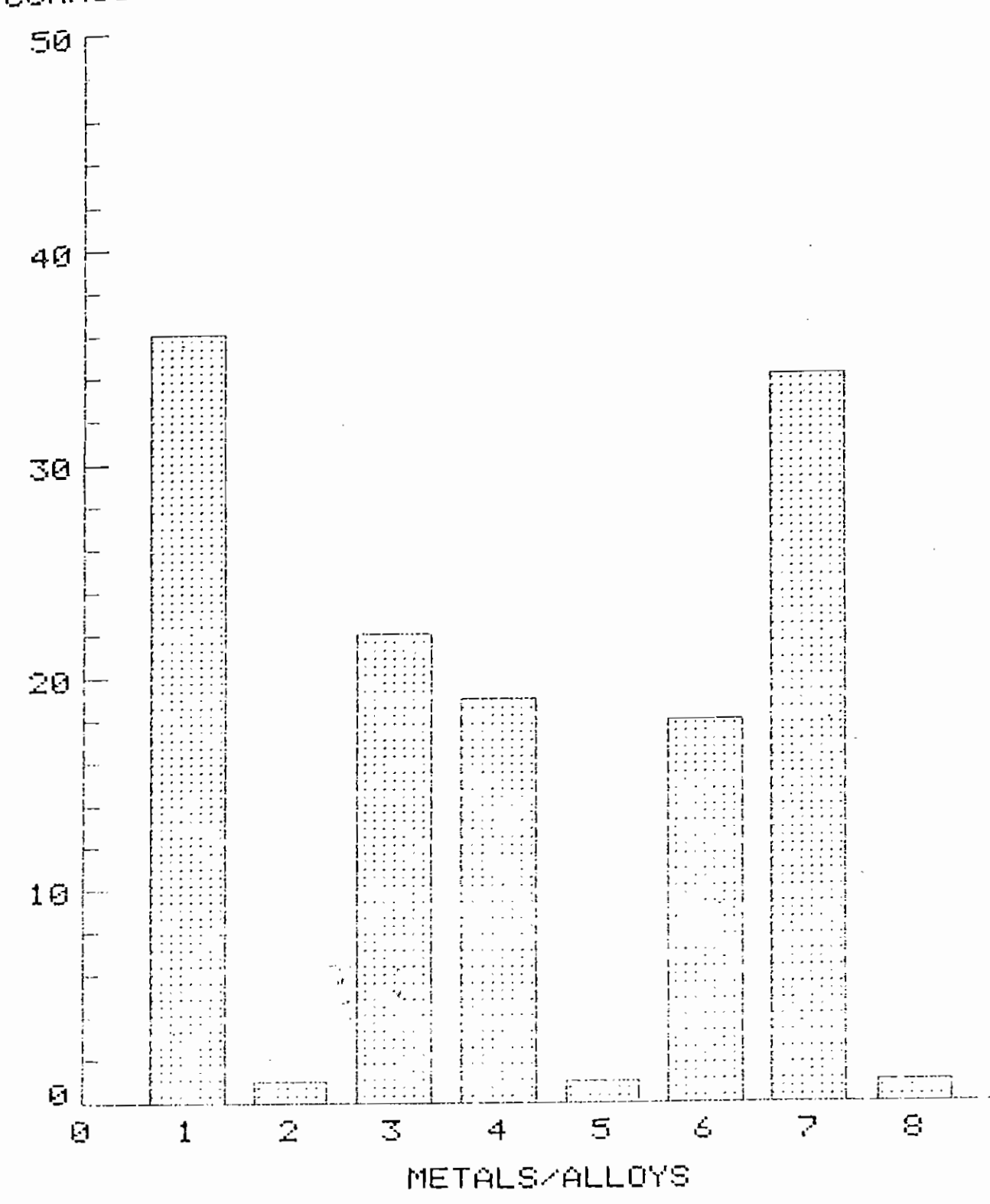
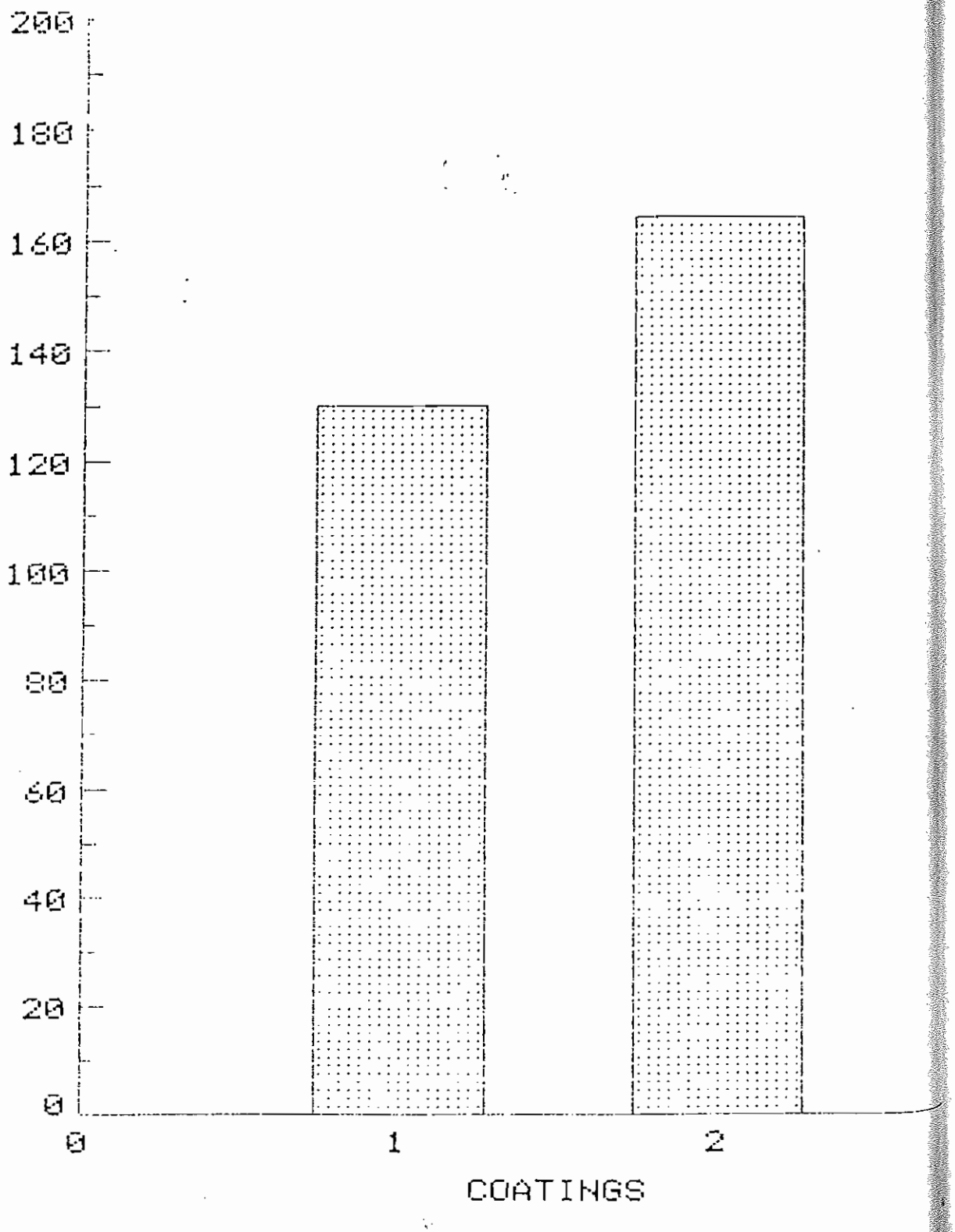


FIGURE 42. CORROSION THICKNESS ON METALS/ALLOYS



1. Copper 2. Titanium 3.. 90/10 Copper-Nickel 4. Cunifer 30
5. Aluminium brass 6. 70/30 Copper-Nickel 7. Cunifer 10 8. stainless steel.

FIGURE 43. BIOFILM THICKNESS ON COATINGS



1. Gel coat.

2. Flow coat.